Current Topics in Microbiology and Immunology

Marc Daëron
Falk Nimmerjahn *Editors*

Fc Receptors

Springer

Current Topics in Microbiology and Immunology

Volume 382

For further volumes:
http://www.springer.com/series/82

Marc Daëron · Falk Nimmerjahn
Editors

Fc Receptors

Responsible Series Editor: Bernard Malissen

Springer

Editors
Marc Daëron
Institut Pasteur
Paris
France

and

Inserm, U1104
Centre d'Immunologie de
Marseille-Luminy
Marseille
France

Falk Nimmerjahn
Department of Biology Institute of Genetics
University of Erlangen-Nürnberg
Erlangen
Germany

ISSN 0070-217X ISSN 2196-9965 (electronic)
ISBN 978-3-319-07910-3 ISBN 978-3-319-07911-0 (eBook)
DOI 10.1007/978-3-319-07911-0
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014943921

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

High amounts of specific antibodies are produced upon antigen stimulation during adaptive immune responses. Antibody production can rapidly resume, long after a primary response, and in higher amounts, during memory responses. Altogether, antibodies of every individual recognize a wide repertoire of antigens encountered during life, including autoantigens. As a result, several grams of antibodies with multiple specificities per liter of plasma circulate throughout the body via the bloodstream. Antibodies can reach remote organs in the periphery within minutes. They are involved in a variety of biological responses in health and disease. They can both protect from infections and induce allergic, autoimmune, or other inflammatory diseases. Genetically engineered monoclonal antibodies are increasingly used in passive immunotherapy, mostly, but not exclusively in cancer. Immunoglobulins pooled from the plasma of thousands of normal donors are injected intravenously (IVIg) as an anti-inflammatory treatment in an increasing number of autoimmune diseases. Actively produced specific antibodies account for the therapeutic effects of the overwhelming majority of protective vaccines, whether prophylactic or therapeutic. How antibodies work, however, is far from being fully understood and appreciated.

Antibodies bind to specific antigens by their Fab portions with a wide range of affinities. Binding is necessary for antibodies to act on antigens. Binding, however, is not sufficient. Antibodies indeed exert little or no effect when binding to antigen only. They have no biological activities per se. Antibodies, however, *mediate* many biological activities. They are mediators rather than effectors of adaptive immunity. Biological activities mediated by antibodies require their Fc portion. The Fc portion of immunoglobulins consists of the C-terminal constant domains of the two heavy chains that are characteristic of antibody classes and subclasses. Antibody-mediated biological activities indeed depend on the class of antibodies. The reason is that the Fc portion of antibodies of different classes differentially interacts with other molecules that can induce a variety of effector functions. These molecules are of two types: soluble molecules such as components of complement, and Fc Receptors (FcRs) expressed on the membrane of various cells.

For long, the existence of FcRs has been inferred from the observed biological effects of so-called “cytophilic” antibodies. In spite of the classical opposition between cell-mediated immunity and humoral immunity, some biological properties of antibodies were indeed found to depend on cells. When binding to

antigens, antibodies called "opsonins"—literally, which prepare the food to be ingested—enabled phagocytes to internalize particulate antigen-antibody complexes. Anaphylaxis and antibody-dependent cell-mediated cytotoxicity were found to result from the release of vasoactive and cytotoxic mediators, respectively, stored in the granules of different cell types. There were "homocytotropic" antibodies, which triggered responses in homologous tissues, and "heterocytotropic" antibodies, which triggered responses in heterologous tissues; there were antibodies whose cytophilic properties were heat-labile and antibodies which were heat-stable; there were washing-resistant and washing-sensitive cell-sensitizing antibodies, all of which could trigger similar responses but under different conditions. There were also enhancing and regulatory antibodies of different IgG subclasses. Although the concept of receptors for the Fc portion of cytophilic antibodies was proposed to account for the enhanced internalization of opsonized antigens by macrophages in the 1960s (Berken and Benacerraf 1966), the term Fc Receptors was not coined until 1972 by Frixos Paraskevas to describe IgG receptors on B lymphocytes (Paraskevas et al. 1972). By being given a name, FcRs gained a material existence. They could be identified on cell membranes and they became susceptible to molecular analysis.

FcRs for the various classes of immunoglobulins were indeed identified using several means to visualize cell-bound antibodies. FcRs with a high affinity were first found on a limited number of cells by assessing the binding of radiolabeled immunoglobulins. Using this approach, IgG and IgE receptors were found on macrophages and mast cells, respectively. Homogeneous cell lines made it possible to assess FcR numbers on single cells, to measure association and dissociation constants, and thus to calculate affinity constants. These were between 10^8 (Unkeless and Eisen 1975) and 10^{10} M^{-1} (Kulczycki and Metzger 1974).

Many more receptors for the same and for other immunoglobulin isotypes were subsequently identified by assessing the binding of red cells sensitized with antibodies under the microscope. These receptors had no measurable affinity for radiolabeled monomeric immunoglobulins, but they could bind multivalent immune complexes with high avidity. Using this "rosetting" procedure, all myeloid cells and some lymphoid cells expressed FcRs, and FcRs for all five immunoglobulin classes were recognized. These findings led to the distinction of high-affinity receptors referred to as FcRI, which bind antibodies as monomers, and of low-affinity receptors referred to as FcRII, which bind antigen-antibody complexes only. FcRs were also found on parasites (Torpier et al. 1979; Vincendeau and Daëron 1989), bacteria (Langone 1982), and even virus-encoded FcRs were described on infected cells (McTaggart et al. 1978; Litwin et al. 1990; Litwin and Grose 1992).

When monoclonal antibodies were raised against FcRs, cell population analysis by flow cytometry confirmed the distinction between high- and low-affinity FcRs and their differential tissue distribution. It also revealed a further heterogeneity amongst low-affinity receptors for IgG expressed by different cell types (Unkeless et al. 1988). Low-affinity FcRs were therefore subdivided into FcγRII and FcγRIII. More recently another high-affinity receptor for IgG found in mice but not in

humans, was named FcγRIV (Nimmerjahn et al. 2005). Human FcRs identified by referenced monoclonal antibodies were given CD numbers and used as phenotypic markers of cell populations: CD16 corresponds to FcγRIII, CD32 to FcγRII, CD64 to FcγRI, CD23 to FcεRII and CD89 to FcαRI. FcεRI have no CD number.

As FcRs were increasing in numbers, biochemical analysis disclosed their molecular heterogeneity. High-affinity IgE receptors first (Holowka et al. 1980), then IgG (Ernst et al. 1993) and IgA receptors (Pfefferkorn and Yeaman 1994) were found to contain several polypeptides. Most are composed of 2–3-extracellular-domain immunoglobulin-binding FcRα subunit noncovalently associated with a widely expressed, highly conserved homodimeric common subunit named FcRγ (Orloff et al. 1990) and, when expressed in mast cells or basophils, with a 4-transmembrane subunit named FcRβ whose expression is restricted to these cells (Kurosaki et al. 1992). cDNAs encoding the various FcR subunits having been cloned and expressed in different cells, their functional roles could be analyzed. FcRγ and FcRβ were found to control both the membrane expression of FcRα (Takai et al. 1994; Kinet 1999) and the ability of membrane FcRs to generate activation signals when engaged by antigen–antibody complexes. FcRγ and FcRβ were indeed shown to contain Immunoreceptor Tyrosine-based activation Motifs (ITAMs) (Reth 1989). Two single-chain low-affinity IgG receptors expressed in humans only also contained one ITAM, whereas another single-chain low-affinity IgG receptor expressed in mice and humans was found to contain an Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) (Daëron et al. 1995). Other receptors triggered neither activation nor inhibition signals, but permitted a strictly controlled internalization of antibodies. The 5 extracellular domain-containing polyIg receptor enables pentameric IgM and dimeric IgA to transcytose through polarized cells (Brandtzaeg 1983), whereas the β2-microglobulin-associated MHC-I-like FcRn not only mediates the intestinal absorption of maternal IgG through the fetal gut epithelium, but protects IgG from degradation in adults (Raghavan et al. 1993; Roopenian et al. 2003).

When FcR genes were cloned, their phylogenetic relationship was established (Qiu et al. 1990) and their heterogeneity was further enriched. Capital letters were added to FcR names to designate human genes and their murine orthologs. More recently, a novel family of FcR-like (FCRL) molecules was disclosed in mice and humans, which dramatically expanded the FcR field (Ehrhardt et al. 2007). Many FCRLs still have no known ligand. Some can bind immunoglobulins. They have similar structures, similar signaling properties and similar genetic organizations as classical FcRs, but also marked differences. Genetic polymorphisms were unraveled in classical human FcRs, some of which were associated with disease, mostly autoimmune diseases, and/or with a differential efficacy of therapeutic antibodies. FcR knockout, knockin, and transgenic mice were genetically engineered that proved to be invaluable analytical tools to assess FcR function in vivo. Special efforts have been made to generate humanized mice in which murine FcRs have been more or less extensively replaced by human FcRs, sometimes with the same tissue distribution as in humans. These unique mice should be a major advance to analyze the contribution of the various FcRs to the protective and the pathogenic roles of antibodies in mouse models of human diseases. They should be also of

interest to tailor and to assess the efficacy of novel therapeutic antibodies for specific purposes. Indeed, nonhuman primates that are often viewed as the best animal models for preclinical vaccine trials, seem not to have the same FcRs as humans (Trist et al. 2014).

As knowledge on FcRs progressed, the complexity of the mechanisms by which antibodies work increased dramatically. The difficulty to understand their biological effects in health and disease and to use them as therapeutic tools increased in parallel. As a consequence, it became difficult for most scientists, including immunologists, to embrace the multifaceted and often antagonistic properties of antibodies. The aim the this issue of Current Topics in Microbiology and Immunology on FcRs was to gather in a single volume the contributions of internationally recognized FcR experts on essential novel aspects of FcR biology in physiology and pathology. To our knowledge, such a book has not been published for many years. This volume is divided into five parts, which, we believe, cover the main aspects of current knowledge on FcRs.

The Part I titled *Old and New FcRs* contains three chapters. It provides novel information on old receptors and information on novel FcRs. Hiromi Kubagawa et al. report their recent findings on human and murine FcμR. The existence of this long suspected receptor for IgM now lies on solid grounds. It also has unexpected properties that other FcRs do not have. Randall Davis et al. provide a state-of-the-art overview of the FCRL family with their known ligands, and they discuss their potential functions. Finally, Leo James describes TRIM21. This intriguing intracellular receptor with an extraordinarily high affinity for IgG and IgM has unique structural and functional properties that endow it with major protective properties, especially against viral infection.

The Part II deals with *FcR Signaling*. It also contains three chapters. Denis Thieffry et al. present their novel bioinformatic approach of FcR signaling, using high-affinity IgE receptors in mast cells as a model. They show how computational modeling can help to integrate the complexity of signaling pathways. Pierre Launay et al. focus their review on calcium channels that have long been known to be critical in FcR signaling. They discuss the role of novel channels that control intracellular calcium and how these channels are tightly regulated. Finally, Michael Huber et al. address the mechanisms by which the lipid phosphatase SHIP1 negatively regulates FcεRI signaling and how both the expression and function of SHIP1 are controlled. This hematopoietic cell-specific phosphatase is a major regulator of many signaling pathways, particularly but not only, in mast cells.

The Part III entitled *FcR Biology* deals with various FcR functions, mostly, but not exclusively under physiological conditions. It contains six chapters. Marc Daëron first discusses how FcRs function as adaptive immunoreceptors (with an adaptive specificity, structure and signaling) that trigger adaptive biological responses with an extensive combinatorial functional diversity, depending on the functional repertoire of FcR-expressing cells selected by antibodies. Pauline Rudd et al. provide a comprehensive overview of the role that glycosylation plays in FcR functions. The glycosylation of antibodies is well known to determine their

binding to FcRs. That of FcRs is much less known. The interplay between carbohydrate–carbohydrate and carbohydrate–protein interactions, in ligands and receptors, opens a novel field of investigation. Birgitta Heyman discusses how, depending on the antigen and on the antibody class, antigen–antibody complexes can exert potent adjuvant effects or, on the contrary, suppress antibody responses. Understanding the mechanisms behind these versatile effects is essential for antibody-based immunotherapy. Renato Monteiro et al. review the anti-inflammatory properties of IgA and IgA receptors. IgA indeed uses several receptors and several mechanisms to regulate inflammatory processes generated during immune responses and the resulting tissue damage observed in autoimmune and inflammatory diseases. Jeffrey Ravetch et al. discuss how humanized mice can be used to assess the role of IgG-FcγR interactions in the in vivo effects of therapeutic antibodies used in the clinic. They focus on such a mouse in which all the FcγRs have been deleted and replaced by human FcγRs with a human tissue distribution. Finally, Sally Ward et al. review the mechanisms used by FcRn to protect IgG from lysosomal degradation through recycling and transcytosis, to deliver antibodies across cellular barriers to sites of pathogen encounter, to maintain and regulate renal filtration and to present antigen. These multifaceted functions open new FcRn-targeted therapies.

The Part IV specifically deals with *FcRs and Disease*. It contains three chapters. Robert Kimberly et al. address the issue of FcR polymorphism in human diseases. They review the single nucleotide polymorphisms, as well as the copy number variations in classical FcRs, including FcRn, but also in FCRLs, and they discuss their roles in infectious and inflammatory diseases associated wit these genetic variations. René Toes et al. focus on the roles of autoantibody–FcR interactions in rheumatoid arthritis. Specifically, they show how anti-citrullinated protein antibodies determine joint damage through the interplay between activating an inhibitory receptors. Finally, Mark Hogarth et al. provide a comparative analysis of human and non-human primate FcγRs, in viral infection. They focus on the polymorphism of macaque FcγRs and HIV infection and discuss how faithful the macaque model is for designing safe and efficient HIV vaccine strategies.

The Part V bears on *FcRs and Therapeutic Antibodies*. It contains three chapters. Mark Cragg et al. first discuss how ITIM-containing inhibitory FcγRIIB, that were shown to decrease the efficacy of therapeutic antibodies such as Rituximab or Trastuzumab can, on the contrary, enhance that of antibodies against members of the TNF Receptor superfamily such as anti-CD40 antibodies. Jantine Bakema and Marjolein van Egmond review the mechanisms involved in FcR-dependent passive immunotherapy of cancer. They specifically focus on therapeutic antibodies of the IgA class, instead of the anti-tumor IgG antibodies that are commonly used. Finally, Falk Nimmerjahn et al. review the anti-inflammatory activity of normal IgG and, specifically, the role of IgG glycosylation in this property. They discuss how IgG sialylation critically determines the therapeutic effects of IVIg in several models of autoimmune diseases by affecting both innate and adaptive immune responses through several receptors and mechanisms.

We hope this volume will interest scientists and clinicians, immunologists and non-immunologists, who are willing to know more about FcRs and to master better antibodies for therapeutic purposes. We also wish they will share with us the pleasure we had to put these chapters together when they read them.

Marc Daëron
Falk Nimmerjahn

References

Berken A, Benacerraf B (1966) Properties of antibodies cytophilic for macrophages. J Exp Med 123(1):119–144

Brandtzaeg P (1983) The secretory immune system of lactating human mammary glands compared with other exocrine organs. Ann N Y Acad Sci 409:353–382

Daëron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S, Fridman WH (1995) The same tyrosine-based inhibition motif, in the intracytoplasmic domain of FcgRIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. Immunity 3:635–646

Ehrhardt GR, Leu CM, Zhang S, Aksu G, Jackson T, Haga C, Hsu JT, Schreeder DM, Davis RS, Cooper MD (2007) Fc receptor-like proteins (FCRL): immunomodulators of B cell function. Adv exp med biol 596:155–162

Ernst LK, Duchemin AM, Anderson CL (1993) Association of the high-affinity receptor for IgG (Fc gamma RI) with the gamma subunit of the IgE receptor. Proc Natl Acad Sci USA 90(13):6023–6027

Holowka D, Hartmann H, Kanellopoulos J, Metzger H (1980) Association of the receptor for immunoglobulin E with an endogenous polypeptide on rat basophilic leukemia cells. Journal of receptor research 1(1):41–68

Kinet JP (1999) The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. Annu Rev Immunol 17:931–972

Kulczycki A, Jr., Metzger H (1974) The interaction of IgE with rat basophilic leukemia cells. II. Quantitative aspects of the binding reaction. J Exp Med 140(6):1676–1695

Kurosaki T, Gander I, Wirthmueller U, Ravetch JV (1992) The beta subunit of the Fc epsilon RI is associated with the Fc gamma RIII on mast cells. J Exp Med 175(2):447–451

Langone JJ (1982) Protein A of Staphylococcus aureus and related immunoglobulin receptors produced by streptococci and pneumonococci. Adv Immunol 32:157–252

Litwin V, Grose C (1992) Herpesviral Fc receptors and their relationship to the human Fc receptors. Immunol Res 11(3-4):226–238

Litwin V, Sandor M, Grose C (1990) Cell surface expression of the varicella-zoster virus glycoproteins and Fc receptor. Virology 178(1):263–272

McTaggart SP, Burns WH, White DO, Jackson DC (1978) Fc receptors induced by herpes simplex virus. I. Biologic and biochemical properties. J Immunol 121(2):726–730

Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV (2005) FcgammaRIV: a novel FcR with distinct IgG subclass specificity. Immunity 23(1):41–51

Orloff DG, Ra CS, Frank SJ, Klausner RD, Kinet JP (1990) Family of disulphide-linked dimers containing the zeta and eta chains of the T-cell receptor and the gamma chain of Fc receptors. Nature 347(6289):189–191

Paraskevas F, Lee ST, Orr KB, Israels LG (1972) A receptor for Fc on mouse B-lymphocytes. J Immunol 108(5):1319–1327

Pfefferkorn LC, Yeaman GR (1994) Association of IgA-Fc receptors (Fc alpha R) with Fc epsilon RI gamma 2 subunits in U937 cells. Aggregation induces the tyrosine phosphorylation of gamma 2. J Immunol 153(7):3228–3236

Qiu WQ, de Bruin D, Brownstein BH, Pearse R, Ravetch JV (1990) Organization of the human and mouse low-affinity Fc gamma R genes: duplication and recombination. Science 248(4956):732–735

Raghavan M, Gastinel LN, Bjorkman PJ (1993) The class I major histocompatibility complex related Fc receptor shows pH-dependent stability differences correlating with immunoglobulin binding and release. Biochemistry 32(33):8654–8660

Reth M (1989) Antigen receptor tail clue. Nature 338(6214):383–384

Roopenian DC, Christianson GJ, Sproule TJ, Brown AC, Akilesh S, Jung N, Petkova S, Avanessian L, Choi EY, Shaffer DJ, Eden PA, Anderson CL (2003) The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. J Immunol 170(7):3528–3533

Takai T, Li M, Sylvestre D, Clynes R, Ravetch JV (1994) FcR gamma chain deletion results in pleiotrophic effector cell defects. Cell 76(3):519–529

Torpier G, Capron A, Ouaissi MA (1979) Receptor for IgG(Fc) and human beta2-microglobulin on S. mansoni schistosomula. Nature 278(5703):447–449

Trist HM, Tan PS, Wines BD, Ramsland PA, Orlowski E, Stubbs J, Gardiner EE, Pietersz GA, Kent SJ, Stratov I, Burton DR, Hogarth PM (2014) Polymorphisms and interspecies differences of the activating and inhibitory FcgammaRII of Macaca nemestrina influence the binding of human IgG subclasses. J Immunol 192(2):792–803

Unkeless JC, Eisen HN (1975) Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages. J Exp Med 142:1520–1532

Unkeless JC, Scigliano E, Freedman VH (1988) Structure and function of human and murine receptors for IgG. Annu Rev Immunol 6:251–281

Vincendeau P, Daëron M (1989) Trypanosoma musculi co-express several receptors binding rodent IgM, IgE, and IgG subclasses. J Immunol 142(5):1702–1709

Contents

Part I Old and New FcRs

The Old but New IgM Fc Receptor (FcμR) 3
Hiromi Kubagawa, Yoshiki Kubagawa, Dewitt Jones, Tahseen H. Nasti, Mark R. Walter and Kazuhito Honjo

Emerging Roles for FCRL Family Members in Lymphocyte Biology and Disease 29
F. J. Li, W. J. Won, E. J. Becker Jr., J. L. Easlick, E. M. Tabengwa, R. Li, M. Shakhmatov, K. Honjo, P. D. Burrows and R. S. Davis

Intracellular Antibody Immunity and the Cytosolic Fc Receptor TRIM21 51
Leo C. James

Part II FcR Signaling

Computational Modeling of the Main Signaling Pathways Involved in Mast Cell Activation 69
Anna Niarakis, Yacine Bounab, Luca Grieco, Romain Roncagalli, Anne-Marie Hesse, Jérôme Garin, Bernard Malissen, Marc Daëron and Denis Thieffry

Calcium Channels in Fc Receptor Signaling 95
Tarik Attout, Andres Floto and Pierre Launay

Regulation of FcεRI Signaling by Lipid Phosphatases 111
Marcel Kuhny, Carolin N. Zorn and Michael Huber

Part III FcR Biology

Fc Receptors as Adaptive Immunoreceptors 131
Marc Daëron

Glycosylation and Fc Receptors 165
Jerrard M. Hayes, Eoin F. J. Cosgrave, Weston B. Struwe, Mark Wormald, Gavin P. Davey, Roy Jefferis and Pauline M. Rudd

Antibodies as Natural Adjuvants 201
Birgitta Heyman

IgA, IgA Receptors, and Their Anti-inflammatory Properties 221
Sanae Ben Mkaddem, Ivy Christou, Elisabetta Rossato, Laureline Berthelot, Agnès Lehuen and Renato C. Monteiro

Humanized Mice to Study FcγR Function 237
Stylianos Bournazos, David J. DiLillo and Jeffrey V. Ravetch

FcRn: From Molecular Interactions to Regulation of IgG Pharmacokinetics and Functions 249
Dilip K. Challa, Ramraj Velmurugan, Raimund J. Ober and E. Sally Ward

Part IV FcRs and Disease

Human FcR Polymorphism and Disease 275
Xinrui Li, Andrew W. Gibson and Robert P. Kimberly

Bridging Autoantibodies and Arthritis: The Role of Fc Receptors 303
Hanane el Bannoudi, Andreea Ioan-Facsinay and René E. M. Toes

The FcγR of Humans and Non-human Primates and Their Interaction with IgG: Implications for Induction of Inflammation, Resistance to Infection and the Use of Therapeutic Monoclonal Antibodies 321
P. Mark Hogarth, Jessica C. Anania and Bruce D. Wines

Part V FcRs and Therapeutic Antibodies

FcγRIIB as a Key Determinant of Agonistic Antibody Efficacy 355
Ann L. White, Stephen A. Beers and Mark S. Cragg

Fc Receptor-Dependent Mechanisms of Monoclonal Antibody Therapy of Cancer 373
Jantine E. Bakema and Marjolein van Egmond

Sweet and Sour: The Role of Glycosylation for the Anti-inflammatory Activity of Immunoglobulin G 393
Sybille Böhm, Daniela Kao and Falk Nimmerjahn

Thematic Index 419

Part I
Old and New FcRs

The Old but New IgM Fc Receptor (FcμR)

Hiromi Kubagawa, Yoshiki Kubagawa, Dewitt Jones, Tahseen H. Nasti, Mark R. Walter and Kazuhito Honjo

Abstract IgM is the first Ig isotype to appear during phylogeny, ontogeny and the immune response. The importance of both pre-immune "natural" and antigen-induced "immune" IgM antibodies in immune responses to pathogens and self-antigens has been established by studies of mutant mice deficient in IgM secretion. Effector proteins interacting with the Fc portion of IgM, such as complement and complement receptors, have thus far been proposed, but fail to fully account for the IgM-mediated immune protection and regulation of immune responses. Particularly, the role of the Fc receptor for IgM (FcμR) in such effector functions has not been explored until recently. We have identified an authentic FcμR in humans using a functional cloning strategy and subsequently in mice by RT-PCR and describe here its salient features and the immunological consequences of FcμR deficiency in mice. Since the FcμR we cloned was identical to Toso or Fas inhibitory molecule 3 (FAIM3), there have been spirited debates regarding the real function of FcμR/Toso/FAIM3 and we will also comment on this topic.

H. Kubagawa (✉) · Y. Kubagawa · D. Jones · K. Honjo
Division of Laboratory Medicine, Department of Pathology,
University of Alabama at Birmingham, Birmingham, AL 35209, USA
e-mail: hiromikubagawa@uab.edu

T. H. Nasti
Department of Dermatology, University of Alabama at Birmingham,
Birmingham, AL 35209, USA

M. R. Walter
Department of Microbiology, University of Alabama at Birmingham,
Birmingham, AL 35209, USA

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_1,

Contents

1 Introduction 4
2 Human FcμR 5
2.1 Functional Cloning, Predicted Structure, and Biochemical Nature of FcμR 5
2.2 Exon Organization of *FCMR* 7
2.3 IgM-Binding (FcμR) Versus Anti-apoptotic (FAIM3/Toso) Functions 8
2.4 Comparison of the Ig-Like Domain Among FcμR, pIgR, and Fcα/μR 9
2.5 Conserved Ser and Tyr Residues in the FcμR Cytoplasmic Tail 11
2.6 Cellular Distribution 11
2.7 FcμR in CLL 13
2.8 Other IgM-Binding Proteins 14
3 Mouse FcμR 15
3.1 Preparation of Mouse FcμR cDNA, Its Stable Transductants and mAbs 15
3.2 IgM Ligand Binding of Mouse FcμR$^+$ Cells 18
3.3 Cellular Distribution 20
3.4 *Fcmr*-Deficient Mice 22
4 Epilogue 25
References 25

1 Introduction

Antibody has dual binding activities: to antigen via its amino terminal variable regions and to effector molecules such as Fc receptors (FcRs) via its carboxyl terminal constant regions. FcRs are expressed by many different cell types in the immune system, and their interaction with antibody can initiate a broad spectrum of effector functions that are important in host defense. These functions include phagocytosis of antibody-coated microbes, lysosomal degradation of endocytosed immune complexes, antibody-dependent cell-mediated cytotoxicity, secretion of cytokine and chemokines, release of potent inflammatory mediators, enhancement of antigen presentation, and regulation of antibody production by B lymphocytes, and plasma cell survival. These diverse regulatory roles depend upon the antibody isotype and cellular distribution of the corresponding FcR. FcRs for IgG (FcγRI to FcγRIV), IgE (FcεRI) or IgA (FcαR) have been extensively characterized at both protein and genetic levels (see other chapters in this volume; Refs. (Ravetch and Kinet 1991; Daëron 1997; Monteiro and Van De Winkel 2003; Nimmerjahn et al. 2005)). IgM is the first antibody isotype to appear during phylogeny, ontogeny and immune responses, and the existence of an FcR for IgM (FcμR) on various cell types (B, T, NK cells, macrophages (Mφs), and granulocytes) has been suggested for decades with conflicting results (see Refs in (Kubagawa et al. 2009)). Thus, it has long been a puzzle why the gene encoding an FcμR has defied identification. In this article, we will describe the identification of an authentic FcμR, its genetic and biochemical features, and the cellular distribution and function of FcμR in both humans and mice.

2 Human FcμR

2.1 Functional Cloning, Predicted Structure, and Biochemical Nature of FcμR

In 1986 during her analysis of B cell activation antigen, Sheila Sanders, then a post-doctoral fellow in the laboratory of Max Cooper, serendipitously identified a single chain polypeptide of ~60 kDa by mouse IgM monoclonal antibodies (mAbs) irrespective of their antigen binding specificities that was expressed on the surface of human blood B cells following activation with phorbol myristate acetate (PMA) (Sanders et al. 1987). The ~60 kDa IgM binding protein was also detectable on freshly isolated chronic lymphocytic leukemia (CLL) B cells (see Sect. 2.7) and on the PMA-activated human pre-B cell line 697 (Sanders et al. 1987; Ohno et al. 1990). The IgM-binding by PMA-activated normal blood B or 697 pre-B cells as well as by CLL B cells could be demonstrated by flow cytometry using highly purified IgM preparations. Several attempts over the years to obtain protein sequence of this molecule to assist its molecular cloning met with failures. It was not until 2009 that we were able to identify the gene encoding the IgM binding protein. Two different cDNA libraries were constructed from CLL B cells and the PMA-activated 697 pre-B cell line and ligated into a retroviral expression vector before transfection into a packaging cell line and transduction into a mouse T cell line BW5147, which lacks IgM binding. The resultant IgM-binding transduced cells were initially present at very low frequency, but could be enriched by magnetic and fluorescence-activated cell sorting (FACS) and were finally subcloned by limiting dilution. Nucleotide sequence analyses of the ~2 kb insert cDNAs responsible for IgM binding in these single cell-derived subclones defined an identical 1,173-bp open reading frame in both cDNA libraries (Kubagawa et al. 2009).

The human FcμR cDNA encodes a 390-aa type I transmembrane protein (17-aa signal peptide, 234-aa extracellular region, 21-aa transmembrane segment, and 118-aa cytoplasmic tail) (Fig. 1a). The amino terminal half of the extracellular region contains a single V-set Ig-like domain with homology to two other IgM-binding receptors (the polymeric Ig receptor (pIgR) and the FcR for IgA and IgM (Fcα/μR)), but the remaining extracellular region has no identifiable domain features, designated the "stalk" region in this article. The core peptide is predicted to have an M_r of ~41 kDa and an isoelectric point (pI) of ~9.9. There are no N-linked glycosylation motifs (NxS/T; single aa letter code and x indicating any aa) in the extracellular region, consistent with results of our previous biochemical characterization of the IgM binding protein (Sanders et al. 1987; Ohno et al. 1990). By using both receptor-specific mAbs and IgM ligands, the surface FcμR expressed on FcμR cDNA-transduced cells, PMA-activated 697 pre-B cells, CLL B cells and blood mononuclear cells has an M_r of ~60 kDa on SDS-PAGE under both reducing and non-reducing conditions, albeit with a more intense signal under

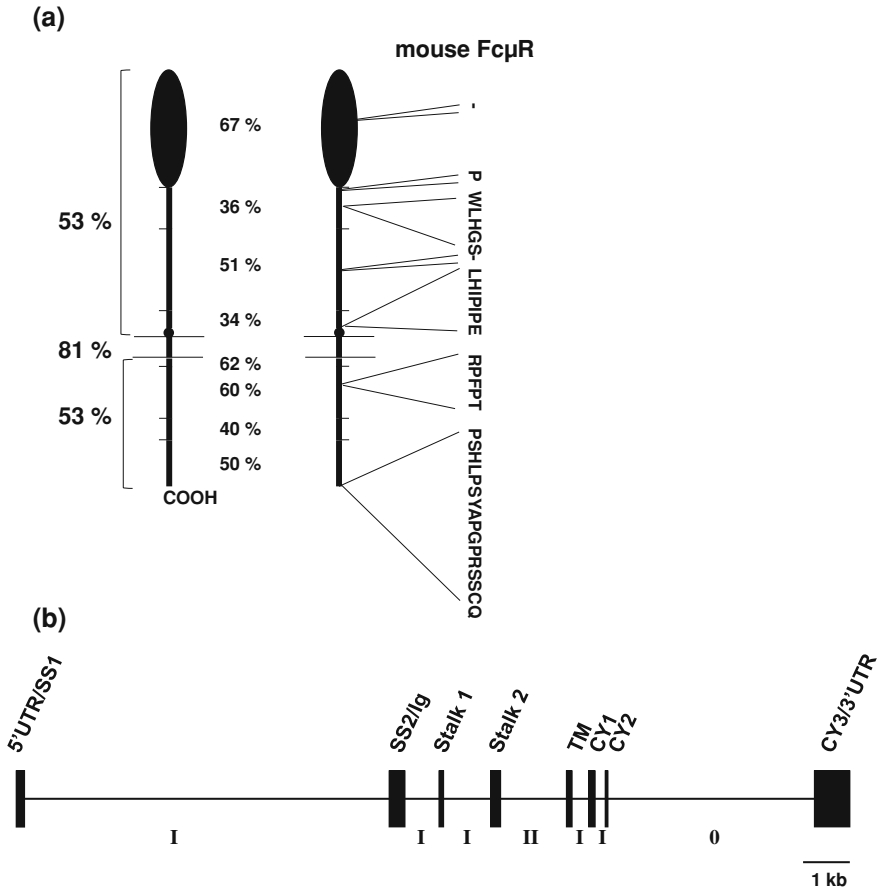


Fig. 1 *Schematic representation of FcμR.* **a** *Homology between human and mouse FcμR proteins. Left* FcμR is depicted as a badminton-like shape: amino terminal Ig-like domain (*black closed oval shape*), stalk region (above the *top line*), transmembrane (between the *two lines*) and cytoplasmic tail (below the *bottom line*). Hatch marks indicate exon boundaries and the small closed circle in the transmembrane region indicates a charged His residue. Numbers indicate the aa identity in the indicated regions between human and mouse receptors. *Right* The sites of additional aa or gaps in the mouse FcμR are shown by the single aa letter code or *dashes* (-), respectively. **b** *Schematic representation of the FCMR gene.* The exon (*black closed boxes*) organization of *FCMR* is drawn to the scale indicated, along with intron phases ("phase 0" indicating between codons; "phase I" between the first and second nucleotide of a codon; "phase II" between the second and third nucleotide). Exons encoding particular regions of the receptor are denoted as follows: the 5′ untranslated (5′UT), the signal peptide (SS1 and 2), the Ig-like domain (Ig), the uncharacteristic extracellular (stalk 1 and 2), the transmembrane (TM), the cytoplasmic (CY1–3), and the 3′ untranslated (3′UT) regions

reducing conditions, and into a spot with a pI of ∼5 on two-dimensional PAGE analysis, suggesting that one third of the M_r of the mature FcμR is made up of carbohydrate moiety containing many sialic acids (Kubagawa et al. 2009).

Removal of sialic acid by neuraminidase treatment of FcμR$^+$ cells slightly enhanced IgM binding, suggesting a role of sialic acid in this interaction.

Unlike our earlier observation that the IgM-binding protein on PMA-activated 697 pre-B cells could be attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) linkage (Ohno et al. 1990), the structure predicted by the FcμR cDNA is of a transmembrane protein. (Notably, Tetsuya Nakamura had previously noticed that the ~60 kDa IgM-binding protein on blood T cells, unlike PMA-activated 697 pre-B cells, was resistant to GPI-specific phospholipase C (GPI-PLC) (Nakamura et al. 1993)). We thus reexamined this issue using a highly purified GPI-PLC. After GPI-PLC treatment, the surface level of FcμR on both the BW5147 transductants and PMA-activated 697 pre-B cell line was unchanged, whereas the expression of the GPI-anchored Thy-1 or CD73 was reduced by ~65 %, indicating that FcμR is an genuine transmembrane protein, consistent with the predicted structure encoded by the FcμR cDNA (Kubagawa et al. 2009). We also searched for a cDNA encoding a potential GPI-linked form of FcμR but failed to identify it. However, the experimental data not relying on GPI-PLC in previous studies by Tatsuharu Ohno (Ohno et al. 1990) could not be ignored because the results were unambiguous and thus worthy of reconsideration. Namely, when cell surface-iodinated, PMA-activated 697 pre-B cells were incubated at 37 °C even without GPI-PLC, significant amounts of the ~60 kDa IgM-binding protein were released into the medium. Furthermore, the ~60 kDa IgM-binding protein was clearly precipitated by IgM-coupled beads from the culture supernatants of metabolically labeled, PMA-activated 697 pre-B cells. For direct comparison of FcμR in the cell lysates and supernatants, NP-40 detergent was also added into the supernatants to obtain equivalent conditions in SDS-PAGE analysis of immunoprecipitated materials (Ohno et al. 1990). Thus, it is quite conceivable that FcμR is released as small vesicles or exosomes from the plasma membrane upon certain types of activation. The physiological relevance of FcμR-containing exosomes, as well as of the soluble form of FcμR of ~40 kDa described in Sect. 2.7, might be to deliver IgM/antigen complexes to other cell types or to remotely modulate IgM-mediated immune regulation as a decoy receptor.

2.2 *Exon Organization of* FCMR

FCMR is a single copy gene located on chromosome 1q32.2, adjacent to two genes encoding other IgM-binding receptors, *PIGR* expressed on mucosal epithelium and *FCAMR* expressed on follicular dendritic cells (FDCs), separated by ~40 Mb from the cluster of genes encoding FcγRs, FcεRI, and FcR-like molecules on 1q21 to 1q23 (Kubagawa et al. 2009). *FCMR* spans ~17.6 kb and is composed of eight exons (Fig. 1b). Unlike the FcRs and most of their relatives, which as a conserved feature have a signal peptide encoded by two separate exons, the second of which is either a 21-bp or 36-bp "mini-exon," *FCMR* lacks this feature as do the *PIGR*

and *FCAMR* genes (Kikuno et al. 2007; Davis et al. 2001) The intron to exon length proportion of *FCMR* is ~8, suggesting that it belongs to the class of genes with relatively short introns, based on the fact that the average intron/exon ratio is ~26 with a median of ~11 (Castillo-Davis et al. 2002). Given the fact that the transcription process in eukaryotes is slow (~20 nucleotide transcribed per second) and biologically expensive (at least two ATP molecules per nucleotide), transcription of genes with short introns is thus less costly than those with long introns, which are particularly common in mammals (Ucker and Yamamoto 1984). Intriguingly, highly expressed genes have substantially shorter introns than genes expressed at low levels. In this regard, natural selection appears to favor short introns in highly expressed genes to minimize the cost of transcription and other molecular processes, such as splicing. Selection favoring short introns is particularly strong for genes that have to be expressed at high levels at short notice, such as stress-induced proteins. The *FCMR*, whose product is selectively expressed on adaptive immune cells, may thus belong to the intermediate response family of genes.

Other fundamental questions regarding the evolution of *FCMR* include: (i) do FcμR and IgM genes coevolve during evolution? If so, *Fcmr* should appear in jawed, but not jawless, vertebrates, i.e., from cartilaginous fish onwards. (ii) Is FcμR structurally conserved? (iii) Which cell types express FcμR? In mammals like humans and mice, do adaptive immune cells exclusively express FcμR (see below)? In this regard, the recent bioinformatics analysis revealed that both FcμR and Fcα/μR were suggested to appear during early mammalian evolution (Akula et al. 2014).

2.3 IgM-Binding (FcμR) Versus Anti-apoptotic (FAIM3/Toso) Functions

When we analyzed our FcμR cDNA sequence using the basic local alignment search technique (BLAST) database, to our surprise, it was identical to that of the previously identified human Fas apoptosis inhibitory molecule 3 (FAIM3), except for one nucleotide difference at a position reported as a synonymous single nucleotide polymorphism. FAIM3 was also identified in a similar cDNA library-based retroviral functional assay as a potent inhibitor of Fas/CD95-induced apoptosis and was originally designated as Toso after a Japanese liquor enjoyed on New Year's Day to celebrate long life and eternal youth (Hitoshi et al. 1998). However, apoptosis in this functional assay was induced by ligation of Fas with an agonistic IgM mAb (CH11 clone), raising the concern that the CH11 mAb bound the Fas receptor via its variable Fabμ region and also to FAIM3/Toso via its constant Fcμ region.

To reconcile the conflicting FcμR functions, IgM Fc binding (FcμR) versus inhibition of Fas apoptosis (FAIM3/Toso), we first examined the $FcμR^+$ BW5147 cells for their Ig-binding specificity. The $FcμR^+$ cells clearly boud IgM, but not other Ig isotype (IgG1-4, IgA1,2, IgD, or IgE), in a dose-dependent manner and the IgM binding was mediated by its Fc5μ fragments consisting mostly of Cμ3/Cμ4 domains, but not by Fabμ fragments, thereby confirming its IgM Fc-binding

activity. FcμR binds IgM pentamers with a strikingly high avidity of ~10 nM as determined by Scatchard plot analysis with the assumption of a 1:1 stoichiometry of FcμR to IgM ligand (Kubagawa et al. 2009). Next, to determine if the FcμR has anti-apoptotic activity, we first repeated the experiment of Hitoshi et al. (1998) and introduced the FcμR cDNA into an apoptosis-prone human T cell line Jurkat. Ligation of Fas with the CH11 IgM mAb induced robust apoptotic cells in the control cells, but not in the FcμR$^+$ cells (Fig. 2), consistent with the reported anti-apoptotic activity of FAIM3/Toso. However, ligation of Fas with an agonistic IgG3 mAb (2R2), which should have the same biological effect as the CH11 mAb, or with the recombinant Fas ligand induced apoptosis in both FcμR$^+$ and control cells, indicating that FAIM3/Toso is not an anti-apoptotic protein (Kubagawa et al. 2009; Honjo et al. 2012a). Since FcμR$^+$ Jurkat cells used in these experiments bound IgM and were reactive with receptor-specific mAbs, whereas control Jurkat cells were not, these results taken together clearly demonstrated that the FAIM3/Toso is an authentic IgM Fc binding protein. This conclusion was recently confirmed by others (Vire et al. 2011; Murakami et al. 2012). Similar results with agonistic IgM versus IgG3 anti-Fas mAb were also observed with Epstein Barr virus-transformed B cell lines expressing both endogenous FcμR and Fas on their cell surface. Thus, the correct functional designation of this gene product should be FcμR and not FAIM3 or Toso.

2.4 *Comparison of the Ig-Like Domain Among FcμR, pIgR, and Fcα/μR*

The molecular mechanisms responsible for FcμR binding specificity will likely require crystal structure analysis of FcμR and the IgM/FcμR complex. Fortunately, the crystal structure of domain 1 (D1) of pIgR, which shares ~31 % aa sequence identity with the Ig-like domain of FcμR, has been solved and suggests the Ig-like domain of FcμR shares the same overall β-sandwich fold observed for the pIgR D1 (Hamburger et al. 2004). Sequence alignments and structural analysis suggest the FcμR Ig-like domain Cys residues (C^{37} and C^{104}) and (C^{49} and C^{58}) pair to form intra-chain disulfide bonds, as observed for pIgR. The Ig-like domain of FcμR also forms a salt bridge, between R^{75} and D^{98}, which is conserved in other Ig domains, including pIgR. However, notably missing from FcμR is the invariant Trp, which is a Leu (L^{48}) in FcμR. Several additional residues are conserved in pIgR and Fcα/μR sequences but not in FcμR (Fig. 3, shown in purple). The greatest difference between FcμR and the other two receptor is in the CDR1 regions. The CDR1 of the pIgR, and Fcα/μR, consists of nine amino acids (PPTSVNRHT for human pIgR). In contrast, the corresponding CDR1 region of FcμR consists of only five amino acids (PEMHV for human FcμR). Furthermore, an Arg found in CDR1 of pIgR, which is solvent exposed and thought to directly interact with polymeric IgA (Hamburger et al. 2004), has been replaced by a noncharged aa residue (M^{42} in humans or L^{42} in

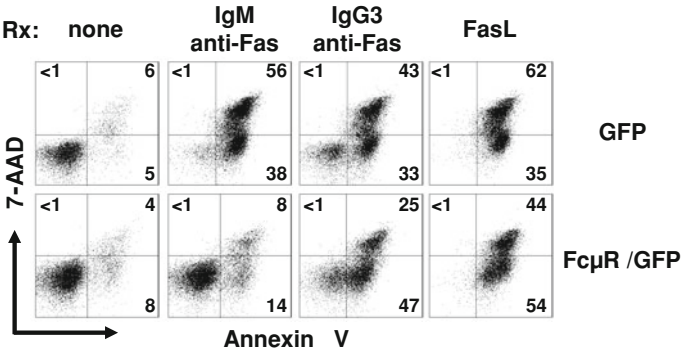


Fig. 2 *Role of FcμR in Fas-mediated apoptosis in Jurkat T cells*. Jurkat cells transduced with the bicistronic construct containing both human FcμR and GFP cDNA (FcμR/GFP) or only GFP (GFP) as a control were cultured at 37 °C for 20 h without (none) or with agonistic mouse anti-human Fas mAb of IgMκ (CH11; 10 ng/ml) or IgG3k isotype (2R2; 1 μg/ml) or with a recombinant human Fas ligand (FasL; 10 ng/ml). Cells were stained with 7-aminoactinomycin D (7-AAD) and allophycocyanin-labeled annexin V to identify early (annexin V^+/7-AAD^-) and late (annexin V^+/7-AAD^+) apoptotic and dead (annexin V^-/7-AAD^+) cells by flow cytometric analysis. Numbers indicate percentages of cells. Note the resistance of the FcμR/GFP transductant to Fas-mediated apoptosis by IgM mAb, but not by IgG3 mAb or FasL

```
                    20        30        40                  50        60
                    ..|....|....|....|....|....|     ....|....|....|....|....|...
Hu FcμR             VSGALRILPEVKVEGELGGSVTIKCPLP----EMHVRIYLCREMAGSGTCGTVVSTTNFI
Mo FcμR             ......V....QLNV.W...II.E....----QL...M....Q..KP.I.S....N.-.V
Hu pIgR             ---KSP.FGPEE.NSVE.N..S.T.YY.PTSVNR.T.K.W..QG-AR.G.I.LI.SEGYV
Mo pIgR             ---KSP.FGPQE.SSIE.D..S.T.YY.DTSVNR.T.K.W..QG-A..M.T.LI.SNGYL
Hu Fcα/μR           ---PNSLKGSRL.S..P..A...Q.HYAPSSVNR.Q.K.W..LGPPRWI.Q.I...NQYT
Mo Fcα/μR           ---TNALRGPRL.T.NT..A...H.HYAPSSVNR.Q.K.W..LGSPLWI.H.....NQYT

                    70        80        90        100       110       120
                    .|....|....|....|....|....|....|....|....|....|....|....
Hu FcμR             KAEYKGRVTLKQYPRKNLFLVEVTQLTESDSGVYACGAGMNTDRGKTQKVTLNVHS
Mo FcμR             .K..ER....TPCLD.K.....M.....N.D.I....V..K..K.....I.....N
Hu pIgR             SSK.A..AN.TNF.ENGT.V.NIA..SQD...R.K..L.I.SR-.LSFD.S.E.--
Mo pIgR             SK..S..AN.INF.EN.T.VINIE...QD.T.S.K..L.TSNR-.LSFD.S.E.--
Hu Fcα/μR           HHR.RD..A.TDF.QRG..V.RLS..SPD.I.C.L...I.SENN-MLFLSMN.TI--
Mo Fcα/μR           HPD.R..AA.TDI.QSG..V.RLLR.SLG.V.L.R...I.DRN.-MLFFS.N.T.--
```

Fig. 3 *Amino acid sequence alignment of IgM-binding receptors*. The Ig-binding domains of FcμR, pIgR and Fcα/μR of human and mouse origin are aligned to each other. The numbers indicate the aa position from the first Met residue of human FcμR (NP_005440). Amino acid identity is indicated by *dots* (·) and gaps by *dashes* (-). Residues conserved in all three receptors and in pIgR and Fcα/μR are highlighted in *yellow* and *purple*, respectively. Accession numbers of these sequences are: mouse FcμR (NP_081252); pIgR of human (P01833) and mouse (070570); Fcα/μR of human (AAL51154) and mouse (NP_659209)

mice) in FcμR. These molecular differences are consistent with the stringent specificity of FcμR for IgM compared with the promiscuous binding of pIgR and Fcα/μR to polymeric IgA and IgM.

2.5 Conserved Ser and Tyr Residues in the FcμR Cytoplasmic Tail

Unlike many paired receptors having a similar extracellular region but transmitting opposite signal potentials, such as FcγRs, NK cell receptors, and paired Ig-like receptors (Kubagawa et al. 1997), FcμR is unique in that a charged His residue (H^{253}) exists in the predicted transmembrane region and the cytoplasmic tail is relatively long (118 aa) and contains conserved residues, three Tyr and five Ser, when compared with FcμR from six different species (Fig. 4; Ref. (Kubagawa et al. 2009)). This suggests that FcμR may have a dual signaling capacity: one from a potential adaptor protein noncovalently associating with FcμR via the H^{253} residue, similar to the association of FcR common γ chain with FcγRI, and the other from its own Tyr and/or Ser residues in the cytoplasmic tail. In our previous studies, an $\sim$40 kDa membrane protein (p40) was often co-precipitated with the 60 kDa ligand-binding chain of FcμR but it remains unclear whether p40 represents another membrane protein non-covalently associated with FcμR or an unglycosylated form of FcμR (Kubagawa et al. 2009). The carboxyl terminal Tyr matches the recently described Ig tail tyrosine (ITT) motif (DYxN) in IgG and IgE isotypes (Engels et al. 2009), but the other two do not correspond to an ITAM ($D/Ex_2Yx_2L/Ix_{6\text{-}8}Yx_2L/I$), ITIM ($I/VxYx_2L/V$) or switch motif ($TxYx_2V/I$). Ligation of Fc$\mu$R with preformed IgM immune complexes induced the phosphorylation of both Tyr and Ser residues of the receptor (Kubagawa et al. 2009). Intriguingly, phosphorylated FcμR migrated on SDS-PAGE faster than the unphosphorylated form, unlike the findings that most proteins usually ran slower when phosphorylated. This observation suggests either that phosphorylation may cause a global structural change of FcμR leading to increased mobility as seen in CD45 on PMA-activated myeloid cells (Buzzi et al. 1992) or that proteolytic cleavage may occur in the cytoplasmic tail of FcμR after receptor ligation as observed in FcγRIIa on platelets (Gardiner et al. 2008). Upon IgM binding, FcμR was rapidly internalized, and this activity was mediated by the two carboxyl terminal Tyr residues, as determined by mutation analysis (Vire et al. 2011). Ligation of FcμR on NK cells with IgM immune complexes was shown to induce phosphorylation of PLCγ and Erk1/2 (Murakami et al. 2012).

2.6 Cellular Distribution

Given the fact that IgM is the first antibody isotype to appear during phylogeny, ontogeny, and immune responses and is the first line of defense against pathogens, it seemed reasonable to assume that FcμR would have a broad cellular distribution. Results from earlier studies with rosette formation using IgM-coated erythrocytes also suggested the existence of an FcμR on various cell types (B, T, NK, and phagocytic cells) in humans and rodents. Contrary to this assumption, however,

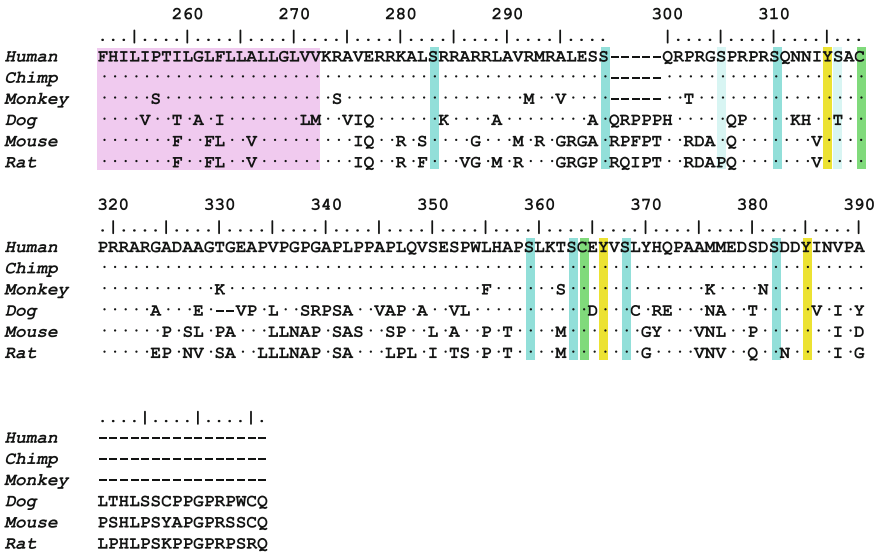


Fig. 4 *Amino acid sequence alignment of the transmembrane and cytoplasmic regions of FcμRs*. The transmembrane and cytoplasmic regions of FcμR from six mammalian species are aligned to each other. Amino acid identity is indicated by *dots* (·) and gaps by *dashes* (-). The predicted transmembrane region is colored in pink. Conserved Tyr, Ser and Cys residues are also highlighted in *yellow*, *dark or light blue*, and *green*, respectively. *Light blue* indicate conservation of Ser residues in five species. The numbers indicate the aa position from the first Met residue of human FcμR. Accession numbers of these sequences are: chimpanzee (chimp; XP_001165341), monkey (XP_001084243), dog (XP_547385), and rat (Q5M871)

current studies using FcμR-specific, RT-PCR primers and mAbs, have shown that FcμR is predominantly expressed by adaptive immune cells, both B and T lymphocytes and, to a lesser extent, NK cells, but not by other hematopoietic cells (i.e., myeloid and erythroid cells and platelets) (Kubagawa et al. 2009). (NK cells are the only known exception for FcμR expression by nonadaptive immune cells, but are now thought to have features of both adaptive and innate immune cells (Vivier et al. 2011)). Treatment of myeloid cells with various stimuli including PMA/ionomycin, LPS, mitogens and several cytokines did not induce the cell surface expression of FcμR. Thus, FcμR is the only FcR constitutively expressed on T cells of all cell types (i.e., αβ T, γδ T, CD4 T, CD8 T, Treg) which are generally negative for the expression of other FcRs. For B cells, FcμR is the only IgM-binding receptor expressed; Fcα/μR was initially thought to be expressed by B cells (Shibuya et al. 2000), but our subsequent analysis revealed that the major cell type expressing Fcα/μR is the FDC in both humans and mice (Kikuno et al. 2007). The restriction of FcμR expression to adaptive immune cells is remarkable, because FcR for the switched Ig isotypes (FcγRs, FcεRI, and FcαR) are expressed by various hematopoietic cells, including phagocytes, and are thought to be central mediators that couple innate and adaptive immune responses (Ravetch and Kinet 1991; Daëron 1997; Monteiro and Van De Winkel 2003; Nimmerjahn et al. 2005). The physiological relevance of such

restricted cellular expression of FcμR may be related to unique features of the IgM ligand, such as its early appearance during immune responses, the pentameric configuration of its secreted form and its potency in complement activation.

Many investigators had previously noticed the instability of IgM binding by B, T and NK cells (Nakamura et al. 1993; Moretta et al. 1977; Pricop et al. 1993). We also found that the cell surface FcμR levels were sensitive to extracellular IgM concentration, tissue milieu and cellular activation status (Kubagawa et al. 2009). This vulnerability could explain why FcμR was limited to an operationally defined entity for such a long time. Short-term culture in IgM-free media enhanced the cell surface expression of FcμR on T cells and, to a lesser extent, on B and NK cells. Remarkably, this phenomenon was much more pronounced with cells from tonsil and spleen; cell surface FcμR was not detectable on freshly isolated B and T cells from these organs, even with receptor-specific mAbs, but was easily demonstrated after overnight culture in IgM-free media. Many other cell surface antigens were detectable in the freshly isolated cell preparations, ruling out an artifact of tissue manipulation. To our knowledge, the IgM concentration in the interstitial spaces of such intact tissues has never been determined. If this in vivo down-modulation of FcμR is solely dependent on the extracellular concentration of IgM and not on the tissue microenvironment (e.g., resident proteases) or cellular activation status, then the interstitial IgM concentration in secondary lymphoid tissues is perhaps much higher than in blood (i.e., $>$ 2 mg/ml). In this regard, it is noteworthy that there are many IgM-producing plasma cells in the immediate vicinity of B and T cells within these lymphoid tissues.

2.7 FcμR in CLL

The association of FcμR with CLL has long been suggested based on the ability of CLL cells to form rosettes with IgM-coated erythrocytes (Pichler and Knapp 1977; Ferrarini et al. 1977; Burns et al. 1979; Rudders et al. 1980; Platsoucas et al. 1980). Unfortunately, this early intriguing suggestion was not pursued thereafter because of uncertainties with such a crude detection procedure. We thus reexamined the expression of FcμR by B and T cells in CLL patients using receptor-specific mAbs (Kubagawa et al. 2009; Li et al. 2011). CLL B cells ($CD5^+/CD19^+$) expressed much higher levels of cell surface FcμR than B cells from healthy donors. This enhanced expression was more evident in Ig heavy chain variable region (*IGHV*)-mutated, better prognostic, $CD38^-$ or early Rai-stage CLL than *IGHV*-unmutated, poor prognostic, $CD38^+$ or advanced Rai-stage CLL. Intriguingly, surface FcμR levels also were significantly elevated in the patients' non-CLL B cells ($CD5^-/CD19^+$) and T cells ($CD5^+/CD19^-$), especially in *IGHV*-mutated CLL, when compared with the corresponding populations in normal individuals. This increase in FcμR expression on T cells in CLL patients is unique, because normal T cells activated ex vivo with anti-CD3 mAb or PMA down-modulate surface FcμR, whereas B cells activated with anti-μ mAb or PMA up-regulate surface FcμR

(Kubagawa et al. 2009). According to the findings from the laboratory of Hassan Jumaa, the CLL-derived BCRs unlike those from other B cell malignancies, ligate each other via interactions between the Ig heavy chain CDR3 of one BCR and an intrinsic motif (W**VRQ**xPG; bold fonts indicating critical aa residues) in the framework region 2 of another BCR, thereby generating antigen-independent cell-autonomous signaling (Duhren-von et al. 2012). Thus, this antigen-independent self-ligation of BCR on CLL cells could account for enhanced expression of cell surface FcμR as well as for the well-known phenomenon of reduced levels of cell surface IgM and IgD on CLL cells. It remains unclear, however, why surface FcμR levels are also elevated on non-CLL B cells and T cells in *IGHV*-mutated CLL patients.

CLL patients also had high serum titers of FcμR compared with healthy donors as determined by sandwich ELISA using two different receptor-specific mAbs. Serum FcμR levels correlated significantly with circulating lymphocyte numbers but not with *IGHV* mutation status or Rai stage. The serum FcμR was resolved as an ~40 kDa protein, distinct from the cell surface FcμR of ~60 kDa, and was produced by both CLL B and non-CLL B cells. Proteomic analysis revealed that the serum FcμR was a soluble form of the receptor encoded by an alternatively spliced FcμR transcript, which resulted from the direct splicing of exon 4 (stalk 2) to exon 6 (CY1), skipping exon 5 (TM), thereby causing a reading frame shift in exon 6 and generating a novel 70-aa hydrophilic carboxyl terminal tail (see Fig. 1b). Collectively, these findings indicate enhanced levels of both membrane-bound and soluble forms of FcμR in CLL patients (Li et al. 2011). The molecular basis for such enhanced soluble FcμR production in CLL patients and possible clinical effects of soluble FcμR on the immune responses of CLL patients remain to be explored. In this regard, it has recently been reported that administration of a recombinant soluble fusion protein consisting of the extracellular portion of human FcμR and the IgG Fc portion ameliorates myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalitis in mice (Brenner et al. 2014). In this regard, various forms of immune-mediated peripheral neuropathy are known to be associated with monoclonal IgM antibodies against myelin-associated glycoproteins, gangliosides or related glycolipids and these demyelinating neuropathies are a clear example of disease association with IgM autoantibodies (Ramchandren and Lewis 2009; Maurer et al. 2012).

2.8 Other IgM-Binding Proteins

In addition to pIgR and Fcα/μR, other proteins have been shown to bind IgM. CD22/siglec-2 is a B cell membrane-bound lectin recognizing glycan ligands containing α2,6-linked sialic acid and was shown to interact with glycan ligands on soluble IgM/antigen complexes, thereby negatively regulating BCR signaling similar to FcγRIIB (Adachi et al. 2012; Poe and Tedder 2012). Other IgM-binding proteins have also been demonstrated in other cell types. For example, tripartite motif-containing protein 21 (TRIM21)/Ro52 (see the chapter by Dr. James) binds

antibody-opsonized pathogens and targets them to proteasomal degradation in phagocytes, and thus, TRIM21/Ro52 behaves as a cytosolic FcR for IgG and IgM (McEwan et al. 2013; Randow et al. 2013). Apoptosis inhibitor of Mφs (AIM) or soluble protein α (Spα) is a member of the group B scavenger receptor cysteine-rich superfamily and is a glycoprotein of ∼45 kDa secreted by Mφs. In addition to support the survival of Mφs, AIM/Spα is shown to bind serum IgM but not IgG or IgA (Tissot et al. 2002; Martinez et al. 2011; Miyazaki et al. 2011). Recent data from analysis of *Aim*-deficient mice suggest that AIM plays an important role in obesity-associated natural IgM autoantibody processes in Fcα/μR-bearing FDCs (Arai et al. 2013).

Another intriguing issue in the FcR field is the inducibility of FcRs by exposure to the corresponding Ig ligands, although such Ig-binding molecules have defied molecular characterization. For example, IgA-binding by murine T cells was induced by exposure to IgA in vivo and in vitro (Hoover et al. 1981). However, this binding must be mediated by a non-FcαR/CD89, because mice lack the human FcαR ortholog gene (Reljic 2006; Maruoka et al. 2004). Similarly, in our previous studies (Ohno et al. 1990), IgM induced an IgM receptor on 697 pre-B cells in a dose-dependent manner without a plateau, and this IgM receptor expression was maximal within 30 min after exposure, in contrast to the much longer exposure to PMA that is required for maximal IgM binding via FcμR. The up-regulation of the IgM receptor was dependent on the continuous presence of the ligand, as the removal of IgM from the culture resulted in a time-dependent decline of IgM-receptor expression on 697 pre-B cells. This ligand-induced IgM receptor seen on 697 pre-B cells is now known to be mediated by a non-FcμR protein as determined by FcμR-specific mAbs.

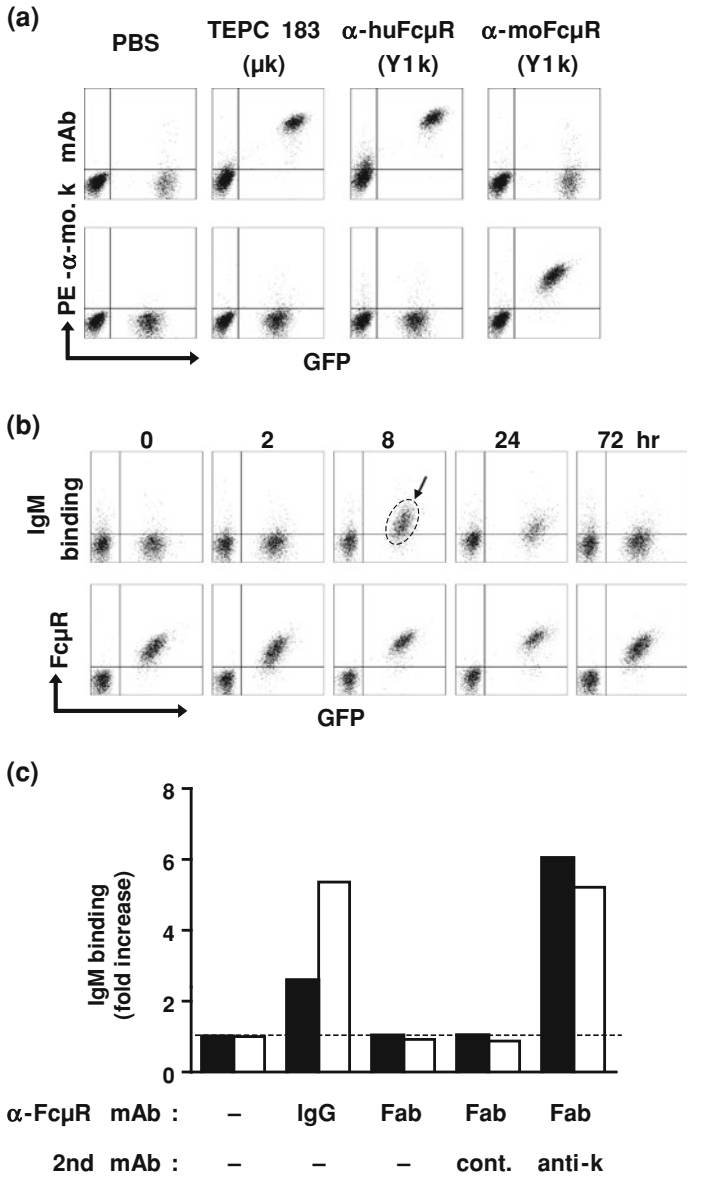
3 Mouse FcμR

3.1 Preparation of Mouse FcμR cDNA, Its Stable Transductants and mAbs

After cloning the human FcμR cDNA as described above, its mouse ortholog with a 1,269-bp open reading frame was identified by BLAST database analysis (GenBank accession no. NM_026976). Surprisingly, the overall aa identity between the 390-aa human and 422-aa mouse FcμRs is not so high, ∼54 %. The mouse receptor contains more additions of 1–16 aa in both the extracellular and cytoplasmic domains, compared to gaps of one aa in the Ig-like and stalk regions (see Fig. 1a right). The core peptide is predicted to have an M_r of ∼47 kDa and a pI of ∼9.6. Like the human receptor, the mouse FcμR has several potential O-linked glycosylation sites but no N-linked glycosylation motifs. *Fcmr* is also a single copy gene located on mouse chromosome 1 (56.89 cM), adjacent to other IgM-binding receptor genes, *Pigr* and *Fcamr*. The exon organization of mouse and human FcμR genes is conserved.

Fig. 5 *IgM binding by FcμR$^+$ cells.* **a** *Difference between human and mouse FcμRs.* Mixture of BW5147 cells stably expressing human (*top panel*) or mouse (*bottom panel*) FcμR along with GFP and control BW5147 cells, both of which were harvested from the mid-exponential stage of cell growth, were first incubated without (PBS) or with an IgMκ myeloma protein (TEPC183), anti-human FcμR (HM14 clone; mouse γ1κ isotype) or anti-mouse FcμR mAb (MM3 clone; mouse γ1κ), washed, and then with PE-labeled anti-mouse κ mAb (187.1 clone; rat γ1κ). Stained cells were analyzed by flow cytometry. Note the clear IgM binding by human FcμR$^+$ cells but not by mouse FcμR$^+$ cells. **b** *Restricted IgM binding activity.* After plating control or mouse FcμR$^+$/GFP$^+$ BW5147 cells at 5×10^4 cells/ml, cells were cultured at 37 °C for the indicated time period and were mixed together before assessment of IgM binding and FcμR expression as described above. Similar results were also obtained with B cell lines (A20 and CH31). Note the IgM binding by mouse FcμR$^+$ cells at an early stage in culture (see *arrow*) compared to the relatively unchanged surface receptor levels. **c** *MAb-induced enhancement of IgM binding.* Mouse FcμR$^+$ cells were first incubated without (-) or with intact (IgG) or Fab fragments (Fab) form of anti-FcμR mAb of either the MM3 (γ1κ isotype; *black column*) or MM4 clones (γ3κ isotype; *white column*), washed, and then with rat anti-mouse κ (anti-κ) or isotype-matched control (cont.) mAb. After washing, cells were assessed for their IgM binding. The results are shown as fold increase in IgM binding by treated cells relative to untreated cells (*broken line*)

The mouse FcμR cDNA was RT-PCR amplified from C57BL/6 lymph nodes using a set of primers corresponding to its translation initiation and termination codons. After verifying the correct nucleotide sequence, the FcμR cDNA was subcloned into an appropriate retroviral expression vector and transduced into BW5147 T cells to generate two stable transductants, one expressing FcμR and the other expressing both FcμR and GFP, as determined by a rat mAb specific for mouse FcμR (4B5 clone, a kind gift from Dr. Hiroshi Ohno (RIKEN, Yokohama); Ref. (Shima et al. 2010)). FcμR$^+$ cells were then used to hyper-immunize *Fcmr*-deficient C57BL/6 mice, thereby generating receptor-specific mAbs. When a mixture of control BW5147 cells and FcμR$^+$/GFP$^+$ cells was incubated with 10 different mAbs against mouse FcμR as well as with the corresponding isotype-matched control mAbs or an IgMκ myeloma protein, the anti-FcμR mAbs specifically reacted with FcμR-bearing GFP$^+$ cells but not control GFP$^-$ cells. Importantly, the mAb reactivity and the GFP intensity (as an indicator of FcμR transgene expression) were well correlated and, like the human FcμR, mAb reactivity was a more sensitive assay for the detection of FcμR than ligand binding using the myeloma IgM and PE-labeled goat anti-mouse Ig antibodies. A glycoprotein of ∼60 kDa was specifically precipitated by anti-FcμR mAbs and IgM ligands from membrane lysates of surface biotinylated, FcμR$^+$ transductants and B cell lines (A20 and CH31). Pre-incubation of membrane lysates of FcμR$^+$ cells with mAbs completely removed the IgM-reactive 60 kDa protein, whereas the reverse, using the IgM ligand, did not efficiently remove the mAb-reactive 60 kDa protein, again suggesting that mAbs are better than IgM ligands in the detection of FcμR (Honjo et al. 2012b).


(a)
PBS
TEPC 183 (μk)
α-huFcμR (Y1k)
α-moFcμR (Y1k)
mAb
PE -α-mo. k
GFP
(b)
0
2
8
24
72 hr
IgM binding
FcμR
GFP
(c)
IgM binding (fold increase)
8
6
4
2
0
α-FcμR mAb : – IgG Fab Fab Fab
2nd mAb : – – – cont. anti-k

3.2 IgM Ligand Binding of Mouse FcμR$^+$ Cells

One of the surprising findings with mouse FcμR is its IgM binding activity compared to human FcμR. Human FcμR$^+$ transductants exhibited IgM-ligand binding irrespective of the growth phase in cell culture (constitutive ligand binding activity) (Fig. 5a). By contrast, mouse FcμR$^+$ transductants bound to IgM during the early, but not exponential, growth phase (transient ligand binding activity), although the cell surface levels of receptors were not significantly changed during an entire period of culture (Fig. 5a, b). This transient ligand binding was not due to IgM ligand configuration, because essentially the same results were obtained with various molar ratios of IgM/antigen immune complexes or to host cells used for transduction, as the same results were obtained with FcμR$^+$ BaF3 pro-B cells. This transient IgM binding activity was also observed with FcμR-bearing B cell lines (A20 and CH31). Treatment of these B cell lines with PMA, but not LPS, enhanced their IgM binding, suggesting that cell activation status affects the ligand binding activity of FcμR on mouse B cells. Pre-incubation of FcμR$^+$ cells with stalk region-specific mAbs also enhanced subsequent IgM-ligand binding and this enhancement was induced by the intact form (divalent), but not Fab fragments (univalent), of anti-FcμR mAbs (Fig. 5c), suggesting that a dimeric or oligomeric configuration of receptor is important, especially in mice, for IgM ligand binding rather than allosteric regulation, e.g., ligand binding regulated by distal portion.

To explore the molecular basis for differences in constitutive versus transient IgM-ligand binding observed in human and mouse FcμRs, respectively, we made constructs encoding a recombinant human and mouse FcμR fusion protein by swapping each functional segment: Ig-like domain, stalk region and transmembrane/cytoplasmic tail, and ligated them into a bicistronic retroviral expression vector. The acronym "HHM" indicates the FcμR consisting of the Ig-like domain and stalk region of human origin and the remaining transmembrane/cytoplasmic region of mouse origin, and the "MMH" indicates the reverse. Cells expressing comparable levels of GFP were enriched from each transductant by FACS and were assessed for their IgM-ligand binding activity as well as for surface FcμR levels using mAbs specific for an extracellular epitope in the Ig-like domain or stalk region of human or mouse origin. (Curiously, we made 10 different murine FcμR-specific mAbs but had no mAbs specific for an epitope in the Ig-like domain, whereas we made 10 different human FcμR-specific mAbs, among which three were directed to the Ig-like domain and seven to the stalk region). As shown in Fig. 6, strong IgM binding is observed with HMM and HHM FcμR-bearing cells, but not with MMH and MHH FcμR-bearing cells, suggesting the correlation of strong IgM binding with the Ig-like domain of human origin. Small subpopulations of MHH FcμR$^+$ cells appear to exhibit weak IgM binding compared to MMH FcμR$^+$ cells, suggesting a minimal contribution of human stalk region to the ligand binding activity of mouse Ig-like domain. These findings suggest that the difference in IgM-ligand binding activity between human and mouse FcμRs is more directly attributed to the ligand-binding, Ig-like domain rather than to allosteric

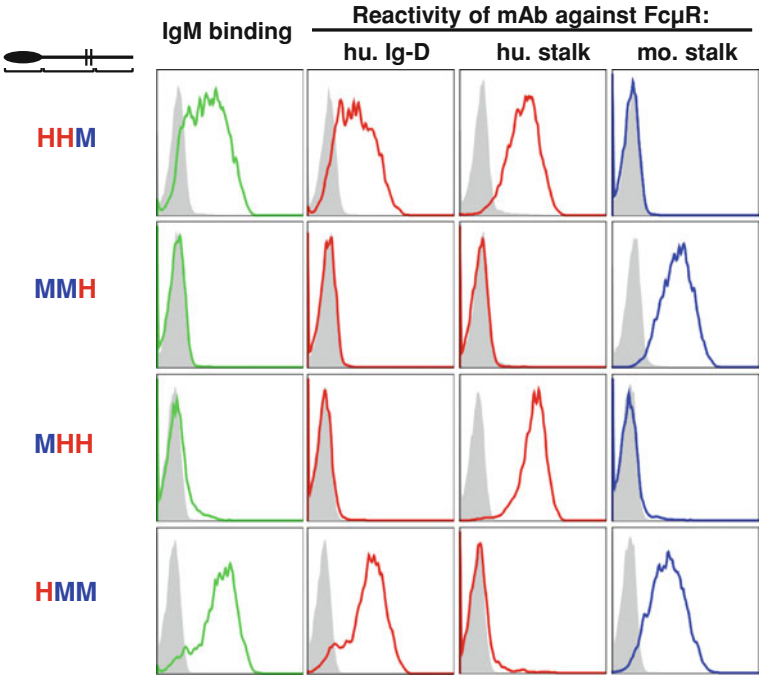


Fig. 6 *IgM binding of human/mouse chimeric FcμR proteins*. BW5147 cells stably expressing FcμR composed of the Ig-like domain, the stalk region and the transmembrane/cytoplasmic tail of either human (H) or mouse (M) origin were incubated with TEPC183 IgMκ for IgM binding, HM7 mAb to the Ig-like domain of human FcμR (hu. Ig-D), HM14 mAb to the stalk region of human FcμR (hu. Stalk) or MM3 mAb to the stalk region of mouse FcμR (mo. Stalk), before developing with PE-labeled goat anti-mouse Ig antibodies. Stained cells were analyzed by flow cytometry

regulation, i.e., indirect influence by other parts of the molecule, e.g., the stalk region or the transmembrane/cytoplasmic tail.

Comparison of mouse and human FcμR Ig-like domains reveals several aa sequence differences, which are localized around the predicted FcμR ligand-binding site and could explain differences in IgM binding properties between human and mouse FcμRs (Fig. 3). In particular, the putative CDR sequences of mouse FcμR differ significantly from those of human FcμR. For example, the negatively charged Glu (E^{41}) found in human FcμR CDR1 is replaced by a Gln in the mouse FcμR. Likewise, murine FcμR CDR2 contains a one residue deletion, relative to the human sequence, that removes Asn (N^{66}) from the CDR2. In addition to the CDRs themselves, murine FcμR residues 79–83 are significantly different from the equivalent human FcμR residues (TPCLD in mouse versus KQYPR in human). Murine sequence differences include two changes in charged residues ($K^{79} \rightarrow$ T and $R^{83} \rightarrow$ D), changes in the positions of Pro, ($Q^{80} \rightarrow$ P and $P^{82} \rightarrow$ L) and the removal of an aromatic residue ($Y^{81} \rightarrow$ C). Based on the structure of pIgR, these residues occur in the DE loop of FcμR, which is directly

adjacent to CDR1. Thus, residues on this loop could stabilize an alternative murine CDR1 structure with reduced affinity for IgM, relative to human FcμR. Further studies are required to determine whether these differences indeed contribute to the constitutive versus transient IgM-ligand binding of human and mouse FcμR.

3.3 Cellular Distribution

Immunofluorescence assessment with receptor-specific mAbs showed the expression of mouse FcμR on B cells, but not on T, Mφs, granulocytes, or dendritic cells in spleen (Fig. 7; Ref. (Honjo et al. 2012)). The restricted expression of FcμR to B cells was also confirmed in lymph nodes, blood, peritoneal cavity, and gut-associated lymphoid tissues. None of the $CD3^-/DX5^+$ NK, $CD3^+/DX5^+$ NKT, $\gamma\delta^+$ T, or innate lymphoid cells expressed FcμR on their cell surface. FcμR expression by T cells or Mφs was not induced after treatment with various stimuli including anti-CD3 mAb (for T cells), PMA, mixed lymphocyte culture supernatants, and LPS (for both T and Mφs). FcμR expression was not observed by freshly prepared, bone marrow (BM) $CD11b^+$ myeloid cells or by M-CSF-induced BM Mφs. Contrary to these results, however, Tak Mak's group recently reported the faint expression of Toso by $Ly6G^+$ BM granulocytes and Mφs (Lang et al. 2013). Strangely, the Ly6G-negative BM cell population, which should contain a significant number of FcμR-bearing B cells, was completely negative with their Toso-specific mAb B68. Because of this important discrepancy, especially for the interpretation of the effect of *Fcmr* ablation on innate immune functions (Lang et al. 2013), we thus extensively reexamined FcμR expression with a panel of mAbs and appropriate controls and found that none of our FcμR-specific mAbs reacted specifically with the surface of myeloid cells in BM or spleen. Furthermore, FcμR transcripts were clearly detectable in B-lineage cells but not in the double sorted $Ly6G^+$ BM granulocytes or B-cell depleted splenocytes as well as in *Rag1*-deficient splenocytes, which are devoid of B and T cells but contain abundant granulocytes and Mφs, even after 35 cycles of amplification (Honjo et al. 2012b, 2013). These experiments provide conclusive evidence that FcμR is not expressed by myeloid or T cells.

Cell surface FcμR levels in each B cell subset in spleen were also assessed with the following hierarchy: $CD21^{int}/CD23^+$ follicular (FO) > $CD21^{hi}/CD23^-$ marginal zone (MZ) > $CD21^-/CD23^-$ newly formed (NF). $CD5^+$ B1 and $CD5^-$ B2 cells expressed comparable levels of FcμR, whereas $GL7^+$ germinal center (GC) B cells expressed much less surface FcμR than $GL7^-$ non-GC B cells, suggesting down-modulation of FcμR during the GC reaction. FcμR was expressed not only by IgM^+ B cells, but also by IgG^+ or IgA^+ B cells, suggesting that the receptor expression is not directly linked with IgM production and is maintained on the switched memory B cells. In BM, FcμR was undetectable on $CD19^+$/surface (s) IgM^- pro-B/pre-B cell compartment, was low on $CD19^+/sIgM^+/sIgD^-$ immature B cells and higher on $CD19^+/sIgM^+/sIgD^+$ mature or recirculating B cells,

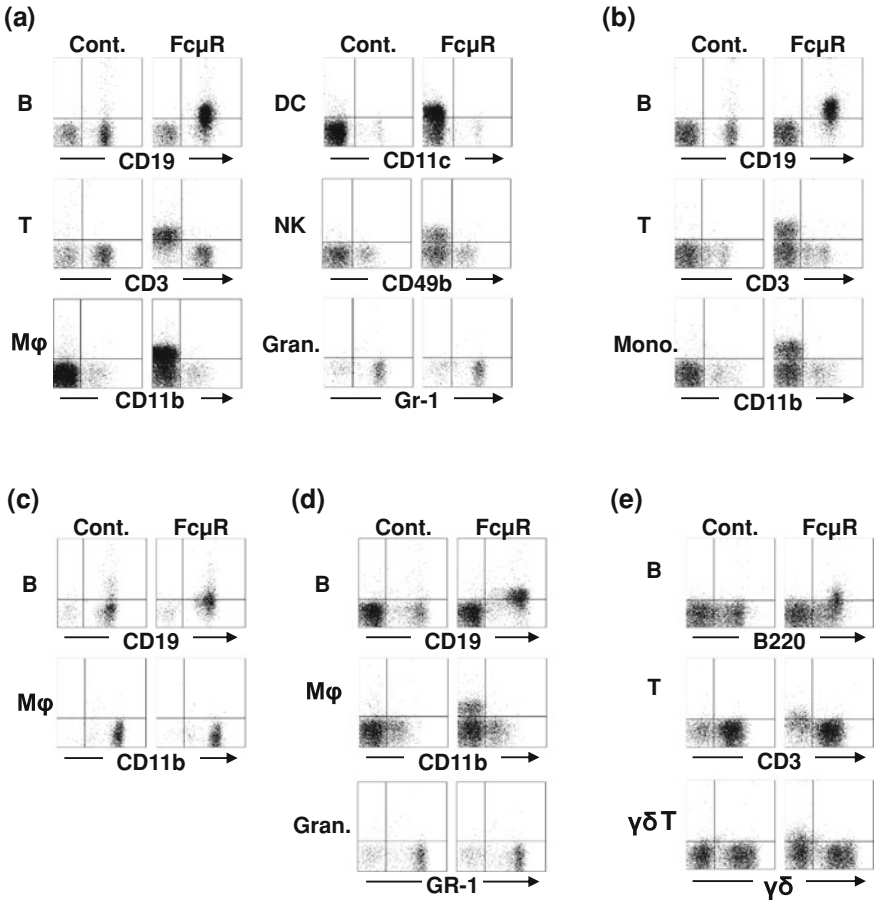


Fig. 7 *Expression of mouse FcμR*. Nucleated cells from WT adult C57BL/6 spleen (**a**), blood (**b**), peritoneal cavity (**c**), bone marrow (**d**) and small intestine (**e**) were first incubated with FcγR-blocking reagents and then with biotin-labeled, anti-FcμR (MM3, γ1κ) or isotype-matched control mAb, before developing with PE-streptavidin. PE-stained cells were counterstained with fluorochrome-labeled mixture of three mAbs with specificity for CD19, CD3, CD11b, CD11c, CD49b, Gr-1, or $\gamma\delta$ TCR as well as with fluorochrome-labeled, corresponding isotype-matched control mAbs for background setting. Stained cells with light scatter characteristics of myeloid or small lymphoid and large mononuclear cells were analyzed by flow cytometry. Note B cell-restricted expression of FcμR

indicating that FcμR expression begins at the immature B cell stage of differentiation. The majority of syndecan-1/CD138$^+$ cells in spleen and lymph nodes and a fraction of the CD138$^+$ cells in BM expressed FcμR as well as CD19 and B220, suggesting the expression of FcμR on plasmablasts but not on mature plasma cells. Collectively, these findings clearly demonstrate that the expression of FcμR in mice is restricted to B-lineage cells, beginning at the early immature B cell stage in

BM and continuing through to the plasmablast stage of differentiation, accompanied by transient down-modulation of FcμR during the GC reaction.

Several distinctions between mouse and human FcμRs in terms of their detection and expression are worth mentioning. (i) For the detection of FcμR on freshly prepared splenocytes or other lymphoid cells by mAbs, pre-incubation of cells in IgM-free media was required for the human (see Sect. 2.6) but not the mouse receptor, suggesting that the mouse FcμR is more resistant to extracellular IgM concentration or tissue milieu. This difference may also be related in part to the fact that laboratory mice are less immunologically stimulated than humans. (ii) Cell surface FcμR levels were indistinguishable between wild type (WT) mice and mutant mice deficient in IgM secretion ($\mu s^{-/-}$), which are able to express surface IgM and other Ig isotypes on B cells and to secrete all other classes of Igs except IgM (Ehrenstein and Notley 2010). This also suggests that secreted IgM does not significantly influence cell surface expression of the FcμR in mice. (iii) However, the ex vivo binding of exogenous IgM to FcμR was more easily demonstrable on B cells in $\mu s^{-/-}$ mice than in WT controls, suggesting that the ligand binding site of FcμR in normal mice could be already occupied with secreted IgM in vivo. It remains unclear if the IgM-bound FcμR is internalized like the human receptor, rapidly retrieved from early endosomes and returned to the cell surface, otherwise the constitutive expression of FcμR cannot easily be explained. Alternatively, IgM-bound FcμR in mice may remain on the cell surface without internalization, as is the case for the interaction of IgE and the high affinity FcεR on mast cells and basophils (Ravetch and Kinet 1991). In this regard, although the mouse receptor contains the Tyr residues that are involved in human FcμR-mediated endocytosis, the mouse FcμR also has an additional 16-aa in its cytoplasmic carboxyl terminal tail that may affect the FcμR-mediated internalization process.

3.4 Fcmr-*Deficient Mice*

Fcmr-deficient (KO) mice have been independently generated by three laboratories: K. H. Lee (Leibniz Center for Medicine and Biosciences, Borstel, Germany), H. Ohno (RIKEN, Yokohama, Japan) and T. Mak (Ontario Cancer Institute, Toronto, Canada) and have recently been characterized by five different groups and there are clear differences in the reported phenotypes (Brenner et al. 2014; Honjo et al. 2012; Lang et al. 2013; Nguyen et al. 2011; Ouchida et al. 2012; Choi et al. 2013). While the basis for these differences requires further investigation, it might be due in part to: (i) different strategies for gene targeting (i.e., deletion of exon 4–7, exon 2–4 versus exon 2–8 and/or the absence versus presence of the *Neo* gene in the mouse genome) as well as the extent of the 129 mouse-origin DNA around the *Fcmr* gene remaining after backcrossing onto C57BL/6, (ii) investigators' concepts of the function of FcμR/Toso as an IgM binding protein versus an anti-apoptotic protein, and/or (iii) other factors (e.g., mouse ages, environments including intestinal microbiota or reagents used). Nevertheless, the abnormal

phenotypes commonly observed in *Fcmr* KO mice are: (i) alterations in B cell subpopulations (Honjo et al. 2012; Ouchida et al. 2012), (ii) dysregulation of humoral immune responses (Honjo et al. 2012; Ouchida et al. 2012; Choi et al. 2013), (iii) impairment of B cell proliferation upon BCR ligation in vitro (Ouchida et al. 2012; Choi et al. 2013), and (iv) predisposition to autoantibody production (Honjo et al. 2012; Ouchida et al. 2012; Choi et al. 2013). Notably, many abnormalities in *Fcmr* KO mice mirror those observed in $\mu s^{-/-}$ mice, suggesting the critical role in normal B cell functions both for secreted IgM and for its interaction with FcμR (Ehrenstein and Notley 2010).

(a) *Alteration of B cell subsets* The ablation of *Fcmr* ablation had no significant effect on overall B- and T-cell development, but led to a fourfold reduction of MZ B cells and a twofold increase in splenic B1 B cells (Honjo et al. 2012). In peritoneal cavity, the total numbers of B1a, B1b, and B2 cells were comparable in both mutant and WT control mice. The numbers of pro-B/pre-B, immature B and recirculating B cells as well as myeloid cells in BM were identical in both groups of mice. In addition to the changes in cell numbers, there were some differences in the density of certain cell surface markers between mutant and WT mice. The CD19, sIgM, and sIgD levels on splenic B cells were indistinguishable, but the CD21 and CD23 levels were slightly lower in mutant mice than in WT controls. Thus, these findings suggest that *Fcmr* ablation does not significantly affect overall B- and T-cell development, but alters B cell subset numbers, accompanied by changes in the surface density of certain markers on B cells.
Contrary to the marked reduction of MZ B cells in *Fcmr* KO mice, the number of MZ B cells in $\mu s^{-/-}$ mice is increased by $\sim$3 fold and this increase can be normalized by passive administration of natural or polyclonal, but not monoclonal, IgM preparations (Baker and Ehrenstein 2002). It remains to be determined, however, whether this normalization results from differentiation of MZ B cells into plasma cells, apoptosis of MZ B cells upon possible engagement of multiple receptors including TLRs with natural IgM, or some other mechanisms. In any case, FcμR and its signals may play an important role in the regulation of MZ B cell responses to blood-borne pathogens or self-antigens.

(b) *Elevation of pre-immune serum IgM* To determine whether *Fcmr* deficiency affects natural antibody levels, we assessed pre-immune serum Ig isotype levels in *Fcmr* KO and WT control mice of the same age (13–18 weeks) and sex by ELISA. Both IgM and IgG3 levels were twofold higher in mutant mice than WT controls: 857 $\pm$ 298 versus 431 $\pm$ 297 μg/ml for IgM (mean $\pm$ 1 SD) and 1,434 $\pm$ 1,292 versus 567 $\pm$ 350 μg/ml for IgG3. Notably, the increase in IgM levels was evident irrespective of gender, whereas significant elevation of IgG3 was observed only in female mutant mice. We also found an elevation of IgM and IgG natural autoantibodies in mutant mice as determined by immunofluorescent analysis of HEp-2 cells and by ELISA using dsDNA and chromatin autoantigens. These findings suggest that FcμR deficiency leads to an elevation of IgM and IgG natural autoantibodies (Honjo et al. 2012).

Since serum IgM levels are not affected in mice with null mutations of other IgM-binding receptors, pIgR or Fcα/μR (Shimada et al. 1999; Honda et al. 2009), the FcμR appears to be the sole receptor in this family that may be involved in maintenance of serum IgM levels within the physiological range. Because the half-life of injected IgM is the same in *Fcmr* KO and WT mice, FcμR may not be involved in IgM catabolism by liver sinusoidal endothelial cells as previously suggested, but rather is involved in the production and/or secretion of IgM by B and/or plasmablasts. The increase in pre-immune natural IgM might be the consequence of exposure to self-antigens associated with cell corpses and B1 B cells are a major source of natural IgM antibodies (Baumgarth et al. 2005), consistent with our findings of increased splenic B1 B cells and elevated natural autoantibodies to nuclear and cytoplasmic components in *Fcmr* KO mice. It will be important to determine whether *Fcmr* ablation facilitates autoimmune processes in autoimmune prone mice.

(c) *Dysregulation of humoral immune responses* To determine the effect of FcμR deficiency on humoral immune responses, we have chosen a live non-encapsulated (avirulent) strain of *Streptococcus pneumonia* (R36A) as a physiologically relevant immunogen. Serum antibodies against phosphorylcholine (PC) as a T-cell independent type 2 (TI-2) antigen and R36A-associated crude proteins and recombinant PspA protein as T-cell dependent (TD) antigens were assessed weekly after immunizing into *Fcmr* KO and WT mice i.p. with a wide range of antigen doses (10^8–10^2 cfu). IgM and IgG3 PC-specific responses were comparable within both groups of mice when immunized with 10^6 or more bacteria. By contrast, at a suboptimal dose of bacteria (10^4 cfu) both IgM and IgG3 PC-specific antibodies were markedly elevated in mutant mice. On the other hand, IgM and IgG responses against R36A-associated proteins or PspA protein were indistinguishable in both groups of mice. These findings suggest a selective role of FcμR on B cells and/or plasmablasts in regulation of TI-2 immune responses (Honjo et al. 2012). It is worth noting that similar selective enhancement of TI-2 immune responses has also been observed in mice with null mutations in components of the BCR complex such as CD19 (Sato et al. 1995), CD81 (Tsitsikov et al. 1997) and μs (Ehrenstein et al. 1998).

Several possible explanations for this selective enhancement are worth consideration. (i) PC-containing polysaccharides may be poorly degraded, and thus retained to stimulate B1 or MZ B cells, in mutant mice compared to WT controls. (ii) Given that the TACI (transmembrane activation calcium modulator and cytophilin ligand interactor) receptor on mature B cells is essential for TI-2 responses (von Bülow et al. 2001; Yan et al. 2001), FcμR on B cells, especially B1 and MZ B cells, might negatively regulate TACI-mediated signaling in response to its ligands, B cell activating factors of the TNF family (e.g., BAFF and APRIL), to maintain homeostasis of humoral immune responses to poorly degradable TI-2 antigens. (iii) Since memory B cells elicited by TI-2 antigens are phenotypically distinct from those elicited by TD antigens and are regulated by antigen-specific IgM or IgG3 antibodies

(Hosokawa 1979; Brodeur and Wortis 1980; Obukhanych and Nussenzweig 2006), FcμR may preferentially affect TI-2 memory B cell responses. (iv) Since FcμR is expressed predominantly by plasmablasts and not by mature plasma cells, FcμR may negatively regulate the transition from activated B to plasmablasts.

4 Epilogue

Although the molecular nature of the FcμR has long been elusive, the recent identification of it as an authentic IgM Fc-binding receptor in humans by functional cloning is unequivocal. Therefore, its original designation as an inhibitor of Fas apoptosis (Toso/FAIM3) was incorrect. In contrast to the constitutive ligand binding activity of the human FcμR, the mouse FcμR exhibits transient IgM binding activity, suggesting the existence of intriguing mechanisms that regulate murine FcμR activity. Moreover, conflicting data on the effects of *Fcmr* ablation have been reported and the investigation of FcμR, especially the mouse receptor, becomes ever more complicated with many unresolved puzzles, some of which have been introduced in this review. If this article ultimately facilitates other researchers in their goal to resolve these puzzles and to understand the dichotomy in human and mouse FcμRs as well as to define their functions, the authors will feel grateful and satisfied. We hope that our contribution will open new avenues of investigation.

Acknowledgments We would like to thank Dr. Peter Burrows and Dr. Sheila Sanders for critical reading and suggestions. This mini-review article is heavily indebted to the early studies of IgM-binding proteins by three outstanding post-doctoral fellows in the laboratory of Dr. Max Cooper: Sheila K. Sanders, Tatsuharu Ohno and Tetsuya Nakamura. HK also would like to dedicate this paper as a token of his immense gratitude to his respected mentor Dr. Max Cooper, Georgia Research Alliance Eminent Scholar, Professor of Pathology and Laboratory Medicine, Emory University School of Medicine, for his great advice and long support.

References

Adachi T, Harumiya S, Takematsu H, Kozutsumi Y, Wabl M, Fujimoto M, Tedder TF (2012) CD22 serves as a receptor for soluble IgM. Eur J Immunol 42:241–247

Akula S, Mohammadamin S, Hellman L (2014) Fc receptors for immunoglobulins and their appearance during vertebrate evolution. PLoS One 9:e96903

Arai S, Maehara N, Iwamura Y, Honda S, Nakashima K, Kai T, Ogishi M, Morita K, Kurokawa J, Mori M, Motoi Y, Miyake K, Matsuhashi N, Yamamura K, Ohara O, Shibuya A, Wakeland EK, Li QZ, Miyazaki T (2013) Obesity-associated autoantibody production requires AIM to retain the immunoglobulin M immune complex on follicular dendritic cells. Cell Rep 3:1187–1198

Baker N, Ehrenstein MR (2002) Cutting edge: selection of B lymphocyte subsets is regulated by natural IgM. J Immunol 169:6686–6690

Baumgarth N, Tung JW, Herzenberg LA (2005) Inherent specificities in natural antibodies: a key to immune defense against pathogen invasion. Springer Semin Immunopathol 26:347–362

Brenner D, Brustle A, Lin GH, Lang PA, Duncan GS, Knobbe-Thomsen CB, St PM, Reardon C, Tusche MW, Snow B, Hamilton SR, Pfefferle A, Gilani SO, Ohashi PS, Lang KS, Mak TW (2014) Toso controls encephalitogenic immune responses by dendritic cells and regulatory T cells. Proc. Natl. Acad. Sci. U. S. A 111:1060–1065

Brodeur PH, Wortis HH (1980) Regulation of thymus-independent responses: unresponsiveness to a second challenge of TNP-Ficoll is mediated by hapten-specific antibodies. J Immunol 125:1499–1505

Burns GF, Cawley JC, Barker CR (1979) Characterization of the receptor for IgM present on human B lymphocytes. Immunology 36:569–577

Buzzi M, Lu L, Lombardi AJ, Posner MR, Brautigan DL, Fast LD, Frackelton AR (1992) Differentiation-induced changes in protein-tyrosine phosphatase activity and commensurate expression of CD45 in human leukemia cell lines. Cancer Res 52:4027–4035

Castillo-Davis CI, Mekhedov SL, Hartl DL, Koonin EV, Kondrashov FA (2002) Selection for short introns in highly expressed genes. Nat Genet 31:415–418

Choi SC, Wang H, Tian L, Murakami Y, Shin DM, Borrego F, Morse HC III, Coligan JE (2013) Mouse IgM Fc receptor, FCMR, promotes B cell development and modulates antigen-driven immune responses. J. Immunol. 190:987–996

Daëron M (1997) Fc receptor biology. Annu Rev Immunol 15:203–234

Davis RS, Wang YH, Kubagawa H, Cooper MD (2001) Identification of a family of Fc receptor homologs with preferential B cell expression. Proc Natl Acad Sci USA 98:9772–9777

Duhren-von MM, Ubelhart R, Schneider D, Wossning T, Bach MP, Buchner M, Hofmann D, Surova E, Follo M, Kohler F, Wardemann H, Zirlik K, Veelken H, Jumaa H (2012) Chronic lymphocytic leukaemia is driven by antigen-independent cell-autonomous signalling. Nature 489:309–312

Ehrenstein MR, Notley CA (2010) The importance of natural IgM: scavenger, protector and regulator. Nat Rev Immunol 10:778–786

Ehrenstein MR, O'Keefe TL, Davies SL, Neuberger MS (1998) Targeted gene disruption reveals a role for natural secretory IgM in the maturation of the primary immune response. Proc Natl Acad Sci USA 95:10089–10093

Engels N, Konig LM, Heemann C, Lutz J, Tsubata T, Griep S, Schrader V, Wienands J (2009) Recruitment of the cytoplasmic adaptor Grb2 to surface IgG and IgE provides antigen receptor-intrinsic costimulation to class-switched B cells. Nat Immunol 10:1018–1025

Ferrarini M, Hoffman T, Fu SM, Winchester R, Kunkel HG (1977) Receptors for IgM on certain human B lymphocytes. J Immunol 119:1525–1529

Gardiner EE, Karunakaran D, Arthur JF, Mu FT, Powell MS, Baker RI, Hogarth PM, Kahn ML, Andrews RK, Berndt MC (2008) Dual ITAM-mediated proteolytic pathways for irreversible inactivation of platelet receptors: de-ITAM-izing FcγRIIa. Blood 111:165–174

Hamburger AE, West AP Jr, Bjorkman PJ (2004) Crystal structure of a polymeric immunoglobulin binding fragment of the human polymeric immunoglobulin receptor. Structure 12:1925–1935

Hitoshi Y, Lorens J, Kitada SI, Fisher J, LaBarge M, Ring HZ, Francke U, Reed JC, Kinoshita S, Nolan GP (1998) Toso, a cell surface, specific regulator of Fas-induced apoptosis in T cells. Immunity 8:461–471

Honda S, Kurita N, Miyamoto A, Cho Y, Usui K, Takeshita K, Takahashi S, Yasui T, Kikutani H, Kinoshita T, Fujita T, Tahara-Hanaoka S, Shibuya K, Shibuya A (2009) Enhanced humoral immune responses against T-independent antigens in Fcα/μR-deficient mice. Proc Natl Acad Sci USA 106:11230–11235

Honjo K, Kubagawa Y, Kubagawa H (2012a) Is Toso an antiapoptotic protein or an Fc receptor for IgM? Blood 119:1789–1790

Honjo K, Kubagawa Y, Jones DM, Dizon B, Zhu Z, Ohno H, Izui S, Kearney JF, Kubagawa H (2012b) Altered Ig levels and antibody responses in mice deficient for the Fc receptor for IgM (FcμR). Proc Natl Acad Sci USA 109:15882–15887

Honjo K, Kubagawa Y, Kubagawa H (2013) Is Toso/IgM Fc receptor (FcμR) expressed by innate immune cells? Proc Natl Acad Sci USA 110:E2540–E2541

Hoover RG, Dieckgraefe BK, Lynch RG (1981) T cells with Fc receptors for IgA: induction of T alpha cells in vivo and in vitro by purified IgA. J Immunol 127:1560–1563

Hosokawa T (1979) Studies on B-cell memory. II. T-cell independent antigen can induce B-cell memory. Immunology 38:291–299

Kikuno K, Kang DW, Tahara K, Torii I, Kubagawa HM, Ho KJ, Baudino L, Nishizaki N, Shibuya A, Kubagawa H (2007) Unusual biochemical features and follicular dendritic cell expression of human Fcα/μ receptor. Eur J Immunol 37:3540–3550

Kubagawa H, Burrows PD, Cooper MD (1997) A novel pair of immunoglobulin-like receptors expressed by B cells and myeloid cells. Proc Natl Acad Sci USA 94:5261–5266

Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW, Gartland GL, Bertoli LF, Mori H, Takatsu H, Kitamura T, Ohno H, Wang JY (2009) Identity of the elusive IgM Fc receptor (FcμR) in humans. J Exp Med 206:2779–2793

Lang KS, Lang PA, Meryk A, Pandyra AA, Boucher LM, Pozdeev VI, Tusche MW, Gothert JR, Haight J, Wakeham A, You-Ten AJ, McIlwain DR, Merches K, Khairnar V, Recher M, Nolan GP, Hitoshi Y, Funkner P, Navarini AA, Verschoor A, Shaabani N, Honke N, Penn LZ, Ohashi PS, Haussinger D, Lee KH, Mak TW (2013) Involvement of Toso in activation of monocytes, macrophages, and granulocytes. Proc Natl Acad Sci USA 110:2593–2598

Li FJ, Kubagawa Y, McCollum MK, Wilson L, Motohashi T, Bertoli LF, Barton JC, Barnes S, Davis RS, Kubagawa H (2011) Enhanced levels of both membrane-bound and soluble forms of IgM Fc receptor (FcμR) in patients with chronic lymphocytic leukemia. Blood 118:4902–4909

Martinez VG, Moestrup SK, Holmskov U, Mollenhauer J, Lozano F (2011) The conserved scavenger receptor cysteine-rich superfamily in therapy and diagnosis. Pharmacol Rev 63:967–1000

Maruoka T, Nagata T, Kasahara M (2004) Identification of the rat IgA Fc receptor encoded in the leukocyte receptor complex. Immunogenetics 55:712–716

Maurer MA, Rakocevic G, Leung CS, Quast I, Lukačišin M, Goebels N, Münz C, Wardemann H, Dalakas M, Lünemann JD (2012) Rituximab induces sustained reduction of pathogenic B cells in patients with peripheral nervous system autoimmunity. J Clin Invest 122:1393–1402

McEwan WA, Tam JC, Watkinson RE, Bidgood SR, Mallery DL, James LC (2013) Intracellular antibody-bound pathogens stimulate immune signaling via the Fc receptor TRIM21. Nat Immunol 14:327–336

Miyazaki T, Kurokawa J, Arai S (2011) AIMing at metabolic syndrome. Towards the development of novel therapies for metabolic diseases via apoptosis inhibitor of macrophage (AIM). Circ J 75:2522–2531

Monteiro RC, Van De Winkel JG (2003) IgA Fc receptors. Annu Rev Immunol 21:177–204

Moretta L, Webb SR, Grossi CE, Lydyard PM, Cooper MD (1977) Functional analysis of two human T-cell subpopulations: help and suppression of B-cell responses by T cells bearing receptors for IgM (Tμ) or IgG (Tγ). J Exp Med 146:184–200

Murakami Y, Narayanan S, Su S, Childs R, Krzewski K, Borrego F, Weck J, Coligan JE (2012) Toso, a functional IgM receptor, is regulated by IL-2 in T and NK cells. J Immunol 189:587–597

Nakamura T, Kubagawa H, Ohno T, Cooper MD (1993) Characterization of an IgM Fc-binding receptor on human T cells. J Immunol 151:6933–6941

Nguyen XH, Lang PA, Lang KS, Adam D, Fattakhova G, Foger N, Kamal MA, Prilla P, Mathieu S, Wagner C, Mak T, Chan AC, Lee KH (2011) Toso regulates the balance between apoptotic and nonapoptotic death receptor signaling by facilitating RIP1 ubiquitination. Blood 118:598–608

Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV (2005) FcγRIV: a novel FcR with distinct IgG subclass specificity. Immunity 23:41–51

Obukhanych TV, Nussenzweig MC (2006) T-independent type II immune responses generate memory B cells. J Exp Med 203:305–310

Ohno T, Kubagawa H, Sanders SK, Cooper MD (1990) Biochemical nature of an Fcμ receptor on human B-lineage cells. J Exp Med 172:1165–1175

Ouchida R, Mori H, Hase K, Takatsu H, Kurosaki T, Tokuhisa T, Ohno H, Wang JY (2012) Critical role of the IgM Fc receptor in IgM homeostasis, B-cell survival, and humoral immune responses. Proc Natl Acad Sci USA 109:E2699–E2706

Pichler WJ, Knapp W (1977) Receptors for IgM-coated erythrocytes on chronic lymphatic leukemia cells. J Immunol 118:1010–1015

Platsoucas CD, Kempin S, Karanas A, Clarkson B, Good RA, Gupta S (1980) Receptors for immunoglobulin isotype on T and B lymphocytes from untreated patients with chronic lymphocytic leukaemia. Clin Exp Immunol 40:256–263

Poe JC, Tedder TF (2012) CD22 and Siglec-G in B cell function and tolerance. Trends Immunol 33:413–420

Pricop L, Rabinowich H, Morel PA, Sulica A, Whiteside TL, Herberman RB (1993) Characterization of the Fcμ receptor on human natural killer cells. Interaction with its physiologic ligand, human normal IgM, specificity of binding, and functional effects. J Immunol 151:3018–3029

Ramchandren S, Lewis RA (2009) Monoclonal gammopathy and neuropathy. Curr Opin Neurol 22:480–485

Randow F, MacMicking JD, James LC (2013) Cellular self-defense: how cell-autonomous immunity protects against pathogens. Science 340:701–706

Ravetch JV, Kinet JP (1991) Fc receptors. Annu Rev Immunol 9:457–492

Reljic R (2006) In search of the elusive mouse macrophage Fcα receptor. Immunol Lett 107:80–81

Rudders RA, Andersen J, Howard JP, Fried R (1980) The binding of IgM to B lymphocytes. I. Studies of the binding of aggregated IgM to normal and leukemic human B lymphocytes. Clin Immunol Immunopathol 15:673–686

Sanders SK, Kubagawa H, Suzuki T, Butler JL, Cooper MD (1987) IgM binding protein expressed by activated B cells. J Immunol 139:188–193

Sato S, Steeber DA, Tedder TF (1995) The CD19 signal transduction molecule is a response regulator of B-lymphocyte differentiation. Proc Natl Acad Sci USA 92:11558–11562

Shibuya A, Sakamoto N, Shimizu Y, Shibuya K, Osawa M, Hiroyama T, Eyre HJ, Sutherland GR, Endo Y, Fujita T, Miyabayashi T, Sakano S, Tsuji T, Nakayama E, Phillips JH, Lanier LL, Nakauchi H (2000) Fcα/μ receptor mediates endocytosis of IgM-coated microbes. Nat Immunol 1:441–446

Shima H, Takatsu H, Fukuda S, Ohmae M, Hase K, Kubagawa H, Wang JY, Ohno H (2010) Identification of TOSO/FAIM3 as an Fc receptor for IgM. Int Immunol 22:149–156

Shimada S, Kawaguchi-Miyashita M, Kushiro A, Sato T, Nanno M, Sako T, Matsuoka Y, Sudo K, Tagawa Y, Iwakura Y, Ohwaki M (1999) Generation of polymeric immunoglobulin receptor-deficient mouse with marked reduction of secretory IgA. J Immunol 163:5367–5373

Tissot JD, Sanchez JC, Vuadens F, Scherl A, Schifferli JA, Hochstrasser DF, Schneider P, Duchosal MA (2002) IgM are associated to Sp alpha (CD5 antigen-like). Electrophoresis 23:1203–1206

Tsitsikov EN, Gutierrez-Ramos JC, Geha RS (1997) Impaired CD19 expression and signaling, enhanced antibody response to type II T independent antigen and reduction of B-1 cells in CD81-deficient mice. Proc Natl Acad Sci USA 94:10844–10849

Ucker DS, Yamamoto KR (1984) Early events in the stimulation of mammary tumor virus RNA synthesis by glucocorticoids. Novel assays of transcription rates. J Biol Chem 259:7416–7420

Vire B, David A, Wiestner A (2011) TOSO, the Fcμ receptor, is highly expressed on chronic lymphocytic leukemia B cells, internalizes upon IgM binding, shuttles to the lysosome, and is downregulated in response to TLR activation. J Immunol 187:4040–4050

Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, Yokoyama WM, Ugolini S (2011) Innate or adaptive immunity? the example of natural killer cells. Science 331:44–49

von Bülow GU, van Deursen JM, Bram RJ (2001) Regulation of the T-independent humoral response by TACI. Immunity 14:573–582

Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T, Tumas D, Grewal IS, Dixit VM (2001) Activation and accumulation of B cells in TACI-deficient mice. Nat Immunol 2:638–643

Emerging Roles for the FCRL Family Members in Lymphocyte Biology and Disease

F. J. Li, W. J. Won, E. J. Becker Jr., J. L. Easlick, E. M. Tabengwa, R. Li, M. Shakhmatov, K. Honjo, P. D. Burrows and R. S. Davis

Abstract Members of the extended Fc receptor-like (FCRL) family in humans and mice are preferentially expressed by B cells and possess tyrosine-based immunoregulatory function. Although the majority of these proteins repress B cell receptor-mediated activation, there is an emerging evidence for their bifunctionality and capacity to counter-regulate adaptive and innate signaling pathways. In light of these findings, the recent discovery of ligands for several of these molecules has begun to reveal exciting potential for them in normal lymphocyte biology and is launching a new phase of FCRL investigation. Importantly, these fundamental developments are also setting the stage for defining their altered roles in the pathogenesis of a growing number of immune-mediated diseases. Here we review recent advances in the FCRL field and highlight the significance of these intriguing receptors in normal and perturbed immunobiology.

F. J. Li · W. J. Won · J. L. Easlick · E. M. Tabengwa · R. Li · M. Shakhmatov · K. Honjo · R. S. Davis (✉)
Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA
e-mail: rsdavis@uab.edu

E. J. Becker Jr. · P. D. Burrows · R. S. Davis
Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, USA

R. S. Davis
Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, USA

P. D. Burrows
Department of Genetics, University of Alabama at Birmingham, Birmingham, AL, USA

P. D. Burrows · R. S. Davis
Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL, USA

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_2,

Contents

1 Introduction 30
2 Discovery and Characteristics of FCRL Family Members 30
3 Cellular Distribution of the FCRLs 32
4 Emerging FCRL Ligands 34
5 Functional and Regulatory Properties 37
5.1 Roles in Adaptive B Cell Signaling 37
5.2 Influence on Innate-Like B Cell Responses 39
5.3 Insight from In Vivo Models 41
6 FCRL Involvement with Disease 42
7 Conclusions 45
References 46

1 Introduction

The identification of a family of Fc receptor-like (FCRL) molecules over 10 years ago revealed a much richer landscape of genes related to the conventional Fc receptors (FCR) for IgG and IgE than was previously anticipated. Although their existence escaped attention for decades, investigation of the FCRLs is uncovering unexpected phylogenetic and immunoregulatory complexity for this ancient molecular cluster. Despite syntenic chromosomal linkage, similar genetic organization, and shared Ig superfamily (IgSF) membership with the classical FCRs, their species-specificity as well as differences in their structural features and expression patterns imply a high degree of evolutionary plasticity for the FCRLs in adaptive immunity. As their ligands and complex tyrosine-based functions become clear, we are realizing that parallel studies in humans, mice, and perhaps other models with be required to better delineate their biologic and pathologic contributions. In this review, we discuss exciting new developments in the FCRL field that are beginning to unearth the biological roles of these molecules in host protection and disease at the nexus of innate and adaptive immunity.

2 Discovery and Characteristics of FCRL Family Members

FCRL genes were discovered by several groups using different strategies and, as a result, a uniform nomenclature to designate them had to be established (Maltais et al. 2006). The first representative reported was a glycosylphosphatidylinositol (GPI)-anchored rat ortholog of FCRL6, initially termed gp42, that was identified in a search for markers of cytotoxic natural killer (NK) lymphocytes induced by IL-2 (Imboden et al. 1989; Seaman et al. 1991). However, it was not until

meticulous work by the Dalla-Favera group nearly 10 years later that the breadth of this family became apparent. In an effort to define the genes joined at a t(1;14)(q21;q32) chromosomal translocation breakpoint in the FR4 multiple myeloma (MM) cell line, the second intron upstream of the exon encoding the C-terminal portion of the *FCRL4* split signal peptide, originally named IgSF receptor translocation-associated gene 1 (*IRTA1*), was found fused to the intron proximal to the transmembrane encoding exon of IgA1 (Hatzivassiliou et al. 2001; Miller et al. 2002). Our bioinformatic approach of searching human genome sequences with a 32 amino acid consensus motif derived from the extracellular Ig-binding region of the classical FCRs yielded discovery of the FCR homolog (FCRH) family (Davis et al. 2001). In silico strategies were also employed by the Taranin group to identify molecules sharing features with the IgSF, FCR, and gp42 proteins (IFGP) (Guselnikov et al. 2002) and the Zhao laboratory to find novel Src homology (SH)-2 domain-containing phosphatase anchoring proteins (SPAP) (Xu et al. 2001). Additionally, using subtractive hybridization methodology, the B cell crosslinked by anti-IgM activation sequence (*BXMAS*) genes were found by Bothwell and colleagues (Nakayama et al. 2001). These studies collectively revealed that the human *FCRL1-5* cluster spans a ~300 kb region of chromosome 1q21–22 at a locus telomeric of the high-affinity FcγRI/CD64 gene (*FCGR1A*) and encodes type I transmembrane glycoproteins with 3–9 extracellular Ig-like domains and cytoplasmic tails with immunoreceptor tyrosine-based activating (ITAM), switch (ITSM), and/or inhibitory (ITIM) motifs (Fig. 1). *FCRL6,* which also codes for a transmembrane receptor with similar features, was identified at a separate locus further telomeric and proximal to the high-affinity IgE (*FCER1A*) gene (Davis et al. 2002a). Finally, two additional relatives termed *FCRLA* and *FCRLB* were located proximal to the genes encoding the low affinity FcγRs (*FCGR2-3*) (Davis et al. 2002b; Facchetti et al. 2002; Mechetina et al. 2002; Masuda et al. 2005; Wilson and Colonna 2005). Not surprisingly, the FCRL proteins encoded by this locus share significant sequence identity with the Ig-like domain subunits of the low affinity FcγRs and CD64/FcγRI. However, in contrast to other FCR/FCRL family members that reside at the cell surface, FCRLA and FCRLB lack transmembrane segments and are intracellular proteins. They also possess unique C-terminal mucin-like regions rich in serine/threonine, proline, and leucine residues.

Significant disparity in gene number as well as genetic and primary amino acid structure is evident for the murine relatives of human FCRLs. Three *FCRL* genes are located in tandem at a syntenic position of mouse chromosome 3 (Davis et al. 2002a, 2004; Guselnikov et al. 2002). Mouse *Fcrl1* and *Fcrl5* encode type I transmembrane proteins with moderately different features from their human cousins. Notably, mouse FCRL5 shares greater structural similarity to human FCRL2 and FCRL3 than its designated name suggests. Its closer relatedness to these receptors may also be supported by the expression patterns and ligands of these proteins (see below). By contrast, *Fcrls,* which is not present in the human genome, encodes a soluble chimeric protein with four Ig-like domains resembling human FCRL2, and a C-terminal type-B scavenger receptor cysteine-rich domain.

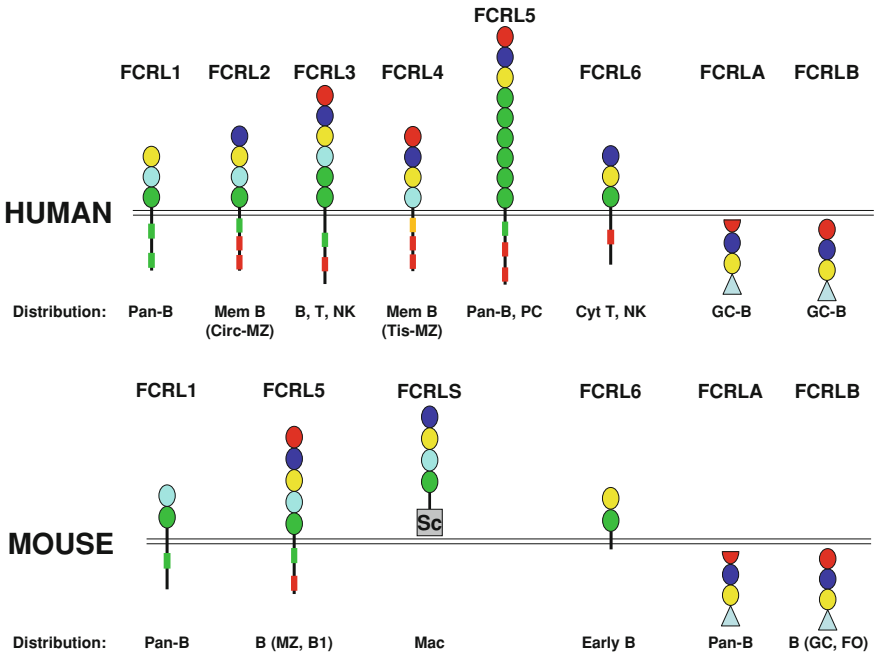


Fig. 1 Protein structure and distribution of human and mouse FCRL family members. Ig-like domains in the schematic diagram are color-coded to highlight their phylogenetic relationships. The first domain of FCRLA appears to be a degenerate Ig-like subunit and is thus truncated in the figure. The type-B cysteine-rich scavenger receptor domain (*Sc*) of mouse FCRLS and mucin-like regions (*triangles*) of FCRLA and FCRLB are also specified. FCRL1-6 cytoplasmic tails possess potential consensus ITIM (L/V/I)-X-Y-X-X-(L/V/I) (*red boxes*), ITAM-like (E/D)-X-X-Y-X-X-(L/I)-X_{6-8}-Y-X-X-(L/I) (*green boxes*), and ITSM (S/T)-X-Y-X-X-(V/I) (*orange box*) sequences. The expression patterns among B cells include memory (*Mem*), circulating (*Circ*) and tissue-based (*Tis*) marginal zone (*MZ*), plasma cell (*PC*), germinal center (*GC*), and follicular (*FO*) subsets. FCRL3 and FCRL6 are both expressed by cytotoxic (*Cyt*) T and NK cells, but FCRL3 is also found on CD4 T regulatory cells. Transcripts for FCRLS have been detected in macrophages (*Mac*)

Three other FCRL relatives, *Fcrl6*, *Fcrla*, and *Fcrlb* are located in syntenic locations on mouse chromosome 1. Although mouse FCRL6 shares greater identity to rat gp42 than human FCRL6, FCRLA, and FCRLB possess the highest interspecies orthology of the family.

3 Cellular Distribution of the FCRLs

FCRL gene expression is almost entirely restricted to lymphocytes and is preferentially concentrated within the B lineage. Transcript analyses from tissues or sorted cells by Northern blot, PCR, and in situ hybridization showed that *FCRL1–5* expression increases as a function of B cell differentiation and peaks among

circulating cells and those localized in secondary lymphoid tissues (Davis et al. 2001; Miller et al. 2002). The development of receptor-specific monoclonal antibodies (mAbs) confirmed these findings and enabled refined examination of their individual and sometimes overlapping expression patterns (see Fig. 1 summary). FCRL1 emerges at the pre-B cell stage and increases with B cell maturation, peaking on naïve and memory subpopulations (Leu et al. 2005; Polson et al. 2006). Although this general distribution implies its practical utility as a pan B cell marker, FCRL1, as well as its other four relatives, are downregulated by activated germinal center (GC) B cells. Evidence that the FCRL1 mouse ortholog exhibits similar broad expression among B cells (Davis et al. 2004) (Won and Davis, unpublished results), suggests that despite their interspecies structural variation, their regulation is strongly conserved. By contrast, FCRL2–5 exhibit subset-specific differences in their expression by B cells. FCRL2 and FCRL3 both peak on memory B cells in the periphery and mark a circulating innate-like marginal zone (MZ) B cell equivalent (Weller et al. 2004; Li et al. 2013). Their presence on this latter subset is noteworthy given the discrete basal regulation of the mouse FCRL5 protein by innate-like MZ and B1 B cells in mice (Won et al. 2006). These specialized B cells are distinguished by their germline-biased Ig repertoires, potential to secrete broadly reactive natural Abs, ability to respond to T cell-independent (TI) antigens, and involvement in primary humoral responses (Martin and Kearney 2000; Cerutti et al. 2013). Consistent with the sensitivity of these cells to innate stimulation, both human FCRL2 and FCRL3 as well as FCRL5 in mice are strongly induced by Toll-like receptor (TLR) agonists (Li et al. 2013) (Won and Davis unpublished results). However, FCRL3 is also individually expressed outside the B lineage by subpopulations of cytotoxic NK and $CD8^+$ T cells as well as a dysfunctional population of $CD4^+$ regulatory T cells (Polson et al. 2006; Nagata et al. 2009; Swainson et al. 2010). FCRL4 defines a subpopulation of tissue-based memory B cells with an activated phenotype and a discriminating transcript profile. These cells occupy sites in mucosa-associated lymphoid tissues (MALT) that correspond to an anatomical equivalent of the MZ (Falini et al. 2003; Ehrhardt et al. 2005, 2008); hence, FCRL4 is typically scarce among circulating B cell populations in healthy donors. FCRL5 has a broader B cell distribution that extends to, and reaches the highest surface density on, terminally differentiated plasma cells (PC) derived from the bone marrow, tonsils, or spleen (Polson et al. 2006). By contrast, human FCRL6 is not expressed by B cells, but is rather a distinguishing surface glycoprotein of perforin-expressing cytotoxic NK and $CD8^+$ T cells as well as a rare $CD4^+$ T lineage subset with similar lytic features (Wilson et al. 2007; Schreeder et al. 2008; Kulemzin et al. 2011). Thus, FCRL3 and FCRL6 are expressed by non-B cells and share overlapping expression on lymphocytes with cytolytic potential. In mice, FCRL6 can be induced in T cells by IL-2, but is constitutively produced by B cell precursors (Won and Davis unpublished results). Human FCRLA is predominantly found in subsets of GC B cells, mainly the proliferating centroblasts, but is expressed at some level by all B cell subsets in the tonsil, with the notable exception of PCs where it is very low/absent, (Davis et al. 2002b; Facchetti et al. 2002; Mechetina et al. 2002; Masir et al. 2004) and in freshly isolated blood B cells (Santiago et al. 2011). In mice,

FCRLA is broadly expressed among peripheral B cells, being highest in PCs, but is downregulated by GC B cells (Wilson et al. 2010; Reshetnikova et al. 2012). FCRLB has been difficult to study because its low-level transcripts are undetectable by Northern blot and even difficult to resolve by RT-PCR; with a single round of amplification, human FCRLB is found in placenta, kidney, and spleen (Wilson and Colonna 2005). Based on analysis of cell lines, FCRLB appears to be restricted among hematopoietic cells to the B lineage. To the extent that it has been examined in primary human tissue, FCRLB is restricted to the GC; however, $FCRLB^{+}$ cells are very rare, small, nonproliferating ($Ki\text{-}67^{-}$) B cells that do not co-express FCRLA (Wilson and Colonna 2005). Thus, despite many shared features, these two proteins appear to have mutually exclusive expression patterns among B cells. Essentially nothing is known about mouse FCRLB, except that the gene knockout has no obvious phenotype (Masuda et al. 2010). Outside of the B lineage, both FCRLA and FCRLB have been reported to be expressed in human melanocytes and melanoma cells (Chikaev et al. 2005; Inozume et al. 2005). The function of FCRLA/B in melanocytes has not been examined, but the expression of these receptors in non-B cells may suggest a more general role for them as ER chaperones.

4 Emerging FCRL Ligands

A major hurdle for understanding the immunologic function of these receptors has been the enigmatic nature of their counterpart ligands. While most FCRLs still remain orphan receptors, ligands for several family members have recently been discovered (see summary in Table 1). There was early anecdotal evidence that FCRL4 and FCRL5 could bind heat-aggregated IgA and IgG (Hatzivassiliou et al. 2001), an anticipated finding given their homology to the classical FCRs. This unpublished data was unconfirmed until Polson et al. also detected interactions between FCRL5 and IgG when staining with a preparation of mixed isotypes, but reactivity was lost when individual subclasses were used (Polson et al. 2006). However, recent work by the Colonna group has confirmed and extended these early findings. By generating FCRL1–6 transient transfectants for flow cytometry-based Ig-binding studies, Wilson et al. confirmed that FCRL4 and FCRL5, but not other FCRLs, can bind heat-aggregated IgA and IgG (Wilson et al. 2012). FCRL5 demonstrated relatively stronger binding to IgG1 and IgG2 aggregates than to IgG4, and reactivity required the three N-terminal Ig-like FCRL domains. The specificity of FCRL4 and FCRL5 interactions with Ig observed in these studies was further supported by blockade studies using receptor-specific mAbs.

The binding specificity and kinetics of FCRL5/IgG associations have now been independently confirmed by surface plasmon resonance (SPR) analyses. Using FCRL5 recombinant protein, the Tolnay laboratory validated interactions with IgG and similarly localized the binding interface with a panel of mAbs reactive with

Table 1 Ligands identified for human and mouse FCRL family members

Receptor	FCRL ligands
Human	
FCRL1	N.D.
FCRL2	N.D.
FCRL3	N.D.
FCRL4	Heat-aggregated IgA—flow cytometry (Wilson et al. 2012)
FCRL5	Heat-aggregated IgG: IgG1, IgG2 > IgG3 > IgG4-flow cytometry (Wilson et al. 2012) Intact IgG; IgG1, IgG4 ($\sim$1 μM) > IgG3 (18 μM) > IgG2 (0.03–205μM)—SPR (Franco et al. 2013)
FCRL6	MHC class II/HLA-DR (Schreeder et al. 2010)
FCRLA	Intracellular IgM and IgG (Wilson et al. 2010) Intracellular IgM, IgG, and IgA (Santiago et al. 2011)
FCRLB	N.D.
Mouse	
FCRL1	N.D.
FCRL5	OMCP (Orthopox MHC class I-like protein) (Campbell et al. 2010)
FCRL6	N.D.
FCRLS	N.D.
FCRLA	N.D.
FCRLB	N.D.

No data (N.D.); surface plasmon resonance (SPR)

the three membrane distal domains of FCRL5 (Franco et al. 2013). Moreover, these studies also revealed several novel and unexpected features of the FCRL5-Ig interaction. First, although binding affinities were estimated overall in the micromolar (μM) range, variation was evident among monoclonal and polyclonal IgG1–4 subclass preparations. IgG1 and IgG4 bound with a K_D of $\sim$1 μM, but the affinity of IgG3 was about a log lower at $\sim$10 μM. However, IgG2 bound over a range of affinities from 35 nM (nanomolar) to 205 μM that varied according to the Ig sample. Second, IgG bound FCRL5 with unusual heterogeneous two-state kinetics that differed among the subclasses. In general, SPR sensorgrams demonstrated a fast initial on rate (K_{a1}) followed by a slow secondary association (K_{a2}), whereas dissociation at the end of the injection was initially fast (K_{d1}) followed by a slower secondary phase (K_{d2}). Notably, these parameters differ from the 1:1 kinetics that typify classical FCR/Ig interactions (Bruhns et al. 2009) and indicate that other properties, beyond the isotype, influence FCRL5's recognition of IgG. Third, in contrast to the FCRs for IgG, IgM, and IgE, which interact strictly with the Fc region, high-affinity binding to FCRL5 required intact Ig molecules. Enzymatic digestion and biochemical strategies to correlate structure–function contributions of IgG1 anatomy with the two-state kinetics evident by SPR confined a primary interaction with the Fc portion and a secondary interaction with the $F(ab')_2$ region. Finally, binding affinities were also strongly dependent on Ig glycosylation status. Sialic acid enrichment of IVIg preparations promoted higher

affinity kinetics, whereas deglycosylation abrogated nearly all binding activity. These intriguing findings introduce a second immunoregulatory IgG-binding receptor on B cells that possesses complex binding properties and potentially higher differential affinity for some intact IgG isotypes than CD32/FcγRIIB, which has a K_A of $\sim 2.5 \times 10^4 - 2 \times 10^5\ M^{-1}$, depending on the IgG isotype (Bruhns et al. 2009).

Evidence for unconventional Ig binding has also been shown for the FCRLA intracellular protein, whose two Ig domains resemble two of the three Ig-like subunits present in the high-affinity CD64/FcγRI. Early studies using chimeric proteins artificially expressed on the cell surface failed to demonstrate interactions with Igs (Facchetti et al. 2002); however, immunoprecipitation of endogenous FCRLA disclosed its co-association with intracellular IgM, IgG, and IgA in cell lines as well as in primary B cells (Wilson et al. 2010; Santiago et al. 2011). The elevated expression of FCRLA in GC B cells, together with its ability to bind multiple isotypes of intracellular Ig, suggests a possible role for FCRLA in Ig retention during affinity maturation. Indeed, FCRLA has been shown to preferentially associate in the ER with the secretory versus membrane form of IgM in the GC-like human B cell line Ramos (Santiago et al. 2011). Although FCRLB has even greater sequence identity with CD64, no evidence of Ig binding has been established for it yet and, like the *Fcrla* knockout mouse (Wilson et al. 2010), the *Fcrlb* knockout mouse had no discernible phenotype (Masuda et al. 2010).

Aside from Ig, MHC-related proteins have been identified as ligands for two other FCRLs, human FCRL6, and mouse FCRL5. FCRL6 is not expressed on B cells but on cytotoxic T cells and NK cells. To search for its ligand(s), we employed a cell line engineered with an NFAT driven GFP reporter that was co-transduced with a construct encoding the human FCRL6 extracellular region fused to the ITAM-bearing mouse CD3ζ cytoplasmic tail (Schreeder et al. 2010). GFP induction was triggered when cells from different sources expressing MHC class II were used for co-culture assays. These studies defined HLA class II as an FCRL6 ligand. Importantly, variability in FCRL6 staining of transductants expressing MHCII heterodimers with different beta subunits indicated that FCRL6 binding affinities may differ according to the MHCII haplotype. These studies thus introduce a novel interaction between FCRL6-expressing cytotoxic NK and T lymphocytes that are critical for maintaining cell-mediated immunity and antigen presenting cells or other cells that upregulate MHCII. Another MHC-related protein was discovered as a mouse FCRL5 ligand. Using a hidden Markov model to identify MHC-like viral proteins that might function as immune decoys, Campbell et al. found a immunoevasin encoded by a cowpox virus termed orthopox MHC class I protein (OMCP). OMCP had first been identified as a ligand for the NK cell activation receptor NKG2D and could suppress its role in cytotoxicity (Campbell et al. 2007). However, beyond NK cells, OMCP also bound innate-like MZ and B1 B cells. An expression cloning approach defined mouse FCRL5 as a second OMCP receptor and the use of blocking mAbs and receptor mutants narrowed the binding interface to FCRL5's three N-terminal domains (Campbell et al. 2010). Although the functional impact of these associations is not yet clear, the tyrosine-based

regulatory potential of these receptors, their interactions with fundamental elements of adaptive immunity, and exploitation as targets of manipulation by pathogens underscore their critical roles in lymphocyte biology.

5 Functional and Regulatory Properties

5.1 Roles in Adaptive B Cell Signaling

In addition to their divergent extracellular Ig-like domain configurations, the cytoplasmic properties of FCRLs are also more complex than those of the classical FCRs. A common theme among IgSF protein families such as the FCR, leukocyte Ig-like receptors (LILR), and paired Ig-like receptors (PIR) is to balance cellular responses by expressing representatives with either activating or inhibitory capacity. These tyrosine-based signals may be transmitted directly via motifs in their cytoplasmic tails or indirectly through noncovalent transmembrane interactions with adaptor proteins harboring cytoplasmic ITAMs. In contrast to this form of bimodal regulation using separate paired receptors, most FCRL cytoplasmic tails possess both ITAM-like and ITIM elements. The possession of these tandem intracellular sequences indicates that the majority of these molecules may be capable of exerting dual-modulation in an autonomous fashion. FCRL1 appears to be an exception to this. It has two ITAM-like sequences and serves as a co-activation receptor. Its ligation by receptor-specific mAbs results in its tyrosine phosphorylation (pTyr) and stimulates human B cell proliferation (Leu et al. 2005). Moreover, crosslinking FCRL1 with the B cell receptor (BCR) augments activation as indicated by enhanced calcium flux and B cell proliferation. Our unpublished observations of the mouse FCRL1 protein show that it has similar activating properties (Won and Davis unpublished results). FCRL1 is also unique among FCRL in humans and mice by virtue of a charged glutamic acid residue in its transmembrane region. This feature indicates that FCRL1 likely co-associates with another partner in *cis*, but what effector proteins are recruited to its intracellular tyrosine-based sequences or its acidic transmembrane region remain under investigation.

Work exploring the contributions of the FCRL2–5 cytoplasmic tyrosine-based motifs on BCR-mediated activation has been carried out by several groups (Ehrhardt et al. 2003; Haga et al. 2007; Kochi et al. 2009; Jackson et al. 2010). Details of these mutagenesis and chimeric receptor analyses have been carefully summarized in a recent review (Ehrhardt and Cooper 2011). Despite the binary potential implied by their composite intracellular regions, in general BCR co-ligation studies have identified a suppressive function for them. While their engagement alone does not appear to impact basal B cell function, crosslinkage with the BCR induces pTyr of FCRL2–5 and coincident docking of the SHP-1 and/or SHP-2 SH-2 domain-containing phosphatases at consensus ITIMs (Fig. 2).

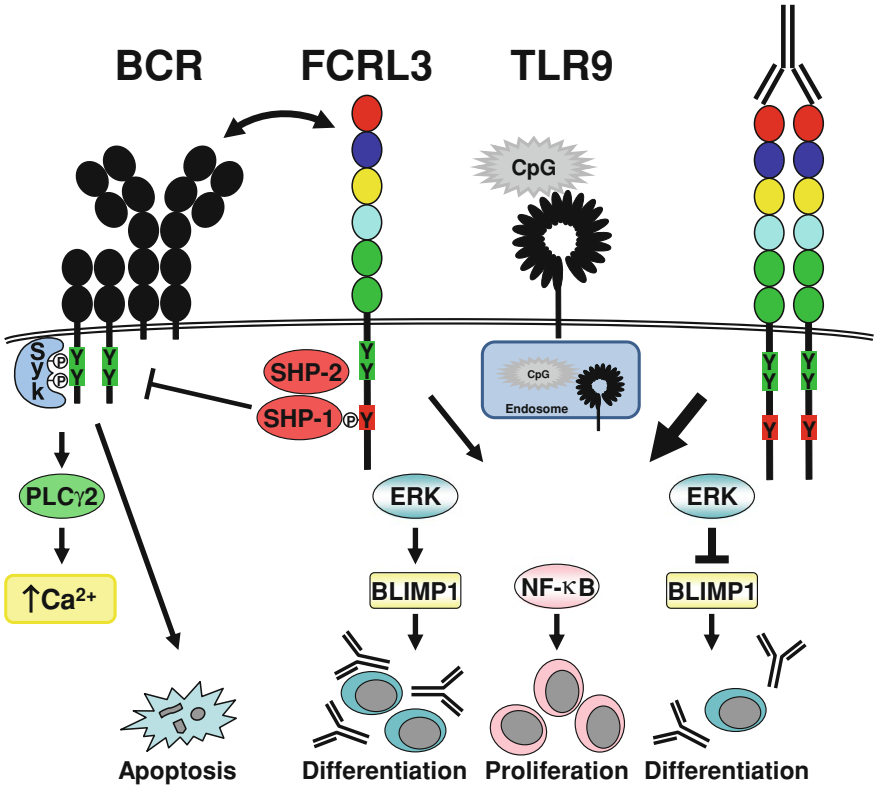


Fig. 2 Differential FCRL3 regulation of adaptive and innate signaling pathways. Co-ligation of FCRL3 with the BCR induces pTyr of the receptor, facilitates the recruitment of SHP-1 and SHP-2 that inhibit Syk and PLCγ2 phosphorylation, and suppresses downstream calcium signaling and apoptosis. Following exposure to the CpG DNA TLR9 agonist, FCRL3 expressing B cells are globally pTyr and activate the NF-κB/p-p65 and MAPK/pERK and p-p38 pathways that drive proliferation. pERK additionally induces expression of the BLIMP1 plasma cell commitment factor that stimulates B cell differentiation and Ab production. By contrast, simultaneous crosslinking of FCRL3 with receptor-specific mAbs in TLR9 activated B cells significantly elevates whole pTyr, p-p65, pERK, and p38 to promote proliferation and survival. However, augmented pERK activation in TLR9/FCRL3 co-stimulated B cells represses BLIMP1 induction and abrogates plasma cell differentiation and Ab production

Accordingly, these repressive components attenuate antigen receptor-mediated calcium mobilization and MAPK activation. However, these studies have also uncovered subtle hints of possible dual functionality. This was initially suggested by the potential of FCRL3 to recruit Syk and ZAP-70 as well as SHP-1 and SHP-2 (Xu et al. 2002), a finding that was recently confirmed (Kochi et al. 2009). Moreover, experiments employing B cell lines transfected with disabled FCRL2-5 ITIM mutants frequently result in enhanced calcium flux compared to BCR engagement alone (Ehrhardt et al. 2003; Haga et al. 2007; Kochi et al. 2009; Jackson et al. 2010).

To more carefully investigate their suspected dualistic function, we dissected the tyrosine-based regulation of the mouse FCRL5 ortholog that, like human FCRL2-5, possesses both cytoplasmic ITAM-like and ITIM consensus sequences. Initial work in WEHI231 and primary MZ B cells showed that FCRL5 also inhibits BCR-mediated calcium signaling (Won et al. 2006), but surprisingly had little impact on activation in innate-like B1 B cells that also discretely express it. As in earlier work (Ehrhardt et al. 2003), we engineered a panel of chimeric receptor mutant constructs comprised of the extracellular and transmembrane portions of mouse FcγRIIB fused to different FCRL5 Y > F tail variants for transduction into the FcγR deficient IgG2a class-switched A20IIA1.6 B cell line (Zhu et al. 2013). This system permits a comparative analysis of downstream signaling pathways engendered by chimeric receptor/BCR co-engagement by using intact anti-mouse IgG versus BCR-only triggering using F(ab')2 fragments (Okazaki et al. 2001). These studies indeed disclosed inverse contributions for the FCRL5 intracellular motifs on BCR driven calcium flux and MAPK activation. Effector recruitment experiments identified binding of SHP-1 at the ITIM and the Lyn Src family kinase at the N-terminal ITAM-like tyrosine residue, but the C-terminal tyrosine was dispensable. These relationships were also confirmed in sorted primary MZ and B1 B cells. To further validate these findings in primary cells and to investigate the apparent impotent effects of FCRL5 on BCR activation in B1 B cells, we utilized mice deficient in Lyn or SHP-1 activity (motheaten/Me^{v}) (Shultz et al. 1993; Chan et al. 1997). Both of these models have expanded B1 B cells, but they express FCRL5 at levels comparable to C57BL/6 wild-type mice. Using B1 B cells from these mutant strains allowed us to directly deconstruct the impact of these opposing signaling proteins on calcium flux and whole-cell pTyr signaling and correlate observations made in A20IIA1.6 cells. These findings revealed a critical role for the SHP-1/Lyn signaling circuit in balancing FCRL5 function. These binary regulatory properties were unique compared to other well-studied inhibitory receptors including CD5, CD22, CD32, and CD70, which failed to acquire enhancing function in SHP-1 deficient B cells. Moreover, the relative activity of SHP-1/Lyn differed in MZ versus B1 B cells. The dominant inhibitory function for FCRL5 directly correlated with a twofold higher level of SHP-1 in MZ B cells, whereas the lack of FCRL5 influence in B1 B cells was ascribed to more balanced SHP-1/Lyn activity in this subset. These data provide robust molecular and functional evidence for novel dual-regulatory features of FCRL molecules and uncover subset-specific differences in their activity in innate-like B cells.

5.2 Influence on Innate-Like B Cell Responses

Although the majority of FCRL signaling work has been focused on the modulation of antigen receptor activation pathways in B cells, more recent studies have begun to explore the effects of human FCRLs on innate-driven cascades. Extending work on FCRL4 signaling, Sohn et al. detected constitutive pTyr and

SHP-1 and SHP-2 binding to FCRL4 in transfected unstimulated Ramos B cells (Sohn et al. 2011). BCR crosslinking did not appear to influence these relationships, but all three tyrosine residues were required for proximal inhibition of Syk activation and the downstream PLCγ2, Vav, and calcium signaling pathways by FCRL4. Consequently, FCRL4 also halted CD69 induction following BCR co-ligation, but its expression alone independently impaired immune synapse formation as determined by time-lapse imaging using TIRF microscopy. However, in addition to adaptive stimulation, the Pierce group also examined the impact of FCRL4 on TLR responses. Remarkably, exposure of FCRL4 expressing B cells to the TLR9 agonist CpG prompted co-localization of these receptors in endosomes and the upregulation of CD23. These results strongly indicate a differential regulatory role for FCRL4 in adaptive versus innate signaling.

Additional evidence for these proteins promoting TLR-mediated signaling has also been observed for FCRL3. We recently examined the impact of this disease-associated receptor (see below) on TLR9-mediated B cell responses. FCRL3 engagement with receptor-specific mAbs augmented TLR9 triggered blood B cell proliferation, survival, and induction of the CD25, CD86, and HLA-DR activation markers (Li et al. 2013) (see Fig. 2). Remarkably though, FCRL3 had inverse effects on Ig production. To examine its role in TI PC generation, we adapted a cord blood differentiation model (Capolunghi et al. 2008). Culturing transitional B cells with CpG 2006 and FCRL3 mAbs promoted B cell proliferation, but halted the differentiation of Ab secreting cells. Flow-based analyses revealed that FCRL3 enhances CpG-mediated NF-κB p65 and MAPK pERK and p38 activation. Because ERK signaling can modulate the expression and regulation of the PC commitment factor BLIMP1 (Rui et al. 2003; Yasuda et al. 2011), we considered this pathway as a mechanistic link for these surprising observations. Exposure of the FCRL3-expressing SUDHL5 B cell line to CpG indeed upregulated BLIMP1 expression, but coincident FCRL3 ligation substantially blocked induction of this repressor protein. Consequently, ERK-dependent BLIMP1 suppression transmitted by FCRL3 could be restored by treatment with a MEK inhibitor. These data provide additional support for counter-regulatory functions of FCRL proteins in adaptive and innate B cell responses. Furthermore, the finding that FCRL3 modulates the differentiation of Ab secreting cells may be important in its implicated role in the pathogenesis of autoimmune (AI) disorders discussed below.

Stimulatory properties for FCRL5 in the interplay of adaptive and innate pathways have also been found by the Tolnay group. Their earlier studies had shown that the viral Notch analog Epstein-Barr virus nuclear antigen 2 (EBNA2) could induce *FCRL5* expression via interactions with the CBF1/RBP-Jκ DNA-binding protein in its promoter (Mohan et al. 2006). In a follow-up analysis they investigated FCRL5 modulation following exposure to different stimuli and explored the consequences of its ligation on B cell responses. Although naïve B cells isolated from blood modestly upregulated protein levels when exposed to CpG, FCRL5 was markedly induced by anti-Ig co-stimulation (Dement-Brown et al. 2012). By contrast, the addition of T cell-dependent (TD) stimuli in the form of anti-CD40 and IL-2 had little effect. However, this combination of stimuli along

with mAb-directed co-ligation of FCRL5 and the BCR enhanced B cell proliferation during TLR9 stimulation. This approach yielded other positive effects including the generation of IgG and IgA isotype switched cells as well as unusual cells that co-expressed several Ig isotypes. This phenomenon has been previously seen in patients with hairy cell leukemia (HCL) (Forconi et al. 2001). It is important to note that, despite similar effects on proliferation, these results concerning a positive role for FCRL5 in driving isotype switched cells may conflict with our recent findings that FCRL3 inhibits PC generation and Ig secretion. Given the alternate experimental strategies, including the use of T cell help that can relieve ERK-mediated BLIMP1 repression (Rui et al. 2006), follow-up studies will be required to carefully dissect the nature of these divergent outcomes.

5.3 Insight from In Vivo Models

Finally, little is known about the in vivo roles of these molecules as only a few genetically deficient mice have been generated. To this point, two transgenic models have now been published describing mice with targeted disruption of *Fcrla* and *Fcrlb*. The Colonna group developed $Fcrla^{-/-}$ mice by cre-mediated ablation of the third and fourth exons that encode the two Ig-like domains in 129 ES cells (Wilson et al. 2010). Lymphocyte development was grossly normal including CD4 and CD8 T cell populations in the thymus and B cells in the bone marrow and spleen. Lymphoid architecture of splenic GCs that formed following challenge with SRBCs was also unremarkable in these mice and the absence of FCRLA protein was confirmed with rabbit polyclonal antisera. However, the availability of pAbs did clarify that intracellular FCRLA expression is restricted to and evident throughout B cell development in wild-type mice. Although primary humoral responses to SRBCs were unperturbed, secondary challenge indicated significantly higher anti-SRBC IgG1 levels in $Fcrla^{-/-}$ animals. However, immunization with another TD antigen NP-KLH did not show alterations in primary or secondary NP-specific IgM or IgG responses as a function of the dose or memory recall time out to 6 months. TI type II responses were also intact. Furthermore, despite the ability of human FCRLA to bind intracellular Ig, the quality and functional activity of Ig produced by $Fcrla^{-/-}$ mice was also normal. Aside from potentially disadvantageous strain-specific regulatory differences (see below), alternative immune challenge strategies or crosses with disease-susceptible models may be required to unveil the subtle phenotype of these mice. However, it is also possible that FCRLA possesses redundant properties to other Ig-binding chaperones. Because FCRLB has similar features it could certainly serve as a candidate, although the evidence to date suggests that these two molecules are not co-expressed, at least in humans (Wilson and Colonna 2005).

The *Fcrlb* gene was targeted by the Burrows group with a construct designed to replace $\sim$1.5 kb of its 5′ upstream sequence including the promoter as well as exons 1, 2, and the 5′ end of exon 3 with neomycin cassette in 129 ES cells

(Masuda et al. 2010). Despite the lack of FCRLB-specific Abs, a PCR-based analysis validated loss of *Fcrlb* expression at the transcript level. Mice were viable and backcrossed with C57BL/6. Similar to *Fcrla* deficiency, there were no global differences in lymphocyte development either from bone marrow and spleen-derived B lineage cells or CD4 and CD8 T cell populations from the thymus or spleen. Although in vitro proliferation studies were unrevealing, TD challenge with NP-CGG indicated enhanced NP-specific IgG1 Ab responses that were more pronounced for those with high-affinity. Moreover, ELISPOT assays showed increased numbers of Ab secreting cells in the spleen and bone marrow. Unfortunately, these results were confounded by a 13 bp deletion in the *Fcgr2b* promoter common to AI mouse strains including 129. This promoter variation disturbs two putative transcription factor binding sites for AP4 and an S box resulting in reduced FcγRIIB expression and inhibitory function (Pritchard et al. 2000). Thus, strains harboring this anomaly have relatively more exuberant TD Ab responses and a greater propensity to develop auto-Abs than mice with intact regulatory regions that are governed by higher FcγRIIB expression and consequent SHIP1 repression (Xiu et al. 2002). Unfortunately, PCR amplification clarified that *Fcrlb* deficient mice indeed possess this deletion making the independent impact of FCRLB difficult to discern. Furthermore, the use of 129 background ES cells for the generation of *Fcrla* knockout mice may also provide rationale for the enhanced SRBC production seen in these mice (Wilson et al. 2010). Investigators will need to be attentive to this issue and craft suitable strategies for accurately assessing humoral responses in FCRL-related transgenic mice.

6 FCRL Involvement with Disease

Given their preferential expression by B cells, it comes as no surprise that since their initial discovery, associations for FCRL family members with immune-mediated disorders have been steadily growing. Not only are they candidate biomarkers for clinical diagnosis and prognosis as well as logical therapeutic targets, but roles for them in disease pathogenesis are also becoming clear. As nearly 75 % of leukemias and lymphomas are B cell-derived, multiple groups have detected their expression and dysregulation in various lymphoproliferative disorders. In fact, their involvement in B cell malignancies first led to the Dalla-Favera laboratory's unearthing of *FCRL4/IRTA1* as a partner joined at a t(1;14)(q21;32) translocation breakpoint in a MM cell line (Hatzivassiliou et al. 2001; Miller et al. 2002). Northern blot analyses of Burkitt lymphoma (BL) cell lines harboring 1q21 abnormalities revealed upregulation of *FCRL5/IRTA2* in the majority of samples. Early searches of *FCRL* expressed sequence tags (ESTs) in the Lymphochip microarray database also showed differential upregulation for the FCRLs among diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), and chronic lymphocytic leukemia (CLL) samples (Alizadeh et al. 2000). The availability of mAbs confirmed FCRL1–5 protein surface expression on DLBCL, FL, CLL, BL,

HCL, and mantle cell lymphoma (MCL) samples (Ise et al. 2005; Polson et al. 2006; Du et al. 2008). However, studies by Ise et al. also detected soluble FCRL5 in the sera of patients with various B lineage malignancies (Ise et al. 2007). Given the lack of diagnostic markers for MZ lymphomas (MZL) and evidence that FCRL4 distinguishes a subset of B cells positioned near the epithelium in MALT corresponding to the MZ, Falini et al. recently investigated its use as a novel histopathologic marker. FCRL4 was identified in the majority of nodal (154/210 73 %) and extranodal (307/329 93 %) MZLs, but was not present in the subtype derived from the spleen (Falini et al. 2012).

Apart from their applicability in diagnosis, the use of FCRLs as prognostic biomarkers has also been under investigation. CLL, the most common leukemia in Western countries, can be segregated into two subtypes that differ in clinical aggressiveness according to the degree of somatic hypermutation in the heavy chain variable region (*IGHV*) gene expressed by the clonally expanded B cells (Damle et al. 1999; Hamblin et al. 1999). To assess whether FCRL1–5 might be useful surrogates for predicting *IGHV* mutation status, we analyzed 107 CLL samples, including 55 mutated-indolent and 52 unmutated-aggressive patients, with a panel of FCRL-specific reagents by flow cytometry (Li et al. 2008). While FCRL1–3 and FCRL5 were all significantly upregulated by the mutated-indolent subtype, FCRL4 was not detected. Remarkably, FCRL2 emerged as 94 % concordant with *IGHV* status and was superior to two established markers of aggression, CD38 and ZAP-70 (Rassenti et al. 2008), in predicting this hallmark feature, and by multivariate analysis was more robust at forecasting first time to progression. Current validation of these initial findings using optimized reagents and expanded samples shows growing promise for FCRL2 as a novel biomarker in CLL.

Strategies to immunotherapeutically target these molecules have also been explored. Because FCRL1 is broadly expressed by B cells it may be a useful candidate. Work by Du et al., who have retained an interest in immunotoxin treatment approaches, found FCRL1 on the majority of CLL, FL, HCL, and MCL samples analyzed and explored the cytotoxicity of anti-FCRL1 toxin conjugated mAbs (Du et al. 2008). Additionally, since FCRL5 is present on PCs, it is being pursued as a tool for MM immunotherapy. An analysis of bone marrow aspirates from MM, monoclonal gammopathy of unknown significance (MGUS), and healthy donors confirmed FCRL5 expression on PCs and the development of antibody–drug conjugates has shown promising preclinical efficacy for targeting it in xenograft models (Elkins et al. 2012).

Several FCRLs have also drawn interest by virtue of their upregulation among lymphocyte populations in individuals with infectious diseases. Perhaps the best investigated representative in this context is the appearance of $FCRL4^{+}$ cells in the circulation of patients afflicted with chronic viral diseases including HIV and Hepatitis C. However, a similar innate-like B cell population has also been found in patients with combined variable immunodeficiency (CVID) (Rakhmanov et al. 2009). Moir et al. identified a subset of $CD19^{+}CD20^{+}CD27^{-}CD21^{-}CD10^{-}$ B cells in the blood of viremic HIV patients that surprisingly co-expressed FCRL4 (Moir et al. 2008). This subpopulation exhibited features of “exhaustion” akin to T

cells in persistent LCMV infections (Zajac et al. 1998; Wherry et al. 2007), which are characterized by the upregulation of inhibitory receptors as well as diminished proliferative potential and replication history. Despite limited Ig diversity, this distinct tissue-like FCRL4$^+$ subset possessed an Ab repertoire enriched for HIV-specific antigens, implying a key role for it in effector humoral responses. To clarify its contribution to their arrested function, a siRNA approach found that knockdown of FCRL4 and several other inhibitory receptors could restore BCR-mediated proliferation, HIV-specific Ab responses, as well as cytokine and chemokine production (Kardava et al. 2011). These findings suggested that lingering tonic signaling by the HIV pathogen might lead to reciprocal dampening mechanisms in these B cells. However, exciting new findings by the Fauci laboratory have provided a novel perspective on the ability of HIV to directly handicap B cell responsiveness. Their earlier work had demonstrated that the gp120 HIV envelope protein can directly bind the α4β7 integrin on NK and T cells (Arthos et al. 2008). In a recent study, gp120 was similarly found to interact with this ligand on B cells and in turn inhibit proliferation and cell cycle progression (Jelicic et al. 2013). Microarray profiling disclosed the upregulation of *TGFβ1* together with related elements of this cascade and FCRL4. Because TGFβ has repressive effects on B cell function (Kehrl et al. 1991), the investigators established that gp120/α4β7 integrin binding initiated an axis of suppression by triggering TGFβ secretion and the autocrine induction of FCRL4, as well as down-modulation of the CD80 co-stimulatory protein. These results have important implications for understanding the humoral dysfunction in HIV patients and could be informative for clarifying why a similar FCRL4$^+$ population materializes in the circulation of individuals infected with malaria and Hepatitis C (Charles et al. 2008; Weiss et al. 2009). How FCRL4 contributes to B cell impairment in these chronic infections and in what way its newfound IgA ligand is integrated will require further study (Wilson et al. 2012).

With regard to tolerance and AI, disease risk associations for single nucleotide polymorphisms (SNP) located in the intergenic noncoding and coding regions of *FCRL* genes have been mounting in a variety of disorders and syndromes. Pioneering work by Kochi et al. surveyed a $\sim$2 Mb region around the *FCRL1-5* locus and identified 41 SNPs in the gene cluster including one that had a peak association among 658 Japanese controls and 830 individuals afflicted with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Grave's disease, and other types of AI (Kochi et al. 2005). The principal variant (rs7528684; $P = 8.5 \times 10^{-7}$; OR = 2.15; 95 % confidence interval = 1.58−2.93) was located in a potential NF-κB consensus binding motif within the *FCRL3* promoter region, 169 bp upstream of the transcription initiation site. Intriguingly, the C susceptibility allele of this −169T → C SNP generated a more orthodox NF-κB binding sequence, fostered higher promoter activity via p50, p65, and c-Rel binding, and exerted a dose-dependent regulatory effect on *FCRL3* transcript and protein expression as well as on auto-Ab production (Kochi et al. 2005; Gibson et al. 2009). This report has stimulated over 80 publications that have focused on this functional SNP as well as others among the FCRLs in a multitude of AI disorders. A recent genome-wide

association study of Grave's disease in a Chinese-Han population has verified the *FCRL3* association and refined the analysis of *FCRL* SNPs in this 1q21 region (Zhao et al. 2013). Accordingly, there is now also evidence that FCRL3 is modulated by this SNP in T cells and is associated with clinical progression in RA (Maehlen et al. 2011; Bajpai et al. 2012). Confirmation that the −169 SNP confers risk or protection in different AI conditions has led to the candidature of *FCRL3* as a general AI susceptibility gene (Chistiakov and Chistiakov 2007). However, there have also been conflicting results for many analyses that find no link with AI disease susceptibility. These incompatible outcomes may reflect differences in racial and ethnic backgrounds. An updated meta-analysis was recently performed to assess the growing number of *FCRL3* case-control association studies and highlight its heterogeneous pathogenic potential in AI (Yang et al. 2013). These intriguing genetic relationships, along with our growing understanding of FCRL3's complex influence on lymphocyte biology in innate versus adaptive responses, have the potential to provide exciting new insight into AI disease pathogenesis.

7 Conclusions

Substantial recent progress has been made in the FCRL field and these discoveries are beginning to unravel fundamental roles for this extended family in the immune system. The recent identification of ligands for several FCRLs presents a new gateway for realizing their biology, but further work will be required to understand the functional consequences of these interactions. How their complex dual-regulation is integrated during these encounters and how they are impacted by innate and adaptive responses will also need to be explored. Another major hurdle has been their evident interspecies differences and a lack of fruitful genetic deficiency models for study in mice. However, several trends are beginning to take shape. For example, the preferential expression of human FCRL2–FCRL3 as well as mouse FCRL5 by innate-like MZ B cells and their capacity to promote TI responses suggests that the regulation of these genes is to a certain extent conserved. Thus, investigating these relatives in parallel will be important. Furthermore, FCRL4's unique distribution among innate-like tissue-based B cells and ability to enhance TI signaling indicates that several FCRLs serve as facilitators of innate stimulation. Determining how their tyrosine-based signaling features modulate TLR versus BCR activation is another area ripe for investigation. Finally, their significance as pathologic, diagnostic, prognostic, and therapeutic agents is showing great promise in a large number of lymphoid malignancies and immune-mediated disorders. In conclusion, recent advances have launched a new phase of exploration for FCRL family members in lymphocyte biology. These intriguing developments portend key roles for these receptor-genes in normal and perturbed immunity and we expect this momentum will accelerate our basic and therapeutic understanding of the FCRLs in the coming years.

Acknowledgments P.D.B. was supported by NIH grant AI100076. R.S.D. was supported by funding from the Lupus Research Institute, the UAB Center for AIDS Research (AI027767), and NIH grants AI097729, CA175912, and CA161731.

References

Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A et al (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403:503–511

Arthos J, Cicala C, Martinelli E, Macleod K, Van Ryk D, Wei D et al (2008) HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. Nat Immunol 9:301–309

Bajpai UD, Swainson LA, Mold JE, Graf JD, Imboden JB, McCune JM (2012) A functional variant in FCRL3 is associated with higher Fc receptor-like 3 expression on T cell subsets and rheumatoid arthritis disease activity. Arthritis Rheum 64:2451–2459

Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S et al (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113:3716–3725

Campbell JA, Davis RS, Lilly LM, Fremont DH, French AR, Carayannopoulos LN (2010) Cutting edge: FcR-like 5 on innate B cells is targeted by a poxvirus MHC class I-like immunoevasin. J Immunol 185:28–32

Campbell JA, Trossman DS, Yokoyama WM, Carayannopoulos LN (2007) Zoonotic orthopoxviruses encode a high-affinity antagonist of NKG2D. J Exp Med 204:1311–1317

Capolunghi F, Cascioli S, Giorda E, Rosado MM, Plebani A, Auriti C et al (2008) CpG drives human transitional B cells to terminal differentiation and production of natural antibodies. J Immunol 180:800–808

Cerutti A, Cols M, Puga I (2013) Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nat Rev Immunol 13:118–132

Chan VW, Meng F, Soriano P, DeFranco AL, Lowell CA (1997) Characterization of the B lymphocyte populations in Lyn-deficient mice and the role of Lyn in signal initiation and down-regulation. Immunity 7:69–81

Charles ED, Green RM, Marukian S, Talal AH, Lake-Bakaar GV, Jacobson IM et al (2008) Clonal expansion of immunoglobulin M + CD27 + B cells in HCV-associated mixed cryoglobulinemia. Blood 111:1344–1356

Chikaev NA, Bykova EA, Najakshin AM, Mechetina LV, Volkova OY, Peklo MM et al (2005) Cloning and characterization of the human FCRL2 gene. Genomics 85:264–272

Chistiakov DA, Chistiakov AP (2007) Is FCRL3 a new general autoimmunity gene? Hum Immunol 68:375–383

Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL et al (1999) Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 94:1840–1847

Davis RS, Dennis G Jr, Odom MR, Gibson AW, Kimberly RP, Burrows PD et al (2002a) Fc receptor homologs: newest members of a remarkably diverse Fc receptor gene family. Immunol Rev 190:123–136

Davis RS, Li H, Chen CC, Wang YH, Cooper MD, Burrows PD (2002b) Definition of an Fc receptor-related gene (FcRX) expressed in human and mouse B cells. Int Immunol 14:1075–1083

Davis RS, Stephan RP, Chen CC, Dennis G Jr, Cooper MD (2004) Differential B cell expression of mouse Fc receptor homologs. Int Immunol 16:1343–1353

Davis RS, Wang YH, Kubagawa H, Cooper MD (2001) Identification of a family of Fc receptor homologs with preferential B cell expression. Proc Natl Acad Sci USA 98:9772–9777

Dement-Brown J, Newton CS, Ise T, Damdinsuren B, Nagata S, Tolnay M (2012) Fc receptor-like 5 promotes B cell proliferation and drives the development of cells displaying switched isotypes. J Leukoc Biol 91:59–67

Du X, Nagata S, Ise T, Stetler-Stevenson M, Pastan I (2008) FCRL1 on chronic lymphocytic leukemia, hairy cell leukemia, and B-cell non-Hodgkin lymphoma as a target of immunotoxins. Blood 111:338–343

Ehrhardt GR, Cooper MD (2011) Immunoregulatory roles for fc receptor-like molecules. Curr Top Microbiol Immunol 350:89–104

Ehrhardt GR, Davis RS, Hsu JT, Leu CM, Ehrhardt A, Cooper MD (2003) The inhibitory potential of Fc receptor homolog 4 on memory B cells. Proc Natl Acad Sci U S A 100:13489–13494

Ehrhardt GR, Hijikata A, Kitamura H, Ohara O, Wang JY, Cooper MD (2008) Discriminating gene expression profiles of memory B cell subpopulations. J Exp Med 205:1807–1817

Ehrhardt GR, Hsu JT, Gartland L, Leu CM, Zhang S, Davis RS et al (2005) Expression of the immunoregulatory molecule FcRH4 defines a distinctive tissue-based population of memory B cells. J Exp Med 202:783–791

Elkins K, Zheng B, Go M, Slaga D, Du C, Scales SJ et al (2012) FcRL5 as a target of antibody-drug conjugates for the treatment of multiple myeloma. Mol Cancer Ther 11:2222–2232

Facchetti F, Cella M, Festa S, Fremont DH, Colonna M (2002) An unusual Fc receptor-related protein expressed in human centroblasts. Proc Natl Acad Sci USA 99:3776–3781

Falini B, Agostinelli C, Bigerna B, Pucciarini A, Pacini R, Tabarrini A et al (2012) IRTA1 is selectively expressed in nodal and extranodal marginal zone lymphomas. Histopathology 61:930–941

Falini B, Tiacci E, Pucciarini A, Bigerna B, Kurth J, Hatzivassiliou G et al (2003) Expression of the IRTA1 receptor identifies intraepithelial and subepithelial marginal zone B cells of the mucosa-associated lymphoid tissue (MALT). Blood 102:3684–3692

Forconi F, Sahota SS, Raspadori D, Mockridge CI, Lauria F, Stevenson FK (2001) Tumor cells of hairy cell leukemia express multiple clonally related immunoglobulin isotypes via RNA splicing. Blood 98:1174–1181

Franco A, Damdinsuren B, Ise T, Dement-Brown J, Li H, Nagata S et al (2013) Human Fc receptor-like 5 binds intact IgG via mechanisms distinct from those of Fc receptors. J Immunol 190:5739–5746

Gibson AW, Li FJ, Wu JM, Edberg JC, Su KH, Cafardi J et al (2009) The FCRL3-169CT promoter single-nucleotide polymorphism, which is associated with systemic lupus erythematosus in a Japanese population, predicts expression of receptor protein on CD19 + B cells. Arthritis Rheum 60:3510–3512

Guselnikov SV, Ershova SA, Mechetina LV, Najakshin AM, Volkova OY, Alabyev BY et al (2002) A family of highly diverse human and mouse genes structurally links leukocyte FcR, gp42 and PECAM-1. Immunogenetics 54:87–95

Haga CL, Ehrhardt GR, Boohaker RJ, Davis RS, Cooper MD (2007) Fc receptor-like 5 inhibits B cell activation via SHP-1 tyrosine phosphatase recruitment. Proc Natl Acad Sci U S A 104:9770–9775

Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK (1999) Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94:1848–1854

Hatzivassiliou G, Miller I, Takizawa J, Palanisamy N, Rao PH, Iida S et al (2001) IRTA1 and IRTA2, novel immunoglobulin superfamily receptors expressed in B cells and involved in chromosome 1q21 abnormalities in B cell malignancy. Immunity 14:277–289

Imboden JB, Eriksson EC, McCutcheon M, Reynolds CW, Seaman WE (1989) Identification and characterization of a cell-surface molecule that is selectively induced on rat lymphokine-activated killer cells. J Immunol 143:3100–3103

Inozume T, Matsuzaki Y, Kurihara S, Fujita T, Yamamoto A, Aburatani H et al (2005) Novel melanoma antigen, FCRL/FREB, identified by cDNA profile comparison using DNA chip are immunogenic in multiple melanoma patients. Int J Cancer 114:283–290

Ise T, Maeda H, Santora K, Xiang L, Kreitman RJ, Pastan I et al (2005) Immunoglobulin superfamily receptor translocation associated 2 protein on lymphoma cell lines and hairy cell leukemia cells detected by novel monoclonal antibodies. Clin Cancer Res 11:87–96

Ise T, Nagata S, Kreitman RJ, Wilson WH, Wayne AS, Stetler-Stevenson M et al (2007) Elevation of soluble CD307 (IRTA2/FcRH5) protein in the blood and expression on malignant cells of patients with multiple myeloma, chronic lymphocytic leukemia, and mantle cell lymphoma. Leukemia 21:169–174

Jackson TA, Haga CL, Ehrhardt GR, Davis RS, Cooper MD (2010) FcR-Like 2 Inhibition of B Cell Receptor-Mediated Activation of B Cells. J Immunol 185:7405–7412

Jelicic K, Cimbro R, Nawaz F, Huang DW, Zheng X, Yang J et al (2013) The HIV-1 envelope protein gp120 impairs B cell proliferation by inducing TGF-beta1 production and FcRL4 expression. Nat Immunol 14(12):1256–1265

Kardava L, Moir S, Wang W, Ho J, Buckner CM, Posada JG et al (2011) Attenuation of HIV-associated human B cell exhaustion by siRNA downregulation of inhibitory receptors. J Clin Invest 121:2614–2624

Kehrl JH, Thevenin C, Rieckmann P, Fauci AS (1991) Transforming growth factor-beta suppresses human B lymphocyte Ig production by inhibiting synthesis and the switch from the membrane form to the secreted form of Ig mRNA. J Immunol 146:4016–4023

Kochi Y, Myouzen K, Yamada R, Suzuki A, Kurosaki T, Nakamura Y et al (2009) FCRL3, an autoimmune susceptibility gene, has inhibitory potential on B-cell receptor-mediated signaling. J Immunol 183:5502–5510

Kochi Y, Yamada R, Suzuki A, Harley JB, Shirasawa S, Sawada T et al (2005) A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. Nat Genet 37:478–485

Kulemzin SV, Zamoshnikova AY, Yurchenko MY, Vitak NY, Najakshin AM, Fayngerts SA et al (2011) FCRL6 receptor: expression and associated proteins. Immunol Lett 134:174–182

Leu CM, Davis RS, Gartland LA, Fine WD, Cooper MD (2005) FcRH1: an activation coreceptor on human B cells. Blood 105:1121–1126

Li FJ, Ding S, Pan J, Shakhmatov MA, Kashentseva E, Wu J et al (2008) FCRL2 expression predicts IGHV mutation status and clinical progression in chronic lymphocytic leukemia. Blood 112:179–187

Li FJ, Schreeder DM, Li R, Wu J, Davis RS (2013) FCRL3 promotes TLR9-induced B-cell activation and suppresses plasma cell differentiation. Eur J Immunol 43:2980–2992

Maehlen MT, Nordang GB, Syversen SW, van der Heijde DM, Kvien TK, Uhlig T et al (2011) FCRL3 -169C/C genotype is associated with anti-citrullinated protein antibody-positive rheumatoid arthritis and with radiographic progression. J Rheumatol 38:2329–2335

Maltais LJ, Lovering RC, Taranin AV, Colonna M, Ravetch JV, Dalla-Favera R et al (2006) New nomenclature for Fc receptor-like molecules. Nat Immunol 7:431–432

Martin F, Kearney JF (2000) B-cell subsets and the mature preimmune repertoire. Marginal zone and B1 B cells as part of a "natural immune memory". Immunol Rev 175:70–79

Masir N, Jones M, Pozzobon M, Marafioti T, Volkova OY, Mechetina LV et al (2004) Expression pattern of FCRL (FREB, FcRX) in normal and neoplastic human B cells. Br J Haematol 127:335–343

Masuda K, Davis RS, Maruyama T, Zhang J, He T, Cooper MD et al (2005) FcRY, an Fc receptor related gene differentially expressed during B lymphocyte development and activation. Gene 363:32–40

Masuda K, Mori H, Ohara O, Nakayama M, Wang JY, Burrows PD (2010) Defining the immunological phenotype of Fc receptor-like B (FCRLB) deficient mice: confounding role of the inhibitory FcgammaRIIb. Cell Immunol 266:24–31

Mechetina LV, Najakshin AM, Volkova OY, Guselnikov SV, Faizulin RZ, Alabyev BY et al (2002) FCRL, a novel member of the leukocyte Fc receptor family possesses unique structural features. Eur J Immunol 32:87–96

Miller I, Hatzivassiliou G, Cattoretti G, Mendelsohn C, Dalla-Favera R (2002) IRTAs: a new family of immunoglobulinlike receptors differentially expressed in B cells. Blood 99:2662–2669

Mohan J, Dement-Brown J, Maier S, Ise T, Kempkes B, Tolnay M (2006) Epstein-Barr virus nuclear antigen 2 induces FcRH5 expression through CBF1. Blood 107:4433–4439

Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA et al (2008) Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med 205:1797–1805

Nagata S, Ise T, Pastan I (2009) Fc receptor-like 3 protein expressed on IL-2 nonresponsive subset of human regulatory T cells. J Immunol 182:7518–7526

Nakayama Y, Weissman SM, Bothwell AL (2001) BXMAS1 identifies a cluster of homologous genes differentially expressed in B cells. Biochem Biophys Res Commun 285:830–837

Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T (2001) PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci U S A 98:13866–13871

Polson AG, Zheng B, Elkins K, Chang W, Du C, Dowd P et al (2006) Expression pattern of the human FcRH/IRTA receptors in normal tissue and in B-chronic lymphocytic leukemia. Int Immunol 18:1363–1373

Pritchard NR, Cutler AJ, Uribe S, Chadban SJ, Morley BJ, Smith KG (2000) Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor FcgammaRII. Curr Biol 10:227–230

Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoenig M, Driessen G et al (2009) Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. Proc Natl Acad Sci U S A 106:13451–13456

Rassenti LZ, Jain S, Keating MJ, Wierda WG, Grever MR, Byrd JC et al (2008) Relative value of ZAP-70, CD38, and immunoglobulin mutation status in predicting aggressive disease in chronic lymphocytic leukemia. Blood 112:1923–1930

Reshetnikova ES, Mechetina LV, Volkova OY, Guselnikov SV, Chikaev NA, Kovesdi D et al (2012) Differential expression of FCRLA in naive and activated mouse B cells. Cell Immunol 272:182–192

Rui L, Healy JI, Blasioli J, Goodnow CC (2006) ERK signaling is a molecular switch integrating opposing inputs from B cell receptor and T cell cytokines to control TLR4-driven plasma cell differentiation. J Immunol 177:5337–5346

Rui L, Vinuesa CG, Blasioli J, Goodnow CC (2003) Resistance to CpG DNA-induced autoimmunity through tolerogenic B cell antigen receptor ERK signaling. Nat Immunol 4:594–600

Santiago T, Kulemzin SV, Reshetnikova ES, Chikaev NA, Volkova OY, Mechetina LV et al (2011) FCRLA is a resident endoplasmic reticulum protein that associates with intracellular Igs, IgM, IgG and IgA. Int Immunol 23:43–53

Schreeder DM, Cannon JP, Wu J, Li R, Shakhmatov MA, Davis RS (2010) Cutting edge: FcR-like 6 is an MHC class II receptor. J Immunol 185:23–27

Schreeder DM, Pan J, Li FJ, Vivier E, Davis RS (2008) FCRL6 distinguishes mature cytotoxic lymphocytes and is upregulated in patients with B-cell chronic lymphocytic leukemia. Eur J Immunol 38:3159–3166

Seaman WE, Niemi EC, Stark MR, Goldfien RD, Pollock AS, Imboden JB (1991) Molecular cloning of gp42, a cell-surface molecule that is selectively induced on rat natural killer cells by interleukin 2: glycolipid membrane anchoring and capacity for transmembrane signaling. J Exp Med 173:251–260

Shultz LD, Schweitzer PA, Rajan TV, Yi T, Ihle JN, Matthews RJ et al (1993) Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. Cell 73:1445–1454

Sohn HW, Krueger PD, Davis RS, Pierce SK (2011) FcRL4 acts as an adaptive to innate molecular switch dampening BCR signaling and enhancing TLR signaling. Blood 118:6332–6341

Swainson LA, Mold JE, Bajpai UD, McCune JM (2010) Expression of the autoimmune susceptibility gene FcRL3 on human regulatory T cells is associated with dysfunction and high levels of programmed cell death-1. J Immunol 184:3639–3647

Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, Traore B et al (2009) Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. J Immunol 183:2176–2182

Weller S, Braun MC, Tan BK, Rosenwald A, Cordier C, Conley ME et al (2004) Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. Blood 104:3647–3654

Wherry EJ, Ha SJ, Kaech SM, Haining WN, Sarkar S, Kalia V et al (2007) Molecular signature of CD8 + T cell exhaustion during chronic viral infection. Immunity 27:670–684

Wilson TJ, Colonna M (2005) A new Fc receptor homolog, FREB2, found in germinal center B cells. Genes Immun 6:341–346

Wilson TJ, Fuchs A, Colonna M (2012) Cutting edge: human FcRL4 and FcRL5 are receptors for IgA and IgG. J Immunol 188:4741–4745

Wilson TJ, Gilfillan S, Colonna M (2010) Fc receptor-like A associates with intracellular IgG and IgM but is dispensable for antigen-specific immune responses. J Immunol 185:2960–2967

Wilson TJ, Presti RM, Tassi I, Overton ET, Cella M, Colonna M (2007) FcRL6, a new ITIM-bearing receptor on cytolytic cells, is broadly expressed by lymphocytes following HIV-1 infection. Blood 109:3786–3793

Won WJ, Foote JB, Odom MR, Pan J, Kearney JF, Davis RS (2006) Fc receptor homolog 3 is a novel immunoregulatory marker of marginal zone and B1 B cells. J Immunol 177:6815–6823

Xiu Y, Nakamura K, Abe M, Li N, Wen XS, Jiang Y et al (2002) Transcriptional regulation of Fcgr2b gene by polymorphic promoter region and its contribution to humoral immune responses. J Immunol 169:4340–4346

Xu MJ, Zhao R, Cao H, Zhao ZJ (2002) SPAP2, an Ig family receptor containing both ITIMs and ITAMs. Biochem Biophys Res Commun 293:1037–1046

Xu MJ, Zhao R, Zhao ZJ (2001) Molecular cloning and characterization of SPAP1, an inhibitory receptor. Biochem Biophys Res Commun 280:768–775

Yang Y, Su X, Zhang K, Zhou R (2013) The Fc receptor-like 3 gene polymorphisms and susceptibility to autoimmune diseases: an updated meta-analysis. Autoimmunity 46:547–558

Yasuda T, Kometani K, Takahashi N, Imai Y, Aiba Y, and Kurosaki T (2011) ERKs induce expression of the transcriptional repressor Blimp-1 and subsequent plasma cell differentiation. Sci Signal 4:ra25

Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD et al (1998) Viral immune evasion due to persistence of activated T cells without effector function. J Exp Med 188:2205–2213

Zhao SX, Liu W, Zhan M, Song ZY, Yang SY, Xue LQ et al (2013) A refined study of FCRL genes from a genome-wide association study for Graves' disease. PLoS ONE 8:e57758

Zhu Z, Li R, Li H, Zhou T, Davis RS (2013) FCRL5 exerts binary and compartment-specific influence on innate-like B-cell receptor signaling. Proc Natl Acad Sci U S A 110:E1282–E1290

Intracellular Antibody Immunity and the Cytosolic Fc Receptor TRIM21

Leo C. James

Abstract Until recently, it was thought that antibody effector mechanisms were mediated purely by Fc receptors expressed on professional cells, following capture of immune complexes in the extracellular space. Recently a new Fc receptor, *TRIM21*, was discovered that is expressed by cells of all histogenetic lineages and which mediates immune responses intracellularly. This new receptor possesses many unique structural and functional properties. *TRIM21* binds both IgG and IgM, interacts primarily with the CH3 rather than CH2 domain and engages two heavy chains simultaneously. This latter property allows *TRIM21* to bind antibodies with a higher affinity than any other Fc receptor. *TRIM21* is cytosolic, has both effector and signalling functions and is exquisitely conserved in mammals. The discovery of this missing part of humoral immunity has important implications for where and how antibodies work.

Contents

1 Introduction 52
2 Origins of *TRIM21* 52
3 *TRIM21* Structure and Antibody Binding Mechanism 53
3.1 *TRIM21* is a Multi-Domain Protein 53
3.2 The PRYSPRY Domain as an Antibody-Binding Protein 55
3.3 Molecular Basis for *TRIM21*:IgG Interaction 57
3.4 Evolutionary Parallels Between PRYSPRY and Ig Protein Families 59
4 *TRIM21* Function 59
4.1 Antibodies Mediate Immune Responses Inside Cells 59
4.2 *TRIM21* Directly Inhibits Viral Infection 61

L. C. James (✉)
MRC Laboratory of Molecular Biology, Cambridge, UK
e-mail: lcj@mrc-lmb.cam.ac.uk

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_3,

4.3 *TRIM21* Targets Cytosolic Antibody-Coated Viruses for Proteasomal Degradation 62
4.4 *TRIM21* is a DAMP Sensor that Activates Innate Immunity 63
5 Conclusions 64
References 64

1 Introduction

The detection of pathogens, in particular viruses, represents perhaps the most significant challenge facing the immune system. This is because viruses are highly adaptive and germline encoded antivirals can be rapidly out-evolved. Antibodies are the only soluble immune molecule whose evolution can hope to keep pace with viruses and recognize an almost limitless diversity of antigen structure. Antibodies are secreted into the extracellular spaces, in fluid compartments and on mucosal surfaces. There they can intercept pathogens as they invade the body. However, viruses are obligate intracellular pathogens meaning that upon infection, they disappear into their target cells. The result is that the body's premier targeting molecules seem excluded from the viruses' most significant habitat.

As it turns out, this is actually not the case. When non-enveloped viruses and intracellular bacteria infect cells they carry antibodies with them (Mallery et al. 2010). These antibodies are attached to the pathogen surface and remain attached during the entry process. Once inside the cytosol, antibody-coated pathogens are rapidly detected by a cytosolic Fc receptor called *TRIM21* (James et al. 2007). *TRIM21* is expressed in most tissues, although as it is regulated by interferon the 'resting' protein levels can be low (Rhodes et al. 2002). Detection by *TRIM21* initiates both an effector and sensing response. *TRIM21* is an E3 ubiquitin ligase and *TRIM21*-dependent ubiquitination targets incoming viral particles for degradation in a system involving both the AAA ATPase VCP and the proteasome (Mallery et al. 2010; Hauler et al. 2012). In addition to this degradation pathway, *TRIM21* also catalyses the formation of K63-ubiquitin chains and activates NFκB, AP-1 and IRF3/5/7 (McEwan et al. 2013). These processes are extremely rapid. For example, within 1–2 h of infecting a cell, a non-enveloped virus like adenovirus has been pulled apart and degraded by the proteasome before it has a chance to begin replicating (Mallery et al. 2010).

2 Origins of *TRIM21*

Before its characterisation as an Fc receptor, *TRIM21* was studied in the context of autoimmunity under the name *Ro52*. Ro52 is an autoantigen in autoimmune diseases rheumatoid arthritis, SLE and Sjorgen's syndrome (Moutsopoulos et al. 1985; Ben-Chetrit et al. 1988, 1990). Anti-Ro52 autoantibodies are diagnostic both

of disease (McCauliffe et al. 1997) and disease progression (Frank et al. 1993) and a pathologic role for *Ro52*:autoantibody immune complex has been suggested (Salomonsson et al. 2005). In 1999, a yeast-two-hybrid screen with *Ro52* identified IgG heavy chain as a hit (Yang et al. 1999). The authors noted that it was unlikely that "the two proteins should exist in the same subcellular compartment" and in follow up work proposed that Ro52:IgG interaction may occur on the surface of apoptosed cells (Yang et al. 2000). Later work has reported interaction between *TRIM21* and a number of different ligands, including *Skp2* (Sabile et al. 2006), *DAXX* (Tanaka and Kamitani 2010), FADD (Young et al. 2011), DDX41 (Zhang et al. 2013), pro-inflammatory regulators *IRF3* (Higgs et al. 2008), IRF5 (Espinosa et al. 2009) and anti-inflammatory IRF8 (Kong et al. 2007). These proteins are functionally unrelated or have opposing affects but have in common that they were all identified as *TRIM21* ligands by immunoprecipitation (IP). In an IP experiment, antibodies are used to pull-down a particular target and anything that co-IPs is considered a potential ligand. *TRIM21*, as an Fc receptor, binds to antibody directly and IPs regardless of antibody specificity. Thus, despite the early identification of heavy chain as a potential *TRIM21* ligand, the focus of *TRIM21* research in the context of autoimmunity has largely shifted onto the IRFs. In this context, *TRIM21* has been reported as both a positive (Kong et al. 2007; Yang et al. 2009) and negative (Wada et al. 2009) regulator of interferon signalling.

3 *TRIM21* Structure and Antibody Binding Mechanism

3.1 TRIM21 *is a Multi-Domain Protein*

TRIM21 is so-called because it is a member of the tripartite motif containing proteins. This family comprises some 100 members in humans and is functionally divergent but related in domain topology. All TRIMs members contain a RING, B Box and coiled-coil domain, the triumvirate that gives the family its name. The RING domain is a zinc finger found in many diverse proteins and hypothesised to function as an E3 ubiquitin ligase. *TRIM*s 5, 18, 21, 25, 32 and 35 have been shown to mediate ubiquitination (Trockenbacher et al. 2001; Urano et al. 2002; Xu et al. 2003; Horn et al. 2004; Kudryashova et al. 2005; Vichi et al. 2005) and this is hypothesised to be a common feature of all *TRIM* proteins. The B Box domain is a CHC3H2 zinc finger exclusive to *TRIM*s, which contain up to two in series. Their function is yet to be determined, however yeast-two-hybrid studies have identified interaction between the microtubule-associated protein phosphatase 2A and the B Box domains of *TRIM1* and *TRIM18* (Short and Cox 2006). The B Box domain is also proposed to mediate higher-order assembly of *TRIM5*, as part of its mechanism of HIV-1 restriction (Diaz-Griffero et al. 2009). Whatever its precise role, studies of *TRIM5* restriction clearly show that the B Box is essential for function. *TRIM* proteins are also all believed to contain a coiled-coil domain, which have recently been re-classified as BBC domains, or 'coiled-coil regions C-terminal to

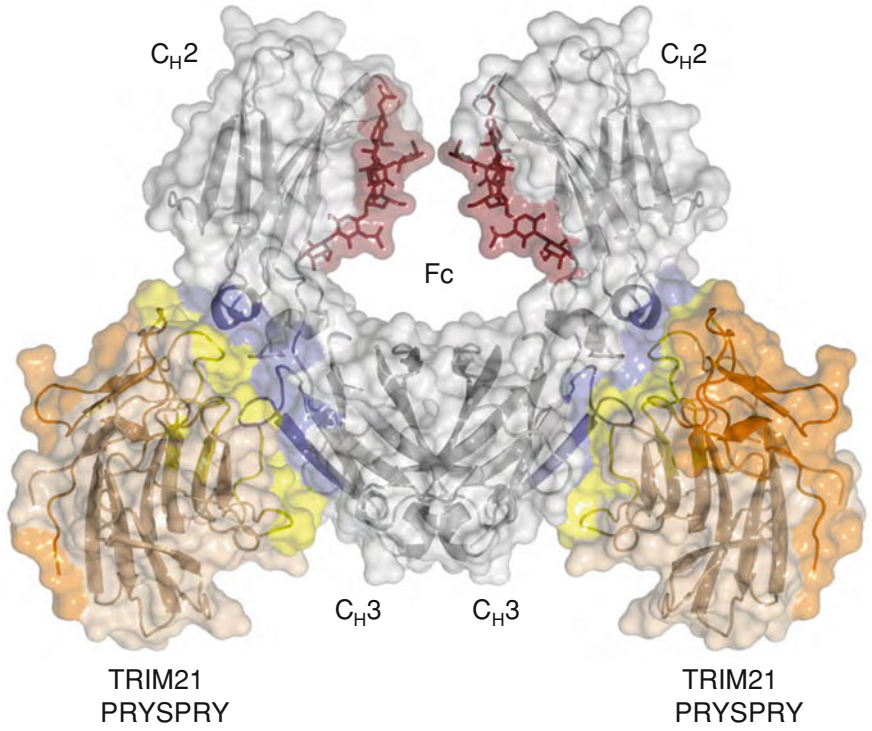


Fig. 1 *Crystal structure of human TRIM21 PRYSPRY bound to human IgG Fc*. A transparent molecular surface with secondary structure visible underneath. IgG Fc is shown in *grey* with two copies of *TRIM21* shown in *orange* ('PRY' element) and wheat ('SPRY' element). The interaction surface on *TRIM21* is shown in *yellow* and on Fc in *blue*. The N-linked glycans are shown in *red*

B Box domains' (Letunic et al. 2006). Coiled-coil domains are known to mediate oligomerisation through α-helical intertwining and have been shown to facilitate *TRIM* dimerisation. The coiled-coil domain is sufficient to facilitate *TRIM21* to dimerise and allows bivalent interaction with IgG.

In addition to these domains, *TRIM21*, as in around a third of *TRIM* proteins, has a C-terminal PRYSPRY domain. It was characterization of this PRYSPRY domain that first revealed *TRIM21* as a new type of Fc receptor. The crystal structure of *TRIM21* PRYPSRY in complex with IgG Fc showed that *TRIM21* engages antibody in a mechanism unlike other antibody receptors. *TRIM21* binds IgG symmetrically, as opposed to the asymmetrical recognition observed with Fcγ receptors. In the *TRIM21* crystal structure, two copies of the PRYSPRY domain are bound per Fc (Fig. 1). Each PRYSPRY makes contacts with both CH2 and CH3 domains although the largest interface is with CH3 (Fig. 2a). *TRIM21* shares Fc epitopes with the neonatal Fc receptor, FcRn. Like FcRn, *TRIM21* interacts with the 'HNHY' motif found in a short loop between the final two β-strands in CH3

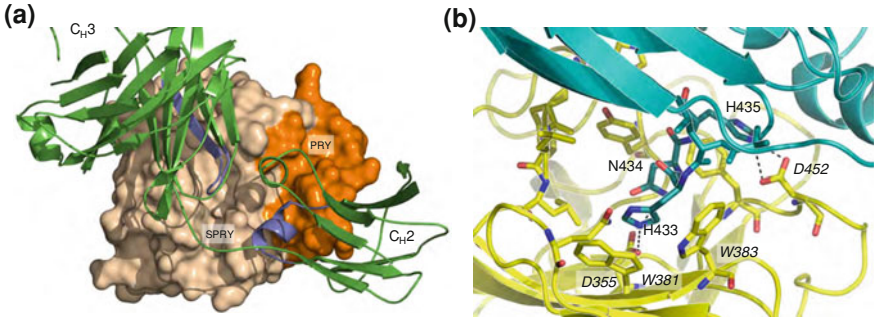


Fig. 2 *Details of TRIM21:Fc interaction*. **a** View into the *TRIM21* PRYSPRY binding site. *TRIM21* is shown as a molecular surface, coloured as in Fig. 1. IgG Fc is shown in a secondary structure representation in *green*, with *TRIM21* contacting areas shown in *blue*. **b** The HNHY motif of IgG Fc (*green*) binds into a hydrophobic pocket in *TRIM21* PRYSPRY (*yellow*)

(Fig. 2b). Unlike FcRn, *TRIM21* binding is not pH-dependent, suggesting that the protonation state of histidines H433 and H435 is not critical. *TRIM21* is a dimeric molecule and when it binds IgG it engages both heavy chains simultaneously, resulting in a 1:1 stoichiometry, unlike the proposed 2:1 stoichiometry for FcRn. The implications of this binding mechanism are that, in contrast to Fcγ receptors, *TRIM21* may not distinguish between free and bound antibodies. The stable 1:1 complex that *TRIM21* forms with IgG suggests that cross-linking does not take place. The simultaneous binding of both heavy chains increases the affinity of *TRIM21* for antibody through avidity. The affinity of a single PRYPSRY monomer for IgG Fc is $\sim$40 nM (depending on salt concentration), whereas the affinity of dimeric *TRIM21* is $\sim$0.5 nM. Thus *TRIM21* is the highest affinity Fc receptor in humans. The functional consequences of these binding characteristics help to distinguish *TRIM21* from other Fc receptors. *TRIM21* has not evolved to mediate a trafficking/transporting function like FcRn (binding is neither transitory nor pH-sensitive) nor does it obviously discriminate between antibody bound states like FcγR (it is not cross-linked or held within a membrane and thus susceptible to clustering). Rather, *TRIM21* can detect antibody that is present even at low concentrations and, once captured, antibody will remain bound.

3.2 The PRYSPRY Domain as an Antibody-Binding Protein

PRYSPRY domains are predicted in 11 families in the human genome but despite this prevalence their function is poorly defined (Rhodes et al. 2005). Within the *TRIM* family, the PRYSPRY domain is believed to function as a protein targeting module: deletion of the PRYSPRY domain abolishes *TRIM* function whereas *TRIM* dysfunction is largely mediated by mutations in the PRYSPRY exon (James et al. 2007). The PRYSPRY domain is a member of the SPRY superfamily, which

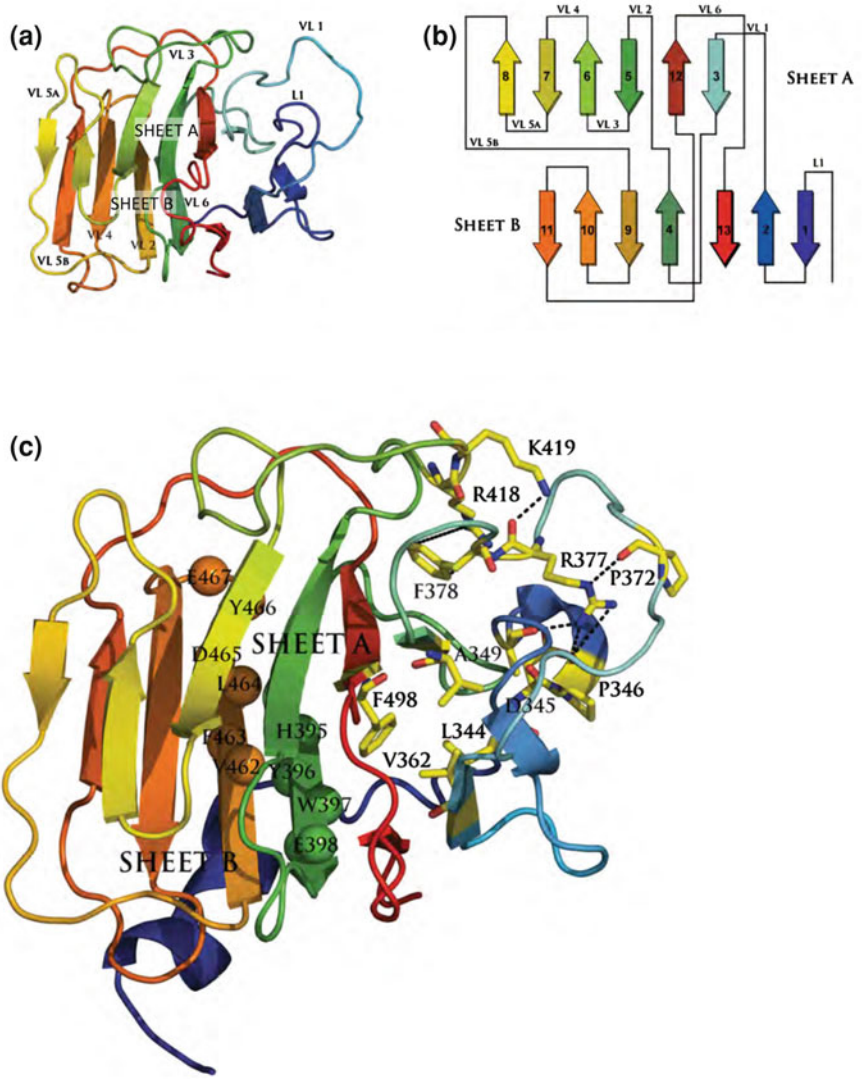


Fig. 3 *The PRYSPRY domain*. **a** Secondary structure representation of the *TRIM21* PRYSPRY domain, indicating variable loops (VLs). **b** Flattened topology diagram. **c** Conserved structural features that define the PRYSPRY fold

has been divided into two distinct groups—SPRY and B30.2. B30.2 proteins have a longer N-terminal sequence, which is defined by SMART, NCBI and PFAM as a separate domain called PRY, hence SPRY members that have a PRY can be referred to as PRYSPRY. PRYSPRY proteins have a β-sandwich topology made up of two twisted β-sheets, one of which forms a concaved surface surrounded by six flexible loops (VL 1–6) (Fig. 3). PRYSPRY proteins have an overall sequence

homology of ~30 % and share three well-conserved motifs LDP, HYWEV and VFLDYE. As can be seen in the *TRIM21* PRYSPRY structure, these last two motifs correspond to neighbouring β-strands that form part of lower β-sheet B (Fig. 3c). In addition to these motifs, there are conserved patches of two or three residues spread throughout the sequence that broadly correspond to other β-strands. SPRY proteins are a more diverse group, with an average sequence identity of <15 % both with respect to each other and to PRYSPRY proteins. SPRY proteins are predicted to lack the N-terminal PRY domain and as a consequence do not have the LDP motif. Homology between SPRY and PRYSPRY is limited to the remaining two motifs, HYWEV and VFLDYE, although even these are significantly more degenerate in SPRY homologs.

As a result of the low sequence homology across the SPRY superfamily, it was suggested that the PRY and SPRY elements form distinct domains with novel topologies. Several SPRY structures—SSB-2 (Masters et al. 2006) and GUSTAVUS (Woo et al. 2006)—and PRYSPRY structures—*TRIM5* (Biris et al. 2012), *TRIM20* (Weinert et al. 2009), *TRIM21* (James et al. 2007) and *TRIM25* (D'Cruz et al. 2013)—have been solved. Contrary to prediction, these proteins all adopt a remarkably similar conformation. The structures also reveal that neither SPRY nor PRY are independent domains with a single globular fold (Fig. 3). In all the structures, the β-sandwich of the SPRY element is completed by three N-terminal β-strands structurally equivalent to PRY. This is true even for GUSTAVUS, which is not annotated as containing a PRY domain. This suggests that the proper domain unit should be 'PRYSPRY' and that most SPRY homologs have an N-terminus that is PRY-like. This PRYSPRY domain definition is supported by the fact that SPRY homologs are often encoded in a single exon containing both SPRY and a preceding N-terminal sequence of PRY length. In humans, PRYSPRY sequences are only found in the *TRIM* and *BTN* families but in other species they are present in unrelated proteins such as guinea pig enterophilin (Gassama-Diagne et al. 2001), 'bloodthirsty' in fish (Yergeau et al. 2005), king cobra snake venom protein ohanin (Pung et al. 2005) and stonefish toxin stonustoxin (Ghadessy et al. 1994). *TRIM* and *BTN* are the largest of the SPRY containing protein families and are only found in higher organisms. This suggests that they may originate from a common SPRY ancestor that has undergone recent and rapid expansion.

3.3 Molecular Basis for TRIM21*:IgG Interaction*

TRIM21 PRYSPRY interacts with IgG Fc using all six of the flexible loops found on sheet A and both PRY and SPRY elements. Residues from the CH2 domain interact with a pocket on the PRY element, while the CH3 domain interacts with a binding pocket in the SPRY (Fig. 2a). The PRY binding site is formed by VL1, which lassoes around a β-hairpin at the beginning of the domain creating an extended planar surface (Fig. 3). The VL1 loop is the site of greatest structural difference between SPRY and PRYSPRY domains. In SPRY homologs such as

GUSTAVUS, VL1 is a short loop connecting strands 2 and 3 and located between the hairpin and VL6 (Fig. 3). The VL1 conformation is highly conserved amongst solved *TRIM* PRYSPRY structures despite having no secondary structure and a low sequence homology. The VL1 loop is constrained primarily by interactions between a conserved 'RF' motif in VL1 and a conserved 'LDP' motif in the β-hairpin (Fig. 3c). The arginine anchors VL1 to the hairpin through hydrogen bonds to main-chain oxygen atoms. The phenylalanine pins VL1 between loop VL3 and strand 3 by forming a cation-π interaction with a conserved arginine in VL3. Cation-π interactions are thought to be particularly important in protein stabilisation; theoretical studies have shown that they may attain a strength of up to 4 kcal/mole and are potentially more stabilising than salt bridges (Gallivan and Dougherty 1999). VL1 is further constrained by hydrogen bonds between main-chain oxygen atoms in the RF motif and a conserved lysine from VL3 (Fig. 3c). Most of the conserved PRYSPRY residues not in β-strands are concerned with maintaining the extended VL1 loop structure. This suggests that the conformation of VL1 is likely to be conserved in most PRYSPRY proteins, with the possible exception of *TRIM5* in which this loop has undergone considerable variation in constitution and length. In *TRIM21*, the PRY binding site creates a pocket for residues from C_H2 α-helix$_{310–314}$ and I253 from loop$_{243–258}$. I253 is one of six hot-spot residues identified by de Lano et al. as a hot-spot for proteins binding at the C_H2–C_H3 Fc interface. However, mutation of *TRIM21* PRYSPRY residues suggests that this part of the interface is not critical for IgG binding. Mutation W299A, within the PRY element, does reduce binding but only weakly.

Most of the important contacts between *TRIM21* PRYSPRY and IgG Fc are located in a concave binding site created by the SPRY element. A short CH3 loop containing the HNHY motif inserts into this site and is surrounded by a ring of hydrophobic residues from VLs 3–6. This ring of hydrophobic residues completely encloses the HNHY loop, effectively creating a solvent-excluding seal (Fig. 2b). The importance of this seal in IgG interaction is illustrated by the fact that mutation of these residues severely diminishes or abolishes binding (James et al. 2007). The nature of this interaction explains why *TRIM21* binds to IgG in a pH independent manner, in contrast to FcRn that is sensitive to the protonation state of the histidines within the HNHY loop. The non-specific nature of hydrophobic burial may also explain why *TRIM21* uniquely binds both IgG and IgM. IgM also has a loop region that is structurally analogous to the HNHY motif, except that it has the sequence PNRV. Thus *TRIM21* may also mediate a hydrophobic interaction with IgM; albeit the affinity for IgM is weaker (monomer PRYSPRY affinity for IgM is ∼10–20 μM, compared to 40nM for monomer PRYSPRY to *TRIM21* under equivalent conditions). In addition to the ring of hydrophobic residues that surrounds the HNHY motif in the *TRIM21* PRYSPRY:Fc crystal structure, an aspartic acid residue at 355 is positioned to form a bifurcated hydrogen bond with the side chains of IgG Fc residues H433 and N434 (Fig. 2b). Mutation of D355 severely diminishes IgG interaction. This bifurcated interaction is not possible with IgM and may be one reason for the lower affinity to this isotype.

3.4 Evolutionary Parallels Between PRYSPRY and Ig Protein Families

The binding between *TRIM21* PRYSPRY and IgG Fc is driven by the six flexible loops that surround the concave β-sheet that forms the binding site. The use of six loops to determine binding is reminiscent of the manner in which IgG Fabs bind their antigen. It also suggests that the use of SPRY domains in multiple protein families with diverse functions may have been achieved by duplication followed by variation in loop sequences to achieve new ligand specificities. To test this possibility the phylogenetic variation of surface residues amongst SPRY sequences was examined. A CLUSTAWL alignment of 1368 SPRY homologs was performed and imported into the program Consurf (Landau et al. 2005). Consurf uses an empirical Bayesian algorithm to calculate a conservation score at every residue position. The stochastic nature of sequence evolution, in particular the different rates of amino acid evolution, is explicitly taken into account to give an accurate estimate of phylogenetic variation. The resulting sequence variability score was then mapped onto the protein surface and coloured from most variable (red) to most conserved (blue). As can be seen in Fig. 4, the residues that are most variable between SPRY homologs extend across both SPRY and PRY binding surfaces as defined by *TRIM21*. Furthermore, these variable surface patches correspond to the six variable loops. This suggests that SPRY domains have a common binding site interface that has been conserved across a billion years of evolution.

TRIM21:IgG complexation has all the hallmarks of a highly specific interaction. It is also exquisitely conserved amongst mammals. Study of these proteins from different mammalian species has shown that interaction is conserved at the most fundamental level (Keeble et al. 2008). Even where there are differences in the PRYSPRY residues between mouse and human *TRIM21*, they are conservative and allow similar interactions with IgG to be made. Indeed the conservation of IgG binding activity in mammalian *TRIM21* is such that structurally equivalent residues within the binding site of different orthologues not only make the same thermodynamic contribution to the binding energy but also the same kinetic contribution (Keeble et al. 2008).

4 *TRIM21* Function

4.1 Antibodies Mediate Immune Responses Inside Cells

As discussed above, the structure of *TRIM21* and its antibody binding mechanism is distinct from other Fc receptors. *TRIM21* is also unique in that it is expressed in the cytosol and not secreted, displayed on the cell surface or found inside endocytic compartments. The purpose of expressing a high affinity Fc receptor in the cytosol is to allow antibodies to exert immune functions inside cells. The fact that

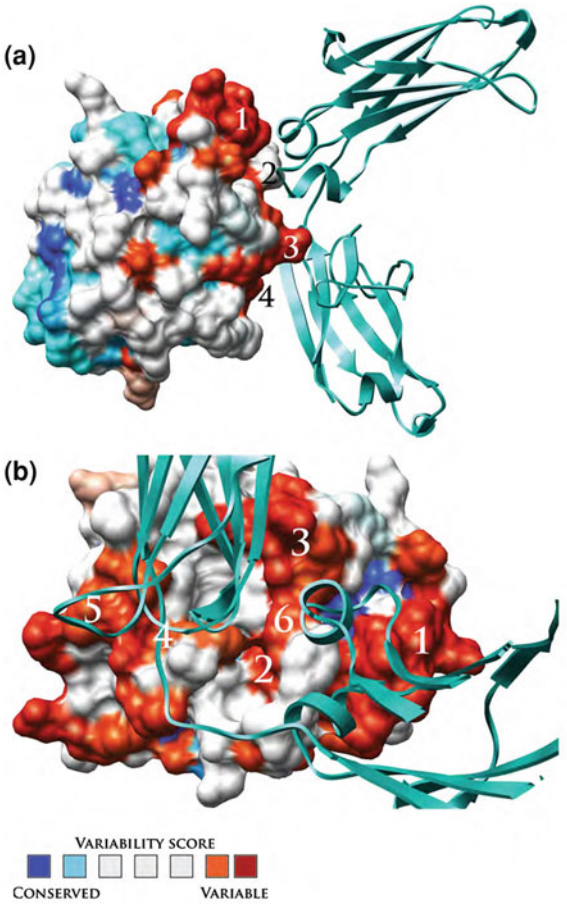


Fig. 4 *Sequence variability in the PRYSPRY domain*. Two views of the complex between *TRIM21* PRYSPRY (molecular surface) and IgG Fc (*green* secondary structure) are shown. The position of the six PRYSPRY VLs are shown. The sequence variability between >1,300 PRYSPRY paralogs has been mapped onto the structure, with conserved regions coloured in *blue* and variable regions in *red*

humoral immunity functions intracellularly, in addition to extracellularly, has only recently been determined and will be discussed in the following section.

In an uninfected host, functional antibodies should not be capable of accessing the cytosol of any cell. This is because antibodies have no mechanism to penetrate the plasma membrane or exit endosomes during endocytosis. Cellular compartmentalization also comprises a barrier for pathogens, preventing access to the nutrient-rich environment of the cytosol (Randow et al. 2013). However, pathogens, including viruses and bacteria, have evolved many diverse strategies to cross cellular membranes and traffic from extracellular to intracellular compartments. The immune system is tasked with recognising when pathogens have invaded a cell and mounting a response that prevents them from replicating. This can be accomplished by detecting molecules or patterns that are unique to a pathogen and absent in the host. However, this strategy exerts selective pressure on the pathogen to change its patterns and avoid recognition. Given that pathogens evolve many

orders of magnitude faster than their hosts, this creates an arms race that is biased in their favour. Antibodies are the only freely diffusing molecule made by mammals whose specificity can be altered on a timescale that is competitive with pathogen evolution. Antibodies are unparalleled in their ability to stick to pathogens once they have entered a host and subsequently mediate a variety of effector mechanisms such as fix complement or trigger phagocytosis. Recently, it was shown that antibodies remain attached to pathogens when they infect cells (Mallery et al. 2010). This provides every cell a way of detecting pathogen invasion without relying on the recognition of a pathogen associated molecular pattern (or PAMP). Antibody-coated pathogens that successfully invade a cell and enter the cytosol are detected by *TRIM21*. Upon recognition of these antibody-coated pathogens, *TRIM21* triggers several different responses acting both as a sensor and effector in antipathogen immunity.

4.2 TRIM21 *Directly Inhibits Viral Infection*

The mechanism of *TRIM21* activity, its regulation and the type of immunity it mediates is distinct from other antibody effector responses. The protection provided by *TRIM21* is fundamentally different because it is not based on immune surveillance but rather on 'ambushing' pathogens inside the cell. Fcγ receptors are expressed largely on professional immune cells, meaning that they will only encounter antibody-pathogen complexes through chance encounter before these pathogens have entered their target cells. *TRIM21* is expressed in almost all tissues and therefore has the potential to detect any antibody-coated pathogen, irrespective of its cellular tropism. Furthermore, recognition occurs during productive infection making this response extremely difficult for pathogens to avoid. For obligate intracellular pathogens, each infection event provides an opportunity for *TRIM21* recognition.

TRIM21 is rapidly recruited to incoming antibody-coated pathogens (Mallery et al. 2010). Parallel infection and electron microscopy experiments have revealed that this process is highly efficient and 1–2 antibodies per pathogen are sufficient (McEwan et al. 2012). *TRIM21* binding to antibody-coated pathogens is thought to activate its ubiquitin ligase activity and *TRIM21*/antibody/pathogen complexes inside the cell also contain ubiquitin (Mallery et al. 2010). *TRIM21* catalyses the formation of ubiquitin chains with different linkages (K48 and K63) *in vitro*. Moreover, *TRIM21*-mediated ubiquitination is functionally essential, as deletion of the RING domain abolishes both effector and sensor activities (Mallery et al. 2010; McEwan et al. 2013). However, it is not known what becomes ubiquitinated upon *TRIM21* recruitment (the pathogen, the antibody or *TRIM21* itself), the order in which different chain types are made or how they are subsequently liberated.

4.3 TRIM21 *Targets Cytosolic Antibody-Coated Viruses for Proteasomal Degradation*

TRIM21 directs incoming viral pathogens for degradation in a process that is dependent upon ubiquitination, the proteasome and the AAA ATPase VCP (Hauler et al. 2012). Proteasomal degradation of virus occurs rapidly, within an hour of infection and before replication has begun. In experiments with replication-deficient human adenovirus 5 (hAdv5), this has the result of reducing infection by 100-fold. In spreading-infection experiments using replication-competent mouse adenovirus (MAV-1), viral replication was shown to be reduced >1,000 fold (Watkinson et al. 2013). These activities are dependent upon the presence of intact, pathogen-specific antibody. Proteolysis of the Fc domain using pepsin, to generate Fab2 fragments, results in a complete loss of *TRIM21* antiviral activity (Mallery et al. 2010). Single point-mutants within the 'HNHY' motif, such as N434D, are also sufficient to abolish activity (McEwan et al. 2012). Importantly, mutations such as N434D also render an otherwise potently neutralising monoclonal antibody, non-neutralising. This demonstrates that the neutralisation activity of antibodies, long thought to simply be a consequence of antibody binding, can be receptor-dependent. Experiments using polyclonal antiviral sera suggest the presence of both entry-blocking and *TRIM21*-dependent antibodies (McEwan et al. 2012). The role of epitope specificity in this dependence is yet to be determined, although antibodies that compete for receptor attachment are likely to be the most entry blocking and *TRIM21*-independent. The efficiency of *TRIM21*-mediated degradation is proportional to both antibody concentration and *TRIM21* expression (McEwan et al. 2012). *TRIM21* is an interferon-induced gene and upon IFN-stimulation, expression levels are substantially increased. Increased expression of *TRIM21* results in increased neutralisation potency and a corresponding reduction in infection.

In addition to ubiquitination and the proteasome, VCP is also required for viral degradation and the block to infection. VCP is an enzyme with unfoldase and segregase activity and is required to process a subset of proteasome substrates (Beskow et al. 2009). These substrates are typically either large, part of multi-component complexes, embedded in membranes or otherwise difficult for the proteasome to degrade unaided. VCP extracts these substrates and unfolds them, allowing proteasomal degradation to proceed. In the case of viral degradation, VCP may be required to extract component proteins from the viral capsid, unfolding them for subsequent degradation in the proteasome. This mechanism is supported by data showing that the requirement for VCP is substrate-specific not constitutive. IgG Fc that is overexpressed in the cytosol is targeted by *TRIM21* and degraded by the proteasome, but this turnover is VCP-independent (Hauler et al. 2012). VCP is recruited to proteasomes that 'stall' upon attempting to process complex substrates (Isakov and Stanhill 2011). This suggests that the recruitment of VCP during *TRIM21*-directed viral degradation is due to a similar stalling of the proteasome when faced with a viral capsid. The importance of VCP and the

proteasome in *TRIM21* antiviral activity is illustrated by the fact that inhibitors of these enzymes (DBeQ or Eerystatin, in the case of VCP, or MG132 or Epoxomicin in the case of the proteasome) prevent neutralisation. Furthermore, the timescale of viral capsid degradation matches the timescale in which these inhibitors are effective in blocking neutralisation (Hauler et al. 2012). The addition of inhibitors at later time points post-infection fails to recover infection, showing that neutralisation occurs within the first few hours of infection, coincident with the observed *TRIM21*, proteasome and VCP-dependent degradation of capsid.

4.4 TRIM21 *is a DAMP Sensor that Activates Innate Immunity*

In addition to recruiting the proteasome, the recognition of antibody-coated pathogens in the cytosol by *TRIM21* activates innate immune signalling. As well as catalyzing the formation of K48-ubiquitin chains, *TRIM21* also forms free K63-ubiquitin chains in concert with the E2 enzyme, UbcH9 (McEwan et al. 2013). Through the production of free K63-ubiquitin chains, *TRIM21* is able to activate the three main innate immune signalling pathways—NFκB, AP-1 and IRF 3/5/7 (McEwan et al. 2013). The activation of these pathways is highly potent, resulting in the upregulation of proinflammatory cytokine transcript levels by >10,000 fold and robust cytokine secretion. In addition to activating cytokine expression, *TRIM21* recognition of cytosolic antibody leads to modulation of cell surface ligands, including upregulation of activating receptor NKG2D and MHC class I and downregulation of inhibitory receptor NKG2A (McEwan et al. 2013). In experiments in primary human lung fibroblast cells it was found that hAdv5 was capable of infecting cells without being detected by either TLRs or nucleic acid PAMP receptors. However, infection experiments in the presence of anti-hAdv5 antibody resulted in strong NFκB induction in a *TRIM21*-dependent manner. The magnitude of cytokine upregulation mediated by *TRIM21* is sufficient to place cells in a highly antiviral state. Interferon secreted from infected cells that were activated by *TRIM21* was sufficient to prevent Sindbis infection of unchallenged cells, in a media transfer experiment. *TRIM21* activation of innate immunity is dependent upon antibody alone and not on additional PAMPs. Antibody-coated beads were shown to provoke a proinflammatory response in a *TRIM21*-dependent manner. The simplicity of this mechanism allows *TRIM21* to detect invasion by both RNA and DNA viruses and the intracellular bacteria Salmonella typhimurium. The antibody molecule behaves as a danger-associated molecular pattern (DAMP) and is sufficient to activate innate immunity. Because antibodies should never be in the cytosol, their presence there can be taken as a clear sign that the sanctity of the cell has been invaded. Furthermore, this system does not require any complex trafficking on behalf of the host. If a pathogen fails to invade a cell, it remains non-infectious. If it crosses the cellular membrane then so do any attached antibodies.

5 Conclusions

Many questions remain to be answered as to the precise molecular events that accompany *TRIM21*-mediated neutralisation and signalling. For instance, it is not clear how binding to antibody activates *TRIM21* ubiquitination activity. Such regulation must take place or otherwise *TRIM21*-expressing cells would exist in a permanent antiviral state. That regulation operates at the level of ubiquitination is suggested by the fact that *TRIM21* ubiquitination cannot be detect in uninfected cells. Furthermore, *TRIM21* has a long cellular half-life and does not appear to be rapidly degraded by the proteasome, suggesting that there is little constitutive autoubiquitination. The full spectrum of pathogens that are susceptible to *TRIM21* also remains to be determined. *TRIM21* has been shown to sense nonenveloped viruses and bacteria and directly neutralise viral infection. However, it is likely that certain viruses will have evolved strategies to evade or antagonize TRM21 activity. One such strategy is for a virus to shed its antibodies as it infects a cell. Enveloped viruses, which fuse at the plasma membrane or inside endosomes, accomplish this very effectively and this is presumably why they have not been shown to activate *TRIM21*. It also remains to be determined in which contexts *TRIM21* is most important during infection. In vivo experiments using MAV-1, suggest that *TRIM21* plays a significant role in the ability of antibodies to protect against lethal infection in both a naïve and protective setting (Vaysburd et al. 2013). A quarter of naïve *TRIM21* knockout mice challenged with MAV-1 succumbed to virally-induced encephalomyelitis within 7 days of infection. In passive transfer experiments, antibody doses that were sufficient to protect all wild-type animals against high-titre viral infection failed to protect most *TRIM21* knockouts. Future studies are required to examine other pathogen models and dissect the contribution of different *TRIM21* functions to humoral immunity in the animal.

References

Ben-Chetrit E, Chan EK et al (1988) A 52-kD protein is a novel component of the SS-A/Ro antigenic particle. J Exp Med 167(5):1560–1571

Ben-Chetrit E, Fox RI et al (1990) Dissociation of immune responses to the SS-A (Ro) 52-kd and 60-kd polypeptides in systemic lupus erythematosus and Sjogren's syndrome. Arthritis Rheum 33(3):349–355

Beskow A, Grimberg KB et al (2009) A conserved unfoldase activity for the p97 AAA-ATPase in proteasomal degradation. J Mol Biol 394(4):732–746

Biris N, Yang Y et al (2012) Structure of the rhesus monkey TRIM5alpha PRYSPRY domain, the HIV capsid recognition module. Proc Natl Acad Sci USA 109(33):13278–13283

D'Cruz AA, Kershaw NJ et al (2013) Crystal structure of the TRIM25 B30.2 (PRYSPRY) domain: a key component of antiviral signalling. Biochem J 456(2):231–240

Diaz-Griffero F, Qin XR et al (2009) A B-box 2 surface patch important for TRIM5alpha self-association, capsid binding avidity, and retrovirus restriction. J Virol 83(20):10737–10751

Espinosa A, Dardalhon V et al (2009) Loss of the lupus autoantigen Ro52/*TRIM21* induces tissue inflammation and systemic autoimmunity by disregulating the IL-23-Th17 pathway. J Exp Med 206(8):1661–1671

Frank MB, Itoh K et al (1993) The mapping of the human 52-kD Ro/SSA autoantigen gene to human chromosome 11, and its polymorphisms. Am J Hum Genet 52(1):183–191

Gallivan JP, Dougherty DA (1999) Cation-pi interactions in structural biology. Proc Natl Acad Sci USA 96(17):9459–9464

Gassama-Diagne A, Hullin-Matsuda F et al (2001) Enterophilins, a new family of leucine zipper proteins bearing a b30.2 domain and associated with enterocyte differentiation. J Biol Chem 276(21):18352–18360

Ghadessy FJ, Jeyaseelan K et al (1994) A genomic region encoding stonefish (Synanceja horrida) stonustoxin beta-subunit contains an intron. Toxicon 32(12):1684–1688

Hauler F, Mallery DL et al (2012) AAA ATPase p97/VCP is essential for *TRIM21*-mediated virus neutralization. Proc Natl Acad Sci USA 109(48):19733–19738

Higgs R, Ni Gabhann J et al (2008) The E3 ubiquitin ligase Ro52 negatively regulates IFN-beta production post-pathogen recognition by polyubiquitin-mediated degradation of IRF3. J Immunol 181(3):1780–1786

Horn EJ, Albor A et al (2004) RING protein Trim32 associated with skin carcinogenesis has anti-apoptotic and E3-ubiquitin ligase properties. Carcinogenesis 25(2):157–167

Isakov E, Stanhill A (2011) Stalled proteasomes are directly relieved by P97 recruitment. J Biol Chem 286(35):30274–30283

James LC, Keeble AH et al (2007) Structural basis for PRYSPRY-mediated tripartite motif (TRIM) protein function. Proc Natl Acad Sci USA 104(15):6200–6205

Keeble AH, Khan Z et al (2008) *TRIM21* is an IgG receptor that is structurally, thermodynamically, and kinetically conserved. Proc Natl Acad Sci USA 105(16):6045–6050

Kong HJ, Anderson DE et al (2007) Cutting edge: autoantigen Ro52 is an interferon inducible E3 ligase that ubiquitinates IRF-8 and enhances cytokine expression in macrophages. J Immunol 179(1):26–30

Kudryashova E, Kudryashov D et al (2005) Trim32 is a ubiquitin ligase mutated in limb girdle muscular dystrophy type 2H that binds to skeletal muscle myosin and ubiquitinates actin. J Mol Biol 354(2):413–424

Landau M, Mayrose I et al (2005) ConSurf 2005: the projection of evolutionary conservation scores of residues on protein structures. Nucleic Acids Res 33(Web Server issue):W299–W302

Letunic I, Copley RR et al (2006) SMART 5: domains in the context of genomes and networks. Nucleic Acids Res 34(Database issue):D257–D260

Mallery DL, McEwan WA et al (2010) Antibodies mediate intracellular immunity through tripartite motif-containing 21 (*TRIM21*). Proc Natl Acad Sci USA 107(46):19985–19990

Masters SL, Yao S et al (2006) The SPRY domain of SSB-2 adopts a novel fold that presents conserved Par-4-binding residues. Nat Struct Mol Biol 13(1):77–84

McCauliffe DP, Wang L et al (1997) Recombinant 52 kDa Ro(SSA) ELISA detects autoantibodies in Sjogren's syndrome sera that go undetected by conventional serologic assays. J Rheumatol 24(5):860–866

McEwan WA, Hauler F et al (2012) Regulation of virus neutralization and the persistent fraction by *TRIM21*. J Virol 86(16):8482–8491

McEwan WA, Tam JC et al (2013) Intracellular antibody-bound pathogens stimulate immune signaling via the Fc receptor *TRIM21*. Nat Immunol 14(4):327–336

Moutsopoulos HM, Skopouli FN et al (1985) Anti-Ro(SSA) positive rheumatoid arthritis (RA): a clinicoserological group of patients with high incidence of D-penicillamine side effects. Ann Rheum Dis 44(4):215–219

Pung YF, Wong PT et al (2005) Ohanin, a novel protein from king cobra venom, induces hypolocomotion and hyperalgesia in mice. J Biol Chem 280(13):13137–13147

Randow F, MacMicking JD et al (2013) Cellular self-defense: how cell-autonomous immunity protects against pathogens. Science 340(6133):701–706

Rhodes DA, de Bono B et al (2005) Relationship between SPRY and B30.2 protein domains. Evolution of a component of immune defence? Immunology 116(4):411–417
Rhodes DA, Ihrke G et al (2002) The 52 000 MW Ro/SS-A autoantigen in Sjogren's syndrome/systemic lupus erythematosus (Ro52) is an interferon-gamma inducible tripartite motif protein associated with membrane proximal structures. Immunology 106(2):246–256
Sabile A, Meyer AM et al (2006) Regulation of p27 degradation and S-phase progression by Ro52 RING finger protein. Mol Cell Biol 26(16):5994–6004
Salomonsson S, Sonesson SE et al (2005) Ro/SSA autoantibodies directly bind cardiomyocytes, disturb calcium homeostasis, and mediate congenital heart block. J Exp Med 201(1):11–17
Short KM, Cox TC (2006) Subclassification of the RBCC/TRIM superfamily reveals a novel motif necessary for microtubule binding. J Biol Chem 281(13):8970–8980
Tanaka M, Kamitani T (2010) Cytoplasmic relocation of Daxx induced by Ro52 and FLASH. Histochem Cell Biol 134(3):297–306
Trockenbacher A, Suckow V et al (2001) MID1, mutated in Opitz syndrome, encodes an ubiquitin ligase that targets phosphatase 2A for degradation. Nat Genet 29(3):287–294
Urano T, Saito T et al (2002) Efp targets 14-3-3 sigma for proteolysis and promotes breast tumour growth. Nature 417(6891):871–875
Vaysburd M, Watkinson RE et al (2013) Intracellular antibody receptor *TRIM21* prevents fatal viral infection. Proc Natl Acad Sci USA 110(30):12397–12401
Vichi A, Payne DM et al (2005) E3 ubiquitin ligase activity of the trifunctional ARD1 (ADP-ribosylation factor domain protein 1). Proc Natl Acad Sci USA 102(6):1945–1950
Wada K, Niida M et al (2009) Ro52-mediated monoubiquitination of IKK{beta} down-regulates NF-{kappa}B signalling. J Biochem 146(6):821–832
Watkinson RE, Tam JC et al (2013) Simultaneous neutralization and innate immune detection of a replicating virus by *TRIM21*. J Virol 87(13):7309–7313
Weinert C, Grutter C et al (2009) The crystal structure of human pyrin b30.2 domain: implications for mutations associated with familial Mediterranean fever. J Mol Biol 394(2):226–236
Woo JS, Imm JH et al (2006) Structural and functional insights into the B30.2/SPRY domain. EMBO J 25(6):1353–1363
Xu L, Yang L et al (2003) BTBD1 and BTBD2 colocalize to cytoplasmic bodies with the RBCC/tripartite motif protein, TRIM5delta. Exp Cell Res 288(1):84–93
Yang K, Shi HX et al (2009) *TRIM21* is essential to sustain IFN regulatory factor 3 activation during antiviral response. J Immunol 182(6):3782–3792
Yang Y, Eversole T et al (1999) Protein-protein interactions between native Ro52 and immunoglobulin G heavy chain. Scand J Immunol 49(6):620–628
Yang YS, Yang MC et al (2000) Autoantigen Ro52 directly interacts with human IgG heavy chain in vivo in mammalian cells. Mol Immunol 37(10):591–602
Yergeau DA, Cornell CN et al (2005) Bloodthirsty, an RBCC/TRIM gene required for erythropoiesis in zebrafish. Dev Biol 283(1):97–112
Young JA, Sermwittayawong D et al (2011) Fas-associated death domain (FADD) and the E3 ubiquitin-protein ligase *TRIM21* interact to negatively regulate virus-induced interferon production. J Biol Chem 286(8):6521–6531
Zhang Z, Bao M et al (2013) The E3 ubiquitin ligase *TRIM21* negatively regulates the innate immune response to intracellular double-stranded DNA. Nat Immunol 14(2):172–178

Part II
FcR Signaling

Computational Modeling of the Main Signaling Pathways Involved in Mast Cell Activation

Anna Niarakis, Yacine Bounab, Luca Grieco, Romain Roncagalli, Anne-Marie Hesse, Jérôme Garin, Bernard Malissen, Marc Daëron and Denis Thieffry

Abstract A global and rigorous understanding of the signaling pathways and cross-regulatory processes involved in mast cell activation requires the integration of published information with novel functional datasets into a comprehensive computational model. Based on an exhaustive curation of the existing literature and using the software CellDesigner, we have built and annotated a comprehensive molecular map for the FcεRI signaling network. This map can be used to visualize and interpret high-throughput expression data. Furthermore, leaning on this map and using the logical modeling software GINsim, we have derived a qualitative

A. Niarakis · L. Grieco · D. Thieffry
Institut de Biologie de l'ENS (IBENS), Ecole Normale Supérieure, Paris, France

A. Niarakis · L. Grieco · D. Thieffry
INSERM, U1024, Paris, France

A. Niarakis · L. Grieco · D. Thieffry (✉)
CNRS, UMR 8197, Paris, France
e-mail: thieffry@ens.fr

Y. Bounab
Unité d'Allergologie Moléculaire et Cellulaire, Département d'Immunologie, Institut Pasteur, Paris, France

Y. Bounab
INSERM, UMS20, Paris, France

R. Roncagalli · B. Malissen
Centre d'Immunologie de Marseille-Luminy (CIML), Université Aix Marseille, Marseille, France

R. Roncagalli · B. Malissen
INSERM, U1104, Marseille, France

R. Roncagalli · B. Malissen
CNRS, UMR7280, Marseille, France

A.-M. Hesse · J. Garin
Laboratoire de Biologie à Grande Echelle, CEA, IRTSV, Grenoble, France

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_4,

dynamical model, which recapitulates the most salient features of mast cell activation. The resulting logical model can be used to explore the dynamical properties of the system and its responses to different stimuli, in normal or mutant conditions.

Contents

1 Introduction ... 69
2 A Comprehensive Map of FcεRI Signaling During Mast Cell Activation ... 72
3 Signaling Events Covered in the CellDesigner Molecular Map ... 75
3.1 FcεRI-Mediated Signaling ... 75
3.2 FcεRI Aggregation and *Lyn*/*Syk*/*Fyn*-Dependent Events ... 75
3.3 FcγRIIB-Mediated Signaling ... 76
3.4 LAT1-Dependent Protein Complex Formation ... 76
3.5 LAT2-Dependent Protein Complex Formation ... 77
4 Access to the Mast Cell Activation Map ... 77
4.1 CellDesigner File ... 77
4.2 Import into Cytoscape ... 78
4.3 REACTOME Database ... 78
4.4 NaviCell ... 78
5 Community-Driven Update of Mast Cell Activation Map ... 79
6 Visualization of Proteomic Data on the CellDesigner Map ... 79
7 Modularization and Decomposition of the Molecular Map Using *BiNoM* ... 81
8 Logical Modeling of Mast Cell Activation Network ... 83
9 Coherence of the Logical Model Behavior with Published Data ... 85
10 Outlook ... 90
References ... 91

1 Introduction

Mast cell activation is a pivotal event in the initiation of inflammatory reactions associated with allergic disorders. It is triggered by the aggregation of high-affinity IgE receptors (FcεRI) on the mast cell surface, which is in turn induced by the binding of a multivalent allergen to FcεRI-bound IgE antibodies. Mast cell

A.-M. Hesse · J. Garin
INSERM, U1038, Grenoble, France

A.-M. Hesse · J. Garin
Laboratoire de Biologie à Grande Echelle, Université de Grenoble Alpes, iRTSV, Grenoble, France

M. Daëron
Institut Pasteur, Paris, France

M. Daëron
Inserm, U1104, Centre d'Immunologie de Marseille-Luminy, Marseille, France

activation is a complex process relying on multiple layers of tightly controlled intracellular signaling molecules, which form an intricate network. Mast cells are crucially important for innate immunity and further play an effective role in the amplification of adaptive immunity. They are key players in biological responses, both in harmless and harmful situations, and it has been long known that they play a central role in type I hypersensitivity reactions and allergic disorders.

A global and rigorous understanding of the signaling pathways and cross-regulatory processes involved in mast cell activation ultimately requires the integration of published information with novel functional genomic datasets, in particular proteomic data, into a comprehensive computational model. In this respect, we engaged into an integrative approach involving two main steps: (i) the construction of a comprehensive and extensively annotated molecular map: (ii) the derivation of a qualitative dynamical model.

The construction of detailed molecular pathway maps is becoming an important component of system biology as attested by the release of various maps dealing, for example, with mTOR signaling (Caron et al. 2010), EGFR (Oda et al. 2005), RB/E2F (Calzone et al. 2008), and MAPKs (Grieco et al. 2013). These maps are integrated in dedicated databases, such as KEGG (Kanehisa and Goto 2000), Transpath (Krull et al. 2006), Reactome (Joshi-Tope et al. 2005), and the Atlas of Cancer Signaling Networks (http://acsn.curie.fr).

The use of graphical representations of complex networks and pathways is spreading as a consequence of the rising of Systems Biology and Synthetic Biology fields. The presence of a consistent, standardized way of representation, and interpretation of molecular maps is of outmost importance to enable exchange of information in a quick, unambiguous and systematic way. A consistent notation also helps improving biological curation (Hucka et al. 2003). An important effort toward this direction has been made recently with the Systems Biology Graphical Notation (SBGN) (Le Novere et al. 2005; Klipp et al. 2007).

To cope with large cellular networks for which precise kinetic data are lacking, logical modeling is increasingly used to derive global qualitative insights about network dynamics (for recent reviews, see Glass and Siegelmann 2010; Bérenguier et al. 2013). Recent applications to mammalian networks include logical models for T-helper cell differentiation (Mendoza 2006; Naldi et al. 2011), T-cell receptor signaling (Saez-Rodriguez et al. 2007), Erb-b receptor signaling (Sahin et al. 2009; Samaga et al. 2009; Helikar et al. 2013), as well as for the main MAPK pathways, including feedbacks and cross-talks (Grieco et al. 2013). Finally, several kinetic models dealing with subparts of the mast cell FcεRI signaling pathway have already been published, especially focusing on the early signaling events mediated by FcεRI (Goldstein et al. 2002; Nag et al. 2010).

If not absolutely required, a detailed molecular map is certainly an excellent basis to build a dynamical model. Perhaps less intuitively, the derivation of a dynamical model forces the biologist to scrutinize the mapped pathways with specific emphases, often provoking the reconsideration of some aspects of the map. Finally, model simulations often result in inconsistencies, thereby fostering the need for further refinements.

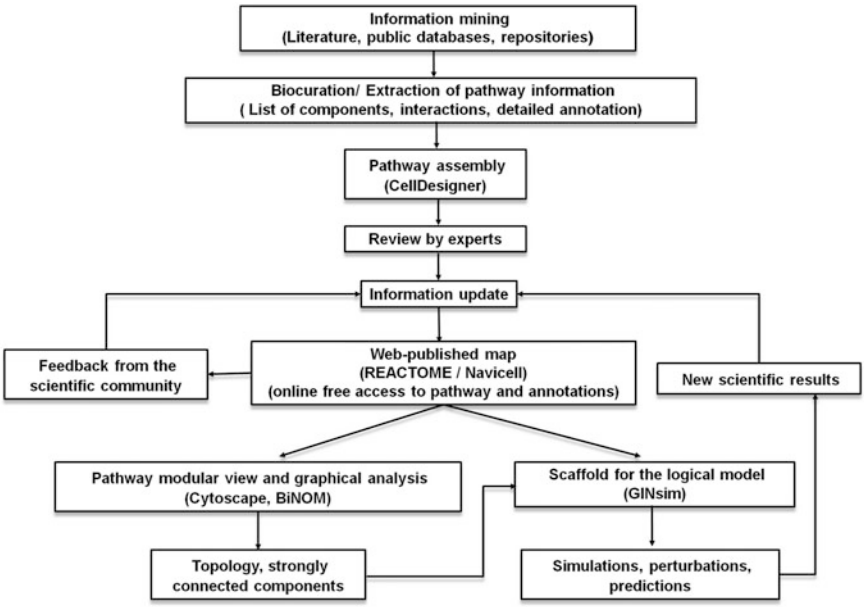


Fig. 1 *Data integration workflow*. The construction of a logical model is a multistep process involving several iterations. First, a map of relevant biological pathways integrating information from literature and public databases is built. Experts then curate this map, leading to the publication of an updated version on the web. Web publication facilitates community feedback and hence further refinements and extensions. Computational biologists can apply graph analysis tools to identify important nodes and pathways, or use it as a scaffold to build dynamical models allowing simulations. Interesting predictions can then be experimentally tested, thereby contributing to the validation and refinement of the map and expanding current knowledge

In short, the computational modeling of complex signaling networks involves various iterations of data curation, molecular mapping and dynamical modeling, until sufficient consistency is reached (see flowchart in Fig. 1).

2 A Comprehensive Map of FcεRI Signaling During Mast Cell Activation

Molecular maps of several pathways associated with FcεRI signaling events during mast cell activation can be found in public databases, with variable levels of detail and different representations (see Table 1). These maps can take the form of simplified graphs focusing on selected components or events, while others are much more comprehensive, encompassing various related pathways. They also differ regarding the number of references, as well as updating frequency.

Table 1 Main entries related to FcεRI signaling in public pathway databases

Database and url	Pathway entry in the corresponding database	Components and references (when specified)
KEGG pathway database http://www.genome.jp/kegg/pathway.html	map04664	33 components 7 references
Pathway interaction database http://pid.nci.nih.gov	Fcer1 pathway2	78 components
Biocarta http://www.biocarta.com	Fc epsilon receptor I signaling in mast cells	32 components
Pathway maps http://pathwaymaps.com/maps/	Immune response_Fc epsilon RI pathway	55 components 13 references

At this stage, however, we felt that existing maps still lack details to support the development of predictive models. Consequently, we engaged in the development of a novel, comprehensive molecular map of the signaling network underlying mast cell activation, based on an extensive analysis of relevant scientific papers and reviews, and taking into account existing pathways available in public databases (Fig. 2). This map has been built using the software CellDesigner (versions 4.2 and 4.3; Funahashi et al. 2003).

The components (vertices) of this map represent proteins and their post-transcriptional variants, protein complexes, metabolites, or genes, while links (arcs) represent molecular reactions (complex formation or dissociation, post-transcriptional modification of proteins, etc.). All nodes and links are extensively annotated with textual information tracing experimental support, along with links to databases, e.g., to relevant scientific papers in PubMed, or to representative entries in genomic databases such as EntrezGene, UniProt or HUGO.

Our current mast cell activation signaling network includes information derived from over 200 peer-reviewed journal articles. We primarily focused on human data, but we incorporated information coming from studies in mice or rats whenever human data were lacking. Similarly, we primarily focused on mast cell-specific data, but we further took into account results of studies dealing with B and T lymphocytes to fill gaps in our current knowledge of mast cell signaling. Inference by homology (from different species or from different cell types) is systematically emphasized in component annotations.

The resulting map encompasses a total of 122 distinct chemical species (proteins, ion channels, ions, receptors, complexes, chemical entities/compounds) and 73 reactions taking place in five main compartments (cytoplasm, plasma membrane, Golgi apparatus, endoplasmic reticulum, nucleus). The reactions encompass 39 state transitions (including catalysis), 30 heterodimer association, 2 transports, and 2 dissociations. Hereafter, we describe the main data integrated in our molecular map in more details (the CellDesigner map is available on request).

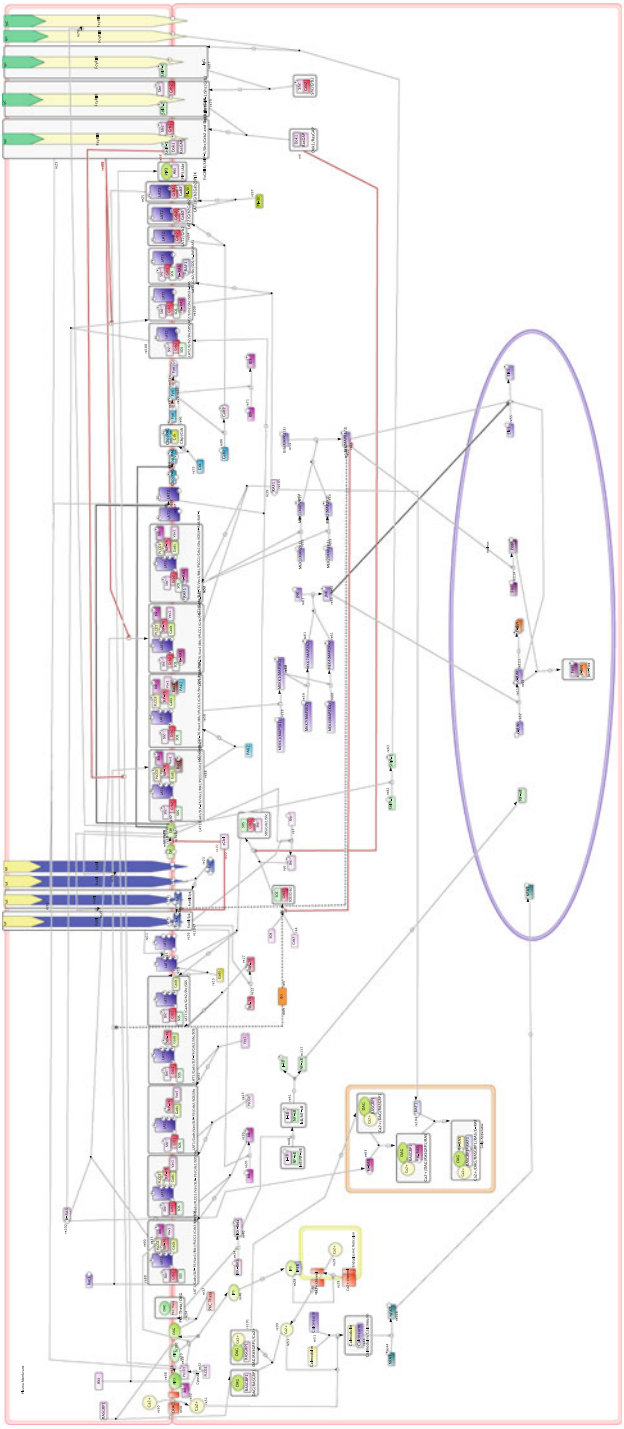


Fig. 2 *Snapshot of the mast cell activation signaling pathway map created with CellDesigner*. Interactions are color coded: *black arrows* denote stimulatory reactions; *red blunt end arrow* denotes inhibitions; *black round head arrows* denote catalysis. Proteins phosphorylation and ubiquitination are depicted by P and Ub labels, respectively. *Dotted contours* emphasize activated entities. Plasma membrane, cytoplasm, endoplasmic reticulum, Golgi apparatus, and nucleus compartments are distinguished

3 Signaling Events Covered in the CellDesigner Molecular Map

3.1 FcεRI-Mediated Signaling

The mast cell receptors FcεRI belong to the Fc receptor family which can bind a of variety antibodies (Ab). On mast cells, Fc receptors bind exclusively IgE (Fcε receptors) or IgG (Fcγ receptors). FcεRI consist of three subunits: an IgE-binding alpha subunit, a signal-amplifying beta subunit, and a signal-initiating homodimeric gamma subunit (Kinet 1999). The beta and gamma subunits contain one tyrosine activation motif (ITAM) each. The phosphorylation of ITAM canonical tyrosine residues initiates a cascade of intracellular events.

3.2 FcεRI Aggregation and Lyn/Syk/Fyn-*Dependent Events*

The Src protein tyrosine kinase Lyn is responsible for the phosphorylation of the ITAM motifs of FcεRI. Like other Src family kinases, Lyn is anchored in the lipid rafts via its palmitoyl or myristoyl moieties. FcεRI stimulation causes an accumulation of these protein tyrosine kinases (PTKs) in lipid raft domains (Kovárová et al. 2001). Moreover, the localization of Lyn in lipid rafts is necessary to sustain FcεRI phosphorylation and to maintain an active Lyn kinase (Young et al. 2003). Following FcεRI aggregation on the mast cell surface, Lyn phosphorylates the ITAMs of the beta and gamma chains. When phosphorylated, these motifs can recruit Syk (Spleen tyrosine kinase). Syk is then phosphorylated by Lyn and autophosphorylates.

Active Syk in turns phosphorylates the transmembrane adaptors LAT1 and LAT2, as well as several cytosolic proteins including Gab2, Clnk, SLP76, Shc, and Btk (Simon et al. 2005).

Activated Lyn further phosphorylates Fyn, which is important for the activation of Btk and Gab2. Fyn is also needed for the activation of PI3K via Fyn-dependent phosphorylation of Gab2. PI3K is essential for the production of PIP3 (Kraft and Kinet 2007). PIP3 is the product of PI3K phosphorylation of phosphatidylinositol (4,5)-bisphosphate (PIP2). Residing on the membrane, this phospholipid causes various proteins with a PH domain to translocate to the plasma membrane and affects their activity accordingly. This includes Akt activation, which in turn activates downstream anabolic signaling pathways required for cell growth and survival.

Fyn activity is regulated by Cbp/PAG adaptor. This adaptor recruits the negatively regulatory kinase Csk, which in turns phosphorylates Fyn and thereby inactivates it (Alvarez-Errico et al. 2009).

3.3 FcγRIIB-Mediated Signaling

The single-chain low-affinity IgG receptors FcγRIIB are widely expressed by hematopoietic cells, including mast cells. They inhibit FcεRI-dependent mast cell activation (Daëron et al. 1995a, b). When IgG-allergen immune complexes co-aggregate FcεRI and FcγRIIB, Lyn phosphorylates the ITAMs of FcεRI and the ITIM (Immune receptor Tyrosine-based Inhibitory Motif) of FcγRIIB (Malbec et al. 1998). Phosphorylation of the FcγRIIB ITIM results in the recruitment of SHIP1, which in turn recruits Dok1 and RasGAP. RasGAP inhibits SOS, thereby interrupting Ras activation, as well as subsequent transcriptional regulation and lipid mediator production. Constitutively bound to Shc in the cytoplasm, Grb2 is then further recruited (Jabril-Cuenod et al. 1996). Moreover, SHIP1 hydrolizes PIP3, resulting in a decrease of PLCγ, IP3, and Ca^{2+} fluxes. FcγRIIB cooperate with FcγRI for the activation of Ca^{2+} fluxes, as well as of MAPK pathway (Kraft and Kinet 2007).

SHIP1 is an important regulator of intracellular levels of PIP3. PtdIns(3,4,5)P3 is dephosphorylated by SHIP1 (SH2-containing inositol phosphatase) on the 5′ position of the inositol ring, producing PI(3,4)P2, thereby downregulating Akt activity.

3.4 LAT1-Dependent Protein Complex Formation

LAT1 is a transmembrane protein that is essential for the propagation of FcεRI-mediated signaling. It acts as an adaptor molecule and enables the association of different proteins such as Grb2, Gads, SLP76, Btk, Vav1, PLCγ (Saitoh et al. 2000). LAT1 undergoes lipid modification such as palmitoylation and acylation, which determines its cellular localization (Gilfillan and Tkaczyk 2006). Syk phosphorylates LAT1 shortly after antigen stimulation. Phosphorylated LAT1 plays the role of a signaling platform. It provides binding sites for the direct binding of Grb2, Gads, and PLCγ (Saitoh et al. 2000). Grb2/SOS complex recruits activated Shc. LAT1 binds SOS and Shc via Grb2. Gads recruits activated SLP76, which in turns recruits Btk and Vav, while Btk phosphorylates PLCγ.

Tyrosine-phosphorylated PLCγ in the membrane hydrolyzes PIP2, forming the second messengers IP3 and 1,2-diacylglycerol, which lead to the release Ca^{2+} from internal stores and activate PKC, respectively. The binding of IP3 to specific receptors in the endoplasmic reticulum results in a depletion of Ca^{2+} stores, which activates store-operated Ca^{2+} entry (ICRAC) from the extracellular medium.

The importance of LAT1 in mast cell activation was demonstrated by experiments in LAT1-deficient mice. LAT1 deficiency did not alter mast cell development and maturation, neither in vivo nor in vitro (Saitoh et al. 2000), but LAT1 deficient mice are resistant to IgE-mediated passive systemic anaphylaxis, while PLCγ and SLP76 phosphorylation is markedly reduced (Alvarez-Errico et al. 2009).

3.5 LAT2-Dependent Protein Complex Formation

Similar to LAT1, LAT2 contains a palmitoylation site adjacent to the transmembrane domain involved in its localization to the lipid rafts. Its exact role in mast cells remains to be elucidated, but it has been shown to affect actin polymerization via Rac and Rho (Tumova et al. 2010). Like LAT1, it is able to recruit PLCγ, but in an indirect way, via Gab2, since LAT2 lacks the PLCγ binding motif contained in LAT1 (Alvarez-Errico et al. 2009). LAT2 further affects degranulation through this indirect recruitment of PLCγ. However, in vivo experiments showed that the response of LAT2-deficient mice to systemic anaphylactic challenge is similar to that of wild type mice (Zhu et al. 2004). LAT2 may also regulate an inhibitory pathway for FcεRI-mediated mast cell degranulation, at least in mouse bone marrow macrophages. LAT2 knock down in human mast cells and the RBL 2H3 rat mast cell line attenuates FcεRI-mediated degranulation through reduction of the calcium signal. Subsequent studies suggested that this interaction may be occurring through SLP76. This maintenance/amplification pathway for antigen-mediated responses in mast cells may be the portal by which signaling pathways initiated by other mast cell receptors allow synergistic potentiation of FcεRI-mediated degranulation and cytokine production (Gilfillan and Beaven 2011). Upon FcεRI aggregation, LAT2 is phosphorylated by Lyn, Syk, and KIT on different tyrosines. Phosphorylated LAT2 likely contributes to the activation of mast cells by providing docking sites for the recruitment of critical signaling molecules into the lipid raft. LAT2 contains about ten tyrosines, five of which are principally phosphorylated by SYK, whereas others are phosphorylated by Lyn and KIT (Iwaki et al. 2008). After proper palmitoylation and phosphorylation, LAT2 recruits Grb2, which in turns recruits phosphorylated Gab2. The LAT2/Grb2/Gab2 complex recruits PI3K. In a different scenario, Grb2 binds to SOS, and the complex recruits activated Shc. The complex Shc/Grb2/SOS associates with LAT2.

4 Access to the Mast Cell Activation Map

4.1 CellDesigner File

As it was initially built using *CellDesigner*, the molecular map integrating all the aforementioned data is provided in the form of an xml file to be open with this software (xml file available on request). In this respect, the user must download, install, and open this *CellDesigner* (http://www.celldesigner.org), and then import the xml file, which will enable navigation through the map with access to all annotations.

4.2 Import into Cytoscape

It is also possible to view the network through the popular software *Cytoscape* (http://cytoscape.org; Shannon et al. 2003) using the plugin *BiNoM* (http://apps.cytoscape.org/apps/binom). This requires to download and install the proper versions of *Cytoscape 2.8.3* and *BiNoM 2.3*. Using these tools, the user can import the *CellDesigner* XML file and perform various kinds of analysis (examples of such analyses are provided below).

4.3 REACTOME Database

REACTOME is an open-source, open-access, manually curated, and peer-reviewed pathway database, which includes an intuitive interface along with various software tools to support the analysis of complex experimental and computational datasets.

An interface based on *SBGN*, *Pathway Browser,* facilitates the visualization of Reactome data and supports zooming, scrolling and event highlighting. It further exploits web services (*PSIQUIC*) to overlay molecular interaction data from the Reactome Functional Interaction Network and external interaction databases, such as *IntAct*, *ChEMBL*, *BioGRID,* and *iRefIndex*.

A first version of the mast cell signaling map has been integrated into *REACTOME* and can thus be browsed and queried directly from the corresponding website (http://www.reactome.org). In the course of the import of our map into *REACTOME*, all documented interactions were re-evaluated, the list of references enriched, and the resulting map was reviewed by experts before public release. Navigation into the *REACTOME* map can be done using a standard web browser, including access to all textual annotations, list of references and links to other databases, thereby avoiding the burden of downloading and installing a dedicated software such as *CellDesigner* or *Cytoscape*. Furthermore, all *REACTOME* maps can be easily exported into *SBML*, *BioPAX,* and other formats to facilitate data exchange with other analysis and modeling tools.

4.4 NaviCell

Finally, the mast cell signaling map has been also integrated in the collection developed by the Cancer Systems Biology team at Institute Curie. This collection is published online using the software *NaviCell*, a web tool for exploring large maps of molecular interactions (https://navicell.curie.fr). *NaviCell* allows easy map navigation and access to the network components through the Google maps engine. Navigation, scrolling, zooming, pop-up bubbles have been adapted from

Google maps. Semantic zooming enables users to explore the maps at different levels of detail. *NaviCell* further contains a web-based blog system (*Wordpress*) to collect feedbacks and facilitate exchange of knowledge between specialists and map managers (Kuperstein et al. 2013). The user can select species and reactions from a panel and get access to all annotations that were manually entered during map construction. Links to relevant PubMed entries are provided. Note that our original map includes protein nodes corresponding to more than one HUGO name (e.g., ERK node denotes both MAPK1 and MAPK3 isoforms); in such cases, we have selected unique representative identifiers.

5 Community-Driven Update of Mast Cell Activation Map

The molecular map for mast cell signaling presented here is more detailed and comprehensive than the previous diagrams published in scientific journals or available in public databases. It should help biologists to better deal with the complexity of mast cell signaling network and help them to identify potential intervention points to block uncontrolled inflammation. Furthermore, as we shall see, this map can be used as a scaffold for systems biologists to derive dynamical models for mast cell activation. However, as novel data will accumulate, regular revisions of the map will be necessary. In this respect, wide availability of the map should foster feedbacks, comments, and suggestions from the scientific community.

6 Visualization of Proteomic Data on the CellDesigner Map

The molecular map for mast cell signaling can be used as a template for the visualization of expression data, such as transcriptomic or proteomic data. For this purpose, *CellDesigner* provides a plugin (*Mapping Array Mass*) (version 4.0 and onward) that allows the import of an attribute file with the corresponding values. The color of the species is set according to the data value. An alternative way to color *CellDesigner* map components with expression data is provided by the *Cytoscape* plugin *BiNoM* (Zinovyev et al. 2008).

We illustrate this approach through the visualization of proteomic data on SLP-76 interactome published by Bounab et al. (2013) for activated BMMCs (Fig. 3). Such coloration facilitates the interpretation of expression and interaction data in the context of the known network.

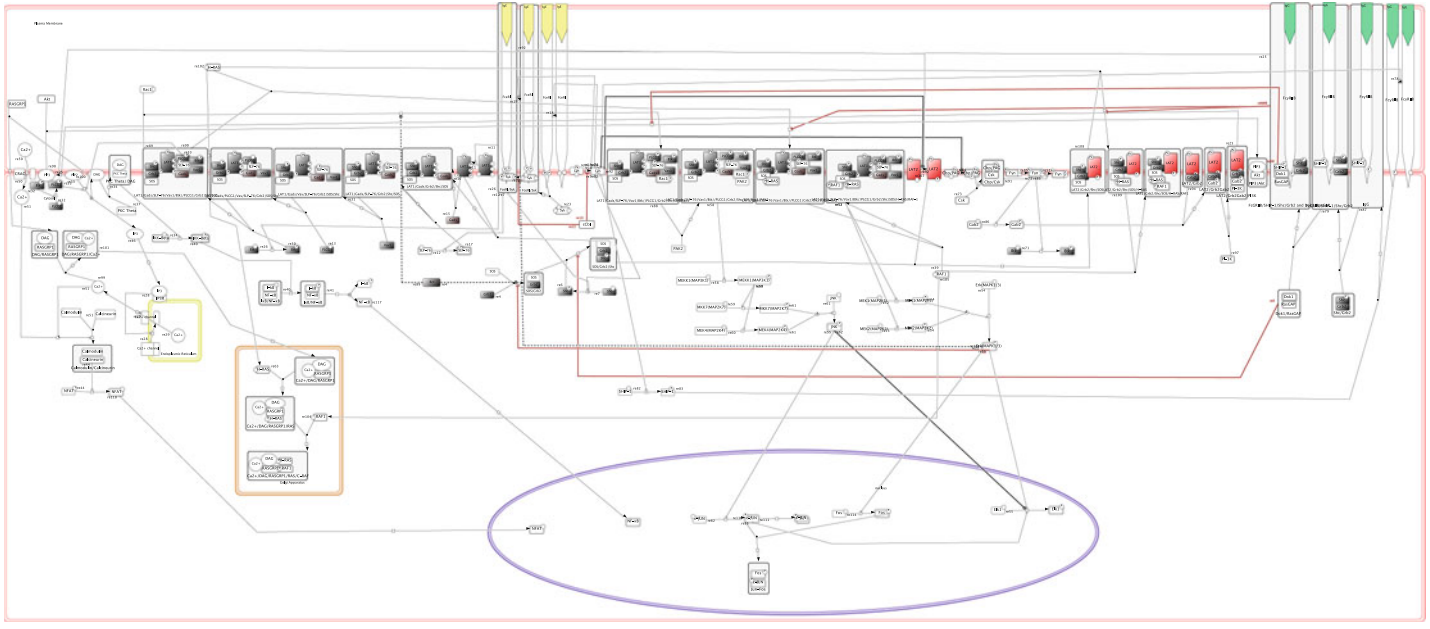


Fig. 3 *Visualization of proteomic data concerning SLP-76 interactome in activated BMMCs on the mast cell signaling map*. A color scale was defined from *light gray* (minimum detection level) to *bright red* (for the most enriched) according to the data values. One can see the high enrichment of LAT2 (*bright red color*) (data from Bounab et al. 2013)

7 Modularization and Decomposition of the Molecular Map Using *BiNoM*

To get insights into the organization of the mast cell signaling network, we used the *Cytoscape* plugin *BiNoM* (Zinovyev et al. 2008). Among other features, *BiNoM* allows the decomposition of complex networks and the generation of several modular views. Such high-level representations are fully based on the underlying detailed map and helps navigation through it. When necessary, the user can easily refer to the detailed mechanisms underlying a given module. A similar approach for modular pathway modeling has been implemented in the *ProMoT* system (Saez-Rodriguez et al. 2006).

Modularization consists in a semi-automatic procedure to delineate modules and ensures their coherence. Modules often represent detailed sequences of events involving either a particular protein or a particular complex. Compressing such sequences into modules enables a simplified and compact representation of complex pathways.

The decomposition of biological networks can be performed in different ways. First, we can separate unconnected network subparts, keeping only the connected components, i.e., decompose the network into (*strongly*) *connected components*. *BiNoM* further implements an algorithm enabling the generation of a *pruned graph* consisting in three main parts: the incoming flux part, from which all paths lead to the central core, the cyclic part that consists of strongly connected components, and the outgoing flux part, devoid of paths leading back to the central core. Figure 4 shows the pruned graph obtained from the application of this algorithm to our mast cell signaling network. Using another feature of *BiNoM,* the central core can be further decomposed into 12 simple cycles.

Still using *BiNoM*, the network can be decomposed into *material components*, each corresponding to one protein, either as a distinct chemical species or as part of a complex. As a protein can participate in different complexes, these subnetworks are usually largely overlapping. Another *BiNoM* function enables a clustering of these components based on common proteins or complexes and on an intersection percentage threshold specified by the user (Bonnet et al. 2013). Automatic decomposition of our molecular map resulted into 45 material components, which were then clustered for an intersection threshold set to 35 %. Manual curation led us to merge some of the 24 resulting components, ultimately defining the 12 modules shown in Fig. 5.

Structural graph analysis gives us a first estimation of the complexity of the biological network and can also reveal important properties that are not obvious at first sight (e.g., nonconnected components, overlapping cycles). The modularization process enables a simplification of the network representation, allowing the user to supervise and check more easily the main events occurring in the network. Especially for very large networks containing hundreds of interactions, modularization greatly facilitates navigation.

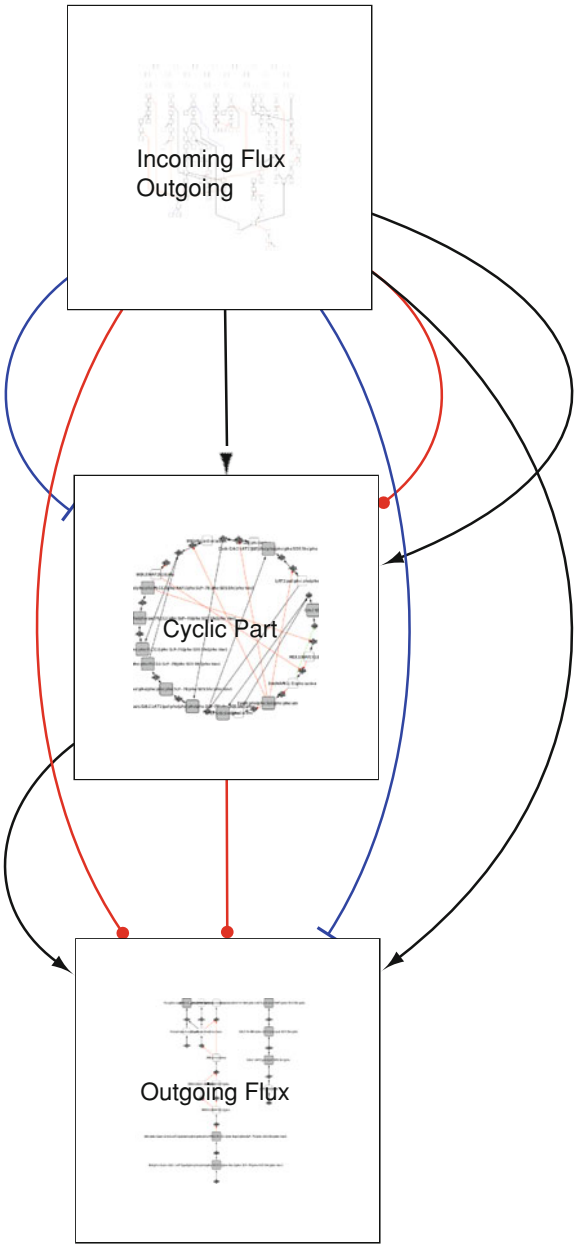


Fig. 4 *Modular view of the pruned graph generated from our molecular map using BiNoM*. Three modules are obtained corresponding to "incoming flux," "cyclic part," and "outgoing flux," while different kinds of connections between these modules are specified: *black arrows* molecular flows; *red ball arrows* catalysis, *blue blunt arrows* inhibitions

Both modular views (pruned graph and material components graph) are useful for the development of a proper dynamical model (cf. following section), in particular to define the main variables and check that no important interaction is missed.

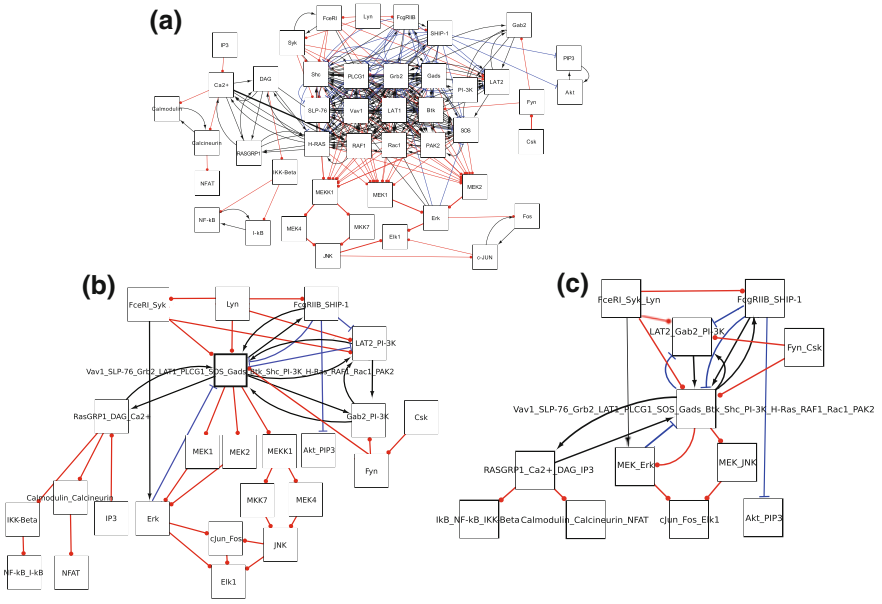


Fig. 5 *Modular views of the mast cell signaling network (cf. Fig. 2) generated with the Cytoscape plugin BiNoM*. Modularization according to material components resulted in 45 modules. Clustering of these networks resulted in a reduction of this number. Manual refinement led to an even more compact representation. *Rectangles* modules with nested networks; *Red arrows* catalysis; *Blue arrows* inhibition; *Black arrows* molecular flow. **a** 45 modules consisting of material components networks. **b** 24 modules deriving from clustering of the material components networks (threshold: 35 % overlapping). **c** 12 resulting modules, after manual curation and merging

8 Logical Modeling of Mast Cell Activation Network

Our mast cell signaling molecular map can be used as a reference to build a predictive dynamical model accounting for the most salient events following mast cell receptor activation. In the absence of detailed kinetic data, we decided to rely upon a sophisticated logical formalism implemented in the software *GINsim* (Chaouiya et al. 2012). In this framework, a regulatory network is modeled in terms of a *regulatory graph*, where nodes represent regulatory components (proteins, complexes, transcription factors, etc.), whereas arcs represent interactions between these components. In addition, each regulatory component is associated with a logical variable denoting its qualitative concentration or level of activity. In most cases, Boolean variables (taking the values 0 or 1) are sufficient to represent the most relevant situations, but whenever needed, multivalued variables can be used.

Based on available data on co-aggregation of FcεRI with the inhibitory receptor FcγRIIB, we abstracted relevant information from the reaction map to define a regulatory graph. Beyond molecular interactions delineated using low-throughput

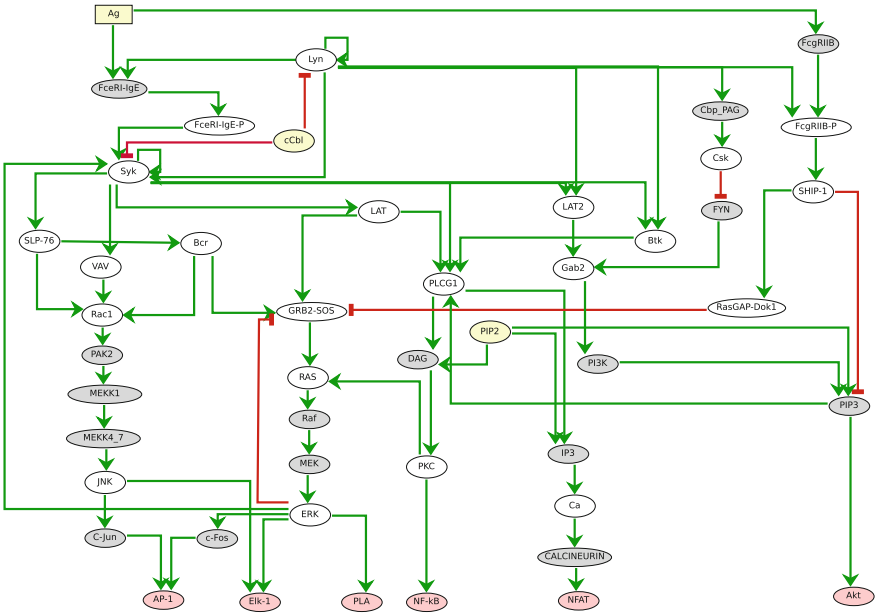


Fig. 6 *Regulatory graph of the mast cell signaling logical model*. The regulatory graph encompasses 47 components. *Green/red arcs* denote activating/inhibitory regulations. Ellipsoid nodes represent Boolean variables, while rectangular nodes represent multilevel variables. Inputs and output nodes are emphasized in *yellow* and *pink*, respectively. Nodes hidden in the reduced version used for simulations are colored in *gray*

approaches, we have used the proteomic data reported in Bounab et al. (2013), which point to novel SLP76 interactants, some previously reported in T or B cell activation processes, but now specifically identified in mastocytes.

Based on the 45 material components extracted with BiNoM (Fig. 5a), we have considered 42 molecular species in the regulatory graph shown in Fig. 6. The following step is the assignment of logical rules for each regulatory component in order to specify its target activity level according to the levels of its regulators. In most cases, this is straightforward, but it becomes tricky when many regulators converge onto a single component. Figure 7 illustrates the relationship between regulatory interactions and logical rules on the one hand, and the underlying molecular subnetworks on the other hand. The structure of the regulatory network and the logical rules are iteratively refined based on the comparison between simulations and documented network properties.

Once a regulatory graph and a set of regulatory rules are defined, the user can select a set of initial values for the components and use *GINsim* to compute a state transition graph, highlighting stable states, and cyclic attractors. However, as the number of components considered increases, such simulations become rapidly challenging from a computational point of view. In this respect, the recent development of a rigorous logical model reduction approach and its

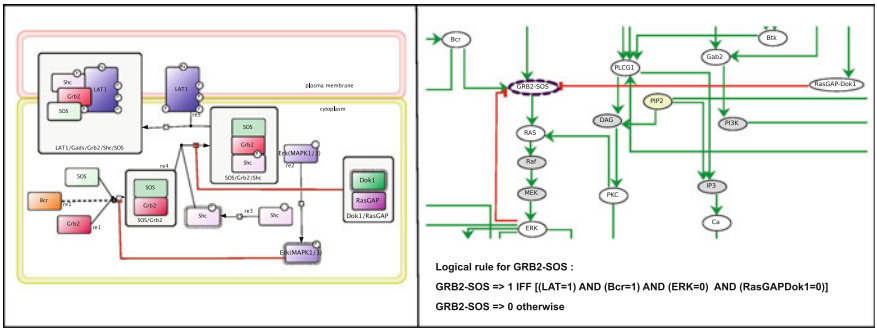


Fig. 7 *From CellDesigner molecular map to GINsim logical model*. *Left* zoom in a section of the *CellDesigner* map. *Right* translation of molecular interactions into regulatory interactions into *GINsim* and delineation of the logical rule for GRB-SOS, whose activity depends on the presence of RasGAP-Dok1, Bcr, LAT, and ERK

implementation into *GINsim* currently allow the simulation and analysis of regulatory networks encompassing hundreds of components (Naldi et al. 2011). The basic idea consists in enabling the user to select a series of components to hide. The software then hides them iteratively one at a time and recomputes the logical rules of their targets. Provided that no regulatory circuit is eliminated in this process (which is forbidden by the algorithm), it has been proven that the most salient dynamical properties are preserved, including all stable states, which typically represent different cellular states.

9 Coherence of the Logical Model Behavior with Published Data

In order to evaluate the coherence of the global behavior of the model with current biological knowledge, we compared its dynamical properties with published data. First, we computed the stable states of the model and compared them with available data. Next, we performed asynchronous simulations for specific initial conditions (input levels, initial states, in the presence of perturbations or not). Inconsistencies were progressively fixed through appropriate modifications of the logical rules and/or adding or removing an interaction or a model component.

Of particular interest are the sets of states forming attractors, i.e., groups of states from which the system cannot escape, which represent potential asymptotic behaviors. Attractors can be classified into two main categories: stable states and cyclic attractors (denoting periodic or homeostatic behavior). From a biological point of view, the asymptotic behavior represents the ultimate cellular outcome induced by an initial configuration.

The three inputs of the model correspond to antigen activity on the receptors (considering three levels: Ag = 0, 1 or 2), cCbl activity, and PIP2 activity, respectively. Ag = 1 corresponds to the aggregation of FcεRI, while Ag = 2 denotes co-aggregation of FcεRI with FcγRIIB. The second input, PIP2 activity is important for the activation of certain pathways (PIP3, Ca^{2+}). Finally, cCbl stands for an ubiquitin-protein ligase targeting Syk and Lyn tyrosine kinases for degradation.

The six outputs of the model and one intermediate component (component Ca, denoting Ca^{2+}) are used to define the functional outcome. AP1, Elk1, Akt, NF-kB, NFAT are associated with cytokine release, PLA with the synthesis of lipidic mediators, and Ca with degranulation.

Table 2 lists the stable states (ss) obtained for the unperturbed model. For wild type conditions, the model shows that, in the absence of antigen, no matter of the values of the other inputs, signaling is abolished (ss1 and ss2). However, the stable state ss3 corresponds to a situation where transient but substantial activation would nevertheless lead to cytokine release, degranulation and lipidic mediator synthesis. This, state can only be attained when Syk is provided in the initial conditions (cf. Model file available in the model repository on GINsim website, at http://www.ginsim.org).

For medium receptor activation (Ag = 1), we have two possible stable states, ss4 and ss5. In the first case (ss4), the FcεRI-dependent pathways are activated provided that Lyn, Syk, and PIP2 are initially present, while in the second case (ss5), in the absence of Lyn and Syk, no matter the values of the other two inputs, FcεRI-dependent pathways are not activated.

For high receptor activation (Ag = 2), we obtain three stable states, ss6, ss7, and ss8. The first one (ss6) is very similar to ss5, meaning again that, in the absence of Lyn and Syk, no matter the values of the other two inputs, we have no activation. In the second case (ss7), the presence of Lyn and Syk in the absence of the other inputs can result in the activation of intermediate components, but we have a final down-regulation of NFkB, NFAT, AP1, PLA, and Elk1. The third case (ss8) corresponds to a full pathway activation in the presence of Lyn, Syk and PIP2.

To further assess the behavior of our model in comparison with published data, we designed and performed a series of in silico experiments (simulations) combining different initial conditions and virtual perturbations (loss or gain of function of selected model components). As already mentioned above, for the simulation part, we reduced our model using a specific function of GINsim, resulting in reduced model version encompassing 31 components.

Table 3 shows the results obtained for simulations corresponding to two different genetic backgrounds.

Analysis of FcεRI signaling pathways in Syk-deficient mast cells indicates that Syk is not required for the activation of Lyn. In contrast, FcεRI-induced rise in intracellular Ca^{2+} and activation of the ERK and JNK MAP kinase pathways is completely abrogated in the absence of Syk. Furthermore, phosphorylation of phospholipase Cγ1 (PLCγ1) and of the Vav1 exchange factor is also Syk-dependent

Table 2 Stable states of the logical model for mast cell activation (wild-type)

Component	ss1	ss2	ss3	ss4	ss5	ss6	ss7	ss8
Ag (input)	0	0	0	1	1	2	2	2
Akt	*	0	0	0	*	*	0	0
AP1	*	*	1	1	*	*	0	1
Bcr	0	0	0	0	0	0	0	0
Btk	0	0	1	1	0	0	0	1
Ca	0	0	1	1	0	0	1	1
CCbl (input)	0	0	1	1	0	0	1	1
Csk	0	0	1	1	0	0	0	1
Elk1	0	1	1	1	0	0	1	1
ERK	0	0	1	1	0	0	0	1
FceRI-IgE_P	0	0	1	1	0	0	0	1
FcgRIIB_P	0	0	0	1	0	0	1	1
Gab2	0	0	0	0	0	0	1	1
GRB2-SOS	0	0	0	0	0	0	0	0
JNK	0	0	0	0	0	0	0	0
LAT	0	0	1	1	0	0	1	1
LAT2	0	0	1	1	0	0	1	1
Lyn	0	0	1	1	0	0	1	1
NFAT	0	1	1	1	0	0	1	1
NFkB	0	0	1	1	0	0	0	1
PIP2 (input)	0	0	1	1	0	0	0	1
PKC	0	0	1	1	0	0	0	1
PLA	0	0	1	1	0	0	0	1
PLCG1	0	0	1	1	0	0	1	1
Rac1	0	0	1	1	0	0	1	1
RAS	0	0	1	1	0	0	0	1
RasGAP-Dok1	0	0	0	0	0	0	1	1
SHIP1	0	0	0	0	0	0	1	1
SLP76	0	0	1	1	0	0	1	1
Syk	0	0	1	1	0	0	1	1
Vav	0	0	1	1	0	0	1	1

All stable states of the model are listed, which differ regarding input (Ag, cCbl, and PIP2) values, and downstream effects
The * refers to both values 0 and 1

(Simon et al. 2005). In Syk knockout experiments, degranulation and cytokine release are both abolished (Gilfillan and Tkaczyk 2006).

To check the consistency of our model with these data, we performed an in silico simulation of Syk knockout, with Lyn and PIP2 present and Ag set to level 2 at the initial state. Note that Lyn level needs to be set at the initial state as we lack information about its upstream regulator(s). The results of this simulation are shown in Table 3 (third column). In this situation, the system reaches a stable state where AP-1, ERK, JNK, Elk-1, NF-kB, NFAT, PKC, PLCG1, $Ca2^{+}$, and Vav are all set to zero, in agreement with the published data.

Table 3 Examples of simulations for full receptor activation

Component	WT	Syk KO	Btk KO
Ag (input)	2	2	2
Akt	0	0	0
AP1	1	0	0
Bcr	1	0	1
Btk	1	0	0
Ca	1	0	0
CCbl (input)	0	0	0
Csk	1	1	1
Elk1	1	0	1
ERK	1	0	0
FceRI-IgE_P	1	1	1
FcgRIIB_P	1	1	1
Gab2	0	0	0
GRB2-SOS	0	0	0
JNK	1	0	1
LAT	1	0	1
LAT2	1	0	1
Lyn	1	1	1
NFAT	1	0	0
NFkB	1	0	0
PIP2 (input)	1	1	1
PKC	1	0	0
PLA	1	0	0
PLCG1	1	0	0
Rac1	1	0	1
RAS	1	0	0
RasGAP-Dok1	1	1	1
SHIP1	1	1	1
SLP76	1	0	1
Syk	1	0	1
Vav	1	0	1

Columns 2, 3, and 4 correspond to wild-type, and Syk and Btk knockouts, respectively
In each case, we start with initial conditions with Ag = 2, Lyn = PIP2 = 1, all other components being set to zero. For both mutants, calcium signaling (hence degranulation), NFkB, NFAT, and AP1 activity (hence cytokine release), and PLA activation (hence membrane synthesis) are impaired, although we observe a broader impact of Syk KO on the activity of signaling components

In the case of Btk knockout, a decrease in degranulation and cytokine release, as well as a decrease of PLCG1, ERK, and JUN levels have been observed (Kawakami et al. 2000; Setoguchi et al. 1998). The simulation of Btk knockout indeed results into a decrease of PLCG1 and ERK (set to zero), in agreement with published data.

Certain mutant phenotypes are not easy to assess because the data are still controversial (e.g., Lyn knockout and its effect on degranulation). Other mutant simulations give self-evident results (e.g., Gab2 knockout results in the blockage

of PI3K dependent pathway). Comparing the results for WT and Gab2 knockout mutant, starting from initial conditions where PI3K and Gab2 are set to zero, we observed that their activation is only transient (data not shown).

In the case of Lyn knockout, it has been observed that PI3K activity is increased, while the phosphorylation of the ITAMs of the beta and gamma chains of FcεRI is decreased, and MAP and JUN phosphorylation prolonged (Gilfillan and Tkaczyk 2006). Our model does not yet account for these results, pointing to gaps in our knowledge regarding Lyn activation and role.

For LAT knockout and knockdown mice, a decrease on degranulation, cytokine release, SLP76 and PLCγ phosphorylation, and MAPK activity have been observed, with no change in Syk and Vav phosphorylation (Gilfillan and Tkaczyk 2006). Our model is partly coherent with experimental data, as the simulation of a LAT knockout mutant points to a decrease in cytokine release (decrease of AP-1, Erk, NFAT, NFkB), in calcium ions and PLCγ, with no change in Syk and Vav activity.

On the other hand, for LAT2 knockout, an increase of degranulation and cytokine release, and a hyperphosphorylation of *ERK1* and 2, PLCγ, and LAT have been observed (Gilfillan and Tkaczyk 2006). Our model does not account for these results, since simulations of LAT2 loss-of-function shows no difference from the WT. This inconsistency can likely be attributed to gaps in our knowledge in the functioning of the inhibitory pathway. As more data will become available, a more refined modeling of this pathway could be established.

Regarding SLP76 knockout, a decrease of degranulation, cytokine release, calcium signaling and PLCγ phosphorylation have been reported. The corresponding simulation leads to a decrease in cytokine release (decrease of AP-1, JNK, NFAT, NFkB), while calcium and PLCγ activity are preserved. Here also, our model could be refined to better take into account the different potential sources of calcium ions, once they will be better characterized.

Loss of SHIP expression in vivo has been shown to lead to high Akt activation in bone marrow-derived mast cells in response to cytokine stimulation (Liu et al. 1999). Simulations of SHIP1 knockout mutants indicates that its absence does not suffice to activate Akt. To reach full activation of Akt, our model suggests that Csk must be also inactive, thereby enabling Fyn to phosphorylate and activate Gab2. The simulation of a double SHIP-1 and Csk knock-out indeed results in the activation of Akt.

Regulatory circuits (or feedback loops) have been reported to play crucial roles in the generation of specific dynamical properties, such as multistability or periodic behavior. Multistationarity is related to the presence of positive circuits, while oscillatory behavior depends on the presence of negative ones (for a review, see Thieffry 2007). In this respect, *GINsim* includes an algorithm enabling the identification of all regulatory circuits embedded in a logical model, along with the delineation of functionality conditions, i.e., conditions on the levels of external regulators enabling a circuit to generate the corresponding property.

In the case of our mast cell activation model, the analysis of circuits emphasizes the functionality of two positive circuits, corresponding to Lyn and Syk

autophosphorylations, and of one negative circuit comprising Raf, MEK, ERK, GRB2-SOS, and RAS, suggesting a mechanism underlying the oscillatory behavior observed for medium Ag levels (data not shown).

10 Outlook

In this article, we have reported a systematic effort to model FcεRI signaling using all the relevant information available.

The molecular map presented here is the result of the integration of information found in the numerous publications. After taking into account suggestions and corrections from experts, the map has been released on the web. The feedback from the scientific community and the publication of novel results will lead to further updating.

Subsequently, the map can be used as a template in order to visualize experimental data, gaining valuable insights about the specific parts of the signaling cascade that play a major role in response to specific stimuli. Using *Cytoscape* and *BiNoM* software, topological and material analyses have been performed to characterize the structure of the underlying network, decompose it into modules and thereby simplify its representation and ease navigation.

The molecular map and its modular representations have in turn been used to build a dynamical model, using a logical formalism. The derivation of a logical model and its calibration (through the specification of the logical rules) led us to reconsider and update specific parts of the map. Furthermore, model simulations resulted in some inconsistencies (e.g., regarding Lyn knockout), thereby emphasizing gaps in our knowledge and the need for further model refinements.

This logical modeling approach enabled the recapitulation of several dynamical properties of an extremely complex biological system, such as Fc receptor signaling. Systematic testing of different initial conditions and stimuli could further lead to predictions regarding the outcomes of single or multiple perturbations (e.g., mutations, use of specific enzymatic inhibitors), as well as potential pharmacological intervention points.

As further experimental data will be gathered regarding the cascades of Fc receptor signaling, our logical model could serve as a template to design continuous or stochastic models enabling more quantitative predictions. Ultimately, dynamical model analyses should help to understand in more details how the different functional outcomes of mast cell activation (degranulation, synthesis of lipidic mediators, induction of cytokine transcription) are articulated at the level of the underlying molecular network, and to what extend it might be possible to uncouple these functions and delineate means to control them separately or collectively.

Acknowledgements This project was supported by the ANR through the MI2 project iSa and by the proteomics ProFI infrastructure, as well as by the French excellence initiatives MemoLife LabEx and PSL* IdEx.

References

Alvarez-Errico D, Lessmann E, Rivera J (2009) Adapters in the organization of mast cell signaling. Immunol Rev 232(1):195–217

Bérenguier D, Chaouiya C, Monteiro PT, Naldi A, Remy E, Thieffry D, Tichit L (2013) Dynamical modeling and analysis of large cellular regulatory networks. Chaos 23(2):025114

Bonnet E, Calzone L, Rovera D, Stoll G, Barillot E, Zinovyev A (2013) BiNoM 2.0, a Cytoscape plugin for accessing and analyzing pathways using standard systems biology formats. BMC Syst Biol 7:18

Bounab Y, Hesse AM, Iannascoli B, Grieco L, Couté Y, Niarakis A, Roncagalli R, Lie E, Lam KP, Demangel C, Thieffry D, Garin J, Malissen B, Daëron M (2013) Proteomic analysis of the SH2domain-containing leukocyte protein of 76 kDa (SLP76) interactome. Mol Cell Proteomics 12:2874–2889

Calzone L, Gelay A, Zinovyev A, Radvanyi F, Barillot E (2008) A comprehensive modular map of molecular interactions in RB/E2F pathway. Mol Syst Biol 4:173

Caron E, Ghosh S, Matsuoka Y, Ashton-Beaucage D, Therrien M, Lemieux S, Kitano H (2010) A comprehensive map of the mTOR signaling network. Mol Syst Biol 6:453

Chaouiya C, Naldi A, Thieffry D (2012) Logical modelling of gene regulatory networks with GINsim. Methods Mol Biol 804:463–479

Chaouiya C, Berenguier D, Keating SM, Naldi A, van Iersel MP, Rodriguez N, Dräger A, Büchel F, Cokelaer T, Kowal B, Wicks B, Gonçalves E, Dorier J, Page M, Monteiro PT, von Kamp A, Xenarios I, de Jong H, Hucka M, Klamt S, Thieffry D, Le Novère N, Saez-Rodriguez J, Helikar T (2013) SBML qualitative models: a model representation format and infrastructure to foster interactions between qualitative modelling formalisms and tools. BMC Syst Biol 7:135

Daëron M, Malbec O, Latour S, Arock M, Fridman WH (1995a) Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. J Clin Invest 95(2):577–585

Daëron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S, Fridman WH (1995b) The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. Immunity 3(5):635–646

Funahashi A, Tanimura N, Morohashi M, Kitano H (2003) Cell Designer: a process diagram editor for gene-regulatory and biochemical networks. BIOSILICO 1:159–162

Gilfillan AM, Tkaczyk C (2006) Integrated signalling pathways for mast-cell activation. Nat Rev Immunol 6(3):218–230

Gilfillan AM, Beaven MA (2011) Regulation of mast cell responses in health and disease. Crit Rev Immunol 31(6):475–529

Glass L, Siegelmann HT (2010) Logical and symbolic analysis of robust biological dynamics. Curr Opin Genet Dev 20(6):644–649

Goldstein B, Faeder JR, Hlavacek WS, Blinov ML, Redondo A, Wofsy C (2002) Modeling the early signaling events mediated by aggregation of FceRI. Mol Immunol 38(16–18):1213–1219

Grieco L, Calzone L, Bernard-Pierrot I, Radvanyi F, Kahn-Perlès B, Thieffry D (2013) Integrative modelling of the influence of MAPK network on cancer cell fate decision. PLoS Comput Biol 9:e1003286

Helikar T, Kochi N, Kowal B, Dimri M, Naramura M, Raja SM, Band V, Band H, Rogers JA (2013) A comprehensive, multi-scale dynamical model of ErbB receptor signal transduction in human mammary epithelial cells. PLoS ONE 8(4):e61757

Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, Arkin AP, Bornstein BJ, Bray D, Cornish-Bowden A, Cuellar AA, Dronov S, Gilles ED, Ginkel M, Gor V, Goryanin II, Hedley WJ, Hodgman TC, Hofmeyr JH, Hunter PJ, Juty NS, Kasberger JL, Kremling A, Kummer U, Le Novère N, Loew LM, Lucio D, Mendes P, Minch E, Mjolsness ED, Nakayama Y, Nelson MR, Nielsen PF, Sakurada T, Schaff JC, Shapiro BE, Shimizu TS, Spence HD, Stelling J,

Takahashi K, Tomita M, Wagner J, Wang J (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 19(4):524–531

Iwaki S, Spicka J, Tkaczyk C, Jensen BM, Furumoto Y, Charles N, Kovarova M, Rivera J, Horejsi V, Metcalfe DD, Gilfillan AM (2008) Kit- and Fc epsilonRI-induced differential phosphorylation of the transmembrane adaptor molecule NTAL/LAB/LAT2 allows flexibility in its scaffolding function in mast cells. Cell Signal 20(1):195–205

Jabril-Cuenod B, Zhang C, Scharenberg AM, Paolini R, Numerof R, Beaven MA, Kinet JP (1996) Syk-dependent phosphorylation of Shc. A potential link between FcepsilonRI and the Ras/mitogen-activated protein kinase signaling pathway through SOS and Grb2. J Biol Chem 271(27):16268–16272

Joshi-Tope G, Gillespie M, Vastrik I, D'Eustachio P, Schmidt E, de Bono B, Jassal B, Gopinath GR, Wu GR, Matthews L, Lewis S, Birney E, Stein L (2005) Reactome: a knowledgebase of biological pathways. Nucleic Acids Res 33:D428–D432

Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30

Kawakami Y, Kitaura J, Satterthwaite AB, Kato RM, Asai K, Hartman SE, Maeda-Yamamoto M, Lowell CA, Rawlings DJ, Witte ON, Kawakami T (2000) Redundant and opposing functions of two tyrosine kinases, Btk and Lyn, in mast cell activation. J Immunol 165(3):1210–1219

Kinet JP (1999) The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. Annu Rev Immunol 17:931–972

Klipp E, Liebermeister W, Helbig A, Kowald A, Schaber J (2007) Systems biology standards—the community speaks. Nat Biotech 25:390–391

Kovárová M, Tolar P, Arudchandran R, Dráberová L, Rivera J, Dráber P (2001) Structure-function analysis of Lyn kinase association with lipid rafts and initiation of early signaling events after Fcepsilon receptor I aggregation. Mol Cell Biol 21(24):8318–8328

Kraft S, Kinet JP (2007) New developments in FcepsilonRI regulation, function and inhibition. Nat Rev Immunol 7(5):365–378

Krull M, Pistor S, Voss N, Kel A, Reuter I, Kronenberg D, Michael H, Schwarzer K, Potapov A, Choi C, Kel-Margoulis O, Wingender E (2006) TRANSPATH: an information resource for storing and visualizing signaling pathways and their pathological aberrations. Nucleic Acids Res 34:D546–D551

Kuperstein I, Cohen DP, Pook S, Viara E, Calzone L, Barillot E, Zinovyev A (2013) NaviCell: a web-based environment for navigation, curation and maintenance of large molecular interaction maps. BMC Syst Biol 7(1):100

Le Novere N, Finney A, Hucka M, Bhalla US, Campagne F, Collado-Vides J, Crampin EJ, Halstead M, Klipp E, Mendes P, Nielsen P, Sauro H, Shapiro B, Snoep JL, Spence HD, Wanner BL (2005) Minimum information requested in the annotation of biochemical models (MIRIAM). Nat Biotech 23:1509–1515

Liu Q, Sasaki T, Kozieradzki I, Wakeham A, Itie A, Dumont DJ, Penninger JM (1999) SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. Genes Dev 13(7):786–791

Malbec O, Fong DC, Turner M, Tybulewicz VL, Cambier JC, Fridman WH, Daëron M (1998) Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. J Immunol 160(4):1647–1658

Mendoza L (2006) A network model for the control of the differentiation process in Th cells. Biosystems 84(2):101–114

Nag A, Faeder JR, Goldstein B (2010) Shaping the response: the role of FcεRI and Syk expression levels in mast cell signaling. IET Syst Biol 4(6):334–347

Naldi A, Berenguier D, Fauré A, Lopez F, Thieffry D, Chaouiya C (2009) Logical modelling of regulatory networks with GINsim 2.3. Biosystems 97(2):134–139

Naldi A, Remy E, Thieffry D, Chaouiya C (2011) Dynamically consistent reduction of logical regulatory graphs. Theor Comput Sci 412(21):2207–2218

Oda K, Matsuoka Y, Funahashi A, Kitano H (2005) A comprehensive pathway map of epidermal growth factor receptor signaling. Mol Syst Biol 1(2005):0010

Saez-Rodriguez J, Mirschel S, Hemenway R, Klamt S, Gilles ED, Ginkel M (2006) Visual setup of logical models of signaling and regulatory networks with ProMoT. BMC Bioinformatics 7:506

Saez-Rodriguez J, Simeoni L, Lindquist JA, Hemenway R, Bommhardt U, Arndt B, Haus UU, Weismantel R, Gilles ED, Klamt S, Schraven B (2007) A logical model provides insights into T cell receptor signaling. PLoS Comput Biol 3(8):e163

Saitoh S, Arudchandran R, Manetz TS, Zhang W, Sommers CL, Love PE, Rivera J, Samelson LE (2000) LAT is essential for Fc(epsilon)RI-mediated mast cell activation. Immunity 12(5):525–535

Samaga R, Saez-Rodriguez J, Alexopoulos LG, Sorger PK, Klamt S (2009) The logic of EGFR/ErbB signaling: theoretical properties and analysis of high-throughput data. PLoS Comput Biol 5(8):e1000438

Sahin O, Fröhlich H, Löbke C, Korf U, Burmester S, Majety M, Mattern J, Schupp I, Chaouiya C, Thieffry D, Poustka A, Wiemann S, Beissbarth T, Arlt D (2009) Modeling ERBB receptor-regulated G1/S transition to find novel targets for de novo trastuzumab resistance. BMC Syst Biol 3:1

Setoguchi R, Kinashi T, Sagara H, Hirosawa K, Takatsu K (1998) Defective degranulation and calcium mobilization of bone-marrow derived mast cells from Xid and Btk-deficient mice. Immunol Lett 64(2–3):109–118

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13(11):2498–2504

Simon M, Vanes L, Geahlen RL, Tybulewicz VL (2005) Distinct roles for the linker region tyrosines of Syk in FcepsilonRI signaling in primary mast cells. J Biol Chem 280(6):4510–4517

Thieffry D (2007) Dynamical roles of biological regulatory circuits. Brief Bioinform 8:220–225

Tůmová M, Koffer A, Simíček M, Dráberová L, Dráber P (2010) The transmembrane adaptor protein NTAL signals to mast cell cytoskeleton via the small GTPase Rho. Eur J Immunol 40(11):3235–3245

Young RM, Holowka D, Baird B (2003) A lipid raft environment enhances Lyn kinase activity by protecting the active site tyrosine from dephosphorylation. J Biol Chem 278(23):20746–20752

Zhu M, Liu Y, Koonpaew S, Granillo O, Zhang W (2004) Positive and negative regulation of FcepsilonRI-mediated signaling by the adaptor protein LAB/NTAL. J Exp Med 200(8):991–1000

Zinovyev A, Viara E, Calzone L, Barillot E (2008) BiNoM: a Cytoscape plugin for manipulating and analyzing biological networks. Bioinformatics 24(6):876–877

Calcium Channels in Fc Receptor Signaling

Tarik Attout, Andres Floto and Pierre Launay

Abstract The calcium ion (Ca^{2+}) is the main common second messenger involved in signaling transduction subsequent to immunoreceptor activation. Its rapid intracellular elevation induces multiple cellular responses, such as secretion, proliferation, mobility, and gene transcription. Intracellular levels of Ca^{2+} need to reach a specific threshold to efficiently transduce the signal to activate transcription factors through the recruitment of Ca^{2+}-binding molecules. However, since Ca^{2+} cannot be metabolized, its intracellular concentration is tightly regulated to avoid the induction of programmed cell death. This highly controlled regulation of Ca^{2+} homeostasis has recently been clarified by the uncovering of new ion channels. The regulation of these channels allows the role of Ca^{2+} in Fc receptor transduction pathways to be more precisely defined.

Contents

1 Regulation of Ca^{2+} Homeostasis in Hematopoietic Cells 96
2 Ca^{2+} Channels in Immune Cells 97
2.1 Orai1 Subunits form the CRAC Channel 98
2.2 STIM1, the Endoplasmic Reticulum Ca^{2+} Sensor 98
2.3 Other Ca^{2+} Channels 99
3 TRP Channels 99
3.1 TRPC Channels 100
3.2 TRPM2 Channels 100
3.3 TRPM4 Channels 101

T. Attout · P. Launay (✉)
Inserm U1149, Bichat Medical School, Paris, France
e-mail: pierre.launay@inserm.fr

T. Attout
e-mail: tarikattout@gmail.com

A. Floto
Cambridge Institute for Medical Research, University Cambridge, Cambridge, UK
e-mail: arf27@cam.ac.uk

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_5,

4 Fc Receptor-Mediated Ca^{2+} Signaling 101
4.1 Activating FcRs 102
4.2 Inhibitory FcRs 103
4.3 ITAMi-Mediated Ca^{2+} Mobilization 106
5 Conclusions 106
References 106

1 Regulation of Ca^{2+} Homeostasis in Hematopoietic Cells

Calcium (Ca^{2+}) is a ubiquitous second messenger in many hematopoietic cells, including myeloid and lymphoid cells (Vig and Kinet 2009). Ca^{2+} mobilization subsequent to receptor activation is involved in multiple cellular functions such as motility, phagocytosis, cytokine secretion, gene expression, and apoptosis. The aggregation of cell-surface immunoreceptors—such as the T cell receptor (TCR), the B cell receptor (BCR) or Fcγ receptors (FcγRs)—by their cognate ligands triggers a rapid increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) within a few seconds that lasts for several minutes before returning to basal levels. The main mechanism responsible of this intracellular Ca^{2+} rise has been described as store-operated calcium entry (SOCE) through the Ca^{2+} release-activated Ca^{2+} (CRAC) channel (Feske 2007; Vig and Kinet 2009). Following aggregation, immunoreceptors recruit several protein kinases and phosphatases to the receptor signaling cluster, which initiates signal transduction. The membrane-proximal localization of a particular repertoire of kinases and phosphatases confers signaling specificity on each type of membrane receptor. However, the downstream transmission of the signal from surface membranes requires the generation of one or more second messengers such as inositol-1,4,5-trisphosphate (IP_3). Among signaling molecules located close to membrane receptors and involved in the early steps of signal transduction, phospholipase C (PLC) plays a key role in the induction of Ca^{2+} mobilization. The activation of PLC family members hydrolyzes the lipid phosphatidylinositol-3,4-bisphosphate (PIP_2) into diacylglycerol (DAG) and IP_3. IP_3 freely diffuses within the cytosol to bind its specific receptor, IP_3R, triggering Ca^{2+} release from the endoplasmic reticulum (ER), the major intracellular Ca^{2+} store. However, Ca^{2+} release from intracellular organelles can only support a moderate and transient increase in intracellular Ca^{2+}, and stored Ca^{2+} release is therefore rapidly followed by a massive influx of Ca^{2+} from the extracellular space (Scharenberg et al. 2007). In resting lymphocytes, the intracellular concentration of Ca^{2+}, which is tightly controlled, is maintained at very low levels (10^{-7} M), while the extracellular Ca^{2+} concentration is 10,000 times higher (10^{-3} M). The depletion of ER Ca^{2+} thus activates store-operated Ca^{2+} channels located in the plasma membrane, leading to Ca^{2+} influx into the cell down its steep concentration gradient, and triggering a number of downstream signaling events such as the dephosphorylation (and subsequent nuclear entry) of the transcription factor NFAT in T cells by the Ca^{2+}-dependent activation of calcineurin (Dolmetsch et al. 1997, 1998).

CRAC is the best described and main SOCE channel in lymphocytes. However, other mechanisms of cytosolic Ca^{2+} modulation that do not rely on store-operated calcium signaling also exist in hematopoietic cells (Vig and Kinet 2009). These channels may offer alternative and stimulus-specific ways for Ca^{2+} mobilization in leukocytes, and the initial cue received by the immune cell could thus determine the strength, amplitude and duration of the Ca^{2+} response.

The Ca^{2+} signaling pathway, however, results from the balance between the positive and negative signals generated by multiple combinations of receptor-ligand interactions that in turn depend on the variety of membrane receptors expressed by a given immune cell (Billadeau and Leibson 2002). The Fc receptor (FcR) family presents great heterogeneity and provides a good example of immune response regulation (Winfield et al. 1977). The FcRs are composed of five main subfamilies, according their binding of specific immunoglobulin isotypes: FcαR, FcγR, FcμR, FcεR, and FcδR (Hulett and Hogarth 1994), and each further consists of different members. FcRs can be expressed in soluble form or integrated into the plasma membrane, and are found on many leukocytes including monocytes, macrophages, mast cells, basophils, neutrophils, natural killer cells, eosinophils, and B cells (Daeron 1997; Amigorena and Bonnerot 1999). The consequences of antibody aggregation on leukocyte function are directly related to the intrinsic structure of the intracellular part of the FcR and its association with the common signal-transducing γ subunit. In fact, FcRs are classified in two groups depending on their cellular effects: activating FcRs and inhibitory FcRs, and the resultant Ca^{2+} mobilization is directly correlated with the status of these receptors. The activating FcRs share the immunoreceptor tyrosine-based activation motif (ITAM) with both the BCR and TCR, and use similar transduction pathways to trigger cell responses (Daeron 1997). In contrast, certain FcRs could deliver an inhibitory signal via the immunoreceptor tyrosine-based inhibition motif (ITIM), resulting in the absence of sustained Ca^{2+} entry. In this review, we aim to describe the mechanisms of Ca^{2+} mobilization subsequent to FcR activation.

2 Ca^{2+} Channels in Immune Cells

Since the increase in cytosolic Ca^{2+} is correlated with the control of a wide variety of cellular processes, its regulation is crucial to leukocyte fate. Under resting conditions, Ca^{2+} levels differ widely depending on cellular compartment (such as the ER or mitochondria) and the external environment. To maintain its extremely low levels in the cytosol when compared to the ER or mitochondria, the cytosolic $[Ca^{2+}]_i$ is tightly regulated, and an efficient return to homeostatic levels must occur following Ca^{2+} mobilization (Robert et al. 2011). Several ion channels and transporters are located at the plasma membrane and on intracellular organelles to maintain intracellular Ca^{2+} homeostasis (Feske et al. 2012). In the following sections, we present various molecules implicated in the movement of Ca^{2+} across

the different cellular membranes in resting or activated cells, and their involvement in the regulation of Ca^{2+} homeostasis.

2.1 Orai1 Subunits form the CRAC Channel

Cellular activation following the engagement of immunoreceptors leads to the generation of IP_3, the release of Ca^{2+} from the ER, and consequently, a massive Ca^{2+} influx across the plasma membrane. This process, known as SOCE, represents the main mechanism by which the intracellular Ca^{2+} concentration increases, and involves the opening of the CRAC channels (Putney 1986). CRAC channels have been extensively characterized in hematopoietic cells, especially in T lymphocytes and mast cells. The existence of CRAC channels was originally demonstrated in 1992 by the characterization of its current (Icrac) (Hoth and Penner 1992). However, almost 15 years were needed to uncover the molecular structure underlying the Icrac current (Vig and Kinet 2007). The hallmarks of the CRAC channel are its extremely high ion selectivity for Ca^{2+} and its low conductance with a characteristic inwardly rectifying current-voltage relationship (Prakriya 2009; Feske et al. 2012). For the molecular identification of the CRAC channel, several laboratories took advantage of the whole-genome RNA interference (RNA_i) screen developed in *Drosophila* (Boutros et al. 2004). By visualizing either intracellular Ca^{2+} mobilization or the translocation of the NFAT-GFP reporter in *Drosophila* S2 cells, the knock-down of the single gene *olf186-F* was found to be crucial for SOCE and Icrac generation (Feske et al. 2006; Vig et al. 2006a, b). *FLJ14466*, the human homolog of *Drosophila olf186-F*, named *ORAI1* (or CRACM1) is a 32.7 kDa four transmembrane-domain glycoprotein with both amino and carboxyl termini located intracellularly (Feske et al. 2012). Two other CRAC channel proteins, *ORAI2 and ORAI3* (also known as CRACM2 and CRACM3), have high sequence homology to *ORAI1*. The molecular structure of the CRAC channel requires the homo-tetramerization of the *ORAI1* molecule (Prakriya et al. 2006; Vig et al. 2006a, b; Yeromin et al. 2006), but hetero-multimerization also can occur with its homologs (*ORAI2 and ORAI3*) as well as some TRPC channel subunits (Liao et al. 2008; Vig and Kinet 2009). CRAC channels are activated by the depletion of the Ca^{2+} store by the coupling of other Ca^{2+} sensitive molecules located in intracellular stores including the ER.

2.2 STIM1, the Endoplasmic Reticulum Ca^{2+} Sensor

Another key set of molecules, the stromal interaction molecules (STIMs), is involved in the regulation of Ca^{2+} mobilization, and has been extensively characterized concomitantly to Orai1. STIM1 and STIM2 are crucial for coordinating Ca^{2+} release and entry signals necessary for the maintenance of cellular Ca^{2+}

homeostasis. STIM1 senses the depletion of ER Ca^{2+} stores through its EF hand-SAM domain, and is directly coupled with *ORAI1* channels to allow *ORAI1*-mediated Ca^{2+} entry (Soboloff et al. 2012). This single transmembrane-domain protein (77 kDa) is localized essentially in the ER and diffusely distributed in resting cells. Consequent to cell activation and store depletion, the STIM1 protein oligomerizes and is redistributed as discrete puncta to create an ER-plasma membrane junction (Vig and Kinet 2009; Feske et al. 2012).

2.3 Other Ca^{2+} Channels

Although CRAC channels, composed of the assembly of *ORAI* and STIM molecules, are the best characterized Ca^{2+} channels in hematopoietic cells, several other channels may also mediate Ca^{2+} influx, including P2X receptors, voltage-gated Ca^{2+} channels, and TRP channels. The P2X receptors are a family of nonselective ion channels that are activated by extracellular ATP and allow the influx of cations, including Ca^{2+}. At least three different P2X receptors have been implicated in Ca^{2+} influx in human T lymphocytes: P2X1, P2X4 and P2X7. Their opening causes Ca^{2+} entry and the activation of downstream signaling molecules such as calcineurin, resulting in cell proliferation and IL-2 production by the autocrine production of ATP (Yip et al. 2009). The second family of channels that is still under investigation for its role in immune cells is the family of voltage-gated Ca^{2+} channels (Ca_v channels). Originally described in excitable cells such as neurons or cardiac cells, there are numerous lines of evidence demonstrating the expression of Ca_v channels in lymphocytes and particularly in T cells (Pelletier and Savignac 2013). Ca_v channels are highly Ca^{2+}-selective channels that mediate Ca^{2+} influx in response to the depolarization of excitable cells. Members of the Ca_v channel family, including $Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$, and $Ca_v1.4$, and their regulatory subunits β3 and β4, have been found to be expressed in human and mouse T cells, and several studies have reported the presence of truncated or alternatively spliced Ca_v isoforms (Feske et al. 2012). Recently, it has been suggested that voltage-gated Ca^{2+} channels might play a different role in T cell biology than CRAC channels (Badou et al. 2013). For example $Ca_v1.4$ modulates naïve T cell survival and antigen-driven immune responses (Omilusik et al. 2011).

Other Ca^{2+} channels thought to be directly involved in extracellular Ca^{2+} entry into leukocytes include those belonging to the TRP family, which were first identified in the early 2000s.

3 TRP Channels

The transient receptor potential (TRP)-related channels are a large superfamily of 30 molecules expressed in several tissues and cell types. Most of them fall under three main subfamilies: the canonical (TRPC), the melastatin-related (TRPM), and

vanilloid-receptor-related (TRPV) subfamilies. The ten remaining TRP channels are subdivided into the TRPP, TRPML, TRPN, and TRPA subfamilies (Fleig and Penner 2004; Pedersen et al. 2005). TRP channels are functionally heterogeneous despite structural similarities (Freichel et al. 2012). Most TRP channels are non-selective and permeable to several cations, including Ca^{2+} and Na^{+}, and show little voltage dependence (Feske et al. 2012; Freichel et al. 2012). In this section, we will present the role of the TRP Ca^{2+} channels that have been shown to be involved in the regulation of Ca^{2+} homeostasis in immune cells.

3.1 TRPC Channels

The seven members of the TRPC family (TRPC1-TRPC7) are nonselective cation channels and are activated by PLCγ stimulation. It has been reported by different groups that TRPC channels increase the $[Ca^{2+}]_i$ through plasma membrane receptor-coupled stimulation or through store depletion in different cell types (Venkatachalam et al. 2001; Philipp et al. 2003; Putney 2005; Vig and Kinet 2009). In particular, DAG can activate TRPC3, TRPC6, and TRPC7, and induce sustained Ca^{2+} mobilization (Venkatachalam et al. 2001; Vig and Kinet 2009). Before the identification of *ORAI1*, the contribution of TRPC channels to SOCE was for a time suggested (Feske 2007; Freichel et al. 2012), but this possibility is still under investigation.

3.2 TRPM2 Channels

TRPM2 is a nonselective cation channel that is widely expressed in mammalian cells including leukocytes (Perraud et al. 2001). TRPM2 is permeable to Ca^{2+} and activated by *ADP*-ribose. Several intracellular factors positively (Ca^{2+}, cADP-ribose, H_2O_2) or negatively (AMP) regulate this channel (Yamamoto et al. 2010; Sumoza-Toledo and Penner 2011). The activation of TRPM2 by excess ADP-ribose production associated with DNA damage and repair may contribute to apoptosis. In T cells, it has been demonstrated that TRPM2 expression is strongly upregulated after cell activation and that TRPM2 contributes to Ca^{2+} influx (Beck et al. 2006; Feske et al. 2012). Furthermore, in murine TRPM2-deficient macrophages, the cytoplasmic Ca^{2+} elevation after H_2O_2 activation is completely abolished, suggesting that the TRPM2 channel may constitute the principal Ca^{2+} pathway during early exposure to H_2O_2 (Zou et al. 2013).

3.3 TRPM4 Channels

TRPM4 is a monovalent cation channel and is not Ca^{2+}-permeable. However, TRPM4, together with the potassium (K^+) channels $K_{Ca}1.3$ and $K_v3.1$, regulates calcium influx by controlling cell membrane potential, and thus, the driving force for Ca^{2+} entry (Feske et al. 2012). TRPM4 is expressed in many cell types including leukocytes. We and others have characterized the TRPM4 protein as a Ca^{2+}-activated, nonselective (CAN) cation channel (Launay et al. 2002; Nilius et al. 2003) that is inhibited by intracellular ATP (Fleig and Penner 2004). Although the TRPM4 channel is not directly required for Ca^{2+} entry, it acts as a powerful regulator of intracellular Ca^{2+} levels after cell activation. Indeed, subsequent to an increase of the $[Ca^{2+}]_i$, the opening of TRPM4 channels allows a massive influx of Na^+ and potent membrane depolarization, which together decrease the electrical driving force for Ca^{2+} entry. Thus, TRPM4 prevents cellular Ca^{2+} overload and is therefore important for SOCE regulation by providing a negative feedback mechanism. In Jurkat T cells, the inhibition of endogenous TRPM4 by RNA_i or the overexpression of a dominant negative mutant form of TRPM4 leads to an increase in Ca^{2+} influx and enhanced IL-2 production (Launay et al. 2004). Bone-marrow-derived dendritic cells from TRPM4-deficient mice show impaired migration after bacteria-induced inflammation, but no alteration of maturation, emphasizing that the maturation and the migration of dendritic cells are independently regulated (Barbet et al. 2008). Moreover, mast cells from TRPM4-deficient mice show an increase in Ca^{2+} influx and more degranulation than wild-type mice after FcεRI activation. As expected, *TRPM4*-deficient mice develop a more severe IgE-mediated passive cutaneous anaphylactic response (Vennekens et al. 2007). Thus, ion channels that control Ca^{2+} influx are key players in the regulation of the signal transduction of receptors expressed at the surface of immune cells, including FcRs (Fig. 1).

4 Fc Receptor-Mediated Ca^{2+} Signaling

The aggregation of Fc receptors activates a battery of signaling molecules, some of which are involved in the mobilization of intracellular Ca^{2+}. However, how antibodies or immune complexes generate Ca^{2+} signals in leukocytes is still not fully understood, mainly due to the structural diversity of receptor subtypes and the different pathways engaged (Nunes and Demaurex 2010). FcRs are versatile molecules that, depending on the nature of the co-aggregated subunits, can deliver a variety of signals to the cell. They share activation motifs with BCRs and TCRs, and under appropriate conditions, can trigger cell responses using the same transduction pathways as the antigen receptors.

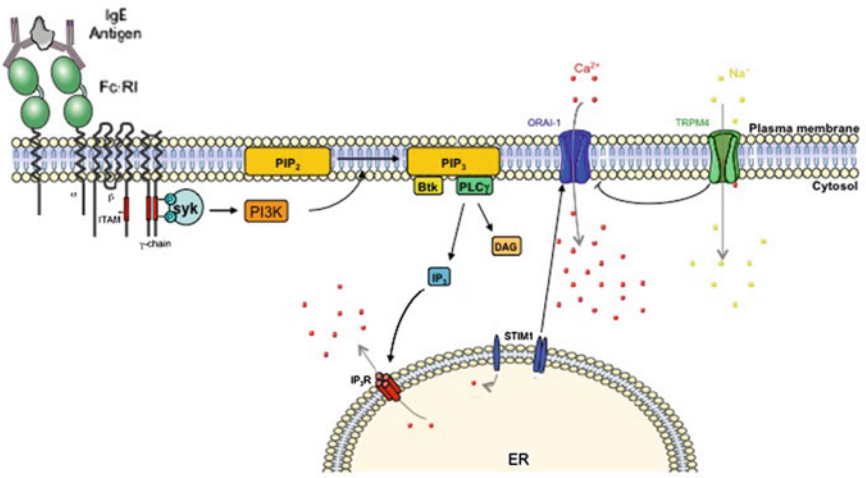


Fig. 1 *FcεRI-mediated* Ca^{2+} *mobilization*. The aggregation of FcεRI by the IgE-antigen complex triggers the liberation of Ca^{2+} stored within the endoplasmic reticulum. Subsequently, the Ca^{2+} ion dissociates from *STIM1*, which allows its relocation to the plasma membrane and the opening of the *ORAI1* channel. The increased intracellular Ca^{2+} concentration activates the TRPM4 channel, leading to massive Na^{+} entry. This potent membrane depolarization lowers the Ca^{2+} driving force, thereby reducing the Ca^{2+} influx and cellular activation

4.1 Activating FcRs

The defining feature of an activating FcR is the presence of an immunoreceptor tyrosine-based activation motif (ITAM) in its intracytoplasmic domain (Reth 1989). Most subunits associated with immunoreceptors contain this ITAM transduction motif including the TCR-associated CD3γ, CD3δ, CD3ε, and ζ chains, the BCR-associated Igα and Igβ chains, DAP12, and several virally encoded transmembrane molecules. This signaling motif has also been found to be carried directly by the FcR α chain in the case of the human FcγRIIA or by transmembrane adaptor molecules (*γ or β chain*) (Blank et al. 1989; Nimmerjahn and Ravetch 2008). The cross-linking of activating FcRs by an immune complex initiates a similar signaling pathway to that observed with the TCR or BCR, and starts by the activation/dephosphorylation of src family protein kinases. These phosphorylate both tyrosine residues of the ITAM, which is the docking site for the tandem SH2 domains of the Syk family kinases. Syk kinase phosphorylation is believed to be central to FcR signal transduction and leads to the recruitment and phosphorylation of various signaling molecules such as *phosphatidylinositide* 3-kinases (PI3K). The production of phosphatidylinositol-3,4,5-trisphosphate (PIP_3) by PI3K allows the membrane recruitment of pleckstrin homology (PH) domain-containing molecules, such as Bruton's tyrosine kinase (*Btk*) and *PLCγ* (Ravetch and Bolland 2001; Billadeau and Leibson 2002; Nimmerjahn and Ravetch 2008). As seen following

TCR activation, the aggregation of FcRs such as FcεRI leads to the phosphorylation of PLCγ, which generates IP_3. IP_3 freely diffused within the cell and increases the Ca^{2+} concentration rapidly (through store release) followed by extracellular Ca^{2+} influx triggered by the membrane association of STIM1 and Orai1 (Fig. 1). The increase in intracellular Ca^{2+} levels is often interpreted as a successful outcome of FcR activation by the immune complex. The signaling pathway described earlier is based on the activation of CRAC channels. Converging data obtained recently using cells from mice or humans in which SOCE induction fails support this idea. Indeed, NK cells purified from patients carrying the R91W single amino acid substitution in Orai1, display defective SOCE after FcγRIIIA cross-linking (Maul-Pavicic et al. 2011). Also, a defect in at least one of the molecules implicated in SOCE affects the activating capacity of FcR and subsequent cellular function. Braun et al. have demonstrated that murine STIM1-deficient macrophages present impaired FcγR-induced Ca^{2+} entry and a strong diminution of IgG2a and IgG2b-mediated phagocytosis (Braun et al. 2009). It has been further confirmed by another study on IgG-opsonized zymosan internalization and phagosomal reactive oxygen species (ROS) production in DMSO-differentiated HL-60 cells and neutrophils, which concludes that FcγR-mediated phagocytosis requires intracellular Ca^{2+} store depletion for the bacterial-internalization phase. In addition, phagosomal ROS production requires extracellular Ca^{2+} entry mediated by Orai1/STIM1 and relayed by the S100A8-A9 Ca^{2+}-binding proteins (Steinckwich et al. 2011).

However, Choi et al. have suggested an alternative pathway that is primarily responsible for Ca^{2+} mobilization in the RBL mast cell line following the binding of the IgE/antigen complex (Choi et al. 1996). The cross-linking of FcεRI activates *sphingosine kinase* (SK), which produces an alternative second messenger, sphingosine-1-phosphate (S1P), for intracellular Ca^{2+} mobilization. The FcεRI-mediated Ca^{2+} signal can be suppressed using a competitive SK inhibitor, without affecting Syk tyrosine kinase activation and the low-level production of IP_3 (Daeron 1997). Furthermore, the aggregation of FcγRI in IFN-γ-treated monocytes mobilizes a transient release of stored Ca^{2+} that involves the activation of *phospholipase D* and results in the downstream activation of SK (Floto et al. 1997; Melendez et al. 1998; Nunes and Demaurex 2010).

4.2 Inhibitory FcRs

FcRs provide a mechanism for lymphoid and myeloid cells to regulate the immune response, especially when activating and inhibitory receptors recognize similar ligands. Inhibitory receptors provide a good example of how cells regulate activating signals by simultaneously triggering an inhibitory signal, thus setting thresholds for cell activation (Ravetch and Lanier 2000). Inhibitory receptors contain an immunoreceptor tyrosine-based inhibition motif (ITIM) in their cytoplasmic domains.

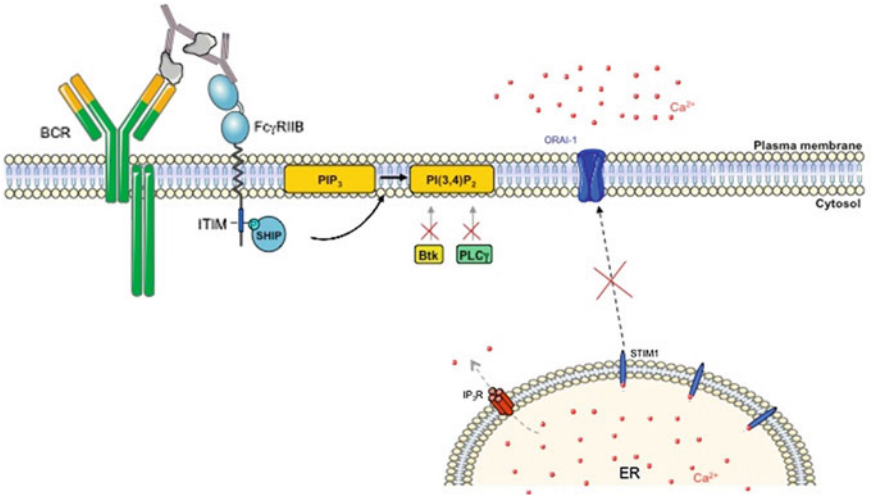


Fig. 2 *FcγRIIB reduces Ca^{2+} mobilization*. The co-aggregation of FcγRIIB and the BCR recruits and activates the phosphatase SHIP, which acts upstream of store-mediated Ca^{2+} release and subsequently modulates Ca^{2+}-dependent signal transduction

The first discovered and only known IgG Fc receptor bearing this inhibitory motif is the FcγRIIB. This receptor is highly conserved between mice and humans and has been extensively studied as an ITIM-containing receptor promoting an inhibitory signal. FcγRIIB is broadly expressed on hematopoietic cells with the exception of NK and T cells (Ravetch and Lanier 2000; Billadeau and Leibson 2002; Mackay et al. 2006; Nimmerjahn and Ravetch 2008; Nunes and Demaurex 2010). Its function is particularly well documented in B cells and its signaling pathway may be extrapolated to other leukocytes bearing ITIM-containing receptors. The simultaneous engagement of the BCR and FcγRIIB results in the phosphorylation of the tyrosine residue of the ITIM sequence by src family protein tyrosine kinases (PTKs). This leads to the recruitment of the SH2-domain-containing inositol 5-phosphatase *SHIP*. This phosphatase catalyzes the conversion of the *PI3K* product PIP_3 into PIP_2. *Btk*, and *PLCγ* are no longer recruited to the plasma membrane, thus inhibiting the signal transduction mediated by the BCR (Scharenberg et al. 2007) (Fig. 2). Choquet and colleagues were the first to demonstrate that the co-cross-linking of membrane Igs and FcγRIIB with an anti-Ig antibodies triggered only a transient Ca^{2+} release from intracellular stores, but not extracellular Ca^{2+} influx into B cells (Choquet et al. 1993). The mechanism of Ca^{2+} influx abrogation via FcγRIIB has been further deciphered by addressing the role of Btk/Tec *kinases* in the regulation of Ca^{2+} homeostasis. As *Btk* plays a key role in signal transduction in B cells, the co-cross-linking of the BCR and FcγRIIB has been shown to abrogate *Btk*-dependent intracellular Ca^{2+} mobilization (Fluckiger et al. 1998) (Fig. 2).

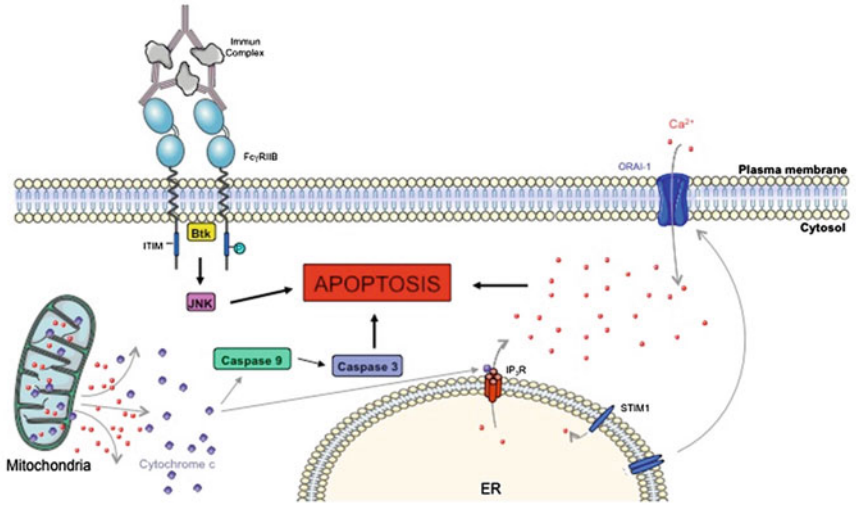


Fig. 3 *Homo-oligomerization of FcγRIIB induces apoptosis*. The oligomerization of FcγRIIB by the immune complex results in increased levels of B cell apoptosis, independently of *SHIP*. Upstream signaling involving the *Btk/JNK* pathway activates cytochrome *c* release and mitochondrial membrane depolarization. Cytochrome *c* can directly bind to the IP_3 receptors, causing sustained Ca^{2+} mobilization. The addition of Ca^{2+} leakage from disrupted mitochondria and Ca^{2+} influx into the cell by Orai1 activation would lead to Ca^{2+} overload and apoptosis

The inhibitory signal mediated by the FcγRIIB receptor is important for the regulation of immune balance. A reduced or absent inhibitory signal in B cells contributes to lowering the threshold for cell activation, and consequently induces stronger B cell activation after BCR cross-linking and accelerates the onset autoimmune disease (Mackay et al. 2006; Nimmerjahn and Ravetch 2008). In the case of systemic lupus erythematosus (SLE), human and animal studies have established a strong correlation between inhibitory FcγRIIB and disease susceptibility (Yuasa et al. 1999; Bolland and Ravetch 2000; Nakamura et al. 2000). Interestingly, SLE B cells exhibit a greater BCR-mediated Ca^{2+} elevation than B cells from normal individuals or patients with other systemic autoimmune rheumatic diseases (Liossis et al. 1996; Enyedy et al. 2001). However, the increase in Ca^{2+} release from intracellular stores in SLE patients is not correlated with a substantial increase in IP_3 production. The molecular mechanism explaining the increased Ca^{2+} mobilization in lymphocytes from SLE patients thus remains obscure, and one can only speculate that there is an upregulation or gain-of-function of *STIM1 and/or ORAI1* in these lymphocytes (Tsokos 2008). In addition to having a regulatory role on cell activation and the immune response, FcγRIIB plays an important role in the development of B cells and has emerged as a late checkpoint for humoral immunity (Nimmerjahn and Ravetch 2008). The BCR-independent aggregation (homo-oligomerization) of FcγRIIB can induce the apoptosis of B cells (Pearse et al. 1999; Nimmerjahn and Ravetch 2008) (Fig. 3).

In DT40 chicken B cells expressing the mouse FcγRIIB, the aggregation of the inhibitory receptor independently of the BCR induces the activation of both caspase 3 and 9 in association with the release of cytochrome *c* from mitochondria, leading to apoptosis (Tzeng et al. 2005). During apoptosis, cytochrome *c* released from mitochondria binds to IP_3 receptors and deregulates Ca^{2+} homeostasis, amplifying the FcγRIIB-mediated programmed cell death (Boehning et al. 2003) (Fig. 3). Indeed, the regulation of Ca^{2+} homeostasis is closely correlated with the proapoptotic signal, and the control of Ca^{2+} mobilization by *STIM1* is directly involved in B cell fate (Melamed et al. 1998; Limnander et al. 2011).

4.3 ITAMi-Mediated Ca^{2+} Mobilization

Besides the inhibitory signal mediated by ITIM, we have described an alternative immune regulatory function mediated by the ITAMs, named ITAMi (Pasquier et al. 2005). In contrast to the effects of ligand mediated co-aggregation, the binding of FcαRI by IgA in the absence of antigen induces a potent SHP-1-dependent inhibition of heterologous receptors (Blank et al. 2009). The intracellular increase in Ca^{2+} influx was also assessed in the context of ITAMi, and although, the aggregation of multiple FcεRIs by IgE/antigen alone induces potent Ca^{2+} mobilization, uncomplexed IgGs or anti-FcγRIII $F(ab')_2$ binding of FcγRIII reduces the Ca^{2+} mobilization mediated by aggregated FcεRI (Aloulou et al. 2012).

5 Conclusions

The aggregation of Fc receptors recruits and activates a cluster of protein tyrosine kinases, resulting in the production of IP_3. In immune cells, the cytoplasmic diffusion of IP_3 induces an initial release of intracellular stored Ca^{2+} followed by a much more potent influx of extracellular Ca^{2+} (through SOCE), which is regulated by the molecular interaction of *STIM1 and ORAI1*. To maintain cellular integrity and the ability of the cell to be activated subsequently, the Ca^{2+} signal returns to basal levels in part through the modulation of the membrane potential by channels such as TRPM4.

References

Aloulou M, Ben Mkaddem S, Biarnes-Pelicot M, Boussetta T, Souchet H, Rossato E, Benhamou M, Crestani B, Zhu Z, Blank U, Launay P, Monteiro RC (2012) IgG1 and IVIg induce inhibitory ITAM signaling through FcgammaRIII controlling inflammatory responses. Blood 119(13):3084–3096

Amigorena S, Bonnerot C (1999) Fc receptor signaling and trafficking: a connection for antigen processing. Immunol Rev 172:279–284

Badou A, Jha MK, Matza D, Flavell RA (2013) Emerging roles of L-type voltage-gated and other calcium channels in T lymphocytes. Front Immunol 4:243

Barbet G, Demion M, Moura IC, Serafini N, Leger T, Vrtovsnik F, Monteiro RC, Guinamard R, Kinet JP, Launay P (2008) The calcium-activated nonselective cation channel TRPM4 is essential for the migration but not the maturation of dendritic cells. Nat Immunol 9(10):1148–1156

Beck A, Kolisek M, Bagley LA, Fleig A, Penner R (2006) Nicotinic acid adenine dinucleotide phosphate and cyclic ADP-ribose regulate TRPM2 channels in T lymphocytes. FASEB J 20(7):962–964

Billadeau DD, Leibson PJ (2002) ITAMs versus ITIMs: striking a balance during cell regulation. J Clin Invest 109(2):161–168

Blank U, Launay P, Benhamou M, Monteiro RC (2009) Inhibitory ITAMs as novel regulators of immunity. Immunol Rev 232(1):59–71

Blank U, Ra C, Miller L, White K, Metzger H, Kinet JP (1989) Complete structure and expression in transfected cells of high affinity IgE receptor. Nature 337(6203):187–189

Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T, Snyder SH (2003) Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. Nat Cell Biol 5(12):1051–1061

Bolland S, Ravetch JV (2000) Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. Immunity 13(2):277–285

Boutros M, Kiger AA, Armknecht S, Kerr K, Hild M, Koch B, Haas SA, Paro R, Perrimon N (2004) Genome-wide RNAi analysis of growth and viability in Drosophila cells. Science 303(5659):832–835 Heidelberg Fly Array Consortium

Braun A, Gessner JE, Varga-Szabo D, Syed SN, Konrad S, Stegner D, Vogtle T, Schmidt RE, Nieswandt B (2009) STIM1 is essential for Fcgamma receptor activation and autoimmune inflammation. Blood 113(5):1097–1104

Choi OH, Kim JH, Kinet JP (1996) Calcium mobilization via sphingosine kinase in signalling by the Fc epsilon RI antigen receptor. Nature 380(6575):634–636

Choquet D, Partiseti M, Amigorena S, Bonnerot C, Fridman WH, Korn H (1993) Cross-linking of IgG receptors inhibits membrane immunoglobulin-stimulated calcium influx in B lymphocytes. J Cell Biol 121(2):355–363

Daeron M (1997) Fc receptor biology. Annu Rev Immunol 15:203–234

Dolmetsch RE, Lewis RS, Goodnow CC, Healy JI (1997) Differential activation of transcription factors induced by Ca2+ response amplitude and duration. Nature 386(6627):855–858

Dolmetsch RE, Xu K, Lewis RS (1998) Calcium oscillations increase the efficiency and specificity of gene expression. Nature 392(6679):933–936

Enyedy EJ, Nambiar MP, Liossis SN, Dennis G, Kammer GM, Tsokos GC (2001) Fc epsilon receptor type I gamma chain replaces the deficient T cell receptor zeta chain in T cells of patients with systemic lupus erythematosus. Arthritis Rheum 44(5):1114–1121

Feske S (2007) Calcium signalling in lymphocyte activation and disease. Nat Rev Immunol 7(9):690–702

Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A (2006) A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 441(7090):179–185

Feske S, Skolnik EY, Prakriya M (2012) Ion channels and transporters in lymphocyte function and immunity. Nat Rev Immunol 12(7):532–547

Fleig A, Penner R (2004) The TRPM ion channel subfamily: molecular, biophysical and functional features. Trends Pharmacol Sci 25(12):633–639

Floto RA, Somasundaram B, Allen JM, Mahaut-Smith MP (1997) Fcgamma receptor I activation triggers a novel Ca2+ -activated current selective for monovalent cations in the human monocytic cell line, U937. J Biol Chem 272(8):4753–4758

Fluckiger AC, Li Z, Kato RM, Wahl MI, Ochs HD, Longnecker R, Kinet JP, Witte ON, Scharenberg AM, Rawlings DJ (1998) Btk/Tec kinases regulate sustained increases in intracellular Ca2+ following B-cell receptor activation. EMBO J 17(7):1973–1985

Freichel M, Almering J, Tsvilovskyy V (2012) The role of TRP proteins in mast cells. Front Immunol 3:150

Hoth M, Penner R (1992) Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 355(6358):353–356

Hulett MD, Hogarth PM (1994) Molecular basis of Fc receptor function. Adv Immunol 57:1–127

Launay P, Cheng H, Srivatsan S, Penner R, Fleig A, Kinet JP (2004) TRPM4 regulates calcium oscillations after T cell activation. Science 306(5700):1374–1377

Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP (2002) TRPM4 is a Ca2+ -activated nonselective cation channel mediating cell membrane depolarization. Cell 109(3):397–407

Liao Y, Erxleben C, Abramowitz J, Flockerzi V, Zhu MX, Armstrong DL, Birnbaumer L (2008) Functional interactions among Orai1, TRPCs, and STIM1 suggest a STIM-regulated heteromeric Orai/TRPC model for SOCE/Icrac channels. Proc Natl Acad Sci USA 105(8):2895–2900

Limnander A, Depeille P, Freedman TS, Liou J, Leitges M, Kurosaki T, Roose JP, Weiss A (2011) STIM1, PKC-delta and RasGRP set a threshold for proapoptotic Erk signaling during B cell development. Nat Immunol 12(5):425–433

Liossis SN, Kovacs B, Dennis G, Kammer GM, Tsokos GC (1996) B cells from patients with systemic lupus erythematosus display abnormal antigen receptor-mediated early signal transduction events. J Clin Invest 98(11):2549–2557

Mackay M, Stanevsky A, Wang T, Aranow C, Li M, Koenig S, Ravetch JV, Diamond B (2006) Selective dysregulation of the FcgammaIIB receptor on memory B cells in SLE. J Exp Med 203(9):2157–2164

Maul-Pavicic A, Chiang SC, Rensing-Ehl A, Jessen B, Fauriat C, Wood SM, Sjoqvist S, Hufnagel M, Schulze I, Bass T, Schamel WW, Fuchs S, Pircher H, McCarl CA, Mikoshiba K, Schwarz K, Feske S, Bryceson YT, Ehl S (2011) ORAI1-mediated calcium influx is required for human cytotoxic lymphocyte degranulation and target cell lysis. Proc Natl Acad Sci USA 108(8):3324–3329

Melamed D, Benschop RJ, Cambier JC, Nemazee D (1998) Developmental regulation of B lymphocyte immune tolerance compartmentalizes clonal selection from receptor selection. Cell 92(2):173–182

Melendez A, Floto RA, Cameron AJ, Gillooly DJ, Harnett MM, Allen JM (1998) A molecular switch changes the signalling pathway used by the Fc gamma RI antibody receptor to mobilise calcium. Curr Biol 8(4):210–221

Nakamura A, Yuasa T, Ujike A, Ono M, Nukiwa T, Ravetch JV, Takai T (2000) Fcgamma receptor IIB-deficient mice develop Goodpasture's syndrome upon immunization with type IV collagen: a novel murine model for autoimmune glomerular basement membrane disease. J Exp Med 191(5):899–906

Nilius B, Prenen J, Droogmans G, Voets T, Vennekens R, Freichel M, Wissenbach U, Flockerzi V (2003) Voltage dependence of the Ca2+ -activated cation channel TRPM4. J Biol Chem 278(33):30813–30820

Nimmerjahn F, Ravetch JV (2008) Fcgamma receptors as regulators of immune responses. Nat Rev Immunol 8(1):34–47

Nunes P, Demaurex N (2010) The role of calcium signaling in phagocytosis. J Leukoc Biol 88(1):57–68

Omilusik K, Priatel JJ, Chen X, Wang YT, Xu H, Choi KB, Gopaul R, McIntyre-Smith A, Teh HS, Tan R, Bech-Hansen NT, Waterfield D, Fedida D, Hunt SV, Jefferies WA (2011) The Ca(v)1.4 calcium channel is a critical regulator of T cell receptor signaling and naive T cell homeostasis. Immunity 35(3):349–360

Pasquier B, Launay P, Kanamaru Y, Moura IC, Pfirsch S, Ruffie C, Henin D, Benhamou M, Pretolani M, Blank U, Monteiro RC (2005) Identification of FcalphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. Immunity 22(1):31–42

Pearse RN, Kawabe T, Bolland S, Guinamard R, Kurosaki T, Ravetch JV (1999) SHIP recruitment attenuates Fc gamma RIIB-induced B cell apoptosis. Immunity 10(6):753–760

Pedersen SF, Owsianik G, Nilius B (2005) TRP channels: an overview. Cell Calcium 38(3–4):233–252

Pelletier L, Savignac M (2013) Ca(2+) signaling in T-cell subsets with a focus on the role of cav1 channels: possible implications in therapeutics. Front Immunol 4:150

Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, Stokes AJ, Zhu Q, Bessman MJ, Penner R, Kinet JP, Scharenberg AM (2001) ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. Nature 411(6837):595–599

Philipp S, Strauss B, Hirnet D, Wissenbach U, Mery L, Flockerzi V, Hoth M (2003) TRPC3 mediates T-cell receptor-dependent calcium entry in human T-lymphocytes. J Biol Chem 278(29):26629–26638

Prakriya M (2009) The molecular physiology of CRAC channels. Immunol Rev 231(1):88–98

Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG (2006) Orai1 is an essential pore subunit of the CRAC channel. Nature 443(7108):230–233

Putney JW Jr (1986) A model for receptor-regulated calcium entry. Cell Calcium 7(1):1–12

Putney JW Jr (2005) Capacitative calcium entry: sensing the calcium stores. J Cell Biol 169(3):381–382

Ravetch JV, Bolland S (2001) IgG Fc receptors. Annu Rev Immunol 19:275–290

Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. Science 290(5489):84–89

Reth M (1989) Antigen receptor tail clue. Nature 338(6214):383–384

Robert V, Triffaux E, Savignac M, Pelletier L (2011) Calcium signalling in T-lymphocytes. Biochimie 93(12):2087–2094

Scharenberg AM, Humphries LA, Rawlings DJ (2007) Calcium signalling and cell-fate choice in B cells. Nat Rev Immunol 7(10):778–789

Soboloff J, Rothberg BS, Madesh M, Gill DL (2012) STIM proteins: dynamic calcium signal transducers. Nat Rev Mol Cell Biol 13(9):549–565

Steinckwich N, Schenten V, Melchior C, Brechard S, Tschirhart EJ (2011) An essential role of STIM1, Orai1, and S100A8-A9 proteins for Ca2+ signaling and FcgammaR-mediated phagosomal oxidative activity. J Immunol 186(4):2182–2191

Sumoza-Toledo A, Penner R (2011) TRPM2: a multifunctional ion channel for calcium signalling. J Physiol 589(Pt 7):1515–1525

Tsokos GC (2008) Calcium signaling in systemic lupus erythematosus lymphocytes and its therapeutic exploitation. Arthritis Rheum 58(5):1216–1219

Tzeng SJ, Bolland S, Inabe K, Kurosaki T, Pierce SK (2005) The B cell inhibitory Fc receptor triggers apoptosis by a novel c-Abl family kinase-dependent pathway. J Biol Chem 280(42):35247–35254

Venkatachalam K, Ma HT, Ford DL, Gill DL (2001) Expression of functional receptor-coupled TRPC3 channels in DT40 triple receptor InsP3 knockout cells. J Biol Chem 276(36):33980–33985

Vennekens R, Olausson J, Meissner M, Bloch W, Mathar I, Philipp SE, Schmitz F, Weissgerber P, Nilius B, Flockerzi V, Freichel M (2007) Increased IgE-dependent mast cell activation and anaphylactic responses in mice lacking the calcium-activated nonselective cation channel TRPM4. Nat Immunol 8(3):312–320

Vig M, Beck A, Billingsley JM, Lis A, Parvez S, Peinelt C, Koomoa DL, Soboloff J, Gill DL, Fleig A, Kinet JP, Penner R (2006a) CRACM1 multimers form the ion-selective pore of the CRAC channel. Curr Biol 16(20):2073–2079

Vig M, Kinet JP (2007) The long and arduous road to CRAC. Cell Calcium 42(2):157–162

Vig M, Kinet JP (2009) Calcium signaling in immune cells. Nat Immunol 10(1):21–27

Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP (2006b) CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry. Science 312(5777):1220–1223

Winfield JB, Lobo PI, Hamilton ME (1977) Fc receptor heterogeneity: immunofluorescent studies of B, T, and "third population" lymphocytes in human blood with rabbit IgG b4/anti-b4 complexes. J Immunol 119(5):1778–1784

Yamamoto S, Takahashi N, Mori Y (2010) Chemical physiology of oxidative stress-activated TRPM2 and TRPC5 channels. Prog Biophys Mol Biol 103(1):18–27

Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD (2006) Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. Nature 443(7108):226–229

Yip L, Woehrle T, Corriden R, Hirsh M, Chen Y, Inoue Y, Ferrari V, Insel PA, Junger WG (2009) Autocrine regulation of T-cell activation by ATP release and P2X7 receptors. FASEB J 23(6):1685–1693

Yuasa T, Kubo S, Yoshino T, Ujike A, Matsumura K, Ono M, Ravetch JV, Takai T (1999) Deletion of fcgamma receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. J Exp Med 189(1):187–194

Zou J, Ainscough JF, Yang W, Sedo A, Yu SP, Mei ZZ, Sivaprasadarao A, Beech DJ, Jiang LH (2013) A differential role of macrophage TRPM2 channels in Ca(2)(+) signaling and cell death in early responses to H(2)O(2). Am J Physiol Cell Physiol 305(1):C61–C69

Regulation of FcεRI Signaling by Lipid Phosphatases

Marcel Kuhny, Carolin N. Zorn and Michael Huber

Abstract Mast cells (MCs) are tissue-resident sentinels of hematopoietic origin that play a prominent role in allergic diseases. They express the high-affinity receptor for IgE (FcεRI), which when cross-linked by multivalent antigens triggers the release of preformed mediators, generation of arachidonic acid metabolites, and the synthesis of cytokines and chemokines. Stimulation of the FcεRI with increasing antigen concentrations follows a characteristic bell-shaped dose-responses curve. At high antigen concentrations, the so-called supra-optimal conditions, repression of FcεRI-induced responses is facilitated by activation and incorporation of negative signaling regulators. In this context, the SH2-containing inositol-5′-phosphatase, SHIP1, has been demonstrated to be of particular importance. SHIP1 with its catalytic and multiple protein interaction sites provides several layers of control for FcεRI signaling. Regulation of SHIP1 function occurs on various levels, e.g., protein expression, receptor and membrane recruitment, competition for protein–protein interaction sites, and activating modifications enhancing the phosphatase function. Apart from FcεRI-mediated signaling, SHIP1 can be activated by diverse unrelated receptor systems indicating its involvement in the regulation of antigen-dependent cellular responses by autocrine feedback mechanisms or tissue-specific and/or (patho-) physiologically determined factors. Thus, pharmacologic engagement of SHIP1 may represent a beneficial strategy for patients suffering from acute or chronic inflammation or allergies.

M. Kuhny · C. N. Zorn · M. Huber (✉)
Institute of Biochemistry and Molecular Immunology, University Clinic, RWTH Aachen University, Pauwelsstr. 30, 52074 Aachen, Germany
e-mail: mhuber@ukaachen.de

M. Kuhny
e-mail: mkuhny@ukaachen.de

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_6,

Contents

1 Introduction 112
1.1 Mast Cells as Orchestrators of the Immune System 112
1.2 FcεRI: Structure and Signaling 112
2 The Lipid Phosphatase SHIP1 Regulates FcεRI Signaling 115
2.1 Recruitment of SHIP1 to the Active FcεRI 116
2.2 MicroRNAs Control SHIP1 Expression 117
2.3 Autocrine Signals Modulate SHIP1 Activity 117
2.4 The Adaptor Function of SHIP1 Provides Another Layer of Regulation 119
2.5 Other Lipid Phosphatases 122
3 Concluding Remarks 122
References 123

1 Introduction

1.1 Mast Cells as Orchestrators of the Immune System

Mast cells (MCs) are hemopoietically derived, tissue-resident cells, which are located in tissues proximal to the external environment, i.e., skin and mucosa of the intestine and airways (Metz and Maurer 2007). One of their main functions is to act as guards, alerting the body to invasion by bacteria, parasites, and viruses, and initiating an inflammatory response (Echtenacher et al. 1996; Malaviya et al. 1996; Pennock and Grencis 2006; Wang et al. 2012). On the pathophysiological side, among others, MCs are involved as central effector cells in acute allergic disorders (Galli et al. 2008). Here, MCs recognize multivalent allergens/antigens (Ags) via IgE immunoglobulins, which are bound to high-affinity IgE receptors (FcεRI) on the surface of the MCs. Ag-induced cross-linking of the FcεRI elicits several pro-inflammatory responses, such as the release of preformed mediators (e.g., histamine, proteoglycans, and proteases, such as chymases, tryptase, granzyme B, and even active caspase-3 (Pejler et al. 2010; Pardo et al. 2007; Garcia-Faroldi et al. 2013; Zorn et al. 2013) from intracellular granules in a process called degranulation, and the de novo production and release of arachidonic acid metabolites (e.g., leukotrienes and prostaglandins) as well as cytokines and chemokines (e.g., IL-6, TNF-α, and MCP-1) (Turner and Kinet 1999). As a result, MCs play a central role in the development of type I hypersensitivity reactions (Costa et al. 1997; Feyerabend et al. 2011).

1.2 FcεRI: Structure and Signaling

The FcεRI on murine MCs and basophils consists of one α-subunit, one β-subunit, and two disulfide-bridged γ-subunits ($\alpha\beta\gamma_2$) (Blank et al. 1989). The FcεRI γ-subunit also associates with other activating Fc receptors, and therefore, is called

FcRγ (Ravetch and Kinet 1991). The α-subunit, which contains only a short cytoplasmic tail, binds to the constant Cε3 region of the IgE molecule via its second extracellular immunoglobulin-like domain (Garman et al. 1998). The β-subunit and the γ-subunits harbor immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic domains (Reth 1989). Upon receptor cross-linking the Src family kinase (SFK) Lyn phosphorylates the ITAMs, which then are able to interact with cytoplasmic proteins containing phosphotyrosine-binding SH2-domains (Turner and Kinet 1999). Lyn has been shown to pre-associate with the β-subunit of the FcεRI (Yamashita et al. 1994; Vonakis et al. 1997). For completeness, it has to be added that the FcεRI on murine MCs requires the $\alpha\beta\gamma_2$ configuration for successful surface expression (Blank et al. 1989), whereas the human FcεRI can be expressed in either $\alpha\gamma_2$ or $\alpha\beta\gamma_2$ configuration on human MCs and basophils (Garman et al. 1998). However, the human β-chain exerts two important amplifier functions. Compared to a human $\alpha\gamma_2$ FcεRI, presence of the β-chain enhances (i) stability and surface expression as well as (ii) the activation mechanisms of the FcεRI (Donnadieu et al. 2000; Dombrowicz et al. 1998). Finally, binding of IgE to the FcεRI is not a mere passive pre-sensitization step to confer Ag specificity to the MCs, but rather it induces Ag-independent signaling events actively promoting MC survival (Asai et al. 2001; Kalesnikoff et al. 2001; Yamaguchi et al. 1997).

As mentioned earlier, the IgE-bound FcεRI has to be cross-linked by multivalent Ag for relevant activation, such as release of preformed mediators, to occur. Initially, the SFK Lyn, which is constitutively bound to the β-chain of the FcεRI, phosphorylates the ITAMs of both β- and γ-chains (Yamashita et al. 1994; Vonakis et al. 1997). This enables the tandem SH2 domains of the cytoplasmic tyrosine kinase Syk to interact with the doubly-phosphorylated ITAMs of the γ-chains. This stabilizes Syk in its active conformation, and initiates amplification of several downstream signaling pathways necessary for MC activation (Costello et al. 1996; Jouvin et al. 1994; Kihara and Siraganian 1994). Intriguingly, in B-lymphocytes Syk acts as a double-specificity kinase. It phosphorylates the ITAM-tyrosines and an adjacent serine residue of the Ig-α subunit of the B cell antigen receptor (BCR) (Heizmann et al. 2010). The serine phosphorylation was shown to attenuate Ag-triggered BCR activation. For FcεRI signaling, threonine phosphorylation of FcRγ was demonstrated to be necessary for full FcRγ tyrosine phosphorylation and Syk activation (Swann et al. 1999). It is tempting to speculate that in MCs, Syk as a double-specificity kinase boosts its own activation via positive feedback regulation.

An additional pathway crucial for MC activation is mediated by the SFK Fyn, which phosphorylates the adaptor protein Gab-2, enabling its subsequent interaction with the lipid kinase phosphatidylinositol-3-kinase (PI3 K) (Parravicini et al. 2002; Gu et al. 2001). PI3 K phosphorylates its substrate, phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2), to produce phosphatidylinositol-3,4,5-trisphosphate (PIP_3) (Fig. 1), an important 2nd messenger for the regulation of different cellular activation pathways (Marone et al. 2008). Deficiencies in Fyn, Gab-2 or the p110δ isoform of PI3 K have been demonstrated to result in abrogation or severe attenuation of Ag-triggered MC degranulation as well as allergic

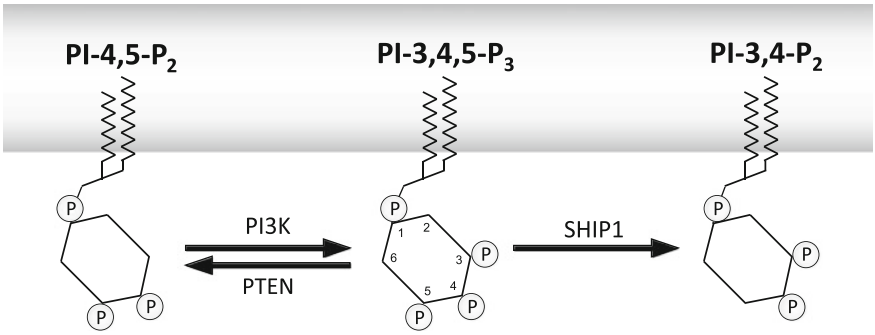


Fig. 1 *Control of the phospholipid composition by lipid kinases and phosphatases*. The phosphatidylinositol-3-kinase (PI3 K) converts PI-4,5-P_2 into the second messenger PI-3,4,5-P_3. Two prominent lipid phosphatases limit the pool of PI-3,4,5-P_3: (i) the inositol-3′-phosphatase, phosphatase and tensin homolog (PTEN), reverses the reaction catalyzed by PI3 K. (ii) the inositol-5′-phosphatase, SHIP1, limits the PI-3,4,5-P_3 pool by removal of the phosphate group at the 5′-position of the inositol ring, thereby generating PI-3,4-P_2

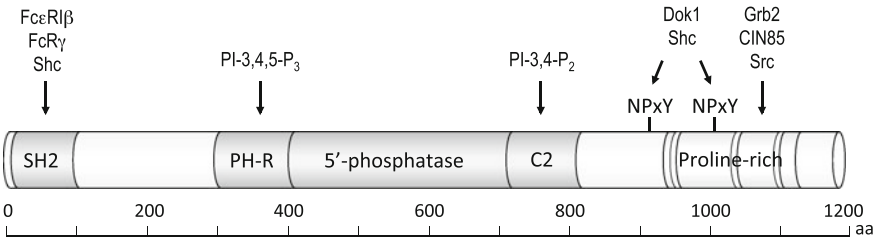


Fig. 2 *Schematic representation of the structure of SHIP1*. The central 5′-phosphatase domain of SHIP1 is N-terminally flanked by a pleckstrin homology-related (PH-R) domain that binds PI-3,4,5-P_3. Located at the C-terminal site of the phosphatase domain is a C2 domain, which enhances the catalytic activity of SHIP1 when bound to PI-3,4-P_2. Protein-protein interactions are facilitated by a Src homology 2 (SH2) domain at the very N-terminus as well as by a C-terminal proline-rich region, which also contains two NPxY motives. For details on the indicated interaction partners see main text

hypersensitivity responses (Parravicini et al. 2002; Gu et al. 2001; Ali et al. 2004). Additional support for the importance of PI3 K-mediated signaling for Ag-triggered MC activation came from the analysis of bone marrow-derived MCs (BMMCs) from mice deficient for the SH2-containing inositol-5′-phosphatase, SHIP1 (Fig. 2), which hydrolyses PIP_3 to yield PI-3,4-P_2 (Fig. 1) (Kalesnikoff et al. 2003). Contrary to p110δ-deficient BMMCs, SHIP1-deficient BMMCs are much more prone to Ag-mediated degranulation than wild-type BMMCs. They even degranulate under conditions where wild-type MCs do not, i.e., following stimulation with Steel Factor (SF; also known as stem cell factor and c-kit ligand) or IgE alone (Huber et al. 1998a, b). These studies established SHIP1 as an important gatekeeper of MC degranulation.

A further signaling pathway important for MC activation is represented by the signaling enzyme PLC-γ, which catalyzes hydrolysis of PI-4,5-P_2 concomitantly yielding the 2nd messengers inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). While IP_3 induces release of calcium ions from intracellular stores, DAG is involved in the activation of various PKC isotypes. In particular, PKC-β has been shown to be crucial for induction of FcεRI-mediated effector functions (Nechushtan et al. 2000; Fehrenbach et al. 2009). Release of calcium ions from the endoplasmic reticulum (ER) is accompanied by a conformational change in the calcium-binding protein STIM1 localized in the ER membrane, which then interacts with and leads to the opening of the store-operated calcium channel ORAI1 in the plasma membrane to allow for sustained calcium entry (Feske 2007). The activity of STIM1 has been shown previously to be indispensible for Ag-triggered degranulation, proinflammatory cytokine production, and anaphylactic responses (Baba et al. 2008).

2 The Lipid Phosphatase SHIP1 Regulates FcεRI Signaling

MC activation, for instance degranulation in response to increasing Ag concentrations follows a bell-shaped dose-response curve, showing weak responses at both low (sub-optimal) and high Ag (supra-optimal) concentrations (Huber 2013). Interestingly, the kinetics of intracellular signaling do not reflect this curve. Indeed, early overall protein tyrosine phosphorylation was slightly enhanced in supra-optimally versus optimally stimulated MCs (Kepley et al. 1998; Gimborn et al. 2005). In particular, tyrosine phosphorylation of SHIP1 increases with rising Ag levels (Gimborn et al. 2005). This suggests that SHIP1 is involved in the regulation of the descending part of the dose-response curve and, in fact, SHIP1-deficient BMMCs only show weak or no reduction of degranulation in response to supra-optimal antigen concentrations (Gimborn et al. 2005). A strong correlation between SHIP1 phosphorylation and supra-optimal Ag signaling has also been reported in human basophils (Gibbs et al. 2006).

Related to this, Lyn has been shown to phosphorylate and activate SHIP1 in MCs and degranulation studies with Lyn-deficient BMMCs have revealed that these cells do also not display the descending part of the bell-shaped degranulation curve (Hernandez-Hansen et al. 2004). Interestingly, Lyn has been shown to tyrosine phosphorylate PKC-δ and complexes of Lyn with SHIP1 and PKC-δ have been reported (Song et al. 1998; Leitges et al. 2002). Relevant to this, Leitges et al. observed augmented Ag-triggered degranulation in PKC-δ-deficient BMMCs, in particular in response to supra-optimal stimulus concentrations (Leitges et al. 2002). These data strongly suggest the existence of an inhibitory signalosome, which appears to take action when MCs are stimulated by supra-optimal Ag concentrations.

2.1 Recruitment of SHIP1 to the Active FcεRI

How would coupling between supra-optimally cross-linked FcεRIs and such an inhibitory signalosome be achieved? Intriguingly, tyrosine phosphorylation of the β-subunit of the FcεRI, which acts as amplifier of FcεRI-mediated activation signals (Dombrowicz et al. 1998; Donnadieu et al. 2000), is strongest in response to high Ag concentrations (Gimborn et al. 2005; Draberova et al. 2004; Xiao et al. 2005). In this respect, in a very thorough study, Xiao et al. demonstrated that Lyn is most active under supra-optimal conditions, resulting in pronounced tyrosine phosphorylation of the β-subunit ITAM as well as SHIP1 along with suppressed degranulation and cytokine production (Xiao et al. 2005). Importantly, there are two differences between the β-chain and FcRγ ITAMs, (i) the β-ITAM has a shorter spacer between its two YXXL sequences and (ii) the β-chain ITAM contains an additional tyrosine residue. Thus, the β-subunit may utilize Lyn to negatively regulate downstream events in response to supra-optimal Ag concentrations via its unique ITAM (Xiao et al. 2005). This puts the FcεRI β-chain and Lyn at the center of initiation of suppressive FcεRI-mediated signaling and SHIP1, by controlling the PI3 K pathway amongst others, as one of the important downstream regulators of this response. However, there are certainly more signaling proteins involved in this process, such as PKC-δ as mentioned earlier (Leitges et al. 2002).

The reports showing that SHIP1 acts as gatekeeper of MC activation in general and that SHIP1 particularly controls MC activation in response to supra-optimal Ag concentrations suggests that the amount and activity of SHIP1 should be decisive for the activity of MCs as well as basophils. In this line, fetal liver-derived MCs from SHIP1 heterozygous mice degranulated significantly stronger in response to FcεRI crosslinking than corresponding wild-type MCs (Huber et al. 2002). Moreover, for a subpopulation of highly allergic donor basophils, termed IgE(+) basophils, hyper-releasability was shown to be associated with low levels of SHIP1 protein (Vonakis et al. 2001). In this line, in an in vivo study SHIP1-deficient mice were found to show augmented anaphylactic response and systemic MC hyperplasia. Importantly, these defects were convincingly demonstrated to be due to loss of SHIP1 in MCs (Haddon et al. 2009). Though not shown for MCs or basophils, several different missense mutations in SHIP1 have been described in acute myeloid leukemia patients resulting in reduced phosphatase activity or altered interaction with receptors and/or adaptor proteins (Luo et al. 2003; Brauer et al. 2012). Assuming that such mutations would govern SHIP1 expression in MCs or basophils, enhanced activation of these cells and thus pro-allergic phenotypes would most likely be the consequence.

2.2 MicroRNAs Control SHIP1 Expression

Several lines of evidence have shown that posttranscriptional regulation is involved in the modulation of SHIP1 expression and function. In particular microRNA-155 (miR-155) was demonstrated to control stability of SHIP1 mRNA thus leading to the reduction of SHIP1 protein content in cells exhibiting increased amounts of miR-155 (O'Connell et al. 2009). SHIP1 has been demonstrated to be a primary target of miR-155, for instance in response to LPS stimulation of primary macrophages (O'Connell et al. 2009). Our preliminary data indicate induction of miR-155 in LPS- and Ag-stimulated BMMCs (data not shown), suggesting a regulatory role for miR-155 in diverse proinflammatory MC responses. Overexpression of miR-155 has been evidenced in several cancers of B-cell origin (Vigorito et al. 2013), suggesting involvement of miR-155 in the process of malignant transformation. Moreover, loss of SHIP1 expression measured in $CD34^+$ bone marrow cells of patients with high-risk myelodysplastic syndromes was associated with enhanced expression of miR-155 and miR-210 (Lee et al. 2012). Interestingly, SHIP1 was proven to be a specific target of miR-210 as well (Lee et al. 2012). In conclusion, these data show that SHIP1 expression can be controlled by different miRs (miR-155 and miR-210) and that dysregulation of these miRs can be involved in pathological developments. Thus, it is tempting to speculate that MC- and basophil-driven proinflammatory and allergic diseases might be aggravated by pathologically enhanced miR-155/-210 expression and concomitant reduction in cellular SHIP1 protein levels.

2.3 Autocrine Signals Modulate SHIP1 Activity

Stimulation of MCs via the FcεRI induces immediate release of preformed mediators from secretory lysosomes as well as production and release of arachidonic acid metabolites (Mencia-Huerta et al. 1983). As shown in Fig. 3, factors secreted in response to Ag stimulation are capable to elicit cytokine production in BMMCs that have not been sensitized with IgE. Interestingly, the released factors trigger a response that is negatively controlled by SHIP1. This experiment clearly shows that the negative regulation by SHIP1 extends beyond FcεRI signaling in MCs. Furthermore, it is a good example for an often-neglected aspect particularly of in vitro experiments: As a consequence of the Ag-triggered, rapid mediator release, autocrine stimulation of receptors that are utterly unrelated to the FcεRI take place and their downstream signaling cooperates and modifies FcεRI-derived cellular responses. For instance, MCs deficient in p110γ, the catalytic subunit of class IB PI3 K, which is not directly involved in FcεRI signaling, show impaired responses to Ag-stimulation. This defect could be attributed to an autocrine mechanism (Laffargue et al. 2002). Interestingly, such autocrine loops might have the potential to bias the signaling cascade originating from the FcεRI e.g. by

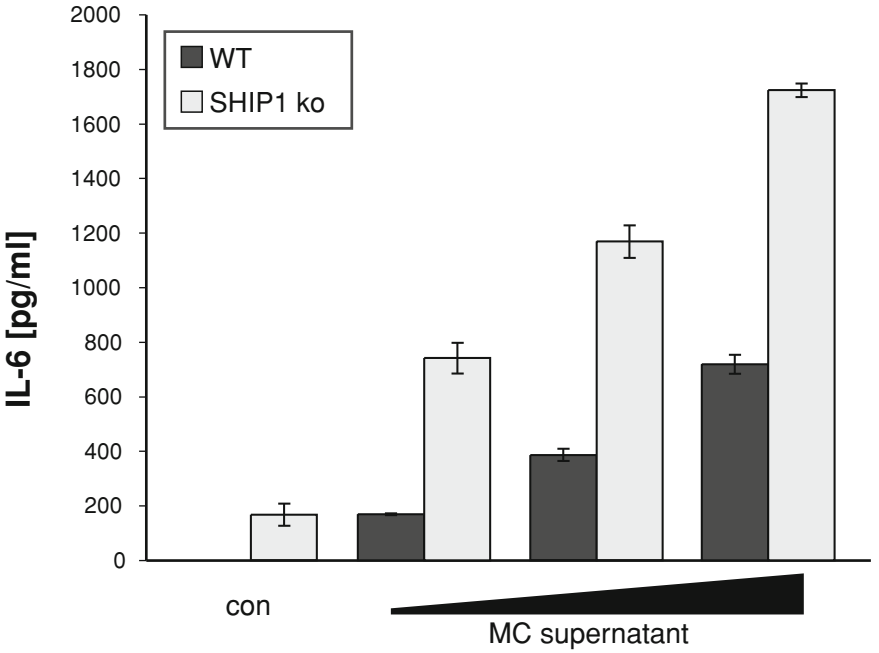


Fig. 3 *Autocrine/paracrine stimulation of mast cells can be controlled by SHIP1*. Soluble factors released from Ag-stimulated MCs elicit the production of the pro-inflammatory cytokine IL-6 in recipient MCs previously not sensitized with IgE and thus blind for Ag. SHIP1-deficient (ko) BMMCs show augmented IL-6 production compared to wild-type (WT) cells. Donor WT BMMCs were preloaded with DNP-specific IgE (SPE7) over night. 20×10^6 cells/ml were stimulated with the Ag DNP-HSA for 30 min, cells were pelleted and different amounts of supernatants were transferred to non-sensitized WT or SHIP1 ko recipient BMMCs. IL-6 production was assessed by ELISA after 4 h. Depicted are mean and SD of one representative experiment

priming the SHIP1 signalosome. The common model for the activation of SHIP1 was based on the recruitment of the molecule to the immediate proximity of the respective receptor and thus to its substrate, PIP_3. Recently, Zhang et al. found that elevated levels of 3′-5′-cyclic adenosine monophosphate (cAMP) resulted in enhanced activity of SHIP1 (Zhang et al. 2009) and subsequently, Ser440 within the phosphatase domain was identified to be the target of cAMP-dependent protein kinase A (PKA) (Zhang et al. 2010). These studies opened up the possibility that stimulation via $G_{\alpha(s)}$-dependent G protein-coupled receptors (GPCRs) might enhance the activity of SHIP1 independent of direct recruitment to the FcεRI-proximal signalosome. cAMP-generation is driven by the adenylyl cyclase, a downstream effector of $G_{\alpha(s)}$-coupled GPCRs. In this context, prostaglandin E_2 (PGE_2) has been proposed as a negative regulator of effector functions in human MCs (Kay et al. 2006). Feng et al. have outlined that the suppression of early and late responses in MCs by PGE_2 is dependent on the $G_{\alpha(s)}$-coupled receptor EP_2,

leading to increased cAMP levels, and activation of PKA which, in turn, leads to reduced MC responses by the induced cAMP early repressor, ICER (Feng et al. 2006). Though not investigated so far, PKA-activated SHIP1 may further attenuate MC functions in this setting. However, conflicting data have been published in murine BMMCs attributing PGE_2 enhancing effects on IgE-mediated effector functions (Gomi et al. 2000). In these cells, PGE_2 appears to act via EP_1 and EP_3, both coupled to $G_{\alpha(q)}$ (and $G_{\alpha(i)}$) proteins, which signal, among others, via direct activation of Btk (Bence et al. 1997). In this setting, PGE_2 signaling via EP_1 and EP_3 would reduce levels of cAMP and thus, PKA-dependent activation of SHIP1. However, attenuated activity of SHIP1 would favor stabilization of PIP_3 levels and, in turn, Btk activity (Bolland et al. 1998). Though means of evidence are still missing, the combination of these data clearly suggests that Ag-stimulated MCs make use of and regulate SHIP1 in at least two waves, a direct one controlled by FcεRI-organized signalosomes and autocrine ones regulated by various receptor types, such as GPCRs (Fig. 4). Though data on SHIP1 function downstream of GPCRs are scarce, with respect to Ag-triggered cytokine secretion, SHIP1 has been demonstrated to control NFκB and $p38^{MAPK}$ activation (Kalesnikoff et al. 2002), both well known to be crucial for gene transcription as well as mRNA stability (Ronkina et al. 2010; Saccani et al. 2002).

2.4 The Adaptor Function of SHIP1 Provides Another Layer of Regulation

How SHIP1 controls Ag-triggered MC activation/degranulation, in particular to supra-optimal Ag concentrations, is not entirely clear. Because of its structure, SHIP1 incorporates catalytic as well as adaptor functions (Fig. 2) (Rohrschneider et al. 2000). Even on the basis of its catalytic activity two mechanisms could be responsible. First, by hydrolyzing PIP_3, SHIP1 would suppress various PIP_3-dependent molecules/pathways, one or more of them critically involved in Ca^{2+} mobilization and degranulation, such as the tyrosine kinase Btk (Bolland et al. 1998). Second, by hydrolyzing PIP_3, PI-3,4-P_2 is generated (Fig. 1), which is known to specifically interact with PH-domains of certain signaling proteins, e.g., the adaptor proteins Bam32/DAPP1, TAPP1, and TAPP2 (Marshall et al. 2002). Indeed, Bam32-deficiency results in augmented Ca^{2+} mobilization and degranulation in response to supra-optimal Ag concentrations, suggesting that it represents an effector of the SHIP1 signalosome (Hou et al. 2010). Intriguingly, compared to wild-type BMMCs Bam32-deficient BMMCs show reduced Lyn and SHIP1 phosphorylation in response to Ag, indicating the presence of so far uncharacterized feedback mechanisms (Hou et al. 2010).

Concerning SHIP1's adaptor or scaffolding function, increasing numbers of interaction partners of SHIP1 have been and continue to be identified (Fig. 2). SHIP1 contains an N-terminal SH2-domain, a centrally located 5′-phosphatase

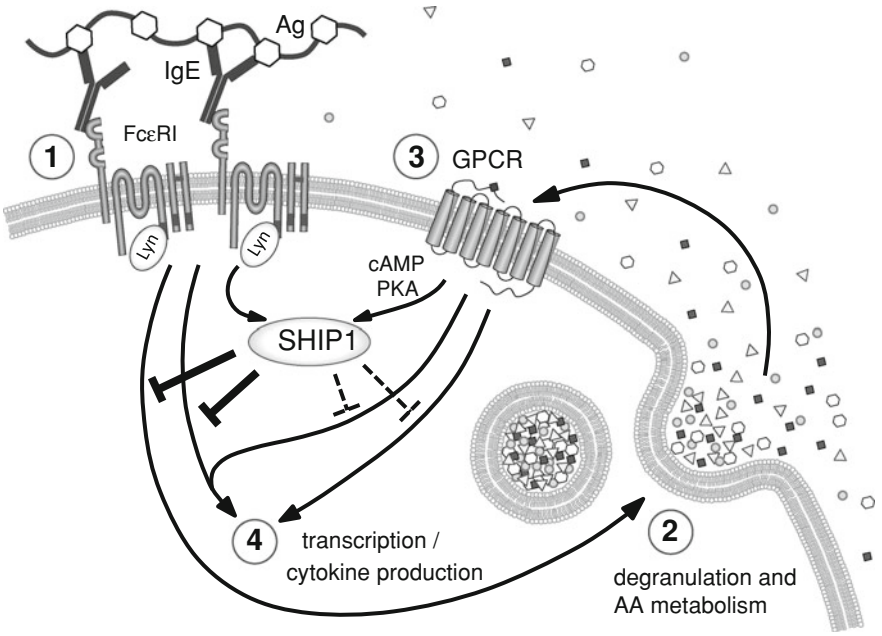


Fig. 4 *SHIP1 as central negative regulator of MC effector functions*. (1) Cross-linking of the FcεRI by multivalent Ag leads to the initiation of multiple signaling cascades. The SFK Lyn is crucial for FcεRI tyrosine phosphorylation within the ITAMs (*black squares*) and thus controls early activation. At supra-optimal Ag concentrations SHIP1 gets recruited to the receptor and activated by Lyn. Lyn and SHIP1 contribute to the down-regulation of MC signaling. (2) The early response of the MCs is triggered by FcεRI signaling, comprising the release of preformed mediators from secretory lysosomes, but also the generation and release of arachidonic acid (AA) metabolites. (3) As of now unidentified factors act back on MCs in an autocrine fashion. These factors utilize, amongst others, G protein-coupled receptors (GPCRs). Depending on the type of GPCR, activation of the adenylyl cyclase will lead to the generation of cAMP and subsequent activation of the cAMP-dependent protein kinase A (PKA), which, in turn can activate SHIP1. Thus, active SHIP1 can negatively regulate the signaling not only of the respective GPCR but, most likely, also of the FcεRI. (4) The late response of MCs comprises the de novo synthesis and release of mediators such as cytokines and chemokines. The signals governing the specific cellular response integrate the initial signals originating from the Ag-triggered FcεRI plus the autocrine feedback loops initiated by the early response. This scheme omits any stimulation that originates from the environment, such as components of the extracellular matrix or microbial products

domain, and a C-terminus containing several proline-rich sequences as well as two NPxY motifs (Rohrschneider et al. 2000). The SH2-domain is able to bind to the phosphorylated ITAMs of the FcεRI β-chain and FcRγ (Osborne et al. 1996; Kimura et al. 1997). Three of the proline-rich motifs in SHIP1's C-terminus show good consensus for binding to SH3-domains of other proteins and several have been experimentally verified, including Grb2, CIN85, and Src kinase (Wisniewski et al. 1999; Kalesnikoff et al. 2003; Buchse et al. 2011). This suggests that Lyn via

its SH3-domain can also interact with SHIP1 in the context of the inhibitory signalosome. Recently, a pleckstrin homology-related domain has been identified positioned N-terminal of the 5′-phosphatase domain of SHIP1, which mediates membrane localization of SHIP1 by binding to PIP_3 (Ming-Lum et al. 2012 6436).

Finally, proteins containing the so-called phosphotyrosine-binding (PTB)-domains, such as the adaptor proteins Shc and p62Dok1, have been found to bind to SHIP1's C-terminal NPxY motifs upon their phosphorylation (Lamkin et al. 1997; Ott et al. 2002; Tamir et al. 2000). Since Shc contains both an N-terminal PTB-domain as well as a C-terminal SH2-domain, it was initially thought that Shc recruited SHIP1 to the FcεRI. However, analysis of SHIP1-deficient BMMCs suggests a different binding order since FcεRI-mediated Shc tyrosine phosphorylation is dependent on SHIP1 expression (Huber et al. 1998a). Thus, upon tyrosine phosphorylation by Lyn, Shc might "tear" SHIP1 away from the receptor thus limiting its suppressive capability. Shc, however, has also been proposed as a linker between SHIP1 and PKC-δ, binding SHIP1 via its PTB-domain and PKC-δ through its SH2-domain (Leitges et al. 2002). Shc is expressed as three isoforms (p46shc, p52shc, and p66shc) with p46shc and p52shc being expressed ubiquitously and p66shc showing a more restricted expression pattern, whereas p46shc and p52shc are involved in Ras-MAPK activation in the context of epidermal growth factor stimulation; p66shc appears to act rather inhibitory on this signaling pathway (Migliaccio et al. 1997). p66shc is also expressed in MCs, and the study of FcεRI signaling in p66shc-deficient BMMCs has revealed that p66shc is limiting Ag-triggered degranulation and pro-inflammatory cytokine secretion (Ulivieri et al. 2011). Furthermore, p66shc was demonstrated to promote SHIP1 recruitment to the trans-membrane adaptor LAT offering a molecular mechanism for p66shc's negative regulatory function (Roget et al. 2008; Ulivieri et al. 2011).

The adaptor protein p62Dok1, a well-known interaction partner of the GTPase-activating protein RasGAP, has also been shown to bind to SHIP1. It inhibits p21Ras and hence, is a negative regulator of the canonical MAPK pathway (Erk1/2) (Ott et al. 2002; Tamir et al. 2000). Conversely, Erk1/2 might positively regulate FcεRI-mediated MC degranulation via two different pathways. Pecht et al. have reported that Erk1 is part of a feed-forward loop positively controlling Syk activity upon FcεRI triggering (Xu et al. 1999). Pharmacologic inhibition of the Erk kinase MEK suppressed Ag-induced MC degranulation (Xu et al. 1999). This effect was recently corroborated with novel MEK inhibitors with higher selectivity (Marschall et al. 2012). Another mechanism by which Erk1/2 could positively regulate MC degranulation is suggested by data from Pozo-Guisado et al. They demonstrated that Erk1/2 phosphorylate STIM1, an important calcium sensor in the membrane of the endoplasmic reticulum, and thereby positively modulate store-operated calcium entry, which is mandatory for degranulation to occur (Baba et al. 2008; Pozo-Guisado et al. 2010). Since the p62Dok1-SHIP1 interaction depends on SHIP1 tyrosine phosphorylation (Tamir et al. 2000) and this tyrosine phosphorylation is enhanced upon supra-optimal FcεRI triggering (Gimborn et al. 2005), Erk1/2 activity could be reduced, contributing to the lack of degranulation under such conditions. Thus, by combining catalytic as well as adaptor functions SHIP1 has the

potential to contribute to the regulation of MC activation under supra-optimal Ag conditions in several ways.

2.5 Other Lipid Phosphatases

Two additional prominent PIP_3 phosphatases are expressed in MCs that participate in regulation of the PI3 K pathway, namely the 5′-phosphatase SHIP2 and the 3′-phosphatase PTEN. SHIP2 was knocked-down by shRNA in BMMCs and this was shown to result in stronger FcεRI-induced degranulation at every Ag concentration tested (Leung and Bolland 2007). However, the effect was most pronounced at optimal Ag concentrations and only marginal at supra-optimal Ag concentrations. PTEN, a prominent tumor suppressor, was also knocked-down by an shRNA approach in human MCs and cells with reduced PTEN expression were shown to react with stronger Ca^{2+} mobilization as well as degranulation in response to FcεRI crosslinking (Furumoto et al. 2006). Unfortunately, titration of crosslinking Ag was not extended to the supra-optimal range and thus, at present no statement can be made on PTEN's role in repressing degranulation at supra-optimal Ag concentrations. This analysis did proof, however, that PTEN (and not SHIP1 nor SHIP2) is crucially involved in the "homeostatic" control of PIP_3 levels in non-stimulated MCs (Furumoto et al. 2006). In conclusion, these results suggest that SHIP1, in particular, is involved in repressing supra-optimal Ag-induced degranulation of MCs. Also of importance, the markedly augmented activation events of Ag-triggered SHIP1-deficient BMMCs were measurable in the presence of normal levels of SHIP2 and PTEN, again indicating the importance of SHIP1 as the gatekeeper of MC activation.

3 Concluding Remarks

SHIP1 has been identified as a central inhibitory signaling protein in MCs as well as basophils, two important allergic effector cells. Thus, addressing SHIP1 pharmacologically could benefit patients with allergic diseases. In this line, small-molecule SHIP1 activators have been identified, which stimulated SHIP1 activity in intact MCs, thereby suppressing PI3 K-mediated signaling events and effector functions. Moreover, these compounds were protective in mouse models of acute and passive cutaneous anaphylaxis (Ong et al. 2007; Stenton et al. 2013). In addition, the knowledge that SHIP1 is involved in the suppression of FcεRI-mediated effector functions, such as degranulation and cytokine production/secretion (Gimborn et al. 2005; Fehrenbach et al. 2009), combined with the information on SHIP1's participation in inhibitory signalosomes in response to supra-optimal Ag concentrations, suggests that the identification of novel SHIP1-interacting proteins will reveal so far unknown regulators/suppressors of MC

activation. In conclusion, the analysis of mast cell signaling triggered by supraoptimal crosslinking is expected to hold great potential for identifying novel targets for pharmacologic therapeutic intervention to benefit patients with acute and chronic allergic diseases.

References

Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C et al (2004) Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. Nature 431(7011):1007–1011

Asai K, Kitaura J, Kawakami Y, Yamagata N, Tsai M, Carbone DP et al (2001) Regulation of mast cell survival by IgE. Immunity 14(6):791–800

Baba Y, Nishida K, Fujii Y, Hirano T, Hikida M, Kurosaki T (2008) Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses. Nat Immunol 9(1):81–88

Bence K, Ma W, Kozasa T, Huang XY (1997) Direct stimulation of Bruton's tyrosine kinase by G(q)-protein alpha-subunit. Nature 389(6648):296–299

Blank U, Ra C, Miller L, White K, Metzger H, Kinet JP (1989) Complete structure and expression in transfected cells of high affinity IgE receptor. Nature 337(6203):187–189

Bolland S, Pearse RN, Kurosaki T, Ravetch JV (1998) SHIP modulates immune receptor responses by regulating membrane association of Btk. Immunity 8(4):509–516

Brauer H, Strauss J, Wegner W, Muller-Tidow C, Horstmann M, Jucker M (2012) Leukemia-associated mutations in SHIP1 inhibit its enzymatic activity, interaction with the GM-CSF receptor and Grb2, and its ability to inactivate PI3 K/AKT signaling. Cell Sig 24(11):2095–2101

Buchse T, Horras N, Lenfert E, Krystal G, Korbel S, Schumann M et al (2011) CIN85 interacting proteins in B cells-specific role for SHIP-1. Mol Cell Proteomics 10(10):M110 006239

Costa JJ, Weller PF, Galli SJ (1997) The cells of the allergic response: mast cells, basophils, and eosinophils. JAMA 278(22):1815–1822

Costello PS, Turner M, Walters AE, Cunningham CN, Bauer PH, Downward J et al (1996) Critical role for the tyrosine kinase Syk in signalling through the high affinity IgE receptor of mast cells. Oncogene 13(12):2595–2605

Dombrowicz D, Lin S, Flamand V, Brini AT, Koller BH, Kinet JP (1998) Allergy-associated FcRbeta is a molecular amplifier of IgE- and IgG-mediated in vivo responses. Immunity 8(4):517–529

Donnadieu E, Jouvin MH, Kinet JP (2000) A second amplifier function for the allergy-associated Fc(epsilon)RI-beta subunit. Immunity 12(5):515–523

Draberova L, Lebduska P, Halova II, Tolar P, Stokrova J, Tolarova H et al (2004) Signaling assemblies formed in mast cells activated via Fcepsilon receptor I dimers. Eur J Immunol 34(8):2209–2219

Echtenacher B, Mannel DN, Hultner L (1996) Critical protective role of mast cells in a model of acute septic peritonitis [see comments]. Nature 381(6577):75–77

Fehrenbach K, Lessmann E, Zorn CN, Kuhny M, Grochowy G, Krystal G et al (2009) Steel factor enhances supraoptimal antigen-induced IL-6 production from mast cells via activation of protein kinase C-beta. J Immunol 182(12):7897–7905

Feng C, Beller EM, Bagga S, Boyce JA (2006) Human mast cells express multiple EP receptors for prostaglandin E2 that differentially modulate activation responses. Blood 107(8):3243–3250

Feske S (2007) Calcium signalling in lymphocyte activation and disease. Nat Rev Immunol 7(9):690–702

Feyerabend TB, Weiser A, Tietz A, Stassen M, Harris N, Kopf M et al (2011) Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmunity. Immunity 35(5):832–844

Furumoto Y, Brooks S, Olivera A, Takagi Y, Miyagishi M, Taira K et al (2006) Cutting Edge: lentiviral short hairpin RNA silencing of PTEN in human mast cells reveals constitutive signals that promote cytokine secretion and cell survival. J Immunol 176(9):5167–5171

Galli SJ, Tsai M, Piliponsky AM (2008) The development of allergic inflammation. Nature 454(7203):445–454

Garcia-Faroldi G, Melo FR, Rönnberg E, Grujic M, Pejler G (2013) Active caspase-3 is stored within secretory compartments of viable mast cells. J Immunol 191(3):1445–1452

Garman SC, Kinet JP, Jardetzky TS (1998) Crystal structure of the human high-affinity IgE receptor. Cell 95(7):951–961

Gibbs BF, Rathling A, Zillikens D, Huber M, Haas H (2006) Initial FcepsilonRI-mediated signal strength plays a key role in regulating basophil signaling and deactivation. J Allergy Clin Immunol 118(5):1060–1067

Gimborn K, Lessmann E, Kuppig S, Krystal G, Huber M (2005) SHIP down-regulates FcepsilonR1-induced degranulation at supraoptimal IgE or antigen levels. J Immunol 174(1):507–516

Gomi K, Zhu FG, Marshall JS (2000) Prostaglandin E2 selectively enhances the IgE-mediated production of IL-6 and granulocyte-macrophage colony-stimulating factor by mast cells through an EP1/EP3-dependent mechanism. J Immunol 165(11):6545–6552

Gu H, Saito K, Klaman LD, Shen J, Fleming T, Wang Y et al (2001) Essential role for Gab2 in the allergic response. Nature 412(6843):186–190

Haddon DJ, Antignano F, Hughes MR, Blanchet MR, Zbytnuik L, Krystal G et al (2009) SHIP1 is a repressor of mast cell hyperplasia, cytokine production, and allergic inflammation in vivo. J Immunol 183(1):228–236

Heizmann B, Reth M, Infantino S (2010) Syk is a dual-specificity kinase that self-regulates the signal output from the B-cell antigen receptor. Proc Natl Acad Sci USA 107(43):18563–18568

Hernandez-Hansen V, Smith AJ, Surviladze Z, Chigaev A, Mazel T, Kalesnikoff J et al (2004) Dysregulated FcepsilonRI signaling and altered Fyn and SHIP activities in Lyn-deficient mast cells. J Immunol 173(1):100–112

Hou S, Pauls SD, Liu P, Marshall AJ (2010) The PH domain adaptor protein Bam32/DAPP1 functions in mast cells to restrain FcvarepsilonRI-induced calcium flux and granule release. Mol Immunol 48(1–3):89–97

Huber M (2013) Activation/Inhibition of mast cells by supra-optimal antigen concentrations. Cell Commun Sig 11(1):7

Huber M, Helgason CD, Damen JE, Liu L, Humphries RK, Krystal G (1998a) The Src Homology 2-Containing Inositol Phosphatase (Ship) Is the Gatekeeper of Mast Cell Degranulation. Proc Natl Acad Sci USA 95(19):11330–11335

Huber M, Helgason CD, Scheid MP, Duronio V, Humphries RK, Krystal G (1998b) Targeted disruption of SHIP leads to Steel factor-induced degranulation of mast cells. EMBO J 17(24):7311–7319

Huber M, Kalesnikoff J, Reth M, Krystal G (2002) The role of SHIP in mast cell degranulation and IgE-induced mast cell survival. Immunol Lett 82(1–2):17–21

Jouvin MH, Adamczewski M, Numerof R, Letourneur O, Valle A, Kinet JP (1994) Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. J Biol Chem 269(8):5918–5925

Kalesnikoff J, Baur N, Leitges M, Hughes MR, Damen JE, Huber M et al (2002) SHIP negatively regulates IgE + antigen-induced IL-6 production in mast cells by inhibiting NF-kappa B activity. J Immunol 168(9):4737–4746

Kalesnikoff J, Huber M, Lam V, Damen JE, Zhang J, Siraganian RP et al (2001) Monomeric IgE Stimulates Signaling Pathways in Mast Cells that Lead to Cytokine Production and Cell Survival. Immunity 14(6):801–811

Kalesnikoff J, Sly LM, Hughes MR, Buchse T, Rauh MJ, Cao LP et al (2003) The role of SHIP in cytokine-induced signaling. Rev Physiol Biochem Pharmacol 149:87–103

Kay LJ, Yeo WW, Peachell PT (2006) Prostaglandin E2 activates EP2 receptors to inhibit human lung mast cell degranulation. Br J Pharmacol 147(7):707–713

Kepley CL, Wilson BS, Oliver JM (1998) Identification of the Fc epsilonRI-activated tyrosine kinases Lyn, Syk, and Zap-70 in human basophils. J Allergy Clin Immunol 102(2):304–315

Kihara H, Siraganian RP (1994) Src homology 2 domains of Syk and Lyn bind to tyrosine-phosphorylated subunits of the high affinity IgE receptor. J Biol Chem 269(35):22427–22432

Kimura T, Sakamoto H, Appella E, Siraganian RP (1997) The negative signaling molecule SH2 domain-containing inositol-polyphosphate 5-phosphatase (SHIP) binds to the tyrosine-phosphorylated beta subunit of the high affinity IgE receptor. J Biol Chem 272(21):13991–13996

Laffargue M, Calvez R, Finan P, Trifilieff A, Barbier M, Altruda F et al (2002) Phosphoinositide 3-kinase gamma is an essential amplifier of mast cell function. Immunity 16(3):441–451

Lamkin TD, Walk SF, Liu L, Damen JE, Krystal G, Ravichandran KS (1997) Shc interaction with Src homology 2 domain containing inositol phosphatase (SHIP) in vivo requires the Shc-phosphotyrosine binding domain and two specific phosphotyrosines on SHIP. J Biol Chem 272(16):10396–10401

Lee DW, Futami M, Carroll M, Feng Y, Wang Z, Fernandez M et al (2012) Loss of SHIP-1 protein expression in high-risk myelodysplastic syndromes is associated with miR-210 and miR-155. Oncogene 31(37):4085–4094

Leitges M, Gimborn K, Elis W, Kalesnikoff J, Hughes MR, Krystal G et al (2002) Protein kinase C-delta is a negative regulator of antigen-induced mast cell degranulation. Mol Cell Biol 22(12):3970–3980

Leung WH, Bolland S (2007) The inositol 5'-phosphatase SHIP-2 negatively regulates IgE-induced mast cell degranulation and cytokine production. J Immunol 179(1):95–102

Luo JM, Yoshida H, Komura S, Ohishi N, Pan L, Shigeno K et al (2003) Possible dominant-negative mutation of the SHIP gene in acute myeloid leukemia. Leukemia 17(1):1–8

Malaviya R, Ikeda T, Ross E, Abraham SN (1996) Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha [see comments]. Nature 381(6577):77–80

Marone R, Cmiljanovic V, Giese B, Wymann MP (2008) Targeting phosphoinositide 3-kinase: moving towards therapy. Biochim Biophys Acta 1784(1):159–185

Marschall JS, Wilhelm T, Schuh W, Huber M (2012) MEK/Erk-based negative feedback mechanism involved in control of Steel Factor-triggered production of Kruppel-like factor 2 in mast cells. Cell Sig 24(4):879–888

Marshall AJ, Krahn AK, Ma K, Duronio V, Hou S (2002) TAPP1 and TAPP2 are targets of phosphatidylinositol 3-kinase signaling in B cells: sustained plasma membrane recruitment triggered by the B-cell antigen receptor. Mol Cell Biol 22(15):5479–5491

Mencia-Huerta JM, Razin E, Ringel EW, Corey EJ, Hoover D, Austen KF et al (1983) Immunologic and ionophore-induced generation of leukotriene B4 from mouse bone marrow-derived mast cells. J Immunol 130(4):1885–1890

Metz M, Maurer M (2007) Mast cells–key effector cells in immune responses. Trends Immunol 28(5):234–241

Migliaccio E, Mele S, Salcini AE, Pelicci G, Lai KM, Superti-Furga G et al (1997) Opposite effects of the p52shc/p46shc and p66shc splicing isoforms on the EGF receptor-MAP kinase-fos signalling pathway. EMBO J 16(4):706–716

Ming-Lum A, Shojania S, So E, McCarrell E, Shaw E, Vu D et al (2012) A pleckstrin homology-related domain in SHIP1 mediates membrane localization during Fcgamma receptor-induced phagocytosis. FASEB J 26(8):3163–3177

Nechushtan H, Leitges M, Cohen C, Kay G, Razin E (2000) Inhibition of degranulation and interleukin-6 production in mast cells derived from mice deficient in protein kinase Cbeta. Blood 95(5):1752–1757

O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D (2009) Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Natl Acad Sci USA 106(17):7113–7118

Ong CJ, Ming-Lum A, Nodwell M, Ghanipour A, Yang L, Williams DE et al (2007) Small-molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. Blood 110(6):1942–1949

Osborne MA, Zenner G, Lubinus M, Zhang X, Songyang Z, Cantley LC et al (1996) The inositol 5'-phosphatase SHIP binds to immunoreceptor signaling motifs and responds to high affinity IgE receptor aggregation. J Biol Chem 271(46):29271–29278

Ott VL, Tamir I, Niki M, Pandolfi PP, Cambier JC (2002) Downstream of kinase, p62(dok), is a mediator of Fc gamma IIB inhibition of Fc epsilon RI signaling. J Immunol 168(9):4430–4439

Pardo J, Wallich R, Ebnet K, Iden S, Zentgraf H, Martin P et al (2007) Granzyme B is expressed in mouse mast cells in vivo and in vitro and causes delayed cell death independent of perforin. Cell Death Differ 14(10):1768–1779

Parravicini V, Gadina M, Kovarova M, Odom S, Gonzalez-Espinosa C, Furumoto Y et al (2002) Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation. Nat Immunol 3(8):741–748

Pejler G, Ronnberg E, Waern I, Wernersson S (2010) Mast cell proteases: multifaceted regulators of inflammatory disease. Blood 115(24):4981–4990

Pennock JL, Grencis RK (2006) The mast cell and gut nematodes: damage and defence. Chem Immunol Allergy 90:128–140

Pozo-Guisado E, Campbell DG, Deak M, Alvarez-Barrientos A, Morrice NA, Alvarez IS et al (2010) Phosphorylation of STIM1 at ERK1/2 target sites modulates store-operated calcium entry. J Cell Sci 123(Pt 18):3084–3093

Ravetch JV, Kinet JP (1991) Fc receptors. Annu Rev Immunol 9:457–492

Reth M (1989) Antigen receptor tail clue [letter]. Nature 338(6214):383–384

Roget K, Malissen M, Malbec O, Malissen B, Daeron M (2008) Non-T cell activation linker promotes mast cell survival by dampening the recruitment of SHIP1 by linker for activation of T cells. J Immunol 180(6):3689–3698

Rohrschneider LR, Fuller JF, Wolf I, Liu Y, Lucas DM (2000) Structure, function, and biology of SHIP proteins. Genes Dev 14(5):505–520

Ronkina N, Menon MB, Schwermann J, Tiedje C, Hitti E, Kotlyarov A et al (2010) MAPKAP kinases MK2 and MK3 in inflammation: complex regulation of TNF biosynthesis via expression and phosphorylation of tristetraprolin. Biochem Pharmacol 80(12):1915–1920

Saccani S, Pantano S, Natoli G (2002) p38-Dependent marking of inflammatory genes for increased NF-kappa B recruitment. Nat Immunol 3(1):69–75

Song JS, Swann PG, Szallasi Z, Blank U, Blumberg PM, Rivera J (1998) Tyrosine phosphorylation-dependent and -independent associations of protein kinase C-delta with Src family kinases in the RBL-2H3 mast cell line: regulation of Src family kinase activity by protein kinase C-delta. Oncogene 16(26):3357–3368

Stenton GR, Mackenzie LF, Tam P, Cross JL, Harwig C, Raymond J et al (2013) Characterization of AQX-1125, a small-molecule SHIP1 activator: Part 2. Efficacy studies in allergic and pulmonary inflammation models in vivo. Br J Pharmacol 168(6):1519–1529

Swann PG, Odom S, Zhou YJ, Szallasi Z, Blumberg PM, Draber P et al (1999) Requirement for a negative charge at threonine 60 of the FcRgamma for complete activation of Syk. J Biol Chem 274(33):23068–23077

Tamir I, Stolpa JC, Helgason CD, Nakamura K, Bruhns P, Daeron M et al (2000) The RasGAP-binding protein p62dok is a mediator of inhibitory FcgammaRIIB signals in B cells. Immunity 12(3):347–358

Turner H, Kinet JP (1999) Signalling through the high-affinity IgE receptor FceR1. Nature 402:B24–B30

Ulivieri C, Fanigliulo D, Masi G, Savino MT, Gamberucci A, Pelicci PG et al (2011) p66Shc is a negative regulator of FcepsilonRI-dependent signaling in mast cells. J Immunol 186(9): 5095–5106

Vigorito E, Kohlhaas S, Lu D, Leyland R (2013) miR-155: an ancient regulator of the immune system. Immunol Rev 253(1):146–157

Vonakis BM, Chen H, Haleem-Smith H, Metzger H (1997) The unique domain as the site on Lyn kinase for its constitutive association with the high affinity receptor for IgE. J Biol Chem 272(38):24072–24080

Vonakis BM, Gibbons S Jr, Sora R, Langdon JM, MacDonald SM (2001) Src homology 2 domain-containing inositol 5' phosphatase is negatively associated with histamine release to human recombinant histamine-releasing factor in human basophils. J Allergy Clin Immunol 108(5):822–831

Wang Z, Lai Y, Bernard JJ, Macleod DT, Cogen AL, Moss B et al (2012) Skin mast cells protect mice against vaccinia virus by triggering mast cell receptor S1PR2 and releasing antimicrobial peptides. J Immunol 188(1):345–357

Wisniewski D, Strife A, Swendeman S, Erdjument-Bromage H, Geromanos S, Kavanaugh WM et al (1999) A novel SH2-containing phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase (SHIP2) is constitutively tyrosine phosphorylated and associated with src homologous and collagen gene (SHC) in chronic myelogenous leukemia progenitor cells. Blood 93(8): 2707–2720

Xiao W, Nishimoto H, Hong H, Kitaura J, Nunomura S, Maeda-Yamamoto M et al (2005) Positive and negative regulation of mast cell activation by Lyn via the FcepsilonRI. J Immunol 175(10):6885–6892

Xu R, Seger R, Pecht I (1999) Cutting edge: extracellular signal-regulated kinase activates syk: a new potential feedback regulation of Fc epsilon receptor signaling. J Immunol 163(3):1110–1114

Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I et al (1997) IgE enhances mouse mast cell Fc(epsilon)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. J Exp Med 185(4):663–672

Yamashita T, Mao SY, Metzger H (1994) Aggregation of the high-affinity IgE receptor and enhanced activity of p53/56lyn protein-tyrosine kinase. Proc Natl Acad Sci USA 91(23):11251–11255

Zhang J, Ravichandran KS, Garrison JC (2010) A key role for the phosphorylation of Ser440 by the cyclic AMP-dependent protein kinase in regulating the activity of the Src homology 2 domain-containing Inositol 5′-phosphatase (SHIP1). J Biol Chem 285(45):34839–34849

Zhang J, Walk SF, Ravichandran KS, Garrison JC (2009) Regulation of the Src homology 2 domain-containing inositol 5′-phosphatase (SHIP1) by the cyclic AMP-dependent protein kinase. J Biol Chem 284(30):20070–20078

Zorn CN, Pardo J, Martin P, Kuhny M, Simon MM, Huber M (2013) Secretory lysosomes of mouse mast cells store and exocytose active caspase-3 in a strictly granzyme B-dependent manner. Eur J Immunol 43(12):3209–3218

Part III
FcR Biology

Fc Receptors as Adaptive Immunoreceptors

Marc Daëron

Abstract Most biological activities of antibodies depend on their ability to engage Receptors for the Fc portion of immunoglobulins (FcRs) on a variety of cell types. As FcRs can trigger positive and negative signals, as these signals control several biological activities in individual cells, as FcRs are expressed by many cells of hematopoietic origin, mostly of the myeloid lineage, as these cells express various combinations of FcRs, and as FcR-expressing cells have different functional repertoires, antibodies can exert a wide spectrum of biological activities. Like B and T Cell Receptors (BCRs and TCRs), FcRs are *bona fide* immunoreceptors. Unlike BCRs and TCRs, however, FcRs are immunoreceptors with an adaptive specificity for antigen, with an adaptive affinity for antibodies, with an adaptive structure and with an adaptive signaling. They induce adaptive biological responses that depend on their tissue distribution and on FcR-expressing cells that are selected locally by antibodies. They critically determine health and disease. They are thus exquisitely adaptive therapeutic tools.

Contents

1 Introduction: Antibodies, for the Best and for the Worst 132
2 FcRs, Immunoreceptors of the Third Type 133
3 FcRs, Immunoreceptors with an Adaptive Specificity for Antigen 134
4 FcRs, Immunoreceptors with an Adaptive Affinity for Antibodies 135
4.1 High-Affinity and Low-Affinity FcRs 136
4.2 Modulation of the Binding Avidity/Affinity with Which Antibodies Bind to FcRs 137
4.3 Affinity Determines the Specificity of FcRs for Immunoglobulins 138

M. Daëron (✉)
Institut Pasteur, Paris, France
e-mail: daeron@pasteur.fr

M. Daëron
Inserm, U1104, Centre d'Immunologie de Marseille-Luminy, Marseille, France

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_7,

5 FcRs, Immunoreceptors with an Adaptive Structure 139
5.1 FcRs as Building Blocks 139
5.2 FcRs as Superstructures 140
6 FcRs, Immunoreceptors with an Adaptive Signaling 141
6.1 ITAM-Containing FcRs Generate Both Activation and Inhibition Signals 142
6.2 ITIM-Containing FcRs Generate Inhibition Signals Only 143
6.3 The Modulation of Signaling by Hetero-Aggregation of FcRs 143
7 FcRs, Immunoreceptors that Induce Adaptive Biological Responses 145
7.1 Biological Responses Induced by Antibodies Depend on the Tissue Distribution of FcRs 145
7.2 Biological Responses Induced by Antibodies Depend on FcR-Expressing Cells that are Selected by Antibodies 147
7.3 Biological Responses Induced by Antibodies Depend on Populations of FcR-Expressing Cells 147
8 FcRs as Adaptive Immunoreceptors in Health and Disease 148
8.1 FcR-Dependent Induction and Inhibition of Immune Responses by Antibodies 149
8.2 FcR-Dependent Prevention and Enhancement of Viral Infection 150
8.3 FcR-Dependent Prevention and Enhancement of Bacterial Infection 150
8.4 FcR-Dependent Induction and Inhibition of Allergic Reactions 151
8.5 FcR-Dependent Induction and Inhibition of Autoimmunity 152
8.6 FcR-Dependent Inhibition and Enhancement of Tumor Growth 152
9 Conclusion: FcRs as Adaptive Therapeutic Tools 153
References 156

1 Introduction: Antibodies, for the Best and for the Worst

In 1888, 8 years after Louis Pasteur showed that chickens can be protected from avian cholera by the inoculation of an attenuated culture of the germs responsible for this disease (Pasteur 1880), Jules Héricourt and Charles Richet found that protective immunity can be transferred to naïve dogs by the serum of dogs immunized with staphylococci (Héricourt and Richet 1888). In 1890, Emil von Behring and Shibasaburō Kitasato found that naïve rabbits can be protected from a lethal dose of diphtheria or tetanus toxin if injected with the serum of rabbits immunized with this toxin (Behring and Kitasato 1890), and in 1901, von Behring was awarded the very first Nobel prize in Medicine or Physiology "for his work on serotherapy."[1] The same year, while on board the yacht of Prince Albert the 1st of Monaco, Charles Richet and Paul Portier discovered anaphylaxis when immunizing dogs with minute amounts of toxins from sea anemones. They published their provocative finding in 1902 (Richet and Portier 1902). Five years later, Richet showed that anaphylactic hypersensitivity can be transferred to naïve dogs by the serum of immunized dogs (Richet 1907). Richet was awarded the 1913 Nobel prize in Medicine or Physiology "for his work on anaphylaxis."[2] It was therefore the same scientist who demonstrated that immune serum can either

[1] http://www.nobelprize.org/nobel_prizes/medicine/laureates/1901/behring-bio.html.

[2] http://www.nobelprize.org/nobel_prizes/medicine/laureates/1913/richet-bio.html.

protect or kill recipients, when challenged with deadly pathogens or harmless doses of toxins, respectively.

One century later, we know that antibodies are responsible for these effects of immune serum, and that antibodies are immunoglobulins present in milligrams per milliliter in serum. Antibodies are the most abundant effector molecules of adaptive immune responses for the best and for the worst. Antibodies, indeed, protect from infectious diseases, they account for the long-term protection conferred by vaccines and they are increasingly used for passive immunotherapy. Antibodies are also responsible for diseases including allergies, hemolytic anemia of the newborn and several autoimmune diseases. How can antibodies exert both protective and pathogenic effects? One reason is that, by themselves, antibodies exert no biological effects.

Antibodies specifically bind to antigens and thereby generate immune complexes, but binding itself does nothing or very little to antigen. It was indeed found recently that both the neutralization of viruses and the neutralization of bacterial toxins, which have long been paradigmatic examples of biological properties of antibodies due to the masking of specific sites on antigens, require more than binding. They require the Fc portion of antibodies and depend on receptors for antibodies (Joller et al. 2010; Mallery et al. 2010). For antibodies to affect antigens, they indeed need not only to bind to antigen epitopes through their Fab portions, but also to interact through their Fc portion with effector systems. These include soluble molecules such as components of the enzymatic cascade of Complement, and cells that express receptors for the Fc portion of antibodies (FcRs).

As FcRs can trigger positive and negative signals, as these signals control a variety of biological activities in a given cell, as FcRs are expressed by cells of many types, as these cells express various combinations of FcRs, and as FcR-expressing cells have different functional repertoires, antibodies can exert a wide spectrum of biological activities. Understanding how antibodies work is not only an exciting endeavor to comprehend the complexity of immune responses, it is also a requirement for whom aims at developing new vaccines or therapeutic antibodies.

I will argue that FcRs are unique immunoreceptors with no predetermined specificity, structure, signaling or biological properties. Actually, FcRs are not functional until they are engaged by immune complexes on cell membranes. Then and there, they can build up a multiplicity of superstructures capable of triggering a wide functional repertoire of adaptive responses to the multitude of antigenic stimuli.

2 FcRs, Immunoreceptors of the Third Type

The term “immunoreceptor” was coined following impassioned discussions at a meeting held in Kecskemét, Hungary, in September 1994, to designate receptors involved in antigen recognition and possessing intracellular tyrosine-based

activation motifs. The term "Immunoreceptor" was rapidly adopted and used to designate not only receptors containing such *Immunoreceptor Tyrosine-based Activation Motifs* (ITAMs) (Reth 1989; Cambier 1995), but also receptors containing *Immunoreceptor Tyrosine-based Inhibition Motifs* (ITIMs) that were soon described (Daëron et al. 1995a). "Immunoreceptor" is a loose term. Its use became even looser when ITIMs were found in innumerable molecules with no evident link with immunity (Daëron et al. 2008).

Several ITAMs and one ITIM having been identified in their intracytoplasmic domains, FcRs were promoted immunoreceptors, just like antigen receptors expressed by B cells (BCRs) and antigen receptors expressed by T cells (TCRs). The word forged the concept. FcRs "recognize" neither native antigens as BCRs do, nor the association of antigen-derived peptides and Major Histocompatibility Complex molecules expressed by antigen-presenting cells as TCRs do. FcRs, however, "recognize" antigen-antibody complexes. Immune complexes are the third form under which any given antigen can interact with and deliver signals to cells of the immune system. FcRs are immunoreceptors of the third type.

Unlike BCRs and TCRs, whose expression is restricted to B and T lymphocytes, respectively, FcRs are widely expressed, including by most cells of the myeloid lineage. Unlike lymphocytes, myeloid cells need neither to proliferate nor to differentiate in order to be functional; they can perform a variety of biological processes; they are abundant in the blood stream and ubiquitous. Myeloid cells are the effectors of innate immunity. They are equipped with a variety of pattern-recognition receptors for molecules that are widely shared by micro-organisms, but they have no antigen receptors. Their FcRs, however, enable them to interact specifically with antigens. When binding to FcRs, antibodies indeed endow these cells with *bona fide* antigen receptors. Through FcRs, antibodies enroll myeloid cells in adaptive immune responses. As a consequence, adaptive immunity uses the same effector cells as innate immunity.

3 FcRs, Immunoreceptors with an Adaptive Specificity for Antigen

Unlike BCRs and TCRs, which contain built-in antigen-specific subunits, FcRs have no antigen-recognition structures. They have therefore no predetermined antigen specificity. They can, however, adopt any antigen specificity. Specificity is provided by antibodies that bind to FcRs (Fig. 1). The fact that antigen specificity is dissociated from receptors enables FcR-expressing cells to act on any antigen the adaptive immune system is confronted to and responds to by the production of antibodies. Although devoid of antigen-recognition capability, FcRs can therefore provide myeloid cells with the whole cognitive repertoire of B cells.

The specificity of individual BCRs and TCRs results from one combination of variable gene segments among the many possible combinations that form the B

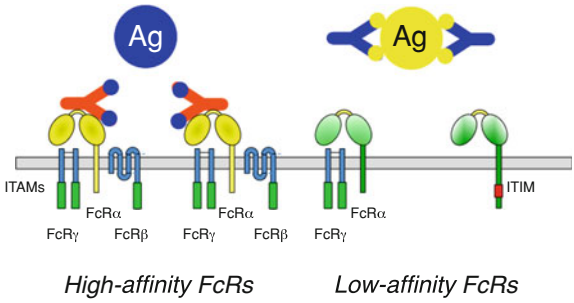


Fig. 1 *Differential binding of antibodies and antigen to high-affinity and to low-affinity FcRs*. Antibodies bind first to high-affinity FcRs, then antigen binds to receptor-bound antibodies (*left*). Antigen binds first to antibodies and form immune complexes that can then bind to low-affinity FcRs (*right*). The order of events is different but the result is the same: FcRs are aggregated

and T cell potential repertoires. The specificity of individual FcRs is that of the subset of antibodies of the B cell actual repertoire, which were synthesized by B cell-derived plasma cells and which happened to bind to FcRs. The specificity of BCRs and TCRs is selected a priori from a potential combinatorial diversity of gene segments, whereas that of FcRs is selected a posteriori from an actual diversity of proteins.

Unlike B and T cells which express BCRs or TCRs of one specificity only, FcR-expressing cells can carry a multiplicity of antibodies of different specificities. Antibody responses are indeed polyclonal. They generate antibodies against several antigens, and antibodies against one antigen can be directed to several epitopes. As antibodies bind to FcRs irrespectively of the specificity of their Fab portions, and as cells express large numbers of FcRs—from a few thousands to one million per cell—every FcR-expressing cell can respond simultaneously to a multitude of antigens.

4 FcRs, Immunoreceptors with an Adaptive Affinity for Antibodies

Antibodies bind to FcRs with a variable affinity. The binding of antibodies to FcRs is reversible and it obeys the mass action law (Kulczycki and Metzger 1974):

$$[\mathrm{Ab}][\mathrm{FcR}] \underset{k_d}{\overset{k_a}{\rightleftharpoons}} [\mathrm{Ab}-\mathrm{FcR}]$$

The affinity of FcRs is characterized by an affinity constant (K_a) which is the quotient of an association constant (k_a) divided by a dissociation constant (k_d). The affinity constant is a characteristic of the FcR.

The binding of antibodies to FcRs depends on the K_a of receptors, but also on the relative concentrations of ligands, i.e, the concentration of antibodies in the environment and the density of receptors on the cell surface. It also depends on the glycosylation of antibodies. Finally, the affinity of FcRs determines their specificity for immunoglobulin classes and subclasses.

4.1 High-Affinity and Low-Affinity FcRs

High-affinity FcRs can bind monomeric immunoglobulins in the absence of antigen, i.e., not as immune complexes (Fig. 1). A proportion of high-affinity FcRs may therefore be occupied in vivo. Antibodies however may dissociate from high-affinity FcRs, which makes receptors available for binding other antibodies. The dissociation constant of high-affinity FcRs therefore critically determines their availability for other antibodies present in the environment. This can be exemplified by the murine high-affinity receptor for IgG2 FcγRIV (K_a for IgG2a $\pm$ 3 $\times$ 10^7 M^{-1}). We found that this receptor is also a low-affinity receptor for IgE ($Ka \pm 5 \times 10^5$ M^{-1}), and we wondered whether this property is biologically meaningful. One indeed expects FcγRIV to be saturated by IgG2 in vivo, and thus unavailable for IgE. We found that the half life of IgG2 on FcγRIV is of a few minutes only at 37 °C, and that IgE immune complexes can perfectly replace IgG2 on FcγRIV in the presence of normal serum (Mancardi et al. 2008). On the contrary, the extremely high affinity constant ($Ka \pm 10^9 - 10^{10}$ M^{-1}) of FcεRI, the high-affinity receptors for IgE expressed by mast cells and basophils in mice and humans, is due to an extremely low dissociation constant (Kulczycki and Metzger 1974). As a consequence, IgE antibodies remain bound to FcεRI for extended periods of time, in spite of their extremely low plasma concentration.

The affinity of low-affinity FcRs (Bruhns et al. 2009) is too low for enabling them to bind monomeric antibodies. They can however bind antibodies as multivalent immune complexes with a high avidity (Fig. 1). As a consequence, in the absence of antigen, low-affinity FcRs (e.g., FcγRs) remain free in spite of the high concentration of circulating immunoglobulins, (e.g., IgG). They are therefore available for immune complexes whenever these come close enough. Immune complexes bind to low-affinity FcR-expressing cells because antibodies are concentrated on multivalent antigens in immune complexes and because many antibodies can bind at the same time to many FcRs on the same cell membrane. Once bound to FcRs, immune complexes remain on low-affinity FcR-expressing cells because antibodies that dissociate from individual FcRs are rapidly replaced by other antibodies present in the complex. Antibodies that dissociate from FcRs and remain bound to antigen can also engage FcRs that were not previously engaged. The interactions of immune complexes with low-affinity FcRs are therefore at the same time labile and stable; they are highly dynamic.

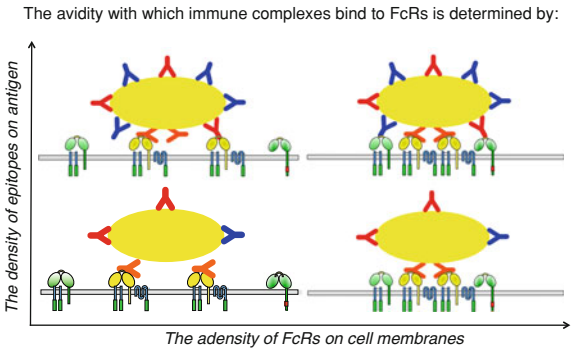


Fig. 2 *Modulation of the binding avidity with which immune complexes bind to FcRs*. The binding avidity varies with the density of epitopes on antigen and with the density of FcRs on cell membranes

4.2 Modulation of the Binding Avidity/Affinity with Which Antibodies Bind to FcRs

When low-affinity FcRs interact with immune complexes, the concentration and the composition of immune complexes therefore determine the avidity with which these bind to receptors. Indeed, what matters for binding is the local concentration of Fc portions. This concentration depends on the density of epitopes that are present on the antigen molecule and that are recognized by antibodies involved in the immune complex. Antibodies of the same specificity can therefore bind with different avidities to the same FcRs when in complex with antigens that have a high density of specific epitopes and when in complex with antigens that have a low density of the same epitopes (Fig. 2).

An interesting situation is the interaction with FcRs of antibodies against cell surface antigens. The possibility that target cells may express FcRs is rarely considered. Antibodies are therefore viewed as binding in *trans*, to target cell antigens via their Fab portions and to effector cell FcRs via their Fc portion. If target cells express FcRs, antibodies can bind in *cis*, i.e., to target antigens via their Fab portions and to FcRs via their Fc portion on the same cell (Fig. 3). Binding to antigens located on the same membrane as FcRs markedly enhances the local concentration of antibodies as they are concentrated in a plane rather than dispersed in a volume. Binding to FcRs is also enhanced by the local concentration, i.e., the density, of specific epitopes on the cell membrane. Such an Fc-FcR *cis* binding was demonstrated to trigger mast cell activation using alloantibodies against MHC class I antigens expressed by the same cells (Daëron et al. 1975; Daëron and Voisin 1978). It was extensively used to inhibit B cell activation using anti-immunoglobulin IgG antibodies (Phillips and Parker 1983). One can expect *cis* binding to apply for many monoclonal antibodies used for passive immunotherapy.

The affinity with which immunoglobulins bind to FcRs further depends on the glycosylation of their Fc portion (Arnold et al. 2007). Thus, each heavy chain of IgG1 contains a single covalently attached biantennary N-glycan at the highly

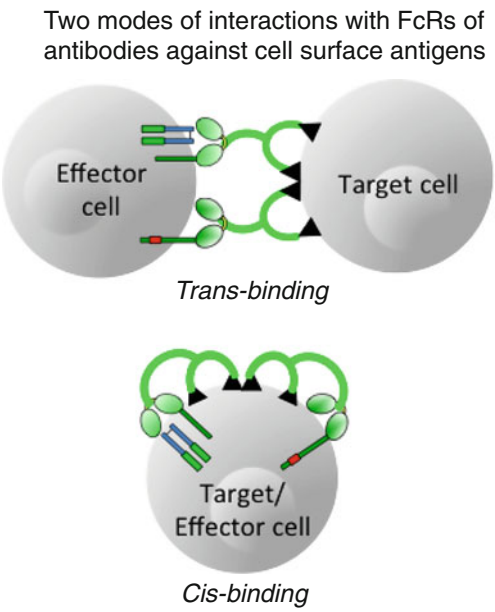


Fig. 3 *Trans- and cis-interactions of antibodies to cell surface antigens with FcR-expressing cells.* Antibodies against antigens expressed by cell membranes can engage FcRs expressed by neighboring cells (trans-binding) or FcRs expressed on the membrane of the same cell (cis-binding)

conserved N_{297} residue in its CH_2 domain. Point mutations of this glycosylation site abrogate the ability of IgG antibodies to bind to FcγRs. If engineered with such a mutation (e.g., $N_{297}Q$), aglycosylated antibodies therefore no longer engage FcγRs and they can be used as blocking-only molecules (Veri et al. 2007). Noticeably, N_{297} mutations do not affect the binding of IgG to neonatal FcRs (FcRn), which protect IgG from degradation.

4.3 *Affinity Determines the Specificity of FcRs for Immunoglobulins*

The *Ka* of the various mouse and human FcRs spans over at least five Logs. High-affinity FcRs, defined operationally by their ability to bind monomeric immunoglobulins, have a *Ka* ranging from 10^7 to 10^{10} M^{-1}. Low-affinity FcRs, defined as being unable to bind monomeric immunoglobulins but as being able to bind immune complexes, have a *Ka* ranging from 10^5 to 10^7 M^{-1} (Bruhns et al. 2009).

Most FcRs do not appear to be specific for one class or subclass of immunoglobulins. Mouse FcγRIIB and FcγRIIIA bind mouse IgG1, IgG2a and IgG2b. Likewise, human FcγRIIA, FcγRIIB, and FcγRIIIA bind all four subclasses of human IgG. However, the affinity of these three receptors is much lower for IgG2 and, to a lower extent, for IgG4 than for IgG1 and IgG3. Noticeably, human FcγRIIB has a lower affinity than any other FcγR for all four subclasses of human IgG (Bruhns et al. 2009). pIgR can bind both dimeric IgA and pentameric IgM

(Bakos et al. 1991). FcγRIV are both high-affinity receptors for mouse IgG2a and IgG2b and low-affinity receptors for mouse IgE (Mancardi et al. 2008). Likewise, the murine low-affinity receptors for mouse IgG FcγRIIB and FcγRIIIA were found to bind also mouse IgE (Takizawa et al. 1992). Whereas the affinity of these receptors for mouse IgG1, IgG2a and IgG2b is between 3×10^5 and 3×10^6 M^{-1}, their affinity for mouse IgE is in the order of 2×10^4 M^{-1} only (Mancardi et al. 2008). This is an extremely low affinity, at the limit of nonspecificity.

This poses the question of the specificity of FcRs. As discussed above, FcRs display a gradient of affinities for the various isotypes of immunoglobulins. This gradient is extremely wide, and it seems continuous. The specificity of FcRs for immunoglobulin classes and subclasses therefore appears more quantitative than qualitative, and what determines the specificity of FcRs is a large enough difference of affinities for different immunoglobulins.

5 FcRs, Immunoreceptors with an Adaptive Structure

The structure of FcRs was determined and refined by biochemical, genetic and proteomic approaches, as they became available. Altogether, these studies provided solid grounds for elaborating widely used 2D- and 3D-models of FcRs (Garman et al. 1998; Maxwell et al. 1999; Sondermann et al. 1999; Ding et al. 2003). As such, however, these models represent FcRs as they are when they are not functional. They show building blocks. Except FcRn, which bind IgG intracellularly in acidified vacuoles (Rodewald and Kraehenbuhl 1984), functional FcRs are receptors engaged on cell membranes by immune complexes. They are superstructures made with these building blocks. FcR engagement indeed associates various numbers of FcRs in various combinations to generate superstructures of various compositions.

5.1 FcRs as Building Blocks

Classically, FcRs have a structure similar to that of BCRs and TCRs. The vast majority of them are made of 2–3 noncovalently associated subunits: a ligand-binding subunit (FcRα) that has an affinity for various classes and subclasses of immunoglobulins, and one or two ITAM-containing signaling subunits (FcRγ and FcRβ) shared by multi-subunit FcRs (Fig. 1).

FcRα altogether form a family of polypeptides with 2–5 extracellular immunoglobulin-binding domains that have a secondary structure typical of Immunoglobulin Superfamily (IgSF) molecules, a hydrophobic transmembrane domain and a nonstructured intracytoplasmic domain of variable length. FcRα with different extracellular domains are the core structure of receptors for IgA (FcαR), IgG (FcγR and FcRn) and IgE (FcεR) (Hulett and Hogarth 1994). Binding involves the

hinge between the two juxta-membrane extracellular domains of FcRs and the penultimate constant domain of immunoglobulin heavy chains. FcRn are unique MHC class I-like molecules that bind the Fc portion of IgG with a high affinity (Burmeister et al. 1994).

FcRγ is a widely expressed homodimer made of two disulfide bond-linked polypeptides highly conserved in mice and humans (Orloff et al. 1990). It is shared by all activating multi-chain FcRs. FcRβ is a 4-transmembrane domain polypeptide that associates with multi-chain FcRs expressed in mast cells and basophils (Kinet et al. 1988). Multi-chain FcRs must associate with at least one specific subunit in order to be expressed. Most need FcRγ (Lobell et al. 1993; Takai et al. 1994; Letourneur et al. 1995). The expression of these receptors therefore depends on the tissue distribution of FcRγ, and FcRγ-deficient mice have no activating FcR (Takai et al. 1994). Mouse FcεRI, but not human FcεRI, also need to associate with FcRβ (Kinet 1999). As FcRβ is expressed by mast cells and basophils only in both species, the expression of FcεRI is restricted to these cells in mice (Kinet et al. 1988), but not in humans (Gounni et al. 1994, 2001; Joseph et al. 1997). FcRn do not associate with FcRγ or with another ITAM-containing subunit, but with β2 microblobulin and this association is mandatory for FcRn to be expressed (Israel et al. 1995).

Few FcRs are single-chain receptors. FcγRIIA and FcγRIIC (in humans) and FcγRIIB (in mice and humans) have two extracellular domains that bind IgG with a low affinity. Human FcγRIIB and FcγRIIC have the same extracellular domains. FcγRIIA and FcγRIIC have the same intracytoplasmic domain (Ravetch and Kinet 1991). FcγRIIA and FcγRIIC contain one ITAM whereas FcγRIIB contain one ITIM. FcγRIIA and FcγRIIC are the only ITAM-containing single-chain FcRs. FcγRIIA were however reported to form homodimers constitutively on cell membranes without delivering activation signals in the absence of ligand (Powell et al. 2006). FcγRIIIB are glycosyl-phosphatidylinositol-anchored single-chain FcRs unique to humans. They bind human IgG with a low affinity. FcRs for polymeric immunoglobulins (pIgR) have 5 extracellular domains that bind dimeric IgA and pentameric IgM. They are not known to associate with ITAM-containing subunits.

Other FcRs that do not associate with FcRγ or FcRβ, do not belong to the IgSF but to the C-type lectin superfamily. These FcRs bind IgE (FcεRII) (Conrad 1990). Binding, however, does not involve the interaction of sugar residues, which are abundant in the Fc portion of IgE, with the extracellular lectin domain of FcεRII. The affinity of FcεRII for IgE is relatively low. However, FcεRII are expressed as homotrimers which can bind IgE immune complexes with a high avidity (Kilmon et al. 2004). They are not known to associate with signaling subunits.

5.2 FcRs as Superstructures

Like BCRs and TCRs, FcRs are aggregated by plurivalent ligands. Unlike BCRs and TCRs, however, FcRs form hetero-aggregates. Because BCRs and TCRs with a single specificity are expressed on B and T cells, they can form homo-aggregates

only, when engaged by naked antigen or peptide-MCH complexes, respectively. FcRs can also form homo-aggregates when engaged by immune complexes made with monoclonal antibodies of a single specificity as they are commonly used in the laboratory. Engaging FcRs under these conditions has been instrumental for establishing the binding parameters of FcRs and for elucidating the mechanisms of signal transduction used by FcRs. This experimental situation, however, is an artifact.

Immune responses are indeed not only polyclonal, but also pluri-isotypic. No immunization procedure induces a single class of antibodies. Depending on the concentration of antigen, depending on the adjuvant, depending on the route of immunization, some isotypes of antibodies can be favored, but in all cases, the overall isotypic pattern of the response is not markedly altered. Even when immunizing mice with protocols that promote strong Th1 responses, such as one injection of a high concentration of proteins in Freund's complete adjuvant followed by repeated injections of the same high dose of antigen in incomplete Freund's adjuvant, IgG1 antibodies remain, by far, the dominant isotype of antibodies, and detectable levels of IgE antibodies can even be observed in serum (Jonsson et al. 2011). As a consequence, immune complexes that form in vivo contain antibodies of several classes and subclasses. As FcRs with different specificities are co-expressed by most FcR-expessing cells, pluri-isotypic immune complexes engage several types of FcRs on cell membranes. When aggregated under physiological conditions, FcRs form hetero-aggregates.

The composition of FcR hetero-aggregates depends on multiple parameters. It depends on the FcRs that are expressed by a given cell at a given time and in a given place. it depends on the respective local concentration of antibodies of the different isotypes. It depends on the nature and on the concentration of antigen present in the environment (Fig. 4). FcR hetero-aggregates of different compositions may have markedly different signaling properties.

6 FcRs, Immunoreceptors with an Adaptive Signaling

FcRs trigger no signal when binding immunoglobulins. They signal when aggregated (Metzger 1992). The sequence of events that lead to receptor aggregation is different for high-affinity and low-affinity FcRs (Fig. 1). Monomeric antibodies bind first to high-affinity FcRs that are aggregated afterwards, when a plurivalent antigen binds to receptor-bound antibodies. Antibodies bind first to antigen, generating immune complexes that can bind to and, therefore, simultaneously aggregate low-affinity FcRs. The result, however, is the same: receptor aggregation. The nature of signals depends primarily, but not exclusively, on molecular motifs contained in the intracytoplasmic domains of FcRs or of their subunits. FcRs can generate activation signals and/or inhibition signals.

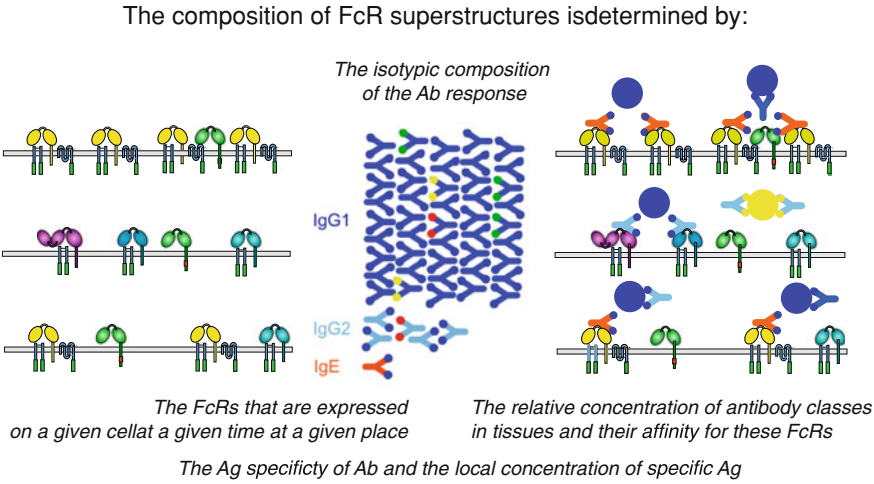


Fig. 4 *Adaptive FcR complexes formed on cell surfaces upon receptor engagement*. FcR complexes with variable compositions and sizes build up on cell membranes, depending on the FcRs expressed on cells, the isotypic composition of the antibody response, the local concentration of antibodies of the various classes and subclasses, the antigen specificity of these antibodies and the local concentration of antigen

6.1 ITAM-Containing FcRs Generate Both Activation and Inhibition Signals

ITAM-containing FcRs generate activation signals. FcRγ-associated FcRs (FcαRI, FcεRI in human neutrophils, eosinophils and monocytes, FcγRI, FcγRIIIA and FcγRIV) contain two ITAMs. FcRs associated with both FcRγ and FcRβ (FcεRI and FcγRIIIA in mast cells and basophils) contain three ITAMs. FcγRIIA and FcγRIIC contain one ITAM only. FcγRIIA, however, contain two ITAMs when dimeric (Powell et al. 2006). The significance of the presence of several ITAMs in immunoreceptors is unknown. One ITAM is enough as the aggregation of single-chain chimeric molecules with the intracytoplasmic domain of FcRγ, FcRβ, TCRζ activated the transfected cells in which they were expressed (Daëron et al. 1995a), although Igα and Igβ triggered different Ca^{2+} signals in B cells (Choquet et al. 1994) Upon receptor aggregation, ITAMs are phosphorylated by src family tyrosine kinases. This intracellular chemical perturbation initiates the constitution of dynamic intracellular signalosomes, in which activation signals are generated. Signalosomes build up on tyrosine-rich transmembrane adapter proteins that reside constitutively in lipid-rich membrane microdomains (Bezman and Koretzky 2007). Signals propagate intracellularly via various metabolic pathways that altogether lead to gene transcription, activation of the lipid metabolism and membrane alterations associated with exocytosis.

ITAM-containing FcRs also generate inhibition signals. Inhibition signals generated by activating FcRs such as FcεRI can be readily observed when challenging mast cells sensitized with IgE antibodies by increasing concentrations of specific antigen. Mast cell degranulation dose-dependently increases up to a maximum. Degranulation thereafter decreases rapidly down to background levels. This inhibition in excess of antigen has long been interpreted as resulting from a reduced ability of antigen to aggregate efficiently FcεRI (Wofsy et al. 1978). Inducible phosphorylation of intracellular proteins, however, keeps increasing beyond the optimal concentration of antigen. Moreover, inhibition in excess of antigen is abrogated in mast cells from mice deficient for the SH2 domain-containing inositol phosphatase SHIP1 (Gimborn et al. 2005). Finally, mast cells from SHIP1-deficient mice display enhanced degranulation responses to optimal and suboptimal antigen concentrations (Huber et al. 1998). FcεRI therefore generate SHIP1-dependent inhibition signals both when suboptimally or optimally aggregated and when supra-optimally aggregated by antigen. Similar effects of SHIP1 deficiency were observed with other activating FcRs (Nakamura et al. 2002). ITAM-containing FcRs therefore generate a mixture of activation and inhibition signals (Malbec et al. 2004). Activation signals are dominant over inhibition signals under physiological conditions.

6.2 *ITIM-Containing FcRs Generate Inhibition Signals Only*

FcγRIIB are the only ITIM-containing FcRs. The same ITIM is present in the intracytoplasmic domain of all murine and human FcγRIIB isoforms (Daëron et al. 1995a). FcγRIIB are not tyrosyl-phosphorylated and they trigger no intracellular signal when aggregated. They trigger negative signals when they are co-aggregated with activating receptors by immune complexes (Daëron et al. 1995b). Under these conditions, the ITIM of FcγRIIB is phosphorylated by the same src-family tyrosine kinase that phosphorylates ITAMs in activating receptors (Malbec et al. 1998). The SH2 domain of SHIP1 having a high affinity for the phosphorylated FcγRIIB ITIM (Bruhns et al. 2000), large amounts of SHIP1 are recruited and brought into signalosomes generated by activating FcRs (Lesourne et al. 2001). FcγRIIB therefore potentiate SHIP1-dependent negative signals generated by ITAM-containing FcRs (Lesourne et al. 2005), and inhibition signals become dominant over activation signals.

6.3 *The Modulation of Signaling by Hetero-Aggregation of FcRs*

Negative regulation of ITAM-containing FcRs by FcγRIIB is one example of hetero-aggregation that critically affects FcR signaling. It is a widely occurring situation. Hetero-aggregation, whether the co-aggregation of different types of

FcRs or the co-aggregation of FcRs with other immunoreceptors, is actually a rule, rather than an exception, under physiological conditions.

Hetero-aggregation can involve activating FcRs only. This happens when pluri-isotypic immune complexes engage FcRs for different immunoglobulin classes on cells that express the corresponding ITAM-containing FcRs. One exemple can be human skin mast cells sensitized with IgE antibodies and exposed to immune complexes containing IgG antibodies against the same antigen. Due to the high concentration of IgG antibodies, allergens are likely to be in complex with IgG when they reach FcεRI-bound IgE on tissue mast cells. Human skin mast cells express FcεRI and FcγRIIA, but no FcγRIIB (Zhao et al. 2006). As a result, FcεRI and FcγRIIA are co-engaged on human mast cells. Activation signals by FcεRI are not identical as signals generated by FcγRIIA because these receptors contain different ITAMs. Both types of signals are expected to be integrated and to lead to cell responses that may differ from signals generated by FcεRI or by FcγRIIA alone.

Hetero-aggregation can involve inhibitory FcRs and activating FcRs of identical or different specificities for immunoglobulins. One example of hetero-aggregation of FcRs of identical specificity is when human or mouse basophils are challenged with IgG immune complexes. Basophils from both species co-express ITAM-containing and ITIM-containing receptors for IgG: human basophils express FcγRIIA and FcγRIIB, whereas mouse basophils express FcγRIIIA and FcγRIIB. Basophils from both species, however, fail to be activated by IgG immune complexes. The reason is that FcγRIIB-dependent inhibition is dominant over FcγRIIA- or FcγRIIIA-dependent activation in these cells (Cassard et al. 2012). An example of hetero-aggregation of FcRs of different specificities is when the same cells are sensitized with IgE and challenged with IgG immune complexes. Under these conditions, IgG immune complexes co-aggregate not only the ITAM- and ITIM-containing FcγRs, but also the ITAM-containing FcεRI. In both cells, FcεRI-dependent responses are negatively regulated by FcγRIIB (Cassard et al. 2012). IgG antibodies therefore control IgE-mediated human and mouse basophil activation.

Hetero-aggregation can involve FcRs and non-FcR immunoreceptors, for instance when cells express one type of FcR only. Immune complexes can co-engage these FcRs, whether inhibitory such as FcγRIIB in B cells or activating such as FcγRIIIA in NK cells, with other immunoreceptors, whether activating such as BCRs in B cells or inhibitory such as Killer cell Inhibitory Receptors with a long intracytoplamsic domain (KIRLs) in NK cells. As a consequence, BCR signaling is negatively regulated by FcγRIIB in B cells (Phillips and Parker 1983; Amigorena et al. 1992) and FcγRIIIA signaling is negatively regulated by KIRLs in NK cells (Moretta et al. 1997).

All the above considered, FcR signaling appears exquisitely susceptible to control and/or to be controlled by other FcRs and/or by other immunoreceptors that are co-engaged in FcR superstructures by immune complexes. Ensuing biological responses may therefore be tightly controlled as a function of antibodies and antigen present in the environment.

7 FcRs, Immunoreceptors that Induce Adaptive Biological Responses

If biological responses triggered by FcRs depend quantitatively on the superstructures built-up on cell membranes by immune complexes, they depend qualitatively on the cell types on which these superstructures form. FcRs do not induce unique biological responses, but biological activities that can be induced by other receptors in the same cell. However, unlike BCRs and TCRs, which induce both cell activation and proliferation, ITAM-containing FcRs induce cell activation only.

7.1 Biological Responses Induced by Antibodies Depend on the Tissue Distribution of FcRs

Superstructures that build-up on cell membranes depend on which FcRs are available for immune complexes on the cell membrane, i.e., on the tissue distribution of FcRs. Cells of hematopoietic origin express a combination of FcRs that is typical of the cell type (Fig. 5).

FcRs are essentially expressed by myeloid cells of all types. Some are expressed by some lymphoid cells such as B cells, NK cells and NKT cells. It is a likely possibility that Innate Lymphoid Cells (ILCs) express FcRs, but which ILCs express which FcRs has not been reported yet. A few nonhematopoietic cells, such as some endothelial cells and some tumor cells (Cassard et al. 2002), also express FcRs. FcRn are expressed by many cells including epithelial cells, monocytes, macrophages, dendritic cells, neutrophils, hepatocytes (Ghetie and Ward 2000).

Activating FcRs are expressed by myeloid cells and by lymphoid cells with no classical antigen receptor, i.e., NK cells (Perussia et al. 1989) and intraepithelial γ/δT cells of the intestine (Deusch et al. 1991; Sandor et al. 1992; Woodward and Jenkinson 2001). They are not expressed by mature T and B lymphocytes. Lymphocytes therefore do not express more than one type of antigen receptor, and activating FcRs do not interfere with lymphocyte activation triggered by clonally expressed antigen receptors. Low levels of FcγRIIIA were however found on a subset of murine CD8 T cells and they efficiently triggered antibody-dependent cell-mediated cytotoxicity (Dhanji et al. 2005).

FcγRIIB are expressed by most myeloid cells and by B lymphocytes. NK cells and T cells, which do not express FcγRIIB, express several other inhibitory receptors involved in cell-cell interactions (Long 1999). FcγRIIB have a more restricted tissue distribution in humans than in mice.

Few cells express one type of FcR only. These are B cells, which express FcγRIIB only in both mice and humans, and NK cells, which typically express FcγRIIIA only in both mice and humans. A SNP in the *FCGR2C* gene, however, determines the expression FcγRIIC by NK cells in ¼ human donors (van der Heijden et al. 2012). Other cells express several FcRs. All express IgG receptors and, in humans, IgA receptors. Monocyte/macrophages express more types of

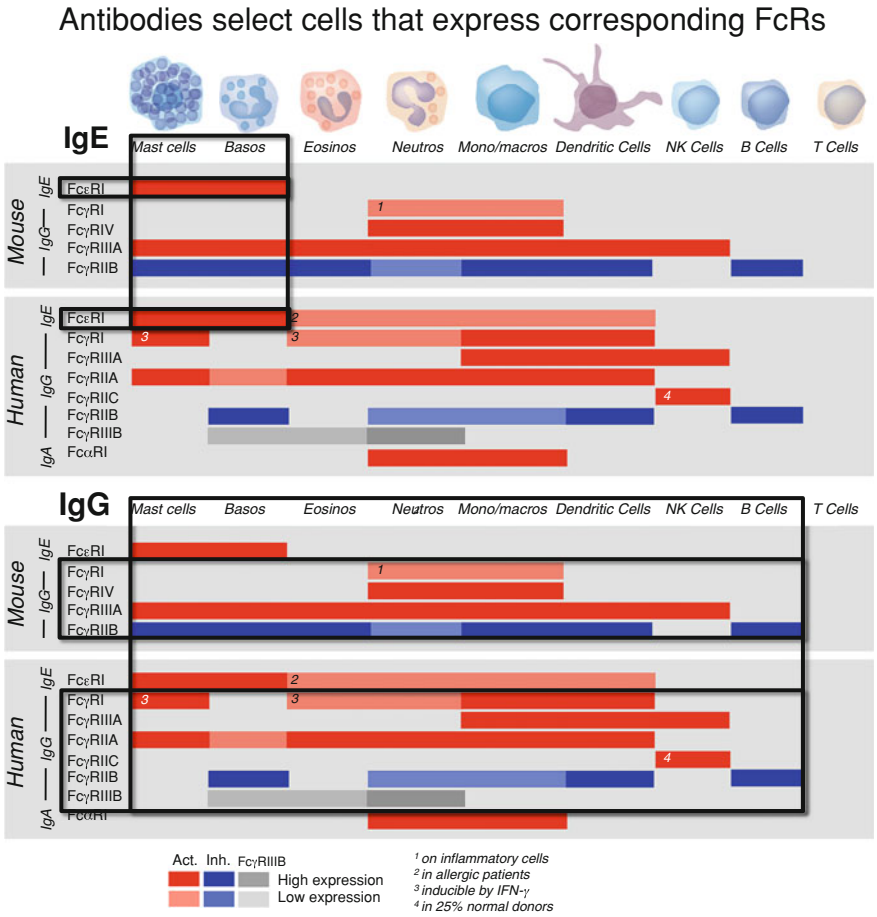


Fig. 5 *Tissue distribution of FcRs and their engagement by antibodies of different classes*. FcRs expressed by the various cell types in mice and humans are differentially engaged by antibodies of different classes. As a consequence, different cell types are involved

FcγRs than other hematopietic cells. FcγRIIA are expressed by all these human cells. Monocytes express higher levels of FcγRIIA than other blood leukocytes. Basophils express much higher levels of FcγRIIB than other blood cells, including B cells (Cassard et al. 2012).

Such a wide tissue distribution of FcRs endows antibodies with a wide spectrum of biological properties. These depend on the functional repertoire of FcR-expressing cells. All cell types can endocytose, some only can phagocytose, and even less can transcytose. The many cells that express FcRn can pinocytose and release IgG molecules that bound to FcRn intracellularly, thereby protecting them from lysosomal degradation. Some cells can expel granules that contain cytotoxic mediators, other cells granules that contain vasoactive or pro-inflammatory mediators and proteases. Many cells can synthesize cytokines, chemokines or growth factors of

different types. FcRs therefore are involved in a variety of biological functions. These include pathogen clearance, toxin neutralization, antigen capture at the initiation of antigen presentation, cytotoxicity, inflammatory responses.

7.2 Biological Responses Induced by Antibodies Depend on FcR-Expressing Cells that are Selected by Antibodies

Due to the differential tissue distribution of FcRs, antibodies select FcR-expressing cells involved in biological responses. FcRs therefore determine which cells are engaged by specific classes and subclasses of antibodies. As discussed above, the outcome depends on the functional repertoire of these cells. This can be exemplified by the analysis of cell types involved in systemic anaphylaxis.

Because IgE-induced passive systemic anaphylaxis (PSA) was abrogated in FcεRI-deficient mice (Dombrowicz et al. 1993), in mast cell-deficient mice (Kalesnikoff and Galli 2010; Feyerabend et al. 2011), and in histidine decarboxylase-deficient mice (Makabe-Kobayashi et al. 2002), histamine release by mast cells triggered by the aggregation of FcεRI upon binding of antigen to receptor-bound IgE became *the* paradigmatic model of anaphylaxis. This widely accepted interpretation forgot IgG-induced passive anaphylaxis described by Zoltan Ovary in the 1950s (Ovary 1952a, b), before IgE antibodies were discovered (Ishizaka et al. 1966). IgG1-induced PSA was later shown to depend on FcγRIIIA (Miyajima et al. 1997). Responsible cells, however, still remain unidentified as this reaction was not abrogated in mast cell (Miyajima et al. 1997) or in basophil deficient mice (Ohnmacht et al. 2010). More recently, we found that mice lacking FcεRI and FcεRII or FcγRIIIA developed active systemic anaphylaxis (ASA) as severe as did wild-type mice, when immunized with antigen in complete Freund's adjuvant and challenged with antigen intravenously. Likewise, using quintuple FcR-deficient (5KO) mice that express one activating FcR only, the high-affinity receptor for IgG2 FcγRIV expressed by monocyte/macrophages and by neutrophils, we unraveled the unexpected role of neutrophils in ASA (Jonsson et al. 2011).

The reason explaining these seemingly discrepant findings is that each class or subclass of antibodies does not select the same cell types (Fig. 5). IgE can engage FcεRI-expressing cells only, i.e., mast cells and basophils in mice, whereas IgG2 can engage FcγRIV- and FcγRI-expressing cells, i.e., monocyte/macrophages and/or neutrophils, and IgG1 can engage the many cells that express FcγRIIIA.

7.3 Biological Responses Induced by Antibodies Depend on Populations of FcR-Expressing Cells

Because immune responses are pluri-isotypic and because cells of different types share receptors for the same isotypes, antibodies select heterogeneous cell populations, rather than homogeneous single-cell populations, when in complex with

antigen. These populations consist of a mixture of various FcR-expressing cells that are either present or recruited by chemokines and/or proliferate in response to growth factors, at the site of the reaction. Biological processes in which FcRs are involved are therefore a resultant of the responses of the many cells that are engaged in the reaction at a given place and at a given time.

If one keeps considering ASA as an example, most cell types that were individually found to contribute to this reaction are present together in the blood stream where antibodies circulate and into which the challenging antigen is injected. The relative contribution of these cells therefore depends on the relative concentrations of the different classes and subclasses of antibodies, on FcRs expressed by these cells and on the interplay between FcRs.

IgG1 is the dominant isotype of antibodies following immunization by antigen in Freund's adjuvant, and FcγRIIIA are the only activating FcRs with an affinity for IgG1. Mouse mast cells (Malbec et al. 2007) and basophils (Cassard et al. 2012) express FcγRIIIA. Mast cells are not expected to be numerous in blood. Basophils are not expected to play a critical role either, as they express high levels of FcγRIIB that prevent IgG1-induced basophil activation (Cassard et al. 2012). Other cells that express FcγRIIIA are neutrophils and monocyte/macrophages. IgG2 is much less abundant than IgG1. FcγRI, FcγRIIIA, and FcγRIV are activating receptors that have an affinity for IgG2. IgG2-induced PSA was observed in 5KO mice that express FcγRIV only and neutrophils was demonstrated to contribute to this shock (Jonsson et al. 2011). IgE are between five hundred thousand- and one million-fold less abundant than IgG1 antibodies. They can bind primarily to FcεRI, but also to FcγRIV. As IgE-induced PSA was abrogated in 5KO mice, FcγRIV are unlikely to contribute to the part of ASA that depends on IgE. Mast cells and basophils remain the likely candidates. These data altogether indicate that IgE, IgG1, and IgG2 can all induce anaphylaxis when engaging FcεRI, FcγRIIIA, and FcγRIV on mast cells, basophils, and neutrophils, respectively.

Selective depletion experiments in wild-type mice could clarify the respective roles of these cells in ASA. Neutrophil depletion markedly reduced ASA in wild-type mice, basophil depletion resulted in a milder but significant reduction, and the depletion of both basophils and neutrophils virtually abrogated the reaction. Noticeably, ASA could be induced in mast cell-deficient mice, confirming the expected insignificant contribution of these cells to ASA. Neutrophils and to a lower extent basophils are therefore the main effectors of ASA, neutrophils being involved via FcγRIIIA by IgG1 and to a lower extend via FcγRIV by IgG2, and basophils being involved via FcεRI by IgE.

8 FcRs as Adaptive Immunoreceptors in Health and Disease

As discussed above, antibodies can trigger the release of potentially harmful—in some cases, life-threatening—inflammatory mediators. They can also induce destructive cytotoxic mechanisms. Antibodies are therefore potentially pathogenic.

This may be the price to pay for having efficient antibodies in protective immunity. The activating properties of antibodies are however (or therefore?) tightly controlled by regulatory mechanisms. As a consequence, immune responses are normally nonpathogenic.

Typically, the induction phase of adaptive immune responses is initiated in the periphery, while effectors are generated centrally and diffuse throughout the body. The antibody response is an example. Although they may be systemic as in anaphylaxis, many biological effects of the effector phase of immune responses take place locally. They therefore depend on local conditions. As a consequence, they are cell- or tissue-specific, pleiotropic and sometimes antagonistic. Below are examples of opposite or unexpected effects, i.e., of the complexity of the FcR-dependent effects of antibodies.

8.1 FcR-Dependent Induction and Inhibition of Immune Responses by Antibodies

Antibodies are potent adjuvants. In spite of their low plasma concentration, IgE antibodies enhance antigen presentation by B cells. IgE immune complexes indeed engage B cell FcεRII, leading to an efficient antigen presentation to T cells (Getahun et al. 2005; Hjelm et al. 2006). As a result, IgE antibodies enhance the production of all classes of antibodies. IgG antibodies also behave as adjuvants through the uptake of antigen-IgG antibody complexes by dendritic cells via activating FcγR. As a consequence, both MHC class II presentation (Heyman 1990) and MHC Class I cross presentation (Machy et al. 2000) are enhanced. Expectedly, activating FcγR-dependent presentation of antigen-antibody complexes is counterbalanced by FcγRIIB expressed by dendritic cells (Kalergis and Ravetch 2002).

FcγRIIB, however, can promote "antigen presentation" to B cells by follicular dendritic cells (Mond et al. 1995). FcγRIIB expressed by these cells can indeed be engaged by the Fc portion of immune complexes and prevent them from co-engaging FcγRIIB with BCRs on B cells (El Shikh et al. 2006). Antigen in immune complexes bound onto follicular dendritic cells are thus more potent inducers of antibody responses than free antigen, whether in vitro (Tew et al. 2001) or in vivo (Wu et al. 2008; El Shikh et al. 2009).

Unlike immune responses to soluble antigen that are markedly enhanced by IgG antibodies, immune responses to particulate antigens are well known to be suppressed by IgG antibodies. Minute amounts of specific IgG can indeed suppress an anti-heterologous erythrocyte immune response, whether primary or secondary. This observation, first made in the 1960s (Henry and Jerne 1968), has been the rationale for injecting Rh^- mothers who have given birth to Rh^+ babies with anti-RhD antibodies, as a preventive treatment of hemolytic disease of the newborn. When FcR-deficient mice became available, it was unexpectedly found that FcγRIIB-dependent negative regulation does not account for this feedback

regulation by antibodies. This regulation was unaltered not only in FcγRIIB-deficient mice (Heyman et al. 2001), but also in mice lacking all FcγR (Karlsson et al. 1999). The mechanism behind inhibition remains unclear.

8.2 FcR-Dependent Prevention and Enhancement of Viral Infection

Antiviral antibodies may profoundly affect viral infection by FcR-dependent mechanisms. It was recently reported that the neutralizing effect of antibodies depends on the interaction of their Fc portion with a unique intracellular FcR named TRIM21, and the subsequent degradation of virus-antibody complexes by the proteasome (Mallery et al. 2010). Classical activating FcRs are also needed to clear influenza virus (Huber et al. 2001). Noticeably, the engagement of activating FcγR by unrelated immune complexes was found to inhibit the replication of HIV-1 in primary human macrophages (David et al. 2006).

Rather than being protective, antibodies can favor or aggravate viral infection. Anti-Spike antibodies, a viral protein which enables the severe acute respiratory syndrome (SRAS) coronavirus to infect epithelial cells, can prevent these cells from being infected. Anti-spike antibodies, however, can enable the infection of human immune cells through their interaction with FcγR (Jaume et al. 2011). Likewise, antibodies may enhance HIV infection. Antibodies in complex with the gp120 protein of HIV indeed bind to FcγR (Fust 1997). Receptor aggregation that ensues enables the internalization of antibody-HIV complexes and, as a consequence, monocytes infection (Jouault et al. 1991).

Interestingly, FcRs may promote viral infection by inducing an antibody-independent immunosuppression. Nucleoplasmid proteins of the measle virus were indeed found to bind to murine and human FcγRII, and this binding was found to inhibit antibody production by human B cells. This mechanism was proposed to account for the well-known immunosuppression associated with measles infection (Ravanel et al. 1997).

8.3 FcR-Dependent Prevention and Enhancement of Bacterial Infection

Specific antibodies are well known to neutralize bacterial toxins. Unexpectedly, the neutralization of *B. anthacis* toxin was recently found to depend on the engagement of FcRs (Abboud et al. 2010).

FcRs are involved in antibody-dependent clearance of bacteria such as *Legionella* (Joller et al. 2010), *Salmonella* (Tobar et al. 2004) or *Toxoplasma* (Joiner et al. 1990) through phagocytosis. FcRγ-deficient mice fail to control *Leishmania major* (Padigel and Farrell 2005) or *Mycobacterium tuberculosis*

(Maglione et al. 2008) infection, whereas FcγRIIB-deficient mice display an enhanced resistance to these bacteria. FcγRIIIA seem to play a predominant role in protection (Thomas and Buxbaum 2008), but FcγRI may contribute to protect from *Bordello pertussis* infection (Ioan-Facsinay et al. 2002).

Like the measles virus, some bacteria can bind to FcRs expressed by immune cells, even when not in complex with antibodies. This interaction facilitates infection. *Escherichia coli* K1 express the outer membrane protein A (OmpA), which binds to FcγRI on macrophages. This binding has two consequences. It facilitates the entry of bacteria into cells and it prevents the phosphorylation of FcRγ (Mittal et al. 2010). FcγRI-deficient mice are resistant to *E. coli* infection.

8.4 FcR-Dependent Induction and Inhibition of Allergic Reactions

IgE antibodies are well-known inducers of allergic reactions when engaging FcεRI expressed by mast cells and basophils in experimental animals and in human patients (Dombrowicz et al. 1993, 1996; Wershil et al. 1987; Arimura et al. 1990; Fung-Leung et al. 1996). One intriguing question is why mast cells, but not basophils, account for IgE-mediated, FcεRI-dependent PSA, and where are the responsible mast cells located. The contributions to allergic symptoms of FcεRI expressed by eosinophils (Tanaka et al. 1995), monocytes (Maurer et al. 1994), alveolar macrophages (Ochiai et al. 1996), neutrophils (Gounni et al. 2001) and platelets (Joseph et al. 1997) in patients with high IgE levels can be expected to be different in allergies that affect different tissues. They remain to be delineated.

Unlike the well-established role of FcγRs in experimental anaphylaxis, the role of FcγRs in human allergies is far from being clear. The ability of human FcγRs to induce allergic reactions was demonstrated using transgenic mice (Jonsson et al. 2012; Mancardi et al. 2013). Both human FcγRI and FcγRIIA triggered IgG-induced PSA and ASA. FcγRIIA expressed by mast cells were also responsible for IgG-induced PCA. Human skin mast cells express FcγRIIA, but no FcγRIIB (Zhao et al. 2006). Interestingly, a mouse deficient for all endogenous FcγR and transgenic for all human FcγR underwent anaphylaxis following an injection of aggregated human IgG (Smith et al. 2012).

Here again, the type of cells that express FcγRs, and especially the FcγRIIA/FcγRIIB ratio, has a decisive influence on the outcome. This ratio is high in human neutrophils and these cells respond robustly to IgG immune complexes. In accordance with this in vitro observation, the transfert of human neutrophils restored anaphylaxis in FcRγ-deficient mice (Jonsson et al. 2011). This ratio is very low in human basophils, and these cells do not respond to the same immune complexes. IgG receptors expressed by human basophils indeed function as inhibitors of cell activation, and IgG immune complexes that co-engaged FcγR with FcεRI on basophils inhibited IgE-dependent basophil activation in all normal donors tested (Cassard et al. 2012).

8.5 FcR-Dependent Induction and Inhibition of Autoimmunity

Autoimune diseases that depend on autoantibodies involve an unbalance between activating and inhibitory FcRs.

Activating FcRs account for the clinical expression of autoimunity in several murine models. FcRγ-deficient mice were protected from multiple sclerosis (Robbie-Ryan et al. 2003), did not develop anti-platelet-induced thrombocytopenic purpura (Fossati-Jimack et al. 1999) and displayed less lesions in a model of Parkinson disease (He et al. 2002). FcγRI (Nimmerjahn and Ravetch 2005), FcγRIIIA (Fossati-Jimack et al. 1999) and FcγRIV (Nimmerjahn et al. 2005) were found to contribute to platelet depletion, to systemic lupus erythematosus (Seres et al. 1998), to experimental hemolytic anemia (Meyer et al. 1998; Syed et al. 2009), to glomerulonephritis (Fujii et al. 2003) and to arthritis (Ioan-Facsinay et al. 2002; Bruhns et al. 2003; Mancardi et al. 2011).

Autoantibodies induced thrombocytopenic purpura (Reilly et al. 1994) or arthritis (Pietersz et al. 2009) in transgenic mice expressing human FcγRIIA, and the expression of human FcγRI in FcRγ-deficient mice restored joint inflammation in the K/BxN model of rheumatoid arthritis (Mancardi et al. 2013) Autoantibodies against myelin found in multiple sclerosis, and autaoantibodies against dopaminergic neurons found in Parkinson's disease (McRae-Degueurce et al. 1988) are thought to induce inflammation by activating FcR-expressing phagocytic cells. Many cells of the central nervous system express FcRs, and immune cells are recruited from the bloodstream into the brain in these disorders.

Conversely, FcγRIIB prevent autoimmunity. FcγRIIB-deficient C57BL/6 mice spontaneously develop autoimmune diseases when ageing, with anti-DNA and anti-chromatin antibodies, and they die of glomerulonephritis (Ravetch and Bolland 2001). Importantly, the partial restoration of FcγRIIB levels on B cells in lupus-prone mouse strains was sufficient to restore tolerance and to prevent disease, suggesting that minor alterations of FcγRIIB expression may be sufficient to induce autoimmunity (McGaha et al. 2005; Mackay et al. 2006). A polymorphism in the transmembrane domain of human FcγRIIB was found to decrease the ability to translocate into lipd rafts and to inhibit BCR signaling (Kono et al. 2005).

8.6 FcR-Dependent Inhibition and Enhancement of Tumor Growth

Anti-tumor antibodies can lead to a significant reduction of tumor mass when injected in wt mice (Nimmerjahn and Ravetch 2005), but not in FcRγ-deficient mice (Clynes et al. 1998). FcγRIIIA (Albanesi et al. 2012), but also FcγRI (Bevaart et al. 2006) and FcγRIV (Nimmerjahn and Ravetch 2005) have been

reported to participate to the reaction. The anti-tumor effects of anti-tumor antibodies were markedly enhanced in FcγRIIB-deficient mice (Clynes et al. 2000). Antibody-dependent cell-mediated cytotoxicity is thought to account for these in vivo effects (Koene et al. 1997) and, as discussed below, it has provided the grounds for passive immunotherapy of cancer. The nature of effector cells is unclear. Cell-depletion experiments suggested a role for monocytes/macrophages (Otten et al. 2008) and possibly other myeloid cells, besides NK cells.

Anti-tumor antibodies can have an opposite effect and enhance tumor growth. An intravenous injection of antibodies against antigen expressed by tumor cells can indeed prevent the rejection of allogeneic tumor cells injected subcutaneously, leading to the death of mice which, otherwise, clear their tumors within 2 weeks (Voisin 1971). In spite of extensive investigation, the mechanism of this long known enhancement phenomenon (Kaliss 1958) has remained largely unknown. We recently found that enhancement is abrogated in FcγRIIB-deficient mice (Getahun et al. unpublished).

9 Conclusion: FcRs as Adaptive Therapeutic Tools

Antibodies appear as potent effector molecules. They are, however, not "magic bullets" as they are sometimes viewed, in reference to Paul Ehrlich's chemical compounds with a selective affinity for pathogens (Strebhardt and Ullrich 2008). As discussed in this review, their action is more subtle. They engage multiple receptors with adaptative structures and signaling on a variety of cells with adaptive functional responses, which enables immune responses to adapt to the infinite varitions of antigenic stimulations. Taking into account the complexity of interactions between antigens, antibodies, FcRs and cells occuring here and there in the body is not only an exciting challenge, it has become a requirement for understanding the pathogenesis of disease and for developing new therapeutic tools. One can indeed exploit this complexity to ameliorate immunotherapy and to conceive new antibody-dependent approaches of a variety of diseases.

In various immune diseases, symptoms are the local manifestations of a systemic process. Allergies and autoimmune disorders, in which antigen is either applied or present locally, are examples of such diseases. They develop at the intersection of a plurality of systemic effectors and of a plurality of tissue effectors. This may also apply to local or tissue-specific infections in which symptoms are primarily due to the anti-pathogen immune response. Thus, if one takes the example of allergies, the clinical manifestations of cutaneous allergies and of respiratory allergies depend on effector cells present in the skin and in the respiratory tract, respectively, and on target organs that do not respond identically to inflammatory mediators secreted by effector cells. Better understanding the polymorphism of allergies is a mean to better treat them.

Antibodies against molecules expressed by target cells have been increasingly used for passive immunotherapy, aiming at engaging FcRs to induce phagocytosis

and/or ADCC by FcR-expressing effector cells and destroy target cells. Thus, the anti-CD20 antibody Rituximab has been used to kill CD20-expressing transformed B cells (Manches et al. 2003), or B cells responsible for the production of pathogenic autoantibodies in rheumatoid arthritis (Shaw et al. 2003; Edwards et al. 2004). When binding to HER2, Trastuzumab not only inhibits the proliferation of breast, ovary or lung cancer cells by preventing receptor dimerization (Yakes et al. 2002), it also induces tumor destruction by engaging activating FcRs on cytotoxic cells (Clynes et al. 2000). Antibodies against molecules expressed by dendritic cells can also enhance antigen presentation and, as a consequence, T-cell dependent cytotoxicity against tumor cells. Unexpectedly, this effect was found to involve FcγRIIB (Li and Ravetch 2011). Indeed, when binding in *trans* to FcγRIIB-expressing cells by their Fc portion, anti-TNF receptors antibodies mimic the effect of multimeric ligands and they aggregate TNF receptors much more efficiently than when they do not (Li and Ravetch 2013).

Likewise, the therapeutic effect of Omalizumab, a monoclonal antibody directed against the FcεRI-binding site of IgE developed to prevent mast cell and basophil sensitization by IgE in allergic patients, happened to be mediated by an unanticipated mechanism. Omalizumab indeed forms IgE-anti-IgE complexes that are rapidly degraded, probably through internalization. As a result, serum IgE become undetectable (Djukanovic et al. 2004). As the half-life of FcεRI is decreased when they are not occupied by IgE, basophils and mast cells have a markedly reduced FcεRI expression.

The efficacy of therapeutic antibodies having been established, one can now aim at enhancing their wanted effects while decreasing their unwanted effects. The situation is simpler than in active immune responses because therapeutic antibodies are directed against a single epitope, because they are mono-isotypic and because most are humanized antibodies made by grafting antigen-specific variable sequences onto the same human backbone (the constant domains of a well-know human IgG1 in many cases). Thus, one can engineer therapeutic antibodies so that they have specific properties. One can mutate the main glycosylation site (e.g., introduce a N297Q point mutation in the Fc portion) to generate antibodies that can bind to target antigens without engaging FcRs except FcRn, which preserves their half life (Veri et al. 2007). One can increase the half life of antibodies by generating mutations that enhance the affinity of the Fc portion for FcRn (Ward and Ober 2009). As a consequence, the plasma concentration of *therapeutic* antibodies is increased (Dall'Acqua et al. 2006). One can either change the glycosylation or generate mutations that enhance the affinity of antibodies for activating FcRs. Thus, mutations that remove fucose residues from the Fc portion of antibodies, enhance their affinity for human FcγRIIIA (Natsume et al. 2005; Niwa et al. 2005). Conversely, one can generate antibodies with mutations in the Fc portion that enhance the affinity of antibodies for inhibitory FcRs. Thus, anti-human CD19 antibodies that bind to FcγRIIB with a several hundred-fold higher affinity than nonmutated antibodies, suppressed BCR-dependent activation of B

cells from healthy donors or from SLE patients, reduced serum IgM, IgG, and IgE levels in SCID mice engrafted with SLE PBMC, and increased survival of mice engrafted with PBMC from a SLE patient (Horton et al. 2011). Likewise, anti-IgE antibodies with an Fc portion having an increased affinity for FcγRIIB further reduced free and total IgE levels by preventing the generation of IgE-secreting plasma cells (Chu et al. 2012).

A step forward may be to know which cell types and which FcRs will be engaged by a given antibody, depending on the location of target cells or molecules, and to use therapeutic antibodies that will preferentially engage the desired FcRs on the appropriate effector cells. Phenotyping FcRs on effector cells in individual patients and assessing their ability to activate these cells would indeed be a progress toward personalized medicine.

Generating new vaccines remains a major challenge for immunologists. Antibodies are responsible for the protective effects of the overwhelming majority of vaccines. Neutralizing antibodies keep being thought to account for protection and, in most cases, FcR-dependent mechanisms are ignored. Different strategies may be necessary for vaccines against systemic infections and for local infections. Also, as exemplified by anti-SARS coronavirus antibodies, one wants to prevent antibodies from enabling the virus to infect FcR-expressing cells that are not infected in the absence of antibodies (Jaume et al. 2011). No protective anti-cancer vaccine is available yet, and efforts are being made to induce and/or amplify cell-mediated cytotoxicity against tumor cells. The efficacy of passively administered therapeutic anti-tumor antibodies, however, suggests that vaccines that would generate such antibodies may be useful. Knowing how to induce antibodies with a therapeutic benefit, but not antibodies with tumor enhancing properties will require that mechanisms of antibody-dependent enhancement are understood.

Finally, if, as discussed here, antibodies can exert a whole array of biological effects, one may stop thinking of vaccines only as a mean to kill, destroy or remove unwanted molecules, cells or pathogens. We recently found that, when co-engaged with a growth factor receptor, FcγRIIB could inhibit the proliferation of transformed tumor cells (Malbec and Daëron 2012). On the basis of this observation, vaccines could aim at co-engaging a variety of target antigens with inhibitory receptors and interfere with pathogenic processes due to cell activation or proliferation. This would extend the field of application of vaccines to diseases other than infectious diseases and cancer, such as inflammatory diseases. Specific immunotherapy of allergy is an example. It was proposed one century ago, and it has being used since then. Its efficacy and indications, however, remain limited. They might be markedly enhanced if the mechanisms behind this empirical maneuver were better known and exploited.

Acknowledgments I am indebted to my mentors, colleagues, collaborators, and students who triggered, encouraged, consolidated and fed my long-lasting interest for Fc receptors. I am grateful to Dr. Fritz Melchers who expressed his interest throughout the years, and who critically discussed a preliminary version of this work.

References

Abboud N, Chow SK, Saylor C, Janda A, Ravetch JV, Scharff MD, Casadevall A (2010) A requirement for FcgammaR in antibody-mediated bacterial toxin neutralization. J Exp Med 207(11):2395–2405

Albanesi M, Mancardi DA, Macdonald LE, Iannascoli B, Zitvogel L, Murphy AJ, Daëron M, Leusen JH, Bruhns P (2012) Cutting edge: FcgammaRIII (CD16) and FcgammaRI (CD64) are responsible for anti-glycoprotein 75 monoclonal antibody TA99 therapy for experimental metastatic B16 melanoma. J Immunol 189:5513–5517

Amigorena S, Bonnerot C, Drake JR, Choquet D, Hunziker W, Guillet JG, Webster P, Sautes C, Mellman I, Fridman WH (1992) Cytoplasmic domain heterogeneity and functions of IgG Fc receptors in B lymphocytes. Science 256(5065):1808–1812

Arimura A, Nagata M, Takeuchi M, Watanabe A, Nakamura K, Harada M (1990) Active and passive cutaneous anaphylaxis in WBB6F1 mouse, a mast cell-deficient strain. Immunol Invest 19(3):227–233

Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA (2007) The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 25:21–50

Bakos MA, Kurosky A, Goldblum RM (1991) Characterization of a critical binding site for human polymeric Ig on secretory component. J Immunol 147(10):3419–3426

Behring E, Kitasato S (1890) Ueber das Zustandekommen der Diphtherie-Immunit€at und der Tetanus-Immunit€at bei Thieren. Deutsch med Wochenschr 16(49):1113–1114

Bevaart L, Jansen MJ, van Vugt MJ, Verbeek JS, van de Winkel JG, Leusen JH (2006) The high-affinity IgG receptor, FcgammaRI, plays a central role in antibody therapy of experimental melanoma. Cancer Res 66(3):1261–1264

Bezman N, Koretzky GA (2007) Compartmentalization of ITAM and integrin signaling by adapter molecules. Immunol Rev 218:9–28

Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, Daëron M (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113(16):3716–3725

Bruhns P, Samuelsson A, Pollard JW, Ravetch JV (2003) Colony-stimulating factor-1-dependent macrophages are responsible for IVIG protection in antibody-induced autoimmune disease. Immunity 18(4):573–581

Bruhns P, Vely F, Malbec O, Fridman WH, Vivier E, Daëron M (2000) Molecular basis of the recruitment of the SH2 domain-containing inositol 5-phosphatases SHIP1 and SHIP2 by fcgamma RIIB. J Biol Chem 275(48):37357–37364

Burmeister WP, Huber AH, Bjorkman PJ (1994) Crystal structure of the complex of rat neonatal Fc receptor with Fc. Nature 372(6504):379–383

Cambier JC (1995) New nomenclature for the Reth motif (or ARH1/TAM/ARAM/YXXL). Immunol Today 16(2):110

Cassard L, Cohen-Solal JF, Galinha A, Sastre-Garau X, Mathiot C, Galon J, Dorval T, Bernheim A, Fridman WH, Sautes-Fridman C (2002) Modulation of tumor growth by inhibitory Fc(gamma) receptor expressed by human melanoma cells. J Clin Invest 110(10):1549–1557

Cassard L, Jonsson F, Arnaud S, Daëron M (2012) Fcgamma receptors inhibit mouse and human basophil activation. J Immunol 189(6):2995–3006

Choquet D, Ku G, Cassard S, Malissen B, Korn H, Fridman WH, Bonnerot C (1994) Different patterns of calcium signaling triggered through two components of the B lymphocyte antigen receptor. J Biol Chem 269(9):6491–6497

Chu SY, Horton HM, Pong E, Leung IW, Chen H, Nguyen DH, Bautista C, Muchhal US, Bernett MJ, Moore GL, Szymkowski DE, Desjarlais JR (2012) Reduction of total IgE by targeted coengagement of IgE B-cell receptor and FcgammaRIIb with Fc-engineered antibody. J Allergy Clin Immunol 129(4):1102–1115

Clynes R, Takechi Y, Moroi Y, Houghton A, Ravetch JV (1998) Fc receptors are required in passive and active immunity to melanoma. Proc Natl Acad Sci U S A 95(2):652–656

Clynes RA, Towers TL, Presta LG, Ravetch JV (2000) Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 6(4):443–446

Conrad DH (1990) Fc epsilon RII/CD23: the low affinity receptor for IgE. Annu Rev Immunol 8:623–645

Daëron M, Duc HT, Kanellopoulos J, Le Bouteiller P, Kinsky R, Voisin GA (1975) Allogenic mast cell degranulation induced by histocompatibility antibodies: an in vitro model of transplantation anaphylaxis. Cell Immunol 20(2):133–155

Daëron M, Jaeger S, Du Pasquier L, Vivier E (2008) Immunoreceptor tyrosine-based inhibition motifs: a quest in the past and future. Immunol Rev 224:11–43

Daëron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S, Fridman WH (1995a) The same tyrosine-based inhibition motif, in the intracytoplasmic domain of FcgRIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. Immunity 3:635–646

Daëron M, Malbec O, Latour S, Arock M, Fridman WH (1995b) Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. J Clin Invest 95(2):577–585

Daëron M, Voisin GA (1978) H-2 antigens, on mast cell membrane, as target antigens for anaphylactic degranulation. Cell Immunol 37(2):467–472

Dall'Acqua WF, Kiener PA, Wu H (2006) Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). J Biol Chem 281(33):23514–23524

David A, Saez-Cirion A, Versmisse P, Malbec O, Iannascoli B, Herschke F, Lucas M, Barre-Sinoussi F, Mouscadet JF, Daëron M, Pancino G (2006) The engagement of activating FcgammaRs inhibits primate lentivirus replication in human macrophages. J Immunol 177(9):6291–6300

Deusch K, Pfeffer K, Reich K, Gstettenbauer M, Daum S, Luling F, Classen M (1991) Phenotypic and functional characterization of human TCR gamma delta + intestinal intraepithelial lymphocytes. Curr Top Microbiol Immunol 173:279–283

Dhanji S, Tse K, Teh HS (2005) The low affinity Fc receptor for IgG functions as an effective cytolytic receptor for self-specific CD8 T cells. J Immunol 174(3):1253–1258

Ding Y, Xu G, Yang M, Yao M, Gao GF, Wang L, Zhang W, Rao Z (2003) Crystal structure of the ectodomain of human FcalphaRI. J Biol Chem 278(30):27966–27970

Djukanovic R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF, Bao W, Fowler-Taylor A, Matthews J, Busse WW, Holgate ST, Fahy JV (2004) Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. Am J Respir Crit Care Med 170(6):583–593

Dombrowicz D, Brini AT, Flamand V, Hicks E, Snouwaert JN, Kinet JP, Koller BH (1996) Anaphylaxis mediated through a humanized high affinity IgE receptor. J Immunol 157(4):1645–1651

Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet JP (1993) Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. Cell 75(5):969–976

Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, Stevens RM, Shaw T (2004) Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 350(25):2572–2581

El Shikh ME, El Sayed R, Szakal AK, Tew JG (2006) Follicular dendritic cell (FDC)-FcgammaRIIB engagement via immune complexes induces the activated FDC phenotype associated with secondary follicle development. Eur J Immunol 36(10):2715–2724

El Shikh ME, El Sayed RM, Szakal AK, Tew JG (2009) T-independent antibody responses to T-dependent antigens: a novel follicular dendritic cell-dependent activity. J Immunol 182(6):3482–3491

Feyerabend TB, Weiser A, Tietz A, Stassen M, Harris N, Kopf M, Radermacher P, Moller P, Benoist C, Mathis D, Fehling HJ, Rodewald HR (2011) Cre-mediated cell ablation contests

mast cell contribution in models of antibody- and T cell-mediated autoimmunity. Immunity 35(5):832–844
Fossati-Jimack L, Reininger L, Chicheportiche Y, Clynes R, Ravetch JV, Honjo T, Izui S (1999) High pathogenic potential of low-affinity autoantibodies in experimental autoimmune hemolytic anemia. J Exp Med 190(11):1689–1696
Fujii T, Hamano Y, Ueda S, Akikusa B, Yamasaki S, Ogawa M, Saisho H, Verbeek JS, Taki S, Saito T (2003) Predominant role of FcgammaRIII in the induction of accelerated nephrotoxic glomerulonephritis. Kidney Int 64(4):1406–1416
Fung-Leung WP, De Sousa-Hitzler J, Ishaque A, Zhou L, Pang J, Ngo K, Panakos JA, Chourmouzis E, Liu FT, Lau CY (1996) Transgenic mice expressing the human high-affinity immunoglobulin (Ig) E receptor alpha chain respond to human IgE in mast cell degranulation and in allergic reactions. J Exp Med 183(1):49–56
Fust G (1997) Enhancing antibodies in HIV infection. Parasitology 115(Suppl):S127–S140
Garman SC, Kinet JP, Jardetzky TS (1998) Crystal structure of the human high-affinity IgE receptor. Cell 95(7):951–961
Getahun A, Hjelm F, Heyman B (2005) IgE enhances antibody and T cell responses in vivo via CD23 + B cells. J Immunol 175(3):1473–1482
Ghetie V, Ward ES (2000) Multiple roles for the major histocompatibility complex class I-related receptor FcRn. Annu Rev Immunol 18:739–766
Gimborn K, Lessmann E, Kuppig S, Krystal G, Huber M (2005) SHIP down-regulates FcepsilonR1-induced degranulation at supraoptimal IgE or antigen levels. J Immunol 174(1):507–516
Gounni AS, Lamkhioued B, Delaporte E, Dubost A, Kinet JP, Capron A, Capron M (1994) The high-affinity IgE receptor on eosinophils: from allergy to parasites or from parasites to allergy? J Allergy Clin Immunol 94(6 Pt 2):1214–1216
Gounni AS, Lamkhioued B, Koussih L, Ra C, Renzi PM, Hamid Q (2001) Human neutrophils express the high-affinity receptor for immunoglobulin E (Fc epsilon RI): role in asthma. FASEB J 15(6):940–949
He Y, Le WD, Appel SH (2002) Role of Fcgamma receptors in nigral cell injury induced by Parkinson disease immunoglobulin injection into mouse substantia nigra. Exp Neurol 176(2):322–327
Henry C, Jerne NK (1968) Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. J Exp Med 128(1):133–152
Héricourt J, Richet C (1888) De la transfusion péritonéale, et de l'immunité qu'elle confère. CR Acad Sci 107:748–750
Heyman B (1990) The immune complex: possible ways of regulating the antibody response. Immunol Today 11(9):310–313
Heyman B, Dahlstrom J, Diaz De Stahl T, Getahun A, Wernersson S, Karlsson MC (2001) No evidence for a role of FcgammaRIIB in suppression of in vivo antibody responses to erythrocytes by passively administered IgG. Scand J Immunol 53(4):331–334 (discussion 339–345)
Hjelm F, Carlsson F, Getahun A, Heyman B (2006) Antibody-mediated regulation of the immune response. Scand J Immunol 64(3):177–184
Horton HM, Chu SY, Ortiz EC, Pong E, Cemerski S, Leung IW, Jacob N, Zalevsky J, Desjarlais JR, Stohl W, Szymkowski DE (2011) Antibody-mediated coengagement of FcgammaRIIb and B cell receptor complex suppresses humoral immunity in systemic lupus erythematosus. J Immunol 186(7):4223–4233
Huber M, Helgason CD, Damen JE, Liu L, Humphries RK, Krystal G (1998) The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. Proc Natl Acad Sci U S A 95(19):11330–11335
Huber VC, Lynch JM, Bucher DJ, Le J, Metzger DW (2001) Fc receptor-mediated phagocytosis makes a significant contribution to clearance of influenza virus infections. J Immunol 166(12):7381–7388
Hulett MD, Hogarth PM (1994) Molecular basis of Fc receptor function. Adv Immunol 57:1–127

Ioan-Facsinay A, de Kimpe SJ, Hellwig SM, van Lent PL, Hofhuis FM, van Ojik HH, Sedlik C, da Silveira SA, Gerber J, de Jong YF, Roozendaal R, Aarden LA, van den Berg WB, Saito T, Mosser D, Amigorena S, Izui S, van Ommen GJ, van Vugt M, van de Winkel JG, Verbeek JS (2002) FcgammaRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. Immunity 16(3):391–402

Ishizaka K, Ishizaka T, Hornbrook MM (1966) Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J Immunol 97(1):75–85

Israel EJ, Patel VK, Taylor SF, Marshak-Rothstein A, Simister NE (1995) Requirement for a beta 2-microglobulin-associated Fc receptor for acquisition of maternal IgG by fetal and neonatal mice. J Immunol 154(12):6246–6251

Jaume M, Yip MS, Cheung CY, Leung HL, Li PH, Kien F, Dutry I, Callendret B, Escriou N, Altmeyer R, Nal B, Daëron M, Bruzzone R, Peiris JS (2011) Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcgammaR pathway. J Virol 85(20):10582–10597

Joiner KA, Fuhrman SA, Miettinen HM, Kasper LH, Mellman I (1990) Toxoplasma gondii: fusion competence of parasitophorous vacuoles in Fc receptor-transfected fibroblasts. Science 249(4969):641–646

Joller N, Weber SS, Muller AJ, Sporri R, Selchow P, Sander P, Hilbi H, Oxenius A (2010) Antibodies protect against intracellular bacteria by Fc receptor-mediated lysosomal targeting. Proc Natl Acad Sci U S A 107(47):20441–20446

Jonsson F, Mancardi DA, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, Shimizu T, Daëron M, Bruhns P (2011) Mouse and human neutrophils induce anaphylaxis. J Clin Invest 121(4):1484–1496

Jonsson F, Mancardi DA, Zhao W, Kita Y, Iannascoli B, Khun H, van Rooijen N, Shimizu T, Schwartz LB, Daëron M, Bruhns P (2012) Human FcgammaRIIA induces anaphylactic and allergic reactions. Blood 119(11):2533–2544

Joseph M, Gounni AS, Kusnierz JP, Vorng H, Sarfati M, Kinet JP, Tonnel AB, Capron A, Capron M (1997) Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. Eur J Immunol 27(9):2212–2218

Jouault T, Chapuis F, Bahraoui E, Gluckman JC (1991) Infection of monocytic cells by HIV1: combined role of FcR and CD4. Res Virol 142(2–3):183–188

Kalergis AM, Ravetch JV (2002) Inducing tumor immunity through the selective engagement of activating Fcgamma receptors on dendritic cells. J Exp Med 195(12):1653–1659

Kalesnikoff J, Galli SJ (2010) Anaphylaxis: mechanisms of mast cell activation. Chem Immunol Allergy 95:45–66

Kaliss N (1958) Immunological enhancement of tumor homografts in mice: a review. Cancer Res 18(9):992–1003

Karlsson MC, Wernersson S, Diaz de Stahl T, Gustavsson S, Heyman B (1999) Efficient IgG-mediated suppression of primary antibody responses in Fcgamma receptor-deficient mice. Proc Natl Acad Sci U S A 96(5):2244–2249

Kilmon MA, Shelburne AE, Chan-Li Y, Holmes KL, Conrad DH (2004) CD23 trimers are preassociated on the cell surface even in the absence of its ligand. IgE. J Immunol 172(2):1065–1073

Kinet JP (1999) The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology. Annu Rev Immunol 17:931–972

Kinet JP, Blank U, Ra C, White K, Metzger H, Kochan J (1988) Isolation and characterization of cDNAs coding for the beta subunit of the high-affinity receptor for immunoglobulin E. Proc Natl Acad Sci U S A 85(17):6483–6487

Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M (1997) Fc gammaRIIIa-158 V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood 90(3):1109–1114

Kono H, Kyogoku C, Suzuki T, Tsuchiya N, Honda H, Yamamoto K, Tokunaga K, Honda Z (2005) FcgammaRIIB Ile232Thr transmembrane polymorphism associated with human

systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. Hum Mol Genet 14(19):2881–2892

Kulczycki A Jr, Metzger H (1974) The interaction of IgE with rat basophilic leukemia cells. II. Quantitative aspects of the binding reaction. J Exp Med 140(6):1676–1695

Lesourne R, Bruhns P, Fridman WH, Daëron M (2001) Insufficient phosphorylation prevents fc gamma RIIB from recruiting the SH2 domain-containing protein-tyrosine phosphatase SHP-1. J Biol Chem 276(9):6327–6336

Lesourne R, Fridman WH, Daëron M (2005) Dynamic interactions of Fc gamma receptor IIB with filamin-bound SHIP1 amplify filamentous actin-dependent negative regulation of Fc epsilon receptor I signaling. J Immunol 174(3):1365–1373

Letourneur F, Hennecke S, Demolliere C, Cosson P (1995) Steric masking of a dilysine endoplasmic reticulum retention motif during assembly of the human high affinity receptor for immunoglobulin E. J Cell Biol 129(4):971–978

Li F, Ravetch JV (2011) Inhibitory Fcgamma receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. Science 333(6045):1030–1034

Li F, Ravetch JV (2013) Antitumor activities of agonistic anti-TNFR antibodies require differential FcgammaRIIB coengagement in vivo. Proc Natl Acad Sci U S A 110(48):19501–19506

Lobell RB, Arm JP, Raizman MB, Austen KF, Katz HR (1993) Intracellular degradation of Fc gamma RIII in mouse bone marrow culture-derived progenitor mast cells prevents its surface expression and associated function. J Biol Chem 268(2):1207–1212

Long EO (1999) Regulation of immune responses through inhibitory receptors. Annu Rev Immunol 17:875–904

Machy P, Serre K, Leserman L (2000) Class I-restricted presentation of exogenous antigen acquired by Fcgamma receptor-mediated endocytosis is regulated in dendritic cells. Eur J Immunol 30(3):848–857

Mackay M, Stanevsky A, Wang T, Aranow C, Li M, Koenig S, Ravetch JV, Diamond B (2006) Selective dysregulation of the FcgammaIIB receptor on memory B cells in SLE. J Exp Med 203(9):2157–2164

Maglione PJ, Xu J, Casadevall A, Chan J (2008) Fc gamma receptors regulate immune activation and susceptibility during Mycobacterium tuberculosis infection. J Immunol 180(5):3329–3338

Makabe-Kobayashi Y, Hori Y, Adachi T, Ishigaki-Suzuki S, Kikuchi Y, Kagaya Y, Shirato K, Nagy A, Ujike A, Takai T, Watanabe T, Ohtsu H (2002) The control effect of histamine on body temperature and respiratory function in IgE-dependent systemic anaphylaxis. J Allergy Clin Immunol 110(2):298–303

Malbec O, Daëron M (2012) Antibodies against growth factor receptors can inhibit the proliferation of transformed cells via a cis-interaction with inhibitory FcR. Immunol Lett 143(1):28–33

Malbec O, Fong DC, Turner M, Tybulewicz VL, Cambier JC, Fridman WH, Daëron M (1998) Fc epsilon receptor I-associated lyn-dependent phosphorylation of Fc gamma receptor IIB during negative regulation of mast cell activation. J Immunol 160(4):1647–1658

Malbec O, Malissen M, Isnardi I, Lesourne R, Mura AM, Fridman WH, Malissen B, Daëron M (2004) Linker for activation of T cells integrates positive and negative signaling in mast cells. J Immunol 173(8):5086–5094

Malbec O, Roget K, Schiffer C, Iannascoli B, Dumas AR, Arock M, Daëron M (2007) Peritoneal cell-derived mast cells: an in vitro model of mature serosal-type mouse mast cells. J Immunol 178(10):6465–6475

Mallery DL, McEwan WA, Bidgood SR, Towers GJ, Johnson CM, James LC (2010) Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21). Proc Natl Acad Sci U S A 107(46):19985–19990

Mancardi DA, Albanesi M, Jonsson F, Iannascoli B, Van Rooijen N, Kang X, England P, Daëron M, Bruhns P (2013) The high-affinity human IgG receptor FcgammaRI (CD64) promotes IgG-

mediated inflammation, anaphylaxis, and antitumor immunotherapy. Blood 121(9):1563–1573
Mancardi DA, Iannascoli B, Hoos S, England P, Daëron M, Bruhns P (2008) FcgammaRIV is a mouse IgE receptor that resembles macrophage FcepsilonRI in humans and promotes IgE-induced lung inflammation. J Clin Invest 118(11):3738–3750
Mancardi DA, Jonsson F, Iannascoli B, Khun H, Van Rooijen N, Huerre M, Daëron M, Bruhns P (2011) Cutting Edge: The murine high-affinity IgG receptor FcgammaRIV is sufficient for autoantibody-induced arthritis. J Immunol 186(4):1899–1903
Manches O, Lui G, Chaperot L, Gressin R, Molens JP, Jacob MC, Sotto JJ, Leroux D, Bensa JC, Plumas J (2003) In vitro mechanisms of action of rituximab on primary non-Hodgkin lymphomas. Blood 101(3):949–954
Maurer D, Fiebiger E, Reininger B, Wolff-Winiski B, Jouvin MH, Kilgus O, Kinet JP, Stingl G (1994) Expression of functional high affinity immunoglobulin E receptors (Fc epsilon RI) on monocytes of atopic individuals. J Exp Med 179(2):745–750
Maxwell KF, Powell MS, Hulett MD, Barton PA, McKenzie IF, Garrett TP, Hogarth PM (1999) Crystal structure of the human leukocyte Fc receptor Fc gammaRIIa. Nat Struct Biol 6(5):437–442
McGaha TL, Sorrentino B, Ravetch JV (2005) Restoration of tolerance in lupus by targeted inhibitory receptor expression. Science 307(5709):590–593
McRae-Degueurce A, Rosengren L, Haglid K, Booj S, Gottfries CG, Granerus AC, Dahlstrom A (1988) Immunocytochemical investigations on the presence of neuron-specific antibodies in the CSF of Parkinson's disease cases. Neurochem Res 13(7):679–684
Metzger H (1992) Transmembrane signaling: the joy of aggregation. J Immunol 149(5):1477–1487
Meyer D, Schiller C, Westermann J, Izui S, Hazenbos WL, Verbeek JS, Schmidt RE, Gessner JE (1998) FcgammaRIII (CD16)-deficient mice show IgG isotype-dependent protection to experimental autoimmune hemolytic anemia. Blood 92(11):3997–4002
Mittal R, Sukumaran SK, Selvaraj SK, Wooster DG, Babu MM, Schreiber AD, Verbeek JS, Prasadarao NV (2010) Fcgamma receptor I alpha chain (CD64) expression in macrophages is critical for the onset of meningitis by Escherichia coli K1. PLoS Pathog 6(11):e1001203
Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ (1997) Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. J Clin Invest 99(5):901–914
Mond JJ, Lees A, Snapper CM (1995) T cell-independent antigens type 2. Annu Rev Immunol 13:655–692
Moretta A, Biassoni R, Bottino C, Pende D, Vitale M, Poggi A, Mingari MC, Moretta L (1997) Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. Immunol Rev 155:105–117
Nakamura K, Malykhin A, Coggeshall KM (2002) The Src homology 2 domain-containing inositol 5-phosphatase negatively regulates Fcgamma receptor-mediated phagocytosis through immunoreceptor tyrosine-based activation motif-bearing phagocytic receptors. Blood 100(9):3374–3382
Natsume A, Wakitani M, Yamane-Ohnuki N, Shoji-Hosaka E, Niwa R, Uchida K, Satoh M, Shitara K (2005) Fucose removal from complex-type oligosaccharide enhances the antibody-dependent cellular cytotoxicity of single-gene-encoded antibody comprising a single-chain antibody linked the antibody constant region. J Immunol Methods 306(1–2):93–103
Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV (2005) FcgammaRIV: a novel FcR with distinct IgG subclass specificity. Immunity 23(1):41–51
Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science 310(5753):1510–1512
Niwa R, Sakurada M, Kobayashi Y, Uehara A, Matsushima K, Ueda R, Nakamura K, Shitara K (2005) Enhanced natural killer cell binding and activation by low-fucose IgG1 antibody

results in potent antibody-dependent cellular cytotoxicity induction at lower antigen density. Clin Cancer Res 11(6):2327–2336
Ochiai K, Kagami M, Umemiya K, Matsumura R, Kawashima T, Tomioka H (1996) Expression of high-affinity IgE receptor (Fc epsilon RI) on human alveolar macrophages from atopic and non-atopic patients. Int Arch Allergy Immunol 111(Suppl 1):55–58
Ohnmacht C, Schwartz C, Panzer M, Schiedewitz I, Naumann R, Voehringer D (2010) Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths. Immunity 33(3):364–374
Orloff DG, Ra CS, Frank SJ, Klausner RD, Kinet JP (1990) Family of disulphide-linked dimers containing the zeta and eta chains of the T-cell receptor and the gamma chain of Fc receptors. Nature 347(6289):189–191
Otten MA, van der Bij GJ, Verbeek SJ, Nimmerjahn F, Ravetch JV, Beelen RH, van de Winkel JG, van Egmond M (2008) Experimental antibody therapy of liver metastases reveals functional redundancy between Fc gammaRI and Fc gammaRIV. J Immunol 181(10):6829–6836
Ovary Z (1952a) Cutaneous anaphylaxis in the albino rat. Int Arch Allergy Appl Immunol 3(4):293–301
Ovary Z (1952b) Quantitative studies in passive cutaneous anaphylaxis of the guinea pig. Int Arch Allergy Appl Immunol 3(2):162–174
Padigel UM, Farrell JP (2005) Control of infection with Leishmania major in susceptible BALB/c mice lacking the common gamma-chain for FcR is associated with reduced production of IL-10 and TGF-beta by parasitized cells. J Immunol 174(10):6340–6345
Pasteur L (1880) Sur les maladies virulentes, et en particulier sur la maladie appelée vulgairement choléra des poules. C R Acad Sci Paris XC (Séance du 9 février 1880):239–248
Perussia B, Tutt MM, Qui WQ, Kuziel WA, Tucker PW, Trinchieri G, Bennett M, Ravetch JV, Kumar V (1989) Murine natural killer cells express functional Fcg receptor II encoded by the FcgRa gene. J Exp Method 170:73–86
Phillips NE, Parker DC (1983) Fc-dependent inhibition of mouse B cell activation by whole anti-mu antibodies. J Immunol 130(2):602–606
Pietersz GA, Mottram PL, van de Velde NC, Sardjono CT, Esparon S, Ramsland PA, Moloney G, Baell JB, McCarthy TD, Matthews BR, Powell MS, Hogarth PM (2009) Inhibition of destructive autoimmune arthritis in FcgammaRIIa transgenic mice by small chemical entities. Immunol Cell Biol 87(1):3–12
Powell MS, Barnes NC, Bradford TM, Musgrave IF, Wines BD, Cambier JC, Hogarth PM (2006) Alteration of the Fc gamma RIIa dimer interface affects receptor signaling but not ligand binding. J Immunol 176(12):7489–7494
Ravanel K, Castelle C, Defrance T, Wild TF, Charron D, Lotteau V, Rabourdin-Combe C (1997) Measles virus nucleocapsid protein binds to FcgammaRII and inhibits human B cell antibody production. J Exp Med 186(2):269–278
Ravetch JV, Bolland S (2001) IgG Fc receptors. Annu Rev Immunol 19:275–290
Ravetch JV, Kinet JP (1991) Fc receptors. Annu Rev Immunol 9:457–492
Reilly AF, Norris CF, Surrey S, Bruchak FJ, Rappaport EF, Schwartz E, McKenzie SE (1994) Genetic diversity in human Fc receptor II for immunoglobulin G: Fc gamma receptor IIA ligand-binding polymorphism. Clin Diagn Lab Immunol 1(6):640–644
Reth M (1989) Antigen receptor tail clue. Nature 338(6214):383–384
Richet C (1907) De l'anaphylaxie en général et de l'anaphylaxie par la mytilocongestive en particulier. Ann Inst Pasteur 21:497–524
Richet C, Portier P (1902) De l'action anaphylactique de certains venins. C R Soc Biol (Paris) 54
Robbie-Ryan M, Tanzola MB, Secor VH, Brown MA (2003) Cutting edge: both activating and inhibitory Fc receptors expressed on mast cells regulate experimental allergic encephalomyelitis disease severity. J Immunol 170(4):1630–1634
Rodewald R, Kraehenbuhl JP (1984) Receptor-mediated transport of IgG. J Cell Biol 99(1 Pt 2):159s–164s

Sandor M, Houlden B, Bluestone J, Hedrick SM, Weinstock J, Lynch RG (1992) In vitro and in vivo activation of murine gamma/delta T cells induces the expression of IgA, IgM, and IgG Fc receptors. J Immunol 148(8):2363–2369

Seres T, Csipo I, Kiss E, Szegedi G, Kavai M (1998) Correlation of Fc gamma receptor expression of monocytes with clearance function by macrophages in systemic lupus erythematosus. Scand J Immunol 48(3):307–311

Shaw T, Quan J, Totoritis MC (2003) B cell therapy for rheumatoid arthritis: the rituximab (anti-CD20) experience. Ann Rheum Dis 62(Suppl 2):ii55–ii59

Smith P, DiLillo DJ, Bournazos S, Li F, Ravetch JV (2012) Mouse model recapitulating human Fcgamma receptor structural and functional diversity. Proc Natl Acad Sci U S A 109(16):6181–6186

Sondermann P, Huber R, Jacob U (1999) Crystal structure of the soluble form of the human fcgamma-receptor IIb: a new member of the immunoglobulin superfamily at 1.7 A resolution. EMBO J 18(5):1095–1103

Strebhardt K, Ullrich A (2008) Paul Ehrlich's magic bullet concept: 100 years of progress. Nat Rev Cancer 8(6):473–480

Syed SN, Konrad S, Wiege K, Nieswandt B, Nimmerjahn F, Schmidt RE, Gessner JE (2009) Both FcgammaRIV and FcgammaRIII are essential receptors mediating type II and type III autoimmune responses via FcRgamma-LAT-dependent generation of C5a. Eur J Immunol 39(12):3343–3356

Takai T, Li M, Sylvestre D, Clynes R, Ravetch JV (1994) FcR gamma chain deletion results in pleiotrophic effector cell defects. Cell 76(3):519–529

Takizawa F, Adamczewski M, Kinet JP (1992) Identification of the low affinity receptor for immunoglobulin E on mouse mast cells and macrophages as Fc gamma RII and Fc gamma RIII. J Exp Med 176(2):469–475

Tanaka Y, Takenaka M, Matsunaga Y, Okada S, Anan S, Yoshida H, Ra C (1995) High affinity IgE receptor (Fc epsilon RI) expression on eosinophils infiltrating the lesions and mite patch tested sites in atopic dermatitis. Arch Dermatol Res 287(8):712–717

Tew JG, Wu J, Fakher M, Szakal AK, Qin D (2001) Follicular dendritic cells: beyond the necessity of T-cell help. Trends Immunol 22(7):361–367

Thomas BN, Buxbaum LU (2008) FcgammaRIII mediates immunoglobulin G-induced interleukin-10 and is required for chronic Leishmania mexicana lesions. Infect Immun 76(2):623–631

Tobar JA, Gonzalez PA, Kalergis AM (2004) Salmonella escape from antigen presentation can be overcome by targeting bacteria to Fc gamma receptors on dendritic cells. J Immunol 173(6):4058–4065

van der Heijden J, Breunis WB, Geissler J, de Boer M, van den Berg TK, Kuijpers TW (2012) Phenotypic variation in IgG receptors by nonclassical FCGR2C alleles. J Immunol 188(3):1318–1324

Veri MC, Gorlatov S, Li H, Burke S, Johnson S, Stavenhagen J, Stein KE, Bonvini E, Koenig S (2007) Monoclonal antibodies capable of discriminating the human inhibitory Fcgamma-receptor IIB (CD32B) from the activating Fcgamma-receptor IIA (CD32A): biochemical, biological and functional characterization. Immunology 121(3):392–404

Voisin GA (1971) Immunological facilitation, a broadening of the concept of the enhancement phenomenon. Prog Allergy 15:328–485

Ward ES, Ober RJ (2009) Chapter 4: multitasking by exploitation of intracellular transport functions the many faces of FcRn. Adv Immunol 103:77–115

Wershil BK, Mekori YA, Murakami T, Galli SJ (1987) 125I-fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin. Demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. J Immunol 139(8):2605–2614

Wofsy C, Goldstein B, Dembo M (1978) Theory of equilibrium binding of asymmetric bivalent haptens to cell surface antibody: application to histamine release from basophils. J Immunol 121(2):593–601

Woodward J, Jenkinson E (2001) Identification and characterization of lymphoid precursors in the murine intestinal epithelium. Eur J Immunol 31(11):3329–3338

Wu Y, Sukumar S, El Shikh ME, Best AM, Szakal AK, Tew JG (2008) Immune complex-bearing follicular dendritic cells deliver a late antigenic signal that promotes somatic hypermutation. J Immunol 180(1):281–290

Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL (2002) Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt Is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. Cancer Res 62(14):4132–4141

Zhao W, Kepley CL, Morel PA, Okumoto LM, Fukuoka Y, Schwartz LB (2006) Fc gamma RIIa, not Fc gamma RIIb, is constitutively and functionally expressed on skin-derived human mast cells. J Immunol 177(1):694–701

Glycosylation and Fc Receptors

Jerrard M. Hayes, Eoin F. J. Cosgrave, Weston B. Struwe, Mark Wormald, Gavin P. Davey, Roy Jefferis and Pauline M. Rudd

Abstract Immunoglobulins and Fc receptors are critical glycoprotein components of the immune system. Fc receptors bind the Fc (effector) region of antibody molecules and communicate information within the innate and adaptive immune systems. Glycosylation of antibodies, particularly in the Fc region of IgG, has been extensively studied in health and disease. The N-glycans in the identical heavy chains have been shown to be critical for maintaining structural integrity, communication with the Fc receptor and the downstream immunological response.

J. M. Hayes · G. P. Davey
School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland
e-mail: jehayes@tcd.ie

G. P. Davey
e-mail: gdavey@tcd.ie

E. F. J. Cosgrave
Pharmaceutical Life Sciences Group, Waters Corporation, 34 Maple St., Milford MA 01757, USA
e-mail: Eoin_Cosgrave@Waters.com

W. B. Struwe
Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Oxford OX1 3TA, UK
e-mail: Weston.Struwe@Chem.ox.ac.uk

M. Wormald
Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK
e-mail: Mark.Wormald@bioch.ox.ac.uk

R. Jefferis
Department of Immunology, University of Birmingham, Birmingham, UK
e-mail: r.jefferis@bham.ac.uk

P. M. Rudd (✉)
NIBRT-Glycoscience Group, NIBRT—The National Institute for Bioprocessing, Research and Training, Foster Av. Blackrock, Dublin 4, Ireland
e-mail: pauline.rudd@nibrt.ie

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_8,

Less is known about glycosylation of the Fc receptor in either healthy or disease states, however, recent studies have implicated an active role for receptor associated oligosaccharides in the antibody-receptor interaction. Research into Fc receptor glycosylation is increasing rapidly, where Fc receptors are routinely used to analyze the binding of therapeutic monoclonal antibodies and where glycosylation of receptors expressed by cells of the immune system could potentially be used to mediate and control the differential binding of immunoglobulins. Here we discuss the glycosylation of immunoglobulin antibodies (IgA, IgE, IgG) and the Fc receptors (FcαR, FcεR, FcγR, FcRn) that bind them, the function of carbohydrates in the immune response and recent advances in our understanding of these critical glycoproteins.

Keywords Immunoglobulin · Fc receptor · Glycosylation · Immunity · N-glycan · O-glycan

Abbreviations

α-gal	Gal(α1,3)-gal
ADCC	Antibody dependent cell mediated cytotoxicity
Asn	Asparagine
CDC	Complement mediated cytotoxicity
DC-SIGN	Dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin
FcαR	Fc alpha receptor
FcεR	Fc epsilon receptor
FcγR	Fc gamma receptor
FcRn	Neonatal Fc receptor
GALT	Gut associated lymphoid tissue
GlcNAc	N-acetylglucosamine
Ig	Immunoglobulin
IgA	Immunoglobulin A
SIgA	Secretory IgA
IgE	Immunoglobulin E
IgD	Immunoglobulin D
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgSF	Immunoglobulin-like super family
ITAM	Immunotyrosine-like activation motif
ITIM	Immunotyrosine-like inhibition motif
Man	Mannose
MBL	Mannan binding lectin
MMR	Macrophage mannose receptor
MHC	Major histocampatibility complex
NGNA	N-glycolylneuraminic acid

NK	Natural killer
PNGase F	*Peptide-N-glycosidase* F
TLR	Toll-like receptor

Contents

1 Introduction 167
2 IgA-Mediated Immunity 170
2.1 FcαR 173
3 IgE-Mediated Immunity 174
3.1 FcεRI: The High Affinity Fc Epsilon Receptor 176
3.2 FcεRII: The Low Affinity Fc Epsilon Receptor 177
4 IgG-Mediated Immunity 178
4.1 The Role of N-Glycans in IgG Biology 178
4.2 Role of Glycosylation in FcγR Biology 181
4.3 FcγRI (CD64) 184
4.4 FcγRII (CD32) 185
4.5 FcγRIII (CD16) 186
5 FcRn: The Neonatal Fc Receptor 189
6 Closing Remarks 190
References 190

1 Introduction

Glycosylation is a key player in the immune response, in antigen recognition of invading microorganisms and in ligand–receptor interactions that lead to cellular and effector activities. Modification of proteins, lipids, and other organic molecules by glycosylation creates a repertoire of glycovariants, some of which may confer orthogonal functions on the proteins to which they are attached. Given the diversity and ubiquity of glycan structures in almost all organisms it is not surprising that the immune system has evolved to detect sugar epitopes from pathogenic sources. Toll-like receptors (TLR) are a particularly good example of this pathogen associated molecular pattern (PAMP) recognition. Endotoxin (lipopolysaccharide), an outer membrane component of gram negative bacteria consisting of a polysaccharide and lipid is sensed by TLR4 which signals to activate the innate immune system (Takeda et al. 2003; Medzhitov et al. 1997; Chow et al. 1999). Mannan binding lectin (MBL) is a C-type lectin that functions in pattern recognition of sugars from pathogenic micro-organisms leading to activation of the lectin pathway and complement system (Petersen et al. 2001). Macrophage mannose receptor (MMR) and dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin

(DC-SIGN) are also C-type lectins which recognize mannose type sugars on bacteria, viruses and fungi (Figdor et al. 2002). Galectins are widely distributed proteins that bind galactose and its derivatives and function in cell–cell and cell–matrix interactions and cell signaling (Barondes et al. 1994a, b). Dectin-1 is another C-type lectin, which mediates various immune functions in response to fungal infection (Brown and Gordon 2001; Ariizumi et al. 2000). This carbohydrate recognition of pathogenic microorganisms by specific sugar binding proteins of the immune system is a critical sensing and defense mechanism. Effective immunity also requires the ability of cells to communicate with each other, to extravasate from blood vessels to sites of infection and to fight the source of infection and maintain normal cellular homeostasis, critical functions mediated by carbohydrates.

Immunoglobulins and their receptors (Fc receptors) are critical glycoprotein components of the immune system that link the innate and adaptive arms of immunity. Immunoglobulins are diverse glycoproteins in terms of their isotype, antigen recognition, distribution/concentration, and effector responses. They can be membrane bound in the form of surface immunoglobulins or soluble. Surface immunoglobulins form part of the B-cell receptor (BCR) and are composed of membrane bound immunoglobulin D (IgD) or immunoglobulin M (IgM). This allows the antibody producing B-cell to detect specific antigens resulting in B-cell activation and antibody production. In humans, there are five types of immunoglobulin antibody: IgA, IgD, IgE, IgG, and IgM, each with specific structure, function and activities. Each antibody monomer consists of two identical heavy chains and two light chains, an Fc domain formed from the constant regions and two antigen binding Fab domains formed from the variable regions but different antibodies have variable numbers of immunoglobulin domains. Somatic hypermutation in the variable regions allows antibodies to recognize a huge repertoire (10^9) of antigens and the constant Fc region allows the antibody to interact with Fc receptors on the surface of innate immune cells such as monocytes, neutrophils, B-cells, macrophages and natural killer (NK) cells. The N-linked glycans present on conserved asparagine containing sequons in the Fc region, particularly IgG are essential for antibody structure and function.

Antibodies communicate with effector cells of the immune system through interactions with membrane bound Fc receptors, which are complex glycoproteins found on many cells types. These interactions induce effector responses including phagocytosis, activation of the complement cascade, and antibody dependent cell-mediated cytotoxicity (ADCC). Even though IgG antibodies bind to complement receptors and C-type lectins for the purpose of this review we focus on the interactions of immunoglobulins with Fc receptors. Fc receptors (FcαR, FcεR, FcγR) are specific for a particular antibody isotype and direct specific immune responses following binding of antibody-immune complexes. Fc receptors have distinct functions but similar structures with familiar domains, architecture and post-translational modifications (see Fig. 1). The IgD receptor is found on B-cells and the recently discovered receptor for IgM (FcμR) is expressed only in lymphoid cells such as B- and T-lymphocytes in humans (Kubagawa et al. 2009; Ouchida et al. 2012). There is an additional Fc receptor known as the neonatal Fc receptor

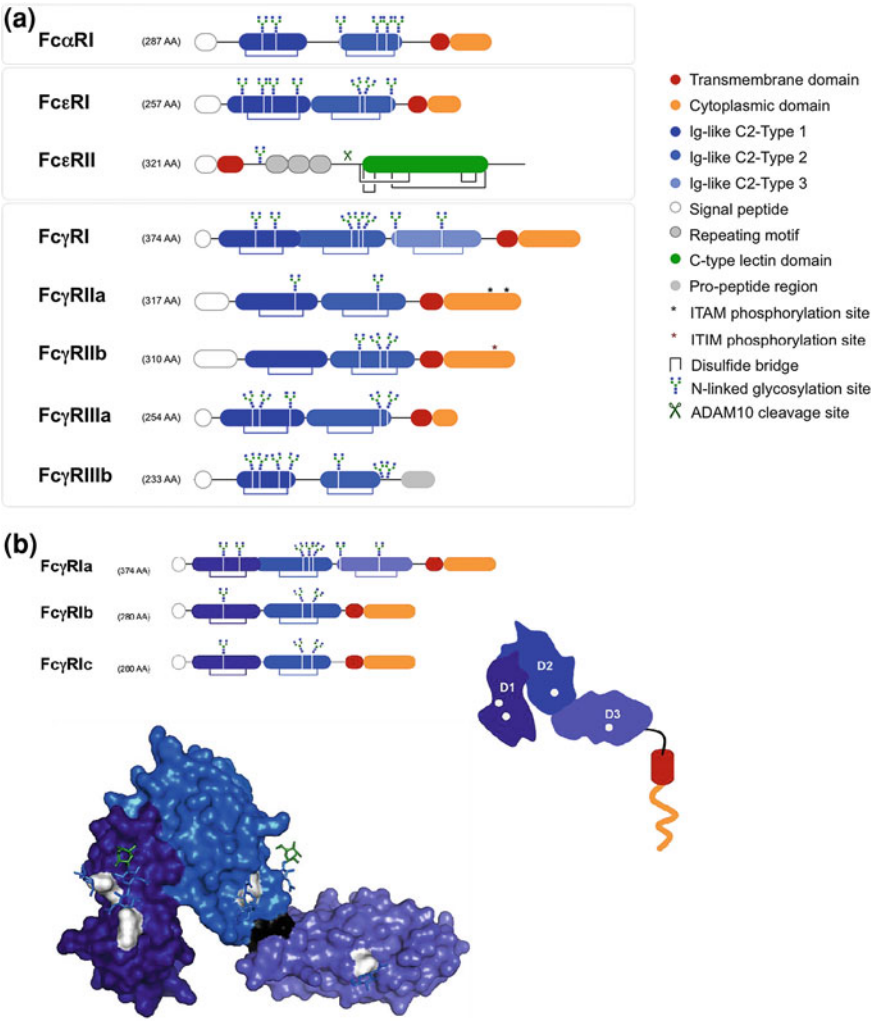


Fig. 1 *Domain architecture of membrane bound Fc receptors*. **a** Schematic representation of Fc receptors showing extracellular, transmembrane and intracellular regions. Annotated features associated with Fc receptors including glycosylation sites, transmembrane regions, intracellular signaling motifs, domain structures and cleavage sites. **b** FcγRI family showing domain structures and annotated features. FcγRIa contains an extra D3 domain, which confers the high-affinity property of the receptor and two additional N-linked sites

(FcRn), which is involved in recycling of IgG, maintaining the serum concentrations of the antibody where high concentrations are required to fight infection (Ward et al. 2003). This receptor is also involved in placental transport of IgG from mother to fetus and is critically important for the transfer of humeral immunity to the baby, which cannot yet make IgG antibodies (Morphis and Gitlin

1970; Brambell 1966). Glycosylation in antibody-receptor interactions has traditionally focused on the function of the N-linked glycans of immunoglobulins, in particular IgG, however, recent exciting discoveries have led to renewed interest and focus on the role of Fc receptor glycosylation in the antibody interaction and subsequent immune response.

Much of the available glycosylation data and information on antibodies and Fc receptors come from studies involving IgG and FcγRs. For this reason, in this chapter we focus mainly on this particular antibody isotype and Fc receptor class. We have chosen not to discuss in any detail IgD or IgM and the corresponding receptors, as there is little glycosylation data available for these receptors. In addition to IgG and FcγRs, we also discuss the extensive glycosylation of IgA and IgE and their receptors: FcαR and FcεR. Where data are available, we discuss the influence of glycosylation on the antibody interactions and immune response. The biopharmaceutical industry is particularly interested in the crucial influence of particular glycans, such as noncore-fucosylated structures, attached to therapeutic monoclonal antibodies. An explosion of genomic, proteomic, and structural data now exists and atomic resolution structures of antibody-receptor complexes are available, which shed light on the mechanisms of interaction and the detailed influence of the associated sugars. We discuss these structures and the ways in which carbohydrates are involved in unique carbohydrate–carbohydrate and carbohydrate–protein interactions.

2 IgA-Mediated Immunity

Antibody-mediated innate and adaptive immunity at mucosal membranes and secretions is largely facilitated by the activity of immunoglobulin A (IgA and secretory IgA), an important class of the immunoglobulin family that provides protection against pathogens at mucosal sites such as the gastrointestinal, genitourinary, and respiratory tracts (Macpherson et al. 2000). IgA comprises approximately 15 % of total body immunoglobulin and, in adults, is produced by the majority of plasma B-cells in larger amounts than all of the other immunoglobulins combined (66 mg/kg/day) (Kerr 1990). IgA, which is unlike other immunoglobulins in that it forms a T-shaped structure exists in a number of forms or subclasses (see Fig. 2a) (Boehm et al. 1999; Furtado et al. 2004). Serum IgA1 and IgA2 which is more abundant in secretions differ by a thirteen amino acid sequence found in the hinge region of IgA1 (van Egmond et al. 2001). This additional hinge region is heavily O-glycosylated, protecting the antibody from bacterial proteolytic degradation (see Fig. 2a). IgA2 does not have these O-linked sites but does contain two additional N-linked sites making it is less susceptible to bacterial proteolysis. Polymeric forms of IgA also exist, composed of 2–4 IgA monomers joined by a 16 kDa chain (J chain) (Kerr 1990). This form of IgA, which is found in secretory fluids such as saliva, tears, colostrum, and gastrointestinal fluids, is known as secretory IgA (SIgA). At mucosal sites such as the gastrointestinal tract, IgA exists

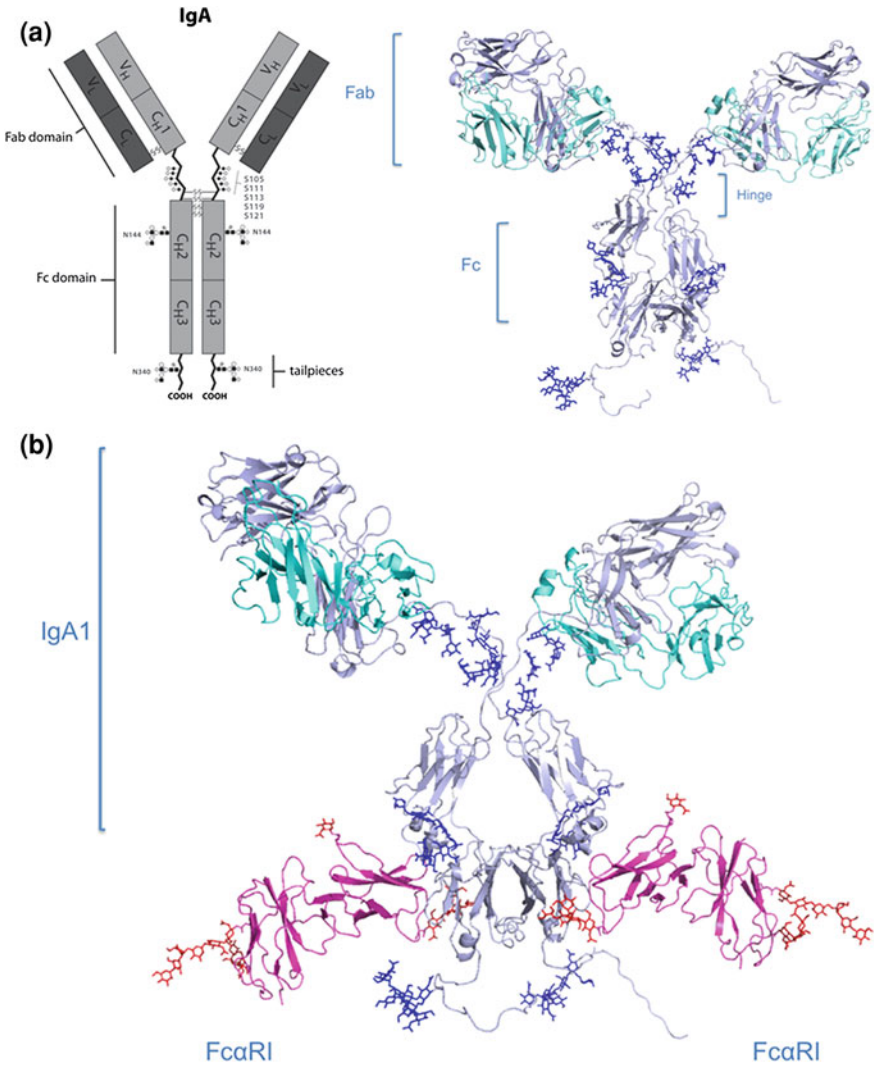


Fig. 2 *Immunoglobulin A, glycosylation and the FcαR complex*. **a** Schematic representation and molecular model of human IgA1 showing glycan sites and glycan site occupancy. The hinge region of IgA is heavily O- and N-glycosylated which protects IgA from bacterial proteolysis. Also shown is Fc glycosylation and extra C-terminal tail region glycosylation containing bi-antennary glycans. **b** IgA1–FcαRI complex. Two FcαR molecules are bound to the IgA antibody in the crystal structure. FcαR glycosylation and site occupancy is shown in *red* and IgA glycosylation is shown in *blue*. IgA glycans (*blue*) appear on the external side of the Cα2 domain of FcαRI. It is believed that the Fcα glycan of IgA comes within 8 Å of the FcαR, indicating a potential protein–carbohydrate or carbohydrate–carbohydrate interaction with the glycans of FcαRI (*red*). The model is based on 1ow0 in the protein data bank

primarily as SIgA and exhibits anti-inflammatory neutralizing properties. This prevents unwanted immune responses against commensal bacteria or other perceived antigens encountered in a normal diet.

SIgA is extensively N- and O-linked glycosylated (see Fig. 2). Both IgA1 and IgA2 contain two N-linked sites per H chain, one in the Cα2 domain (Asn263) and one in the C-terminal region (Asn459). The secretory component (SC) is also heavily glycosylated with seven N-linked sites and an additional site in the J chain. The IgA1 hinge region is heavily O-glycosylated. Comprehensive analysis of the N- and O-linked glycans of IgA were described in several reports which identified significant heterogeneity in both the N- and O-linked foms (Pierce-Cretel et al. 1981, 1982, 1989; Mizoguchi et al. 1982; Hughes et al. 1999). The total glycosylation of SIgA was first reported by Royle et al. in (2003) from the antibody H, J and SC peptide chains from pooled human serum IgA. A large variety of N- and O-linked carbohydrate epitopes were described in the SC and hinge region of the H chain respectively such as galactose in β(1,3) and β(1,4) linkage to GlcNAc, fucose in α(1,2) linkage to galactose and α(1,3) and α(1,4) linkage to GlcNAc and α(2,3) and α(2,6)-linked sialic acids (Royle et al. 2003). An abundance of sialylated N-linked structures in the SC were identified with shorter truncated glycans within the heavy chain region. Notably, all of the Lewis and sial-Lewis glycan epitopes were identified conferring potential binding sites for lectins and bacterial adhesins. These carbohydrate epitopes serve a dual role in protecting SIgA from bacterial proteolysis and in ligand binding. In addition, SIgA glycans facilitate binding to the lectin Mac-1(CD11b/CD18), which plays a role in FcαR-mediated SIgA signaling (van Spriel et al. 2002). Glycosylation of the Fab regions of IgA was reported by Mattu et al. (1998). Bi-antennary N-linked glycans were mainly present in the Fc whilst N- and O-linked sugars with extensive sialylation (30 %) were reported for the Fab regions (Mattu et al. 1998).

Serum IgA, which is present throughout the body following its synthesis by plasma cells in the spleen, is distinct from SIgA which is present at secretory sites such as gut associated lymphoid tissue (GALT). Glycosylation of the various forms of IgA is also different, probably due to the differences in the 3D structures of the individual proteins and the levels of the glycan processing enzymes at their sites of production. Polymeric SIgA has several binding sites for antigen and due to the differences in carbohydrate composition such as exposed GlcNAc and mannose residues it can be internalized by dendritic cells following binding to mannose receptor, whereas serum IgA cannot (Royle et al. 2003; Heystek et al. 2002). Large, complex, sialylated O-glycans in the hinge region differ between serum IgA and SIgA and are also likely to play different roles in interactions with bacterial adhesins. Interestingly, Royle et al. show that N-glycans on the H chains of SIgA contain exposed mannose and GlcNAc residues that can be masked by the SC (Royle et al. 2003). Disruption of the SC–H chain can reveal these exposed terminal monosaccharides, which can then be recognized by lectin receptors on dendritic cells and phagocytes to promote opsonisation and phagocytosis.

2.1 FcαR

IgA antibodies communicate with the immune system via interaction with FcαRs, integral membrane proteins specific for the Fc region of the IgA molecule (see Fig. 2b). Five structurally unrelated FcαRs have been described (Monteiro and van de Winkel 2003). The polymeric IgA receptor (pIgR) is a member of the immunoglobulin superfamily, which binds dimeric SIgA and is expressed by mucosal epithelial cells. Following SIgA binding, translocation of the IgA–pIgR receptor complex across the epithelial cell delivers the antibody to the mucosal surface (Mostov 1994). At the mucosal surface, the SC (part of the epithelial cell) is cleaved and the remaining complex (IgA–J chain–SC) is secreted. Interestingly, this peptide complex is assembled from two different cell types and represents one of the few examples where a protein is assembled from two distinct cell types. The polymeric IgA receptor also facilitates the secretion of IgM antibodies (Johansen et al. 1999).

FcαRI (CD89) is a specific Fc receptor for IgA1 and IgA2 found on cells of myeloid lineage such as macrophages, neutrophils, dendritic cells, and eosinophils (Geissmann et al. 2001; van Egmond et al. 2000; Monteiro et al. 1993). Due to alternative splicing, the FcαRI protein can exist in three different forms or splice variants in vivo (Morton et al. 1996; Pleass et al. 1996). The FcαRI (a.1) isoform is the full-length protein and is a 32 kDa single pass transmembrane receptor (Maliszewski et al. 1990). However, due to extensive glycosylation on six potential N-linked sites and seven potential O-linked sites and further heterogeneity which exists on these glycosylation sites the mature protein exists with a molecular weight of between 50 and 100 kDa in vivo (Morton et al. 1996). FcαRI can range in mass from between 55 and 75 kDa in the case of monocytes and neutrophils to as high as 100 kDa in the case of eosinophils due to differential glycosylation (Morton et al. 1996; van Egmond et al. 2001). Little information exists as to the exact nature of the glycans found on FcαRI, however, deglycosylation experiments using *endoglycosidases* confirmed the presence of extensive glycosylation and also suggested that FcαR1 was differentially glycosylated. All of the six potential N-linked sites have been the focus of recent investigations. Site-directed mutagenesis of each N-linked site revealed no impact on FcαR binding of IgA, with the exception of Asn58 (Xue et al. 2010). Mutagenesis of Asn58 to Glu58 resulted in a near twofold increase in binding of IgA. This study also investigated the role of sialylation on IgA binding and demonstrated a near fourfold increase in affinity following *neuraminidase* treatment of mutagenized FcαR and provided a clear indication that glycosylation of FcαR at position 58 is a key factor in the binding affinity for IgA. The full role of FcαR glycosylation is currently unclear but these recent experiments indicate that different glycans play a role in the IgA interaction and that particular cell types glycosylate the receptor in a cell-type specific manner. It remains a challenge to characterize FcαR glycosylation, define its role and mechanism in binding IgA and control of downstream immune responses.

Serum IgA principally exists as a monomer and interacts with FcαRI leading to immune effector responses (see Fig. 2b). The precise role of FcαR, however, is still debated. FcαRI exists as a low affinity Fc receptor for IgA with an approximate K_a 10^6 M^{-1} that rapidly dissociates from the FcαRI–IgA complex (Wines et al. 1999). In common with IgG binding to Fcγ receptors, IgA immune complexes bind more tightly to FcαR and with higher avidity than monomeric IgA (van Egmond et al. 2001). Soluble glycosylated forms of FcαRI also exist with molecular weights of approximately 30 kDa (25 kDa peptide sequence) and 50–70 kDa. The latter tightly associates with polymeric IgA, however, the function of soluble FcαRI is unclear (van Zandbergen et al. 1999; van der Boog et al. 2002). The crystal structure of the FcαR–IgA complex indicates that two FcαR molecules bind one molecule of IgA (Herr et al. 2003) (see Fig. 2b). Unlike IgG or IgE, IgA glycans appear on the external side of the Cα2 domain as opposed to residing within the interstitial space of the Cα2 dimer. Herr et al., postulate that the Fcα glycan comes within eight angstroms of the FcαR, indicating a potential protein–carbohydrate or carbohydrate–carbohydrate interaction (see Fig. 2b). Structurally, FcαR resembles the Fc gamma receptors by having two extracellular Ig-like domains, a single transmembrane pass, and a short cytoplasmic region. FcαRI associates with the FcR gamma chain that contains an ITAM (immunoreceptor tyrosine-based activation motif) (Morton et al. 1995). Signaling of FcαR is dependent upon crosslinking of IgA immune complexes leading to receptor clustering and localization to lipid rafts resulting in ITAM phosphorylation causing increases in intracellular calcium levels and induction of *NADPH oxidase* activity in neutrophils (Lang et al. 1999). Signaling via FcαRI following antigen recognition by IgA leads to a multitude of effector responses such as ADCC, phagocytosis of bacteria and yeast, superoxide generation and release of cytokines and inflammatory mediators.

3 IgE-Mediated Immunity

When the body loses tolerance to commonly encountered foreign material hypersensitivity can result and manifest as a number of disorders including atopic dermatitis, allergic rhinitis, asthma, and food allergies. Hypersensitivity is largely driven through immunoglobulin E (IgE) and mast cells, although debate exists whether these two factors contribute to long term tissue rearrangement associated with prolonged exposure to allergens (Galli and Tsai 2012). In addition to allergic responses of the immune system IgE is also involved in anti-parasitic responses such as in defense against helminths and parasitic worms (Gounni et al. 1994). IgE is the least abundant immunoglobulin in human serum, typically found at very low levels (150–300 ng/ml) and is most likely complexed with the high affinity receptor for IgE, FcεRI on mast cells and basophils (Dorrington and Bennich 1978). Like other immunoglobulins, IgE exists as a structure consisting of two heavy and two light chains, however, it has a different domain structure and the

hinge regions are more rigid than in other immunoglobulin classes (see Fig. 3a). IgE is also heavily glycosylated with seven potential N-linked sites where carbohydrates comprise approximately 12 % of the antibody mass, making it the most heavily glycosylated immunoglobulin (Arnold et al. 2004; Dorrington and Bennich 1978) (see Fig. 3a, b). Arnold et al. described the N-glycosylation of human serum IgD and IgE and identified extensive oligomannose glycosylation, including Man_3, Man_4, Man_5, Man_6, Man_7, Man_8, Man_9, and additional hybrid structures. The total glycan pool of IgE contained approximately 14 % high mannose structures, which mediate extensive interaction with MBL (Arnold et al. 2004). A significant proportion of glycans were found to be sialylated; mono-sialylated glycans accounted for 39 % and di-sialylated glycans 36 % of the total glycan pool. This agreed with oligomannose glycans and sialylated glycans previously reported on myeloma IgE (Dorrington and Bennich 1978; Baenziger and Kornfeld 1974a, b). Complex glycans were predominantly bi-antennary (97 %) with significant amounts of core-fucosylation (68 %). Bisecting N-acetylglucosamine residues were present on approximately 15 % of N-glycan structures. Site-specific glycosylation analysis of IgE has been performed recently by Plomp et al. who describe an extensive analysis of polyclonal IgE from three different sources. In all three samples of IgE isolated from pooled serum of myeloma patients, myeloma nondiseased and hyperimmune donors the Asn275 N-glycan site contained exclusively oligomannose structures (Man_2–Man_9). The remaining Asn21, Asn49, Asn99, Asn146 and Asn252 residues contained complex glycans which were bi-antennary core fucosylated (98.5–100 %) mono-and di-sialylated structures for the non-myeloma donors and interestingly, contained higher proportions of tri- and tetra-antennary structures and lower bi-secting GlcNAc residues in the IgE myeloma patients. The Asn264 N-glycan site was found to be unoccupied and Asn99, Asn252 and Asn275 only partially occupied (Plomp et al. 2013).

IgE N-glycans have functional importance, particularly the oligomannose carbohydrate at Asn275 which is homologous to Asn297 in IgG where the site contains a complex-type oligosaccharide. Mutation of Asn275 resulted in the loss of binding to the high affinity FcεR1, indicating the importance of this N-linked site (Nettleton and Kochan 1995). Enzymatic deglycosylation of IgE using PNGase F severely decreased the reactivity of IgE for FcεRI, suggesting that IgE glycosylation affects both structure and function of the antibody molecule (Bjorklund et al. 1999). Glycosylation also affects the binding of IgE to the low affinity FcεRII, particularly the glycan at Asn252 (Sondermann et al. 2013). However, in other reports, glycosylation of IgE has been shown to have no effect on the activity of either FcεRI or FcεRII and IgE deglycosylation was shown to have the same activity as mock deglycosylated IgE, suggesting that antibody glycosylation has only a limited affect (Vercelli et al. 1989; Basu et al. 1993). An additional nondirect function of IgE glycosylation can be found in the activity of galectins, a family of lectins that specifically bind β-galactoside. High affinity binding of galectins to the glycans of IgE regulate the activity of IgE and have an anti-allergic effect by blocking antigen complex formation (Niki et al. 2009).

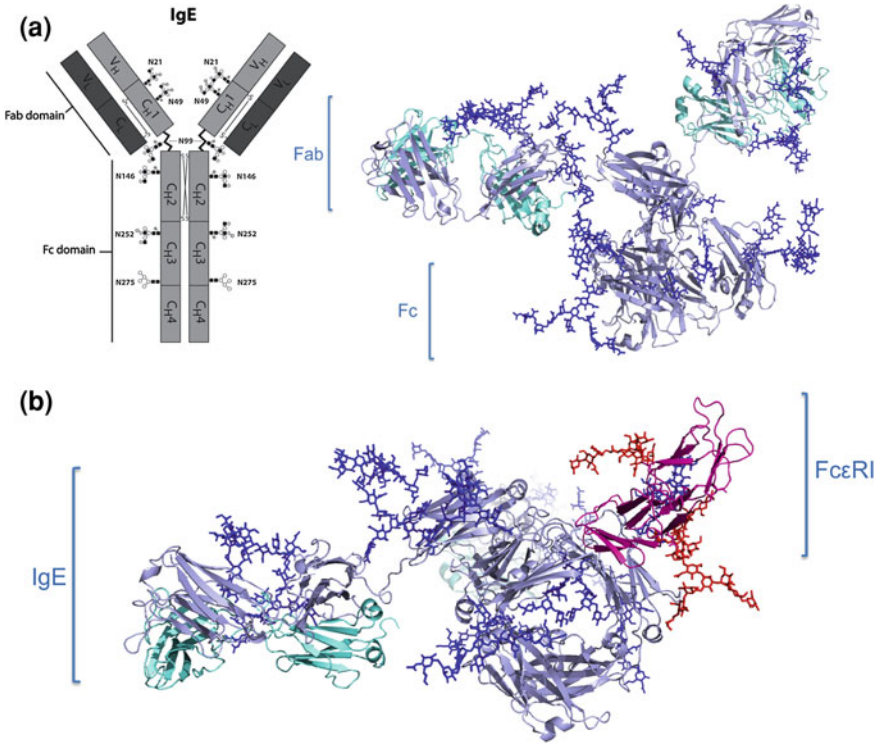


Fig. 3 *Immunoglobulin E, glycosylation and the FcεR complex*. **a** Schematic representation and molecular model of human IgE showing glycan sites and glycan site occupancy. IgE is the most heavily gycosylated immunoglobulin with extensive glycosylation (shown in *blue*) in the Fc and Fab regions. The role of IgE glycosylation in the interaction with the IgE receptor is debated with some reports saying it is important and others suggesting it has minimal impact on the interaction. **b** IgE–FcεRI complex. In the case of FcεRI glycans (*red*) do not appear to be involved at the interaction site with IgE and may not participate in the binding of antibody but are instead suggested to be involved in solubility and protein folding in the endoplasmic reticulum. Shown in *red* is FcεRI glycosylation and in *blue* is IgE glycosylation. The model is based on 1f6a in the protein databank with hinge regions based on 1o0v and Fab regions from IgG

3.1 FcεRI: The High Affinity Fc Epsilon Receptor

IgE-mediated immune responses are controlled via the Fc epsilon receptor (FcεR). The high affinity FcεRI (K_a 10^{10} M^{-1}) is a member of the immunoglobulin superfamily and is found on mast cells, basophils, eosinophils, and Langerhans cells where it triggers effector responses (Kraft and Kinet 2007). This can cause allergic reactions to allergens following interaction of IgE immune complexes with the FcεRI on mast cells through degranulation and release of histamine, *serine proteases*, proteoglycans, and inflammatory mediators. Human FcεRI exists in two forms, an $\alpha\beta\gamma 2$ tetramer and $\alpha\gamma 2$ trimer in which the α subunits bind IgE and the $\beta\gamma$

subunits signal within the effector cell. FcεRI is unique to Fc receptors; in that, it contains the additional beta chain, which acts to amplify the signal (Kraft et al. 2004). Apart from this, it has a typical interaction with the FcR gamma chain required for signaling within the effector cell. There are seven N-linked sites on FcεRI although little information exists as the occupation of these sites or the exact nature of the glycans that reside here (see Fig. 3b). It was shown by Kanellopoulos et al. and La Croix and Froese that the receptor expressed on basophilic cells contains approximately 40 % of its weight due to carbohydrates, decreasing to approximately 28 kDa, following PNGase F treatment (Kanellopoulos et al. 1980; LaCroix and Froese 1993). Experiments using glycosylation inhibitors and *endoglycosidase* enzymes suggested that both the high affinity FcεRI and low affinity FcεRII receptors are composed of mainly complex glycans with only a single oligomannose site. Even though O-linked sites exist these experiments suggest that few O-linked glycans are present on either FcεRI or FcεRII. In the crystal structure of FcεRI carbohydrate density is indicated at three of the potential seven N-glycan sites (Asn21, Asn42, and Asn166) although there is little information as to the function of the glycans present at these sites (Garman et al. 2000). However, studies have suggested that carbohydrates on FcεRI are not required for the binding interaction with IgE and are instead needed for efficient folding and solubility of the receptor (Letourneur et al. 1995; Robertson 1993). In the crystal structure, the glycans do not extend toward the top surface of the receptor where the interaction with IgE is proposed to take place (Garman et al. 2000) (see Fig. 3b). Mutation of the N-linked sites causes misfolding and absence of N-glycosylation due to enzymatic deglycosylation affects neither the stability nor IgE binding capacity of FcεRI, again indicating that FcεRI glycans do not participate in the interaction with IgE (Letourneur et al. 1995).

FcεRI can also bind galectin-3; a known regulator of immune responses through carbohydrate interactions, resulting in crosslinking of receptor bound IgE or FcεR and activation of mast cells and basophils (Liu 2005). Galectin-3 is a lectin that specifically binds β-galactose oligosaccharides and is a low affinity IgE receptor, which can be found extracellular, cytoplasmic or nuclear where it can regulate IgE mediated immune responses with anti-allergic activities and also inflammatory mediator release (Chen et al. 2006; Frigeri et al. 1993). It has also been shown that galectin-9 specifically binds IgE via a lactose residue and prevents IgE-immune complex formation and mast cell degranulation and reduction of allergic responses (Niki et al. 2009).

3.2 FcεRII: The Low Affinity Fc Epsilon Receptor

FcεRII (CD23) is the low affinity IgE receptor of B-cells, macrophages and dendritic cells where it regulates the production of IgE and participates in the elimination of intracellular pathogens (Maeda et al. 1992; Vouldoukis et al. 1995). Unlike other FcRs CD23 is a C-type lectin. It is present either as a membrane bound

trimer or can be released from the cell surface by proteases such as ADAM10 to yield soluble forms (sCD23) (Dhaliwal et al. 2012). Alone, the soluble form of CD23 has a low affinity for IgE compared to FcεRI but when complexed as a soluble trimer can significantly increase the avidity and binding levels. FcεRII has a distinct role in IgE regulation due to its expression on B-cells and it is thought to contribute to both positive and negative regulation and differentiation of B-cells. Little is known about FcεRII glycosylation. There is a single N-linked glycosylation site at Asn63 and potential O-linked sites. It was shown that a 45 kDa component of CD23 was a glycoprotein containing a complex N-linked carbohydrate, several O-linked carbohydrates, and several sialic acid residues (Letellier et al. 1988). In addition, Letellier et al. show, by the use of N-glycosylation inhibitors, that the production of IgE-binding factors, derived from proteolytic cleavage of FcεRII are increased in the absence of N-glycosylation, indicating that degradation of FcεRII is inhibited in the presence of N-glycans. Safrati et al. show that N-glycosylation controlled the activity of sCD23 and when inhibited with tunicamycin switched IgE binding factors from IgE potentiators to IgE suppressors (Sarfati et al. 1984, 1992).

4 IgG-Mediated Immunity

Immunoglobulin G (IgG) antibodies are critical glycoprotein components of the immune system which detect invading microorganisms and tumor-associated antigens and communicate this information to the innate and adaptive immune systems. The IgG antibody is the most abundant antibody isotype found in serum where it comprises approximately 75 % of total serum immunoglobulins. IgG antibodies are further divided into different subclasses (IgG1, IgG2, IgG3, IgG4) based on their abundance in serum. The subclasses differ particularly in the structure of their hinge regions. Each forms a typical Y-shaped structure composed of two heavy chains and two light chains organized into two Fab regions that bind antigen and an Fc region that is recognized by the Fc receptor specific for IgG (FcγR) (see Fig. 4a). Interaction with the FcγR on innate immune cells allows the IgG molecule to communicate with the immune system and induce immune effector functions such as ADCC, complement-dependent cytotoxicity (CDC) and phagocytosis following opsonisation of target antigens (see Fig. 4b).

4.1 The Role of N-Glycans in IgG Biology

Like other immunoglobulins, IgG is glycosylated and the addition of N-linked glycans to two conserved asparagine residues in each of the C_H2 domains of the Fc region is critical for the structure and function of the antibody (Krapp et al. 2003; Arnold et al. 2007) (see Fig. 4a). In addition to glycosylation of the two canonical Asn297 residues in the Fc region, N-linked glycans are present in some 20 % of

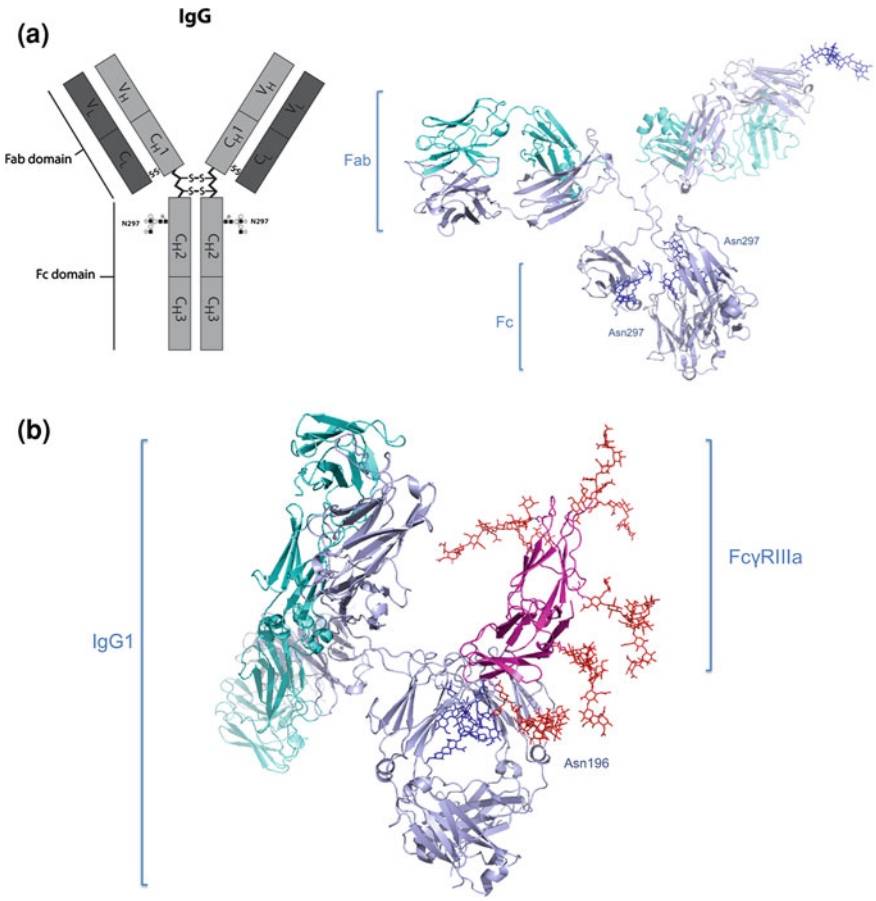


Fig. 4 *Immunoglobulin G, glycosylation and the FcγR complex*. **a** Schematic and molecular model representation of human IgG1 showing glycan sites and glycan site occupancy (shown in *blue*). Shown is Fc glycosylation on conserved Asn297 residues containing bi-antennary glycans. The bi-antennary glycans found on these conserved canonical asparagine residues are believed to maintain the structural integrity of the Fc region of the antibody for binding and communication with the FcγR and also to participate in carbohydrate–carbohydrate interactions with the N-glycans of the receptor. Fab glycosylation found in 20 % of IgG1 in the form of hyper-galactosylated, fucosylated and extensively sialylated bi-antennary glycoforms is also shown. **b** The IgG–FcγRIIIa complex showing Fc glycans on Asn297 of human IgG1 (*blue*) and receptor glycans on Asn196 of human FcγRIIIa (*red*). Potential carbohydrate–carbohydrate interactions are involved at the binding interface between antibody and receptor and participate in the interaction, as has been shown for the soluble domain of FcγRIIIa and afucosylated IgG. The model is based on 1e4k in the protein data bank

the Fab regions in human IgG (see Fig. 4a). These glycans include bi-antennary structures that are hyper-galactosylated, fucosylated, and extensively sialylated (Mimura et al. 2007). N-linked glycans described in the Fc region of polyclonal

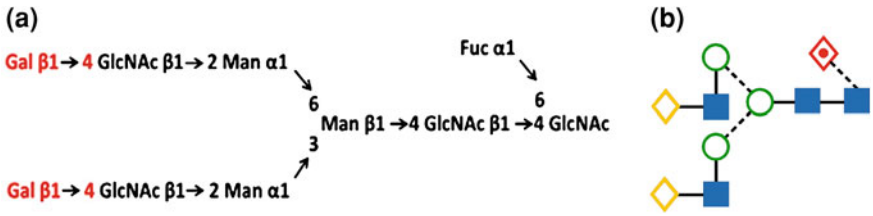


Fig. 5 *The most common core-fucosylated bi-antennary glycan found in the Fc region of IgG.* **a** Schematic representation of IgG bi-antennary glycan. **b** Cartoon representation of IgG bi-antennary glycan. Symbols and linkage positions correspond to those shown in the schematic representation. Human serum IgG has been found with up to 36 different N-glycan structures, predominantly in the form of core-fucosylated bi-antennary structures, as shown in **a** and **b**. Typically the N-glycan contains zero (IgG G0), one (IgG G1) or two (IgG G2) galactose residues (shown in *red* in the schematic representation). The glycan shown in **a** and **b** is the fully galactosylated/hypergalactosylated (IgG G2) form, however, in serum IgG1 it is the IgG G1 form which predominates. In certain conditions such as rheumatoid arthritis IgG G0 forms have been been detected in higher abundance which can effect downstream FcγR binding and immune function

IgG typically display heterogeneity and are composed of a core heptasaccharide defined by a chitobiose core ($Man_1GlcNAc_2$) with branching mannose residues in both α(1–3) and α(1–6) linkage. Further processing by the addition of GlcNAc residues in β(1–2) linkage, fucose, galactose, sialic acid, and bi-secting GlcNAc forms the mature bi-antennary IgG Fc N-glycan (see Fig. 5). In total, human serum IgG has been shown to contain up to 36 different N-glycan structures (Wormald et al. 1997). Unusual glycosylation has also been observed on IgG in the form of galactose linked β(1–4) to bisecting GlcNAc (Harvey et al. 2008). Microheterogeneity is also observed, where glycans on identical but opposing C_H2 domains have been shown to vary (Jefferis et al. 1990; Masuda et al. 2000). Thus, in an otherwise symmetrical molecule, asymmetry can be introduced by differential N-linked glycosylation at individual Asn297 sites.

Glycosylation of IgG plays a number of important roles. The α(1–6) arm of the bi-antennary glycan extends along the hydrophobic face of the C_H2 amino acid backbone where the polar nature of the carbohydrate protects the underlying hydrophobic polypeptide (Lund et al. 1995). The α(1–3) arm of the glycan extends toward the interstitial space formed by the C_H2–C_H3 dimer. Here, the N-linked glycans on opposite Asn297 residues interact and maintain the conformation of the Fc domain and changes in Fc glycosylation can alter the Fc conformation and affect the binding to Fc receptors (Jefferis et al. 1998; Krapp et al. 2003; Radaev and Sun 2001). Several lines of investigation have clearly indicated that changes to Fc glycosylation affect binding affinity and loss of Fc glycosylation abrogates binding altogether. Absence of glycosylation disrupts the structural integrity of the Fc region, which is required for optimal binding to the Fc receptor. In addition to maintenance of Fc structural integrity N-glycan monosaccharides play additional roles. For instance, IgG glycoforms lacking galactose (IgG G0) bind to MBL to

activate complement (Malhotra et al. 1995), galactose residues are involved in placental transport of IgG and IgG galactosylation is increased in pregnant women (Simister 2003). Sialic acids have been implicated in the anti-inflammatory effects of intravenous IgG (IVIg) and desialylation abrogates this property in knockout mice (Kaneko et al. 2006). However, recently the mechanism of IVIg has been questioned and it has been shown that the interaction between DC-SIGN and IgG is not glycan dependent where Yu et al. challenge the idea that DC-SIGN directly binds to IVIG, suggesting that DC-SIGN is unlikely to be the receptor. (Yu et al. 2013).

To fucosylate or not to fucoyslate?
Core-fucosylation of IgG has been the subject of intensive research ever since afucosylated IgG was shown to exhibit improved binding to activating Fc gamma receptor and enhanced ADCC (Shields et al. 2002; Okazaki et al. 2004; Mori et al. 2004; Yamane-Ohnuki et al. 2004; Kanda et al. 2007; Natsume et al. 2005; Iida et al. 2006; Satoh et al. 2006; Ferrara et al. 2006a, b). With such a significant impact on immune effector function, the biopharmaceutical industry has vigorously pursued the generation of afucosylated monoclonal antibody therapeutics. Genetic elimination of *fucosyltransferase* 8 (*FUT* 8) in Chinese hamster ovary cells has been a successful approach for the prevention of core fucosylated IgG (Yamane-Ohnuki et al. 2004). Alternative methods such as the overexpression of β(1,4)-N-acetylglucosaminyltransferase III (*GnT*III) as a means of eliminating the substrate for *fucosyltransferase* and modification of enzymes involved in the N-linked glycan biosynthetic pathway have also been developed with significant success (Umana et al. 1999; von Horsten et al. 2010; Zhou et al. 2008). The majority of approved mAb therapeutics that target ADCC have been engineered for use in anti-cancer therapies where the mAb targets a cell surface receptor associated with a particular tumor. In this situation, the absence of core α(1–6)-linked fucose has been associated with improved cytolytic activity and efficacy of the mAb (Shields et al. 2002; Nimmerjahn and Ravetch 2005). This has been most commonly studied in B-cell lymphomas where the monoclonal antibody (rituximab) recognizes the cell surface receptor CD20 on B-cells and induces natural killer (NK) mediated ADCC. Another very successful anti-tumorigenic mAb is herceptin, which targets the Her2 receptor expressed on some breast cancer tumors. Due to the remarkable success of this approach, industry has moved toward generation of cell lines with genetic modifications that directly influence core fucosylation.

4.2 Role of Glycosylation in FcγR Biology

IgG antibodies communicate with the immune system via interaction with the plasma membrane bound Fcγ receptors found on innate immune cells. FcγRs are typically single pass transmembrane glycoproteins belonging to the immunoglobulin-like superfamily (IgSF), defined by characteristic domains based on two

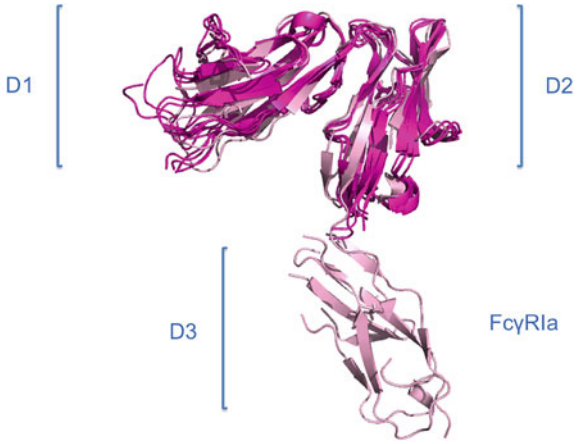


Fig. 6 *FcγRs are structurally homologous with an extra D3 domain in FcγRIa*. Structural alignment of human FcγRs shows structural homology between family members (FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb). Glycosylation site number varies significantly between two and seven sites between family members with two extra N-linked sites in the D3 domain of FcγRIa. This extra domain of FcγRIa is responsible for its high affinity nature and the two N-liked sites within this domain may also contribute to the high affinity nature of the receptor

sheets of antiparallel β-strands (Williams and Barclay 1988) (see Fig. 6). The FcγR family is broadly categorized into three groups: FcγRI, FcγRII, and FcγRIII. Variability is observed within each group at the genomic, transcriptomic, and proteomic level where multiple genes, transcripts, and polymorphic variants all contribute to FcγR complexity. Functionally, the individual FcγR groups coordinate distinct functions. The FcγRI family are characterized by their high affinity ($10^9\ M^{-1}$) for IgG while the remaining families are low affinity ($10^6\ M^{-1}$) receptors. Extensive variability is also observed in the cytoplasmic domain of each receptor, where signaling can be either through association with the γ-chain dimer (for FcγRI and FcγRIII) or through integrated signaling motifs (for FcγRII). Signaling occurs through either an immunotyrosine-like activation motif (ITAM) for activating FcγRI, FcγRIIa, FcγRIII or an immunotyrosine-like inhibitory motif (ITIM) for inhibitory FcγRIIb.

Emerging from the shadows of IgG and Fc glycosylation is the growing complexity of Fcγ receptor biology and the role of these receptors in health and disease. While intensive efforts have been directed towards manipulating IgG glycosylation for enhanced biological activity, Fcγ receptors have traditionally been used in a limited role to investigate monoclonal antibody safety and efficacy. However, this is no longer the case and the complexity of Fcγ receptor biology and the role of glycosylation is becoming increasingly evident. Research into the glycosylation of Fcγ receptors was initiated over three decades ago, however it is only now that are we beginning to appreciate the precise function of the carbohydrate moieties in relation to IgG binding and associated biological activity. Surprisingly, due to the

difficulty in obtaining sufficient amounts of material for analysis very little is known about the glycosylation of Fcγ receptors as they occur in their natural environments, bound to the cell surface membranes of lymphocytes such as neutrophils, monocytes, macrophages, B-cells and NK cells. Each Fcγ receptor contains at least two N-linked glycosylation sites with up to seven potential sites observed in the high affinity FcγRIa. The vast majority of glycan data exists for FcγRIIIa. We now have structural information which shows how human FcγRIIIa associated sugars are involved in carbohydrate–carbohydrate interactions with the Asn297-linked sugars of afucosylated human IgG1 (Ferrara et al. 2011). Glycosylation data have also been reported for recombinant soluble human FcγRIIa and FcγRIIIa (Takahashi et al. 1998, 2002) and recently glycan data have been described for the family of recombinant receptors: FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb expressed in the murine cell line NS0 (Cosgrave et al. 2013). Interestingly, aglycosylated Fcγ receptors expressed in *E. coli* and purified from inclusion bodies still appear to retain the ability to bind IgG (Sondermann and Jacob 1999; Sondermann et al. 2000; Maenaka et al. 2001) which brings into question the exact nature of Fcγ receptor glycosylation in IgG binding.

In the light of the emerging importance of Fcγ receptor glycosylation in IgG function there is a critical need for detailed knowledge of the glycosylation state of natural FcγRs of immune cells. One likely possibility is that an Fcγ receptor demonstrates differential glycosylation depending on the cell type. Ample evidence of this was provided by Edberg et al. (1990); (Edberg and Kimberly 1997). Knowledge that Fcγ receptor glycosylation changes depending on the immune cell will help to understand the associated immunological outcomes. Following cytokine-mediated immune activation, innate effector cells are known to up-regulate Fcγ receptors as has been shown with IFN-γ and monocytes (Fleit and Kobasiuk 1991; Fairchild et al. 1996). In this case, changes in Fcγ receptor glycosylation due to immune cell activation may shed light on glycoform preferences for improved IgG binding. Resting or inactive innate effector cells may glycosylate Fcγ receptors in a manner that promotes dissociation as a mechanism to avoid inadvertent activation. Upon stimulation with appropriate mediators, Fcγ receptor glycosylation may change to recruit and retain circulating antibodies. Rapid upregulation of Fcγ receptor expression is likely to have an impact on glycosylation. The question is does this "activated" Fcγ receptor glycoform have an improved or dampened interaction with IgG or does a change in FcγR glycosylation disrupt or modify cell surface interactions? It is therefore of paramount importance to characterize natural Fcγ receptor glycosylation. Of equal importance is to learn how immune responses alter Fcγ receptor glycosylation. Immune activation may induce the expression of Fcγ receptors with improved binding, possibly through altered glycosylation. The glycosylation may also promote engagement of other molecules that work to extend the presence of receptors on the cell surface. An example of this could be the role of galectin in cellular distribution and trafficking of the epidermal growth factor receptor (EGFR) (Merlin et al. 2011; Liu et al. 2012). During an immune response, the preferable scenario for the innate effector cell is to express the Fcγ receptor on the cell surface to scavenge for IgGs. In fact, it is

most likely that glycosylation changes to promote cross-linking of antibodies. If binding improves and dissociation is reduced, then Fcγ receptors will become occupied by antibodies which may not be involved with a specific immune response. Perhaps more likely is a situation in which clustering of the Fcγ receptors is improved as this will more readily lead to immune cell activation. Another possibility is that glycosylation of inhibitory FcγRs may change to reduce interaction with IgGs. This would effectively eliminate inhibitory Fcγ receptors from cluster points and in the process alter the A/I ratio and induce cell activation. Alternatively, situations of immune tolerance may see glycosylation of inhibitory FcγRIIb change to promote binding and retention/clustering of autoantibodies.

4.3 FcγRI (CD64)

The FcγRI family is the high affinity Fcγ receptor and is structurally distinct from FcγRII and FcγRIII by the presence of a third extracellular D3 domain (see Fig. 6). This additional domain has been shown to account for the high affinity property of the FcγRI family (Harrison and Allen 1998; Allen and Seed 1989; Lu et al. 2011). FcγRIa is primarily expressed by monocytes and macrophages although myeloid cell lines induced with cytokines such as IFN-γ are capable of FcγRI expression (Fairchild et al. 1996). The FcγRI family is the only Fcγ receptor class capable of appreciably binding monomeric IgG in vivo (Bruhns et al. 2009). Similar to the FcγRIII family, FcγRI requires the interaction with the γ-chain homodimer for cell surface expression and signal transduction (van Vugt et al. 1996; Ernst et al. 1993).

FcγRIa is a 374 amino acid protein with seven potential N-glycosylation sites. In the absence of post-translational modifications, FcγRI is a 42.6 kDa single pass transmembrane protein with short cytoplasmic region. At present, little is known about the site occupancy of these N-glycosylation sites or the carbohydrates that reside there. A study of neutrophil FcγRIa did, however, reveal that the receptor is heavily glycosylated with approximately 30 % of its weight due to carbohydrates. There are a larger number of glycosylation sites associated with this receptor in comparison to other members of the FcγR family, largely due to the extra D3 domain, which contains two putative N-linked sites. The crystal structure of soluble extracellular FcγRIa observed and modeled N-glycans at six asparagine residues (Asn59, Asn78, Asn152, Asn159, Asn163, Asn195) but little information as to the exact nature of the carbohydrates exists (Lu et al. 2011). As this is the high affinity receptor for IgG it is likely that the glycans that reside on the N-glycosylation sites in this extra domain will play a role in the interaction with IgG. Further studies are needed to investigate this assumption. Recombinant FcγRIa expressed in murine cells was heavily glycosylated with multiantennary structures, core and outer arm fucosylation with predominantly neutral and mono-sialylated glycans and smaller amounts of di- and tri-sialylated structures. In addition, large amounts of the immunogenic carbohydrates gal-α(1,3)-gal(α-gal) and N-glycolylneuraminic acid (NGNA) were discovered (Cosgrave et al. 2013).

4.4 FcγRII (CD32)

The FcγRII family of Fc receptors are structurally and functionally distinct from FcγRI and FcγRIII primarily due to an integrated signaling motif located in the C-terminal cytoplasmic region of the protein. FcγRII is categorized into FcγRIIa, FcγRIIb, and FcγRIIc, where six transcripts in total have been described (Warmerdam et al. 1993; Qiu et al. 1990). Two transcripts arise from the FcγRIIa gene (FcγRIIa1 and FcγRIIa2), where FcγRIIa2 is believed to be a soluble form of FcγRIIa. Three separate transcripts arise from FcγRIIb (FcγRIIb1, FcγRIIb2, and FcγRIIb3) and only one arises from FcγRIIc. With the exception of FcγRIIa2, all transcripts give rise to single pass transmembrane glycoproteins. Due to the significant differences in biological function between FcγRIIa and FcγRIIb, each will be discussed separately.

4.4.1 FcγRIIa

FcγRIIa is a 40 kDa single pass transmembrane glycoprotein capable of potent inflammatory response activation. The FcγRIIa family is comprised of FcγRIIa1 and FcγRIIa2, where the latter is believed to represent a soluble form of the receptor (van den Herik-Oudijk et al. 1994). Structurally, FcγRIIa demonstrates significant homology to FcγRIIb in the extracellular domain with approximately 92 % amino acid identity, however, significant differences are present in both the transmembrane and cytoplasmic domains where FcγRIIa contains an activatory ITAM motif and FcγRIIb has an inhibitory ITIM motif. FcγRIIa is the most widely expressed FcγR, found on neutrophils, eosinophils, B lymphocytes, platelets (Rosenfeld et al. 1985), mast cells (Sylvestre and Ravetch 1996), Langerhans cells (Schmitt et al. 1990), placental endothelial cells (Sedmak et al. 1991), and dendritic cells (Sallusto and Lanzavecchia 1994).

The low affinity FcγRIIa is a 317 amino acid protein with two N-linked glycosylation sites. Glycosylation of this receptor expressed in insect cells was reported to be uncharged suggesting no sialylation of N-glycans (Sondermann et al. 1999). Glycosylation was also reported to have no effect on binding of IgG. Powell et al. reported that expression of FcγRIIa in CHO cells was found to display micro-heterogeneity resulting from extensive sialylation on both N-linked sites while the same receptor derived from baculovirus infected inset cells contained only simple Man_3 glycan structures (Powell et al. 1999). FcγRIIa expressed in NS0 cells contained complex glycosylation with multiantennary structures, extensive core and outer-arm fucosylation and immunogenic α-gal and NGNA, similar to FcγRIa (Cosgrave et al. 2013). However, little is known as to the site-occupancy of the N-linked sites or the function of FcγRIIa glycosylation. In addition, there is no information currently available relating to natural FcγRIIa glycosylation.

4.4.2 FcγRIIb

FcγRIIb is a 34 kDa (peptide mass) single pass membrane glycoprotein and is the most widely expressed FcγR. In contrast to other FcγRs, FcγRIIb contains an inhibitory signaling motif (ITIM) in its cytoplasmic tail. FcγRIIb has a rare polymorphism resulting from an SNP, causing an amino acid change at position 232 to an isoleucine (Li et al. 2003). The functional consequence of this variation is the failure of FcγRIIb to associate in lipid rafts, thereby losing the ability to dampen immune cell activation through activatory Fc receptors. This has been shown in SLE (Floto et al. 2005; Kono et al. 2005). Two isoforms of FcγRIIb, referred to as FcγRIIb-1 and FcγRIIb-2, exist as a result of alternative splicing and are expressed differentially depending on the cell type, FcγRIIb-1 is exclusively expressed on B-cells and FcγRIIb-2 is expressed on all other FcγR cell types, except NK cells (Nimmerjahn and Ravetch 2008). FcγRIIb is a 310 amino acid protein containing three potential N-glycosylation sites. Similar to other FcγRs little information exists as to the site occupancy or nature of glycosylation at these sites. To our knowledge, the only FcγRIIb glycan data come from a recombinant form expressed in murine cells. In common with other FcγRs expressed from this source FcγRIIb contained multiantennary structures, limited sialylation and extensive immunogenic carbohydrate epitopes (Cosgrave et al. 2013). No information is currently available relating to natural FcγRIIb glycosylation.

4.4.3 FcγRIIc

FcγRIIc is believed to have occurred from a unequal genetic cross-over event that effectively brought the 5′ region of FcγRIIb to the 3′ region of FcγRIIa (Warmerdam et al. 1993). Interestingly, functional FcγRIIc has been identified on NK cells, where four separate mRNA transcripts were isolated from NK cells (Metes et al. 1998). Four distinct isoforms of the protein exist with molecular weights ranging from 25.9 to 35.5 kDa and 234–323 amino acids. There are also three potential N-linked sites although no information exists as to the glycosylation of this protein.

4.5 FcγRIII (CD16)

4.5.1 FcγRIIIa

The biology of the FcγRIII family has been the subject of intensive focus, largely due to the role of activating FcγRIIIa in NK cell activity and ADCC. FcγRIIIa demonstrates unique structural characteristics by requiring association with the γ-chain dimer (similar to FcγRI) and further association with the ζ-chain when expressed by NK cells, where it has been demonstrated that FcγRIII expression requires association with an accessory chain (Kinet 1992; Hibbs et al. 1989).

FcγRIIIa expresses two immunoglobulin-like C2-type domains (similar to FcγRII). Perhaps most interesting is the diversity within the two FcγRIII family members: FcγRIIIa and FcγRIIIb. The extracellular domains of both receptors are nearly homologous with the exception of six amino acids. Interestingly, a key residue at position 203 influences whether FcγRIII becomes a single pass membrane bound receptor (Phe203) or membrane-associated through a GPI-anchor (Ser203). FcγRIIIa is widely distributed across the hematopoietic system, found on cells such as monocytes and NK cells but has different properties depending on the cell such as altered sensitivity to trypsin (Perussia and Ravetch 1991). There is emerging evidence that FcγRIIIa is expressed by γδT cells and contributes to disease progression of multiple sclerosis by ADCC dependent means (Chen and Freedman 2008), although FcγR expression in any form by T-lymphocytes is a widely debated subject.

Most of our knowledge of FcγR glycobiology comes from the low affinity activating FcγRIIIa of monocytes and NK cells. FcγRIIIa is a 254 amino acid protein with five potential glycosylation sites. Early seminal work on FcγRIIIa glycosylation was performed by Edberg et al. who showed that cell type specific glycoforms of the receptor existed that exhibited differential ligand binding (Edberg et al. 1989, 1990). Using lectin-binding experiments FcγRIIIa from NK cells was shown to display N-glycans of predominantly oligomannose type while the same receptor expressed by monocytes displayed complex type oligosaccharides. Furthermore, removal of high mannose oligosaccharides from FcγRIIIa resulted in a change in the binding of aggregated IgG (Kimberly et al. 1989). Edberg et al. also showed that the receptor expressed by monocytes was distinct from that displayed by NK cells (Edberg and Kimberly 1997) and that FcγRIIIa on NK cells has the ability to bind monomeric IgG whereas FcγRIIIa on monocytes lacks this ability. Interestingly, at physiological IgG concentrations, FcγRIIIa is saturated but can more easily be displaced by competing molecules (Mab3G8) than the NK cell equivalent. This data suggests that monocytes utilize FcγRIIIa differently than NK cells and it is convincing to attribute this difference in FcγRIIIa and IgG binding to glycosylation. Interestingly, these discoveries suggest that differential glycosylation potentially plays a role in influencing Fc-FcγR affinity, and therefore immune cell activation.

More recently, a huge amount of proteomic and glycomic information has been described for recombinant forms of FcγRIIIa and importantly the N-linked glycan site occupancy of the receptor. Two N-linked sites of FcγRIIIa directly regulate the binding of IgG. Mutagenesis of the N-linked glycan at Asn162 significantly reduced the binding of IgG1, demonstrating a dependence of this interaction on FcγRIIIa glycosylation at this particular site (Ferrara et al. 2006b) (see Fig. 4b). In addition, the N-linked glycan at position Asn45 was shown to have an inhibitory role in IgG binding, where removal of the N-linked site at this position dramatically improved the binding of IgG1 (Shibata-Koyama et al. 2009). It has been proposed that the glycan at position 45 within the D1 domain of FcγRIIIa acts to stabilize the D2 domain and protect it from intracellular proteolytic degradation. As a consequence, the Asn45 occupied site exhibits a reduced binding affinity of afucosylated IgG1 to FcγRIIIa. A site-specific analysis of recombinant FcγRIIIa

expressed in HEK293 and CHO cells was performed by Zeck et al., who showed that specific types of glycans were found on particular N-linked sites including multi-antennary structures, sialylation, and core-fucosylation. Significant characteristics of the expression host were reported, such as LacdiNAc (HexNAc–HexNAc) structures from HEK293 cells, and the authors also report on the effect on IgG binding of larger HEK293 glycans compared to smaller CHO glycans located on the same sites (Zeck et al. 2011). Although these are non-natural sources it does provide evidence for site-specific glycans, which may mediate the IgG interaction. Recently, it was shown at the molecular level that a unique carbohydrate–carbohydrate interface was involved in the interaction between afucosylated IgG1 and FcγRIIIa, which explained the increased affinity for afucosylated antibody through lack of steric hindrance, which is present for core-fucosylated forms (Ferrara et al. 2011) (see Fig. 4b). Glycosylation of recombinant FcγRIIIa from NS0 cells has also been described (Cosgrave et al. 2013).

4.5.2 FcγRIIIb

FcγRIIIb is a GPI-anchored protein present on macrophages and neutrophils and has no currently known cytosolic signaling domain. FcγRIIIb activation and crosslinking on neutrophils have been shown to cause neutrophil degranulation and generation of reactive oxygen intermediates, which can in turn increase FcγRIIa activation by increasing avidity and efficiency (Salmon et al. 1995). These cooperative FcγR effects show the potential for synergistic FcγR activation. In addition, FcγRIIIb has been shown to associate with complement receptor 3 (CR3) in fibroblast transfectants, highlighting the potential for inter-receptor interactions and activation (Poo et al. 1995).

More information is available for FcγRIIIb glycosylation than for the other FcγRs, with the exception of FcγRIIIa. FcγRIIIb expressed on neutrophils is a 233 amino acid protein with six potential N-linked sites that contain oligomannose glycans that influence the properties of the IgG-receptor binding interactions (Kimberly et al. 1989). The apparent molecular weight of FcγRIIIb varies from 50 kDa to 80 kDa due to differential glycosylation (Ravetch and Perussia 1989; Scallon et al. 1989; Edberg et al. 1989; Huizinga et al. 1990). Furthermore, the NA polymorphic variations (NA1 and NA2) of FcγRIIIb are also believed to demonstrate heterogeneity in glycosylation (Kimberly et al. 1989). Galon et al. (1997) expressed soluble human FcγRIIIb in both *E. coli* and baby hamster kidney (BHK) cells and observed a decrease in binding affinity due to the presence of N-linked glycans (Galon et al. 1997). However, N-linked carbohydrates were not determined. N-linked glycans were reported for FcγRIIIb from BHK cells by Takahashi et al., who described multi-antennary structures containing up to four GlcNAc residues and minimal sialic acid capping (Takahashi et al. 2002). Glycosylation of recombinant FcγRIIIb from murine cells was also described and similar to BHK cells consisted of multiantennary structures and incomplete sialic acid capping and similar to other receptors expressed in murine cells with extensive immunogenic α-

gal and N-glycolylneuraminic acid carbohydrate epitopes (Cosgrave et al. 2013). Site-specific and natural glycosylation information is currently not available for FcγRIIIb.

5 FcRn: The Neonatal Fc Receptor

The neonatal Fc receptor (FcRn) is unlike the other Fc receptors and is more similar in structure to MHC class I. The mature FcRn receptor consists of a complex of two subunits: *p*51 and *p*14 (β2-microglobulin) and forms an MHC class-l-like heterodimer (Simister and Mostov 1989; Burmeister et al. 1994a, b). The receptor is located in numerous tissues and organs, such as the vascular endothelium and myeloid derived antigen presenting cells (APC), such as monocytes, macrophages, and dendritic cells where it functions in the transfer of IgG from mother to fetus (Simister 2003; Simister and Mostov 1989). This process is critical for transferring humeral immunity from mother to child. The receptor is found in the placenta to help facilitate this critical process. FcRn is also involved in recycling of IgG and regulates its serum half-life, maintaining its serum concentration and regulating IgG homeostasis (Ward et al. 2003; Antohe et al. 2001; Roopenian and Akilesh 2007). Only at acidic pH, such as in endocytic vacuoles will FcRn bind IgG, releasing it at physiological pH. By recycling IgG from acidic endosomes and releasing it back at the cell surface FcRn increases the half-life of IgG, which is needed at high serum concentration to fight infection. FcRn can also bind IgG immune complexes resulting in transport to lysosomes in dendritic cells for antigen presentation (Qiao et al. 2008; Yoshida et al. 2004).

FcRn contains a single N-linked glycosylation site in the α2 domain in the 365 amino acid large subunit (*p*51). Human and rat FcRn differ by the number of N-glycan sites, rat FcRn has four sites in the α1, α2, α3 domains, whereas the human receptor has a single glycosylation site (Asn125). The crystal structure of rat FcRn revealed that the carbohydrate on Asn128 is at the interaction site for IgG and is possibly involved in the IgG interaction (Burmeister et al. 1994a). The glycan moiety makes contact with the Fc region of the IgG molecule and has been postulated to help stabilize the complex formation through a carbohydrate interaction with IgG (Vaughn and Bjorkman 1998). Recently, information became available on the glycosylation of human and rat FcRn expressed in canine MDCK II cells which showed that the receptor from both sources contained both oligomannose and complex glycans and that following EndoH removal of high mannose structures the size of the glycoprotein was reduced from 52 to 37 kDa (Kuo et al. 2009). However, the exact monosaccharide compositions were not reported. Kuo et al. report increased surface expression of FcRn following the introduction of additional N-glycan sites to the protein and show that carbohydrates are involved in the direction of IgG transport by FcRn and that direction of transport mediated by human FcRn is reversed by the addition of N-glycan sites, making it more like the rodent form.

6 Closing Remarks

Immunoglobulins and Fc receptors are complex glycoproteins and key components of both the innate and adaptive immune systems. Glycosylation of immunoglobulins (IgA, IgD, IgE, IgG) has been well studied and the sugars attached to the conserved asparagine residues in the Fc region are undeniably critical to the antibodies function and its communication with the immune system. Exciting discoveries that show individual monosaccharide residues of Fc N-glycans modulate binding of antibodies to immunoglobulin receptors on immune effector cells and drive particular immune responses is intriguing and has led to an explosion of research into the influence of glycosylation in antibody mediated responses. Core-fucosylation and its influence on activating FcγRs and antibody mediated anti-tumor activities have led to huge industrial interest into antibody glycosylation. Molecular mechanisms at atomic level for such interactions are now being elucidated. Therapeutic antibodies have had remarkable success in the treatment of many diseases, from cancer to autoimmune disorders and glycosylation is integral and crucial to past and future successes. Emerging now from the shadows of antibody glycosylation are the Fc receptors. It is true to say that we do not yet fully understand the role of these critical receptors in health and disease, particularly the role of glycosylation. Glycosylation of Fc receptors is far more complex than for antibodies and we have very little information as to how these receptors are glycosylated in their natural environment, by cells such as macrophages, B-cells and NK cells. Even though significant information on how these receptors are glycosylated was revealed more than 30 years ago it is fair to say that we are just beginning to understand the true importance of receptor glycosylation in the interaction with antibody and downstream immunological response. It is intriguing to consider the role that sugars could play when a particular immune cell encounters antibody immune complexes and the potential control mechanisms mediated by carbohydrates. It remains a challenge for the future to gain a more complete understanding of the glycosylation of antibody Fc receptors in both activated and dormant states and in both healthy and disease situations. Fc receptors for IgA at mucosal surfaces, for IgE in allergic reactions and defense against parasites, IgG in infection and anti-tumor activities and other antibody-mediated responses is becoming more and more critical to successful future clinical therapies and successes.

References

Allen JM, Seed B (1989) Isolation and expression of functional high-affinity Fc receptor complementary DNAs. Science 243:378–381

Antohe F, Radulescu L, Gafencu A, Ghetie V, Simionescu M (2001) Expression of functionally active FcRn and the differentiated bidirectional transport of IgG in human placental endothelial cells. Hum Immunol 62:93–105

Ariizumi K, Shen GL, Shikano S, Xu S, Ritter R, Kumamoto T 3rd, Edelbaum D, Morita A, Bergstresser PR, Takashima A (2000) Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. J Biol Chem 275:20157–20167

Arnold JN, Radcliffe CM, Wormald MR, Royle L, Harvey DJ, Crispin M, Dwek RA, Sim RB, Rudd PM (2004) The glycosylation of human serum IgD and IgE and the accessibility of identified oligomannose structures for interaction with mannan-binding lectin. J Immunol 173:6831–6840

Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA (2007) The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 25:21–50

Baenziger J, Kornfeld S (1974a) Structure of the carbohydrate units of IgA1 immunoglobulin. I. Composition, glycopeptide isolation, and structure of the asparagine-linked oligosaccharide units. J Biol Chem 249:7260–7269

Baenziger J, Kornfeld S (1974b) Structure of the carbohydrate units of IgA1 immunoglobulin. II. Structure of the O-glycosidically linked oligosaccharide units. J Biol Chem 249:7270–7281

Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, et al (1994a) Galectins: a family of animal beta-galactoside-binding lectins. Cell 76:597–598

Barondes SH, Cooper DN, Gitt MA, Leffler H (1994b) Galectins. Structure and function of a large family of animal lectins. J Biol Chem 269:20807–20810

Basu M, Hakimi J, Dharm E, Kondas JA, Tsien WH, Pilson RS, Lin P, Gilfillan A, Haring P, Braswell EH, et al (1993) Purification and characterization of human recombinant IgE-Fc fragments that bind to the human high affinity IgE receptor. J Biol Chem 268:13118–13127

Bjorklund JE, Karlsson T, Magnusson CG (1999) N-glycosylation influences epitope expression and receptor binding structures in human IgE. Mol Immunol 36:213–221

Boehm MK, Woof JM, Kerr MA, Perkins SJ (1999) The Fab and Fc fragments of IgA1 exhibit a different arrangement from that in IgG: a study by X-ray and neutron solution scattering and homology modelling. J Mol Biol 286:1421–1447

Brambell FW (1966) The transmission of immunity from mother to young and the catabolism of immunoglobulins. Lancet 2:1087–1093

Brown GD, Gordon S (2001) Immune recognition. A new receptor for beta-glucans. Nature 413:36–37

Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, Daeron M (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113:3716–3725

Burmeister WP, Gastinel LN, Simister NE, Blum ML, Bjorkman PJ (1994a) Crystal structure at 2.2 A resolution of the MHC-related neonatal Fc receptor. Nature 372:336–343

Burmeister WP, Huber AH, Bjorkman PJ (1994b) Crystal structure of the complex of rat neonatal Fc receptor with Fc. Nature 372:379–383

Chen HY, Sharma BB, Yu L, Zuberi R, Weng IC, Kawakami Y, Kawakami T, Hsu DK, Liu FT (2006) Role of galectin-3 in mast cell functions: galectin-3-deficient mast cells exhibit impaired mediator release and defective JNK expression. J Immunol 177:4991–4997

Chen Z, Freedman MS (2008) CD16 + gammadelta T cells mediate antibody dependent cellular cytotoxicity: potential mechanism in the pathogenesis of multiple sclerosis. Clin Immunol 128:219–227

Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F (1999) Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem 274:10689–10692

Cosgrave EF, Struwe WB, Hayes JM, Harvey DJ, Wormald MR, Rudd PM (2013) N-Linked glycan structures of the human Fcgamma receptors produced in NS0 cells. J Proteome Res 12(8):3721

Dhaliwal B, Yuan D, Pang MO, Henry AJ, Cain K, Oxbrow A, Fabiane SM, Beavil AJ, McDonnell JM, Gould HJ, Sutton BJ (2012) Crystal structure of IgE bound to its B-cell receptor CD23 reveals a mechanism of reciprocal allosteric inhibition with high affinity receptor FcepsilonRI. Proc Natl Acad Sci U S A 109:12686–12691

Dorrington KJ, Bennich HH (1978) Structure-function relationships in human immunoglobulin E. Immunol Rev 41:3–25

Edberg JC, Barinsky M, Redecha PB, Salmon JE, Kimberly RP (1990) Fc gamma RIII expressed on cultured monocytes is a N-glycosylated transmembrane protein distinct from Fc gamma RIII expressed on natural killer cells. J Immunol 144:4729–4734

Edberg JC, Kimberly RP (1997) Cell type-specific glycoforms of Fc gamma RIIIa (CD16): differential ligand binding. J Immunol 159:3849–3857

Edberg JC, Redecha PB, Salmon JE, Kimberly RP (1989) Human Fc gamma RIII (CD16). Isoforms with distinct allelic expression, extracellular domains, and membrane linkages on polymorphonuclear and natural killer cells. J Immunol 143:1642–1649

Ernst LK, Duchemin AM, Anderson CL (1993) Association of the high-affinity receptor for IgG (Fc gamma RI) with the gamma subunit of the IgE receptor. Proc Natl Acad Sci U S A 90:6023–6027

Fairchild KD, Hudson RG, Douglas SD, McKenzie SE, Polin RA (1996) Effect of gamma interferon on expression of Fc gamma receptors in monocytes of newborn infants and adults. Clin Diagn Lab Immunol 3:464–469

Ferrara C, Brunker P, Suter T, Moser S, Puntener U, Umana P (2006a) Modulation of therapeutic antibody effector functions by glycosylation engineering: influence of Golgi enzyme localization domain and co-expression of heterologous beta1, 4-N-acetylglucosaminyltransferase III and Golgi alpha-mannosidase II. Biotechnol Bioeng 93:851–861

Ferrara C, Grau S, Jager C, Sondermann P, Brunker P, Waldhauer I, Hennig M, Ruf A, Rufer AC, Stihle M, Umana P, Benz J (2011) Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcgammaRIII and antibodies lacking core fucose. Proc Natl Acad Sci U S A 108:12669–12674

Ferrara C, Stuart F, Sondermann P, Brunker P, Umana P (2006b) The carbohydrate at FcgammaRIIIa Asn-162. An element required for high affinity binding to non-fucosylated IgG glycoforms. J Biol Chem 281:5032–5036

Figdor CG, van Kooyk Y, Adema GJ (2002) C-type lectin receptors on dendritic cells and Langerhans cells. Nat Rev Immunol 2:77–84

Fleit HB, Kobasiuk CD (1991) The human monocyte-like cell line THP-1 expresses Fc gamma RI and Fc gamma RII. J Leukoc Biol 49:556–565

Floto RA, Clatworthy MR, Heilbronn KR, Rosner DR, Macary PA, Rankin A, Lehner PJ, Ouwehand WH, Allen JM, Watkins NA, Smith KG (2005) Loss of function of a lupus-associated FcgammaRIIb polymorphism through exclusion from lipid rafts. Nat Med 11:1056–1058

Frigeri LG, Zuberi RI, Liu FT (1993) Epsilon BP, a beta-galactoside-binding animal lectin, recognizes IgE receptor (Fc epsilon RI) and activates mast cells. Biochemistry 32:7644–7649

Furtado PB, Whitty PW, Robertson A, Eaton JT, Almogren A, Kerr MA, Woof JM, Perkins SJ (2004) Solution structure determination of monomeric human IgA2 by X-ray and neutron scattering, analytical ultracentrifugation and constrained modelling: a comparison with monomeric human IgA1. J Mol Biol 338:921–941

Galli SJ, Tsai M (2012) IgE and mast cells in allergic disease. Nat Med 18:693–704

Galon J, Robertson MW, Galinha A, Mazieres N, Spagnoli R, Fridman WH, Sautes C (1997) Affinity of the interaction between Fc gamma receptor type III (Fc gammaRIII) and monomeric human IgG subclasses. Role of Fc gammaRIII glycosylation. Eur J Immunol 27:1928–1932

Garman SC, Wurzburg BA, Tarchevskaya SS, Kinet JP, Jardetzky TS (2000) Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc epsilonRI alpha. Nature 406:259–266

Geissmann F, Launay P, Pasquier B, Lepelletier Y, Leborgne M, Lehuen A, Brousse N, Monteiro RC (2001) A subset of human dendritic cells expresses IgA Fc receptor (CD89), which mediates internalization and activation upon cross-linking by IgA complexes. J Immunol 166:346–352

Gounni AS, Lamkhioued B, Ochiai K, Tanaka Y, Delaporte E, Capron A, Kinet JP, Capron M (1994) High-affinity IgE receptor on eosinophils is involved in defence against parasites. Nature 367:183–186

Harrison PT, Allen JM (1998) High affinity IgG binding by FcgammaRI (CD64) is modulated by two distinct IgSF domains and the transmembrane domain of the receptor. Protein Eng 11:225–232

Harvey DJ, Crispin M, Scanlan C, Singer BB, Lucka L, Chang VT, Radcliffe CM, Thobhani S, Yuen CT, Rudd PM (2008) Differentiation between isomeric triantennary N-linked glycans by negative ion tandem mass spectrometry and confirmation of glycans containing galactose attached to the bisecting (beta1-4-GlcNAc) residue in N-glycans from IgG. Rapid Commun Mass Spectrom 22:1047–1052

Herr AB, Ballister ER, Bjorkman PJ (2003) Insights into IgA-mediated immune responses from the crystal structures of human FcalphaRI and its complex with IgA1-Fc. Nature 423:614–620

Heystek HC, Moulon C, Woltman AM, Garonne P, van Kooten C (2002) Human immature dendritic cells efficiently bind and take up secretory IgA without the induction of maturation. J Immunol 168:102–107

Hibbs ML, Selvaraj P, Carpen O, Springer TA, Kuster H, Jouvin MH, Kinet JP (1989) Mechanisms for regulating expression of membrane isoforms of Fc gamma RIII (CD16). Science 246:1608–1611

Hughes GJ, Reason AJ, Savoy L, Jaton J, Frutiger-Hughes S (1999) Carbohydrate moieties in human secretory component. Biochim Biophys Acta 1434:86–93

Huizinga TW, de Haas M, Kleijer M, Nuijens JH, Roos D, von dem Borne AE (1990) Soluble Fc gamma receptor III in human plasma originates from release by neutrophils. J Clin Invest 86:416–423

Iida S, Misaka H, Inoue M, Shibata M, Nakano R, Yamane-Ohnuki N, Wakitani M, Yano K, Shitara K, Satoh M (2006) Nonfucosylated therapeutic IgG1 antibody can evade the inhibitory effect of serum immunoglobulin G on antibody-dependent cellular cytotoxicity through its high binding to FcgammaRIIIa. Clin Cancer Res 12:2879–2887

Jefferis R, Lund J, Mizutani H, Nakagawa H, Kawazoe Y, Arata Y, Takahashi N (1990) A comparative study of the N-linked oligosaccharide structures of human IgG subclass proteins. Biochem J 268:529–537

Jefferis R, Lund J, Pound JD (1998) IgG-Fc-mediated effector functions: molecular definition of interaction sites for effector ligands and the role of glycosylation. Immunol Rev 163:59–76

Johansen FE, Pekna M, Norderhaug IN, Haneberg B, Hietala MA, Krajci P, Betsholtz C, Brandtzaeg P (1999) Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice. J Exp Med 190:915–922

Kanda Y, Yamada T, Mori K, Okazaki A, Inoue M, Kitajima-Miyama K, Kuni-Kamochi R, Nakano R, Yano K, Kakita S, Shitara K, Satoh M (2007) Comparison of biological activity among nonfucosylated therapeutic IgG1 antibodies with three different N-linked Fc oligosaccharides: the high-mannose, hybrid, and complex types. Glycobiology 17:104–118

Kaneko Y, Nimmerjahn F, Ravetch JV (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313:670–673

Kanellopoulos JM, Liu TY, Poy G, Metzger H (1980) Composition and subunit structure of the cell receptor for immunoglobulin E. J Biol Chem 255:9060–9066

Kerr MA (1990) The structure and function of human IgA. Biochem J 271:285–296

Kimberly RP, Tappe NJ, Merriam LT, Redecha PB, Edberg JC, Schwartzman S, Valinsky JE (1989) Carbohydrates on human Fc gamma receptors. Interdependence of the classical IgG and nonclassical lectin-binding sites on human Fc gamma RIII expressed on neutrophils. J Immunol 142:3923–3930

Kinet JP (1992) The gamma-zeta dimers of Fc receptors as connectors to signal transduction. Curr Opin Immunol 4:43–48

Kono H, Kyogoku C, Suzuki T, Tsuchiya N, Honda H, Yamamoto K, Tokunaga K, Honda Z (2005) FcgammaRIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. Hum Mol Genet 14:2881–2892

Kraft S, Kinet JP (2007) New developments in FcepsilonRI regulation, function and inhibition. Nat Rev Immunol 7:365–378

Kraft S, Rana S, Jouvin MH, Kinet JP (2004) The role of the FcepsilonRI beta-chain in allergic diseases. Int Arch Allergy Immunol 135:62–72

Krapp S, Mimura Y, Jefferis R, Huber R, Sondermann P (2003) Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. J Mol Biol 325:979–989

Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW, Gartland GL, Bertoli LF, Mori H, Takatsu H, Kitamura T, Ohno H, Wang JY (2009) Identity of the elusive IgM Fc receptor (FcmuR) in humans. J Exp Med 206:2779–2793

Kuo TT, de Muinck EJ, Claypool SM, Yoshida M, Nagaishi T, Aveson VG, Lencer WI, Blumberg RS (2009) N-glycan moieties in neonatal Fc receptor determine steady-state membrane distribution and directional transport of IgG. J Biol Chem 284:8292–8300

Lacroix EL, Froese A (1993) The N-linked oligosaccharides of the Fc epsilon receptors of rat basophilic leukemia cells. Mol Immunol 30:321–330

Lang ML, Shen L, Wade WF (1999) Gamma-chain dependent recruitment of tyrosine kinases to membrane rafts by the human IgA receptor Fc alpha R. J Immunol 163:5391–5398

Letellier M, Nakajima T, Delespesse G (1988) IgE receptor on human lymphocytes. IV. Further analysis of its structure and of the role of N-linked carbohydrates. J Immunol 141:2374–2381

Letourneur O, Sechi S, Willette-Brown J, Robertson MW, Kinet JP (1995) Glycosylation of human truncated Fc epsilon RI alpha chain is necessary for efficient folding in the endoplasmic reticulum. J Biol Chem 270:8249–8256

Li X, Wu J, Carter RH, Edberg JC, Su K, Cooper GS, Kimberly RP (2003) A novel polymorphism in the Fcgamma receptor IIB (CD32B) transmembrane region alters receptor signaling. Arthritis Rheum 48:3242–3252

Liu FT (2005) Regulatory roles of galectins in the immune response. Int Arch Allergy Immunol 136:385–400

Liu W, Hsu DK, Chen HY, Yang RY, Carraway KL, Isseroff RR 3rd, Liu FT (2012) Galectin-3 regulates intracellular trafficking of EGFR through Alix and promotes keratinocyte migration. J Invest Dermatol 132:2828–2837

Lu J, Ellsworth JL, Hamacher N, Oak SW, Sun PD (2011) Crystal structure of Fcgamma receptor I and its implication in high affinity gamma-immunoglobulin binding. J Biol Chem 286:40608–40613

Lund J, Takahashi N, Goodall M, Pound JD, Jefferis R (1995) Oligosaccharide-protein interactions in IgG antibody molecules: structural and functional consequences. Biochem Soc Trans 23:102S

Macpherson AJ, Gatto D, Sainsbury E, Harriman GR, Hengartner H, Zinkernagel RM (2000) A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 288:2222–2226

Maeda K, Burton GF, Padgett DA, Conrad DH, Huff TF, Masuda A, Szakal AK, Tew JG (1992) Murine follicular dendritic cells and low affinity Fc receptors for IgE (Fc epsilon RII). J Immunol 148:2340–2347

Maenaka K, van der Merwe PA, Stuart DI, Jones EY, Sondermann P (2001) The human low affinity Fcgamma receptors IIa, IIb, and III bind IgG with fast kinetics and distinct thermodynamic properties. J Biol Chem 276:44898–44904

Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB (1995) Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nat Med 1:237–243

Maliszewski CR, March CJ, Schoenborn MA, Gimpel S, Shen L (1990) Expression cloning of a human Fc receptor for IgA. J Exp Med 172:1665–1672

Masuda K, Yamaguchi Y, Kato K, Takahashi N, Shimada I, Arata Y (2000) Pairing of oligosaccharides in the Fc region of immunoglobulin G. FEBS Lett 473:349–357
Mattu TS, Pleass RJ, Willis AC, Kilian M, Wormald MR, Lellouch AC, Rudd PM, Woof JM, Dwek RA (1998) The glycosylation and structure of human serum IgA1, Fab, and Fc regions and the role of N-glycosylation on Fcalpha receptor interactions. J Biol Chem 273:2260–2272
Medzhitov R, Preston-Hurlburt P, Janeway CA Jr (1997) A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 388:394–397
Merlin J, Stechly L, de Beauce S, Monte D, Leteurtre E, van Seuningen I, Huet G, Pigny P (2011) Galectin-3 regulates MUC1 and EGFR cellular distribution and EGFR downstream pathways in pancreatic cancer cells. Oncogene 30:2514–2525
Metes D, Ernst LK, Chambers WH, Sulica A, Herberman RB, Morel PA (1998) Expression of functional CD32 molecules on human NK cells is determined by an allelic polymorphism of the FcgammaRIIC gene. Blood 91:2369–2380
Mimura Y, Ashton PR, Takahashi N, Harvey DJ, Jefferis R (2007) Contrasting glycosylation profiles between Fab and Fc of a human IgG protein studied by electrospray ionization mass spectrometry. J Immunol Methods 326:116–126
Mizoguchi A, Mizuochi T, Kobata A (1982) Structures of the carbohydrate moieties of secretory component purified from human milk. J Biol Chem 257:9612–9621
Monteiro RC, Hostoffer RW, Cooper MD, Bonner JR, Gartland GL, Kubagawa H (1993) Definition of immunoglobulin A receptors on eosinophils and their enhanced expression in allergic individuals. J Clin Invest 92:1681–1685
Monteiro RC, van de Winkel JG (2003) IgA Fc receptors. Annu Rev Immunol 21:177–204
Mori K, Kuni-Kamochi R, Yamane-Ohnuki N, Wakitani M, Yamano K, Imai H, Kanda Y, Niwa R, Iida S, Uchida K, Shitara K, Satoh M (2004) Engineering Chinese hamster ovary cells to maximize effector function of produced antibodies using FUT8 siRNA. Biotechnol Bioeng 88:901–908
Morphis LG, Gitlin D (1970) Maturation of the maternofoetal transport system for human gamma-globulin in the mouse. Nature 228:573
Morton HC, Schiel AE, Janssen SW, van de Winkel JG (1996) Alternatively spliced forms of the human myeloid Fc alpha receptor (CD89) in neutrophils. Immunogenetics 43:246–247
Morton HC, van den Herik-Oudijk IE, Vossebeld P, Snijders A, Verhoeven AJ, Capel PJ, van de Winkel JG (1995) Functional association between the human myeloid immunoglobulin A Fc receptor (CD89) and FcR gamma chain. Molecular basis for CD89/FcR gamma chain association. J Biol Chem 270:29781–29787
Mostov KE (1994) Transepithelial transport of immunoglobulins. Annu Rev Immunol 12:63–84
Natsume A, Wakitani M, Yamane-Ohnuki N, Shoji-Hosaka E, Niwa R, Uchida K, Satoh M, Shitara K (2005) Fucose removal from complex-type oligosaccharide enhances the antibody-dependent cellular cytotoxicity of single-gene-encoded antibody comprising a single-chain antibody linked the antibody constant region. J Immunol Methods 306:93–103
Nettleton MY, Kochan JP (1995) Role of glycosylation sites in the IgE Fc molecule. Int Arch Allergy Immunol 107:328–329
Niki T, Tsutsui S, Hirose S, Aradono S, Sugimoto Y, Takeshita K, Nishi N, Hirashima M (2009) Galectin-9 is a high affinity IgE-binding lectin with anti-allergic effect by blocking IgE-antigen complex formation. J Biol Chem 284:32344–32352
Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science 310:1510–1512
Nimmerjahn F, Ravetch JV (2008) Fcgamma receptors as regulators of immune responses. Nat Rev Immunol 8:34–47
Okazaki A, Shoji-Hosaka E, Nakamura K, Wakitani M, Uchida K, Kakita S, Tsumoto K, Kumagai I, Shitara K (2004) Fucose depletion from human IgG1 oligosaccharide enhances binding enthalpy and association rate between IgG1 and FcgammaRIIIa. J Mol Biol 336:1239–1249

Ouchida R, Mori H, Hase K, Takatsu H, Kurosaki T, Tokuhisa T, Ohno H, Wang JY (2012) Critical role of the IgM Fc receptor in IgM homeostasis, B-cell survival, and humoral immune responses. Proc Natl Acad Sci U S A 109:E2699–E2706

Perussia B, Ravetch JV (1991) Fc gamma RIII (CD16) on human macrophages is a functional product of the Fc gamma RIII-2 gene. Eur J Immunol 21:425–429

Petersen SV, Thiel S, Jensenius JC (2001) The mannan-binding lectin pathway of complement activation: biology and disease association. Mol Immunol 38:133–149

Pierce-Cretel A, Decottignies JP, Wieruszeski JM, Strecker G, Montreuil J, Spik G (1989) Primary structure of twenty three neutral and monosialylated oligosaccharides O-glycosidically linked to the human secretory immunoglobulin A hinge region determined by a combination of permethylation analysis and 400-MHz 1H-NMR spectroscopy. Eur J Biochem 182:457–476

Pierce-Cretel A, Pamblanco M, Strecker G, Montreuil J, Spik G (1981) Heterogeneity of the glycans O-glycosidically linked to the hinge region of secretory immunoglobulins from human milk. Eur J Biochem 114:169–178

Pierce-Cretel A, Pamblanco M, Strecker G, Montreuil J, Spik G, Dorland L, van Halbeek H, Vliegenthart JF (1982) Primary structure of the N-glycosidically linked sialoglycans of secretory immunoglobulins A from human milk. Eur J Biochem 125:383–388

Pleass RJ, Andrews PD, Kerr MA, Woof JM (1996) Alternative splicing of the human IgA Fc receptor CD89 in neutrophils and eosinophils. Biochem J 318(Pt 3):771–777

Plomp R, Hensbergen PJ, Rombouts Y, Zauner G, Dragan I, Koeleman CA, Deelder AM, Wuhrer M (2013) Site-specific N-glycosylation analysis of human immunoglobulin E. J Proteome Res 13(2):536

Poo H, Krauss JC, Mayo-Bond L, Todd RF 3rd, Petty HR (1995) Interaction of Fc gamma receptor type IIIB with complement receptor type 3 in fibroblast transfectants: evidence from lateral diffusion and resonance energy transfer studies. J Mol Biol 247:597–603

Powell MS, Barton PA, Emmanouilidis D, Wines BD, Neumann GM, Peitersz GA, Maxwell KF, Garrett TP, Hogarth PM (1999) Biochemical analysis and crystallisation of Fc gamma RIIa, the low affinity receptor for IgG. Immunol Lett 68:17–23

Qiao SW, Kobayashi K, Johansen FE, Sollid LM, Andersen JT, Milford E, Roopenian DC, Lencer WI, Blumberg RS (2008) Dependence of antibody-mediated presentation of antigen on FcRn. Proc Natl Acad Sci U S A 105:9337–9342

Qiu WQ, de Bruin D, Brownstein BH, Pearse R, Ravetch JV (1990) Organization of the human and mouse low-affinity Fc gamma R genes: duplication and recombination. Science 248:732–735

Radaev S, Sun PD (2001) Recognition of IgG by Fcgamma receptor. The role of Fc glycosylation and the binding of peptide inhibitors. J Biol Chem 276:16478–16483

Ravetch JV, Perussia B (1989) Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. J Exp Med 170:481–497

Robertson MW (1993) Phage and Escherichia coli expression of the human high affinity immunoglobulin E receptor alpha-subunit ectodomain. Domain localization of the IgE-binding site. J Biol Chem 268:12736–12743

Roopenian DC, Akilesh S (2007) FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 7:715–725

Rosenfeld SI, Looney RJ, Leddy JP, Phipps DC, Abraham GN, Anderson CL (1985) Human platelet Fc receptor for immunoglobulin G. Identification as a 40,000-molecular-weight membrane protein shared by monocytes. J Clin Invest 76:2317–2322

Royle L, Roos A, Harvey DJ, Wormald MR, van Gijlswijk-Janssen D, Redwan el-RM, Wilson IA, Daha MR, Dwek RA, Rudd PM (2003) Secretory IgA N- and O-glycans provide a link between the innate and adaptive immune systems. J Biol Chem 278:20140–20153

Sallusto F, Lanzavecchia A (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 179:1109–1118

Salmon JE, Millard SS, Brogle NL, Kimberly RP (1995) Fc gamma receptor IIIb enhances Fc gamma receptor IIa function in an oxidant-dependent and allele-sensitive manner. J Clin Invest 95:2877–2885

Sarfati M, Bettler B, Letellier M, Fournier S, Rubio-Trujillo M, Hofstetter H, Delespesse G (1992) Native and recombinant soluble CD23 fragments with IgE suppressive activity. Immunology 76:662–667

Sarfati M, Rector E, Sehon AH, Delespesse G (1984) In vitro synthesis of IgE by human lymphocytes. IV. Suppression of the spontaneous IgE synthesis by IgE-binding factors secreted by tunicamycin-treated RPMI 8866 cells. Immunology 53:783–790

Satoh M, Iida S, Shitara K (2006) Non-fucosylated therapeutic antibodies as next-generation therapeutic antibodies. Expert Opin Biol Ther 6:1161–1173

Scallon BJ, Scigliano E, Freedman VH, Miedel MC, Pan YC, Unkeless JC, Kochan JP (1989) A human immunoglobulin G receptor exists in both polypeptide-anchored and phosphatidyl-inositol-glycan-anchored forms. Proc Natl Acad Sci U S A 86:5079–5083

Schmitt DA, Bieber T, Cazenave JP, Hanau D (1990) Fc receptors of human Langerhans cells. J Invest Dermatol 94:15S–21S

Sedmak DD, Davis DH, Singh U, van de Winkel JG, Anderson CL (1991) Expression of IgG Fc receptor antigens in placenta and on endothelial cells in humans. An immunohistochemical study. Am J Pathol 138:175–181

Shibata-Koyama M, Iida S, Okazaki A, Mori K, Kitajima-Miyama K, Saitou S, Kakita S, Kanda Y, Shitara K, Kato K, Satoh M (2009) The N-linked oligosaccharide at Fc gamma RIIIa Asn-45: an inhibitory element for high Fc gamma RIIIa binding affinity to IgG glycoforms lacking core fucosylation. Glycobiology 19:126–134

Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SH, Presta LG (2002) Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 277:26733–26740

Simister NE (2003) Placental transport of immunoglobulin G. Vaccine 21:3365–3369

Simister NE, Mostov KE (1989) An Fc receptor structurally related to MHC class I antigens. Nature 337:184–187

Sondermann P, Huber R, Oosthuizen V, Jacob U (2000) The 3.2-A crystal structure of the human IgG1 Fc fragment-Fc gammaRIII complex. Nature 406:267–273

Sondermann P, Jacob U (1999) Human Fcgamma receptor IIb expressed in Escherichia coli reveals IgG binding capability. Biol Chem 380:717–721

Sondermann P, Jacob U, Kutscher C, Frey J (1999) Characterization and crystallization of soluble human Fc gamma receptor II (CD32) isoforms produced in insect cells. Biochemistry 38:8469–8477

Sondermann P, Pincetic A, Maamary J, Lammens K, Ravetch JV (2013) General mechanism for modulating immunoglobulin effector function. Proc Natl Acad Sci U S A 110:9868–9872

Sylvestre DL, Ravetch JV (1996) A dominant role for mast cell Fc receptors in the Arthus reaction. Immunity 5:387–390

Takahashi N, Cohen-Solal J, Galinha A, Fridman WH, Sautes-Fridman C, Kato K (2002) N-glycosylation profile of recombinant human soluble Fcgamma receptor III. Glycobiology 12:507–515

Takahashi N, Yamada W, Masuda K, Araki H, Tsukamoto Y, Galinha A, Sautes C, Kato K, Shimada I (1998) N-glycan structures of a recombinant mouse soluble Fcgamma receptor II. Glycoconj J 15:905–914

Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. Annu Rev Immunol 21:335–376

Umana P, Jean-Mairet J, Moudry R, Amstutz H, Bailey JE (1999) Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity. Nat Biotechnol 17:176–180

van den Herik-Oudijk IE, Westerdaal NA, Henriquez NV, Capel PJ, van de Winkel JG (1994) Functional analysis of human Fc gamma RII (CD32) isoforms expressed in B lymphocytes. J Immunol 152:574–585

van der Boog PJ, van Zandbergen G, de Fijter JW, Klar-Mohamad N, van Seggelen A, Brandtzaeg P, Daha MR, van Kooten C (2002) Fc alpha RI/CD89 circulates in human serum covalently linked to IgA in a polymeric state. J Immunol 168:1252–1258

van Egmond M, Damen CA, van Spriel AB, Vidarsson G, van Garderen E, van de Winkel JG (2001) IgA and the IgA Fc receptor. Trends Immunol 22:205–211

van Egmond M, van Garderen E, van Spriel AB, Damen CA, van Amersfoort ES, van Zandbergen G, van Hattum J, Kuiper J, van de Winkel JG (2000) FcalphaRI-positive liver Kupffer cells: reappraisal of the function of immunoglobulin A in immunity. Nat Med 6:680–685

van Spriel AB, Leusen JH, Vile H, van de Winkel JG (2002) Mac-1 (CD11b/CD18) as accessory molecule for Fc alpha R (CD89) binding of IgA. J Immunol 169:3831–3836

van Vugt MJ, Heijnen AF, Capel PJ, Park SY, Ra C, Saito T, Verbeek JS, van de Winkel JG (1996) FcR gamma-chain is essential for both surface expression and function of human Fc gamma RI (CD64) in vivo. Blood 87:3593–3599

van Zandbergen G, Westerhuis R, Mohamad NK, van de Winkel JG, Daha MR, van Kooten C (1999) Crosslinking of the human Fc receptor for IgA (FcalphaRI/CD89) triggers FcR gamma-chain-dependent shedding of soluble CD89. J Immunol 163:5806–5812

Vaughn DE, Bjorkman PJ (1998) Structural basis of pH-dependent antibody binding by the neonatal Fc receptor. Structure 6:63–73

Vercelli D, Helm B, Marsh P, Padlan E, Geha RS, Gould H (1989) The B-cell binding site on human immunoglobulin E. Nature 338:649–651

von Horsten HH, Ogorek C, Blanchard V, Demmler C, Giese C, Winkler K, Kaup M, Berger M, Jordan I, Sandig V (2010) Production of non-fucosylated antibodies by co-expression of heterologous GDP-6-deoxy-D-lyxo-4-hexulose reductase. Glycobiology 20:1607–1618

Vouldoukis I, Riveros-Moreno V, Dugas B, Ouaaz F, Becherel P, Debre P, Moncada S, Mossalayi MD (1995) The killing of Leishmania major by human macrophages is mediated by nitric oxide induced after ligation of the Fc epsilon RII/CD23 surface antigen. Proc Natl Acad Sci U S A 92:7804–7808

Ward ES, Zhou J, Ghetie V, Ober RJ (2003) Evidence to support the cellular mechanism involved in serum IgG homeostasis in humans. Int Immunol 15:187–195

Warmerdam PA, Nabben NM, van de Graaf SA, van de Winkel JG, Capel PJ (1993) The human low affinity immunoglobulin G Fc receptor IIC gene is a result of an unequal crossover event. J Biol Chem 268:7346–7349

Williams AF, Barclay AN (1988) The immunoglobulin superfamily–domains for cell surface recognition. Annu Rev Immunol 6:381–405

Wines BD, Hulett MD, Jamieson GP, Trist HM, Spratt JM, Hogarth PM (1999) Identification of residues in the first domain of human Fc alpha receptor essential for interaction with IgA. J Immunol 162:2146–2153

Wormald MR, Rudd PM, Harvey DJ, Chang SC, Scragg IG, Dwek RA (1997) Variations in oligosaccharide-protein interactions in immunoglobulin G determine the site-specific glycosylation profiles and modulate the dynamic motion of the Fc oligosaccharides. Biochemistry 36:1370–1380

Xue J, Zhao Q, Zhu L, Zhang W (2010) Deglycosylation of FcalphaR at N58 increases its binding to IgA. Glycobiology 20:905–915

Yamane-Ohnuki N, Kinoshita S, Inoue-Urakubo M, Kusunoki M, Iida S, Nakano R, Wakitani M, Niwa R, Sakurada M, Uchida K, Shitara K, Satoh M (2004) Establishment of FUT8 knockout Chinese hamster ovary cells: an ideal host cell line for producing completely defucosylated antibodies with enhanced antibody-dependent cellular cytotoxicity. Biotechnol Bioeng 87:614–622

Yoshida M, Claypool SM, Wagner JS, Mizoguchi E, Mizoguchi A, Roopenian DC, Lencer WI, Blumberg RS (2004) Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. Immunity 20:769–783

Yu X, Vasiljevic S, Mitchell DA, Crispin M, Scanlan CN (2013) Dissecting the molecular mechanism of IVIg therapy: the interaction between serum IgG and DC-SIGN is independent of antibody glycoform or Fc domain. J Mol Biol 425:1253–1258

Zeck A, Pohlentz G, Schlothauer T, Peter-Katalinic J, Regula JT (2011) Cell type-specific and site directed N-glycosylation pattern of FcgammaRIIIa. J Proteome Res 10:3031–3039

Zhou Q, Shankara S, Roy A, Qiu H, Estes S, McVie-Wylie A, Culm-Merdek K, Park A, Pan C, Edmunds T (2008) Development of a simple and rapid method for producing non-fucosylated oligomannose containing antibodies with increased effector function. Biotechnol Bioeng 99:652–665

Antibodies as Natural Adjuvants

Birgitta Heyman

Abstract Antibodies in complex with specific antigen can dramatically change the antibody response to this antigen. Depending on antibody class and type of antigen, >99 % suppression or >100-fold enhancement of the response can take place. IgM and IgG3 are efficient enhancers and operate via the complement system. In contrast, IgG1, IgG2a, and IgG2b enhance antibody and $CD4^+$ T cell responses to protein antigens via activating Fcγ-receptors. IgE also enhances antibody and $CD4^+$ T cell responses to small proteins but uses the low-affinity receptor for IgE, CD23. Most likely, IgM and IgG3 work by increasing the effective concentration of antigen on follicular dendritic cells in splenic follicles. IgG1, IgG2a, IgG2b, and IgE probably enhance antibody responses by increasing antigen presentation by dendritic cells to T helper cells. IgG antibodies of all subclasses have a dual effect, and suppress antibody responses to particulate antigens such as erythrocytes. This capacity is used in the clinic to prevent immunization of Rhesus-negative women to Rhesus-positive fetal erythrocytes acquired via transplacental hemorrage. IgG-mediated suppression in mouse models can take place in the absence of Fcγ-receptors and complement and to date no knock-out mouse strain has been found where suppression is abrogated.

Contents

1 Introduction 202
2 IgM and Enhancement of Antibody Responses 203
2.1 Complement and IgM-Mediated Enhancement 204
2.2 Fc-Receptors and IgM-Mediated Enhancement 204
2.3 Summary 205

B. Heyman (✉)
Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden
e-mail: birgitta.heyman@imbim.uu.se

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_9,

3 IgG-Mediated Suppression of Antibody Responses 205
3.1 Complement and IgG-Mediated Suppression 205
3.2 Fc-Receptors and IgG-Mediated Suppression 206
3.3 Summary 206
4 IgG1-, IgG2a-, and IgG2b-Mediated Enhancement of Antibody Responses 207
4.1 Complement and IgG1-, IgG2a-, and IgG2b-Mediated Enhancement 207
4.2 Fc-Receptors and IgG1-, IgG2a-, and IgG2b-Mediated Enhancement 208
4.3 Summary 210
5 IgG3-Mediated Enhancement of Antibody Responses 210
5.1 Complement and IgG3-Mediated Enhancement 210
5.2 Fc-Receptors and IgG3-Mediated Enhancement 210
5.3 Summary 211
6 IgE-Mediated Enhancement of Antibody Responses 211
6.1 Fc-Receptors and IgE-Mediated Enhancement 212
6.2 Summary 213
7 Concluding Remarks 214
References 214

1 Introduction

Antibodies have been known to regulate antibody responses against the antigens with which they form complexes since the 1890s (von Behring and Wernicke 1892). All classes and subclasses, except IgD, have been reported to exert immunoregulatory functions (reviewed in Uhr and Möller (1968), Heyman (2000), Hjelm et al. (2006)). In studies of antibody-mediated feedback regulation, the most common protocol is to immunize control mice with antigen and the experimental group with preformed specific antibodies followed by antigen. Immunizations are usually done intravenously without adjuvants. Typically, mice immunized with both antibodies and antigen have a considerably enhanced or suppressed antibody response against the antigen in question (Fig. 1). Although antibody feedback regulation is antigen-specific, the response not only to the epitopes recognized by the antibody but also to other epitopes present on the same antigen will be modulated. The ability of low doses of allogeneic antibodies to suppress specific antibody responses has been used successfully in Rhesus prophylaxis. Women with the RhD-negative blood group, carrying RhD-positive fetuses, can become immunized against fetal erythrocytes acquired via transplacental hemorrhage. Since maternal IgG-antibodies are actively transported across the placenta, IgG anti-RhD may damage fetal erythrocytes, causing hemolytic disease of the newborn. Passive administration of IgG anti-RhD to RhD-negative women during pregnancy or immediately after delivery inhibits active production of IgG anti-RhD. This treatment has decreased the incidence of hemolytic disease of the newborn dramatically since its introduction in the 1960s (Clarke et al. 1963; Urbaniak and Greiss 2000). Antibodies can also have the opposite effect and act as natural adjuvants. Enhancement of antibody responses can be caused by IgM, IgE,

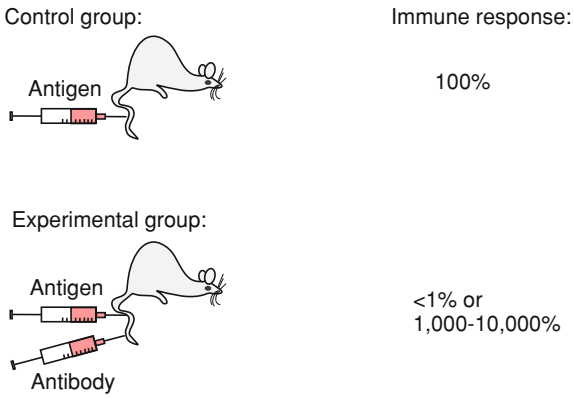


Fig. 1 Antibody feedback regulation. The most commonly used protocol for studying antibody feedback regulation is to immunize mice with preformed antibodies in close temporal relationship to the specific antigen. Depending on antibody class and type of antigen, the active antibody response in the experimental group can either be completely suppressed or enhanced several hundred-fold

and IgA. IgG has a dual effect and enhances antibody responses to proteins and suppresses responses to erythrocytes. Current knowledge suggests that IgM and IgG3 primarily depend on complement activation for their adjuvant effects whereas IgG1, IgG2a, IgG2b, and IgE depend on FcRs (Fc-receptors). The mechanism behind IgA-mediated enhancement is not understood and owing to the limited number of studies (Klaus 1979; Coulie and Van Snick 1985) will not be discussed further. This review will focus on the adjuvant effect of antibodies in murine in vivo models.

2 IgM and Enhancement of Antibody Responses

The ability of specific IgM to enhance antibody responses to erythrocytes was discovered in the 1960s (Henry and Jerne 1968). Small amounts of IgM administered intravenously to mice together with suboptimal doses of SRBC (sheep red blood cells) causes enhanced SRBC-specific primary and memory responses (Henry and Jerne 1968; Heyman et al. 1982; Heyman and Wigzell 1985; Youd et al. 2002; Ding et al. 2013). IgM preferentially enhances responses to large antigens such as erythrocytes (Henry and Jerne 1968; Heyman et al. 1982; Ding et al. 2013; Dennert 1971), malaria parasites (Harte et al. 1983) and KLH (keyhole limpet hemocyanine) (Coulie and Van Snick 1985; Youd et al. 2002; Ding et al. 2013; Enriquez-Rincon and Klaus 1984), whereas responses to small proteins like OVA (ovalbumin) are difficult to enhance. Antigens administered with IgM are localized to splenic follicles more efficiently than antigens administered alone (Dennert 1971; Link et al. 2012). IgM administered with SRBC-OVA does not

enhance proliferation and activation of OVA-specific transgenic $CD4^+$ T cells (Ding et al. 2013) whereas IgM enhanced T helper cell induction after immunization with malaria parasites (Harte et al. 1983). Specific IgM, was recently shown to influence B cell selection in germinal centers and to govern the affinity maturation (Zhang et al. 2013).

2.1 Complement and IgM-Mediated Enhancement

A likely explanation for why IgM only enhances responses to large antigens is that complement-activation is required. In order for IgM to bind C1q and start the classical complement cascade, it must change its conformation from the planar form assumed in serum to a staple form. This conformation change presumably requires binding to a large antigen. The conclusion that IgM-mediated enhancement requires complement activation is based on several observations. First, it was found that monoclonal IgM with a pointmutation in the IgM heavy chain leading to inability to bind C1q, lost its enhancing effect (Heyman et al. 1988). This was confirmed using IgM from a knock-in mouse (Cμ13) carrying the same point mutation as the monoclonal IgM (Ding et al. 2013; Rutemark et al. 2011). Additionally, monomeric IgM, which cannot activate complement, lost its ability to enhance (Youd et al. 2002). Indirect evidence for the importance of complement activation is that depletion of C3 or lack of CR1/2 (complement receptors 1 and 2) abolished IgM-mediated enhancement (Heyman et al. 1988; Applequist et al. 2000; Rutemark et al. 2012).

2.2 Fc-Receptors and IgM-Mediated Enhancement

An alternative to the complement pathway for IgM to influence immune responses would be via Fc-receptors for IgM. A FcμR (TOSO/FAIM3), expressed in mice exclusively on B cells, was recently identified (Kubagawa et al. 2009; Shima et al. 2010). Mice lacking FcμR have reduced antibody responses, reduced development of germinal centers and elevated levels of autoantibodies (Ouchida et al. 2012; Honjo et al. 2012). Importantly, IgM from Cμ13 mice, unable to activate complement and to enhance antibody responses, bound equally well to FcμR as did wild-type IgM (Ding et al. 2013). This observation strongly argues against that enhancement of antibody responses by specific IgM is mediated by FcμR.

Non-immune IgM, present in naïve mice, appears to play a role for the generation of normal antibody responses. This is evidenced by the finding that mice lacking secretory IgM have impaired antibody responses which can be rescued by transfusion of IgM from normal mouse serum (Ehrenstein et al. 1998; Baumgarth et al. 2000). Such non-immune IgM does not seem to enhance antibody responses

via complement activation since Cμ13 mice have normal antibody responses (Rutemark et al. 2011). Possibly, natural IgM enhances antibody responses through FcμRs.

2.3 Summary

The most likely mechanism behind IgM-mediated enhancement of antibody responses is increased localization of antigen on FDC (follicular dendritic cells) in germinal centers. This idea is supported by a number of observations, the first being the correlation between the antigen amount detected in the spleen and the magnitude of the IgM-enhanced antibody response (Dennert 1971). Marginal zone (MZ) B cells continuously shuttle between the MZ and the B cell follicles (Cinamon et al. 2008; Arnon et al. 2013) and have been shown to deposit antigen onto FDC (Link et al. 2012; Ferguson et al. 2004; Heesters et al. 2013). B cells express high levels of CR1/2 and FDC express CR1 (Donius et al. 2013). As mentioned above, IgM-mediated enhancement of antibody responses is dependent on complement activation by IgM as well as on the presence of CR1/2 (Youd et al. 2002; Ding et al. 2013; Heyman et al. 1988; Applequist et al. 2000; Rutemark et al. 2012). Recently, it was directly demonstrated in vivo that passively administered SRBC-specific IgM binds to SRBC in the circulation and causes massive deposition of C3 on the erythrocytes as early as 60 s after immunization (Ding et al. 2013). These observations are compatible with the hypothesis that MZ B cells bind the complement-opsonized antigen via complement receptor 2 and transport it to follicles where it is captured by $CR1^+$ FDC and presented to germinal center B cells.

3 IgG-Mediated Suppression of Antibody Responses

IgG, passively administered together with erythrocytes, can completely suppress a primary antibody response (Henry and Jerne 1968; Enriquez-Rincon and Klaus 1984; Heyman and Wigzell 1984; Karlsson et al. 1999). Priming of T helper cells does not seem to be affected (Karlsson et al. 1999; Getahun and Heyman 2009). Interestingly, administration of IgG several days after the antigen can terminate a primary antibody response (Chan and Sinclair 1973; Karlsson et al. 2001).

3.1 Complement and IgG-Mediated Suppression

IgG2a, IgG2b, and IgG3 can activate complement. Murine IgG1 is generally a poor complement activator. However, some monoclonal IgG1 antibodies do activate complement and when the suppressive ability of one complement-activating and

one non-complement-activating IgG1 clone was compared, they were equally efficient (Heyman et al. 1988). Moreover, IgG can suppress in mice lacking C1q or CR1/2 (B.H. unpublished data). Therefore it seems unlikely that complement-activation by IgG is required for its capacity to suppress antibody responses.

3.2 Fc-Receptors and IgG-Mediated Suppression

Whether or not the Fc part of the IgG molecule is required for suppression has been a matter of debate. Some investigators find that suppression is abrogated when $F(ab')_2$ fragments are used (Enriquez-Rincon and Klaus 1984; Sinclair 1969; Brüggemann and Rajewsky 1982) whereas others report efficient suppression by $F(ab')_2$ fragments (Karlsson et al. 1999; Tao and Uhr 1966; Cerottini et al. 1969). As an alternative way to determine whether FcRs were required, mice lacking various FcγRs were used. IgG can suppress anti-SRBC responses equally well in wild-type controls as in mice lacking FcγRIIB ($FcγRIIB^{-/-}$), FcγRI + FcγRIII + FcγRIV ($FcR\gamma^{-/-}$), FcγRII + FcγRI + FcγRIII + FcγRIV (FcγRIIB × FcRγ double knock-outs), or the neonatal Fc-receptor, FcRn ($\beta 2\ m^{-/-}$) (Karlsson et al. 1999, 2001; Brinc et al. 2008). These observations suggest that FcγRs do not play a major role for the ability of IgG to suppress antibody responses.

3.3 Summary

Although used since the 1960s to prevent Rhesus immunization in RhD-negative women (Clarke et al. 1963), the mechanism behind IgG-mediated suppression of responses to erythrocytes remains poorly understood. No knock-out mouse strain where IgG-mediated suppression is impaired has been found and from observations in mouse models it appears that neither FcγRs nor complement are required. Epitope masking and antigen elimination are two mutually not exclusive possibilities for how IgG can suppress antibody responses. IgG bound to an antigen may competitively inhibit binding of naïve low affinity B cells to the same epitope and thus prevent them from being activated. Alternatively or in parallel, antigen with IgG as a "tag" may be subjected to increased (FcγR- and complement-independent) phagocytosis or be transported to areas where immune recognition is ineffective. Mathematical modelling of antibody feedback suppression suggests that epitope masking together with rapid elimination of antigen best explain the experimental data (Na et al. 2006). More extensive reviews of IgG-mediated suppression can be found in (Heyman 2000, 2003, 2013; Brinc and Lazarus 2009).

4 IgG1-, IgG2a-, and IgG2b-Mediated Enhancement of Antibody Responses

Early studies demonstrated that hyperimmune sera, presumably mainly containing IgG, could enhance both primary antibody responses and induction of memory (Uhr and Möller 1968; Heyman 2000). Thus, in addition to the more well-known role of IgG as an immunosuppressor, IgG can enhance antibody responses. The outcome of the regulation seems to depend on the structure of the antigen with which IgG forms an immune complex. A given hapten-specific monoclonal IgG, administered with haptenated erythrocytes, suppressed the erythrocyte-specific antibody response while the same antibody administered with haptenated proteins, enhanced the protein-specific response (Enriquez-Rincon and Klaus 1984; Wiersma et al. 1989). The adjuvant effects of IgG antibodies has mainly been demonstrated against protein antigens, including KLH which is a large protein with a molecular weight >1,000 kD.

Studies with monoclonal IgG demonstrated that all murine subclasses can enhance primary antibody responses to e.g. KLH, OVA, and BSA (bovine serum albumin) (Coulie and Van Snick 1985; Enriquez-Rincon and Klaus 1984; Wiersma et al. 1989, 1990; Wernersson et al. 1999; Whited Collisson et al. 1984; Diaz de Ståhl 2001, 2003; Getahun et al. 2004; Hjelm et al. 2005). Since IgG3 has a different mode of operation than the other subclasses, it will not be discussed further in this section. Also secondary responses and induction of memory are enhanced by IgG1, IgG2a, and IgG2b (Klaus 1979; Coulie and Van Snick 1985; Diaz de Ståhl 2001) (Fig. 2). The magnitude of the enhancement is impressive, sometimes being more than a 1,000-fold (Coulie and Van Snick 1985; Wernersson et al. 1999). Both IgG1- and IgG2a-responses are upregulated, indicating that there is no Th1/Th2 scewing (Diaz de Ståhl 2001). IgG also increases the frequency of somatic hypermutation in germinal centers (Nie et al. 1997) and the proliferation and activation of antigen specific $CD4^+$ T cells (Getahun et al. 2004; Terres et al. 1974; Hamano et al. 2000; de Jong et al. 2006).

4.1 Complement and IgG1-, IgG2a-, and IgG2b-Mediated Enhancement

A correlation between the ability of IgG to enhance antibody responses and the ability of individual monoclonal IgG antibodies to activate complement has been reported (Klaus 1979; Coulie and Van Snick 1985; Wiersma et al. 1989). Although this suggested that IgG-mediated enhancement depended on complement activation, subsequent studies show that IgG antibodies unable to activate complement can enhance as efficiently as complement-activating control IgG (Wiersma et al. 1990). Moreover, IgG2b can enhance in mice depleted of C3 (Wiersma et al. 1990) and IgG2a can enhance in mice lacking CR1/2 (Applequist et al. 2000). These data

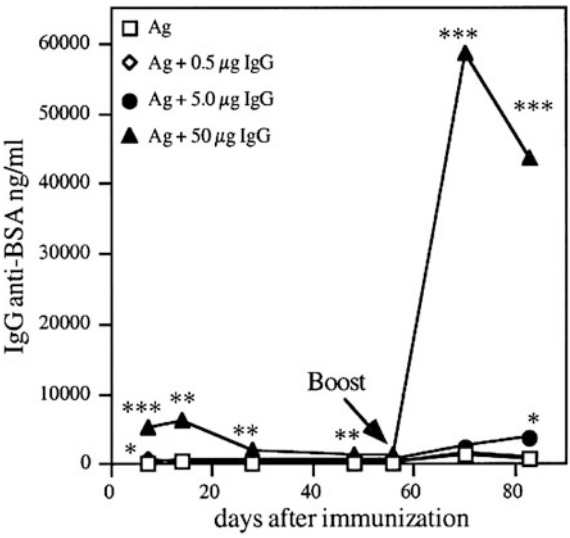


Fig. 2 IgG-mediated enhancement of primary responses and priming for a recall response. Mice were immunized intravenously with 20 μg BSA-TNP (Ag) alone or with a mixture of 20 μg BSA-TNP and the indicated amounts of TNP-specific monoclonal IgG2a. After 56 days, all animals were boosted subcutaneously with 20 μg BSA in phosphate buffered saline. IgG anti-BSA was measured in ELISA (*From* Diaz de Ståhl 2001)

point to a complement-independent pathway for IgG-mediated enhancement. An alternative explanation for the weak enhancement by IgG1, interpreted to depend on lack of complement activation (Coulie and Van Snick 1985; Wiersma et al. 1989), could be that this subclass has a stronger binding to the inhibitory FcγRIIB than do IgG2a and IgG2b (Nimmerjahn and Ravetch 2005). Therefore, an enhancing effect via activating FcγRs may have been downregulated by FcγRIIB and therefore disguised.

4.2 Fc-Receptors and IgG1-, IgG2a-, and IgG2b-Mediated Enhancement

Neither IgG1, IgG2a, nor IgG2b are able to enhance antibody or $CD4^+$ T cell responses in $FcR\gamma^{-/-}$ mice (Wernersson et al. 1999; Diaz de Ståhl 2001; Getahun et al. 2004; Hamano et al. 2000). Since these mice have a normal complement system, the observations support the conclusion that FcγRs rather than complement is of primary importance for the enhancement. Nevertheless, complement may play a role in situations where the aggregation status/size of the antigen favours complement activation.

An interesting question is which role FcγRIIB plays in IgG-mediated enhancement. This receptor negatively regulates receptors which operate via ITAMs (immunoreceptor tyrosine-based activation motifs), e.g. FcγRI, FcγRIII, FcγRIV, and FcεRI (Daëron and Lesourne 2006). However, FcγRIIB is expressed on FDC (Qin et al. 2000) where it may capture IgG-immune complexes, possibly leading to enhancement of antibody responses. Interestingly, $Fc\gamma RIIB^{-/-}$ mice

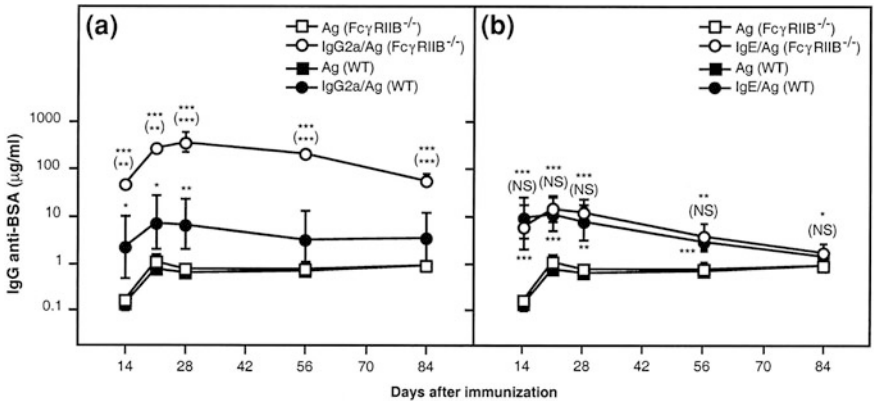


Fig. 3 Enhanced IgG2a-mediated enhancement in FcγRIIB-deficient mice. Wild-type and FcγRIIB-deficient mice were immunized intravenously with 20 μg BSA-TNP alone or with 50 μg of TNP-specific IgG2a (**a**) or IgE (**b**) and 20 μg BSA-TNP. IgG anti-BSA was measured in ELISA. Asterisks indicate statistical differences between mice of the same strain given antigen alone and mice given antibody/antigen complexes. Asterisks within parantheses indicate statistical differences between FcγRIIB-deficient mice and wild-type mice given antibody/antigen complexes (*From* Getahun et al. 2004 (Copyright 2004. The American Association of Immunologists, Inc.))

given TNP-specific IgG1, IgG2a, or IgG2b together with BSA-TNP have a significantly higher antibody response than corresponding wild-type animals (Wernersson et al. 1999; Getahun et al. 2004). Thus, IgG-mediated enhancement does not require FcγRIIB. Moreover, FcγRIIB negatively regulates responses to IgG-antigen complexes as revealed by the enhanced enhancement seen in FcγRIIB$^{-/-}$ mice (Fig. 3). This observation further strengthens the conclusion that IgG1, IgG2a, and IgG2b enhance via activating FcγRs, as these can be negatively regulated by FcγRIIB. In contrast, IgG3- and IgE-mediated enhancement of antibody responses is not further enhanced in FcγRIIB$^{-/-}$ mice (Wernersson et al. 1999; Getahun et al. 2004; Diaz de Ståhl et al. 2003) (Fig. 3b). This is to be expected since IgG3- and IgE-mediated enhancement is mediated by CR1/2 and CD23 respectively and neither of these receptors signal via ITAMs. Not only primary antibody responses, but also recall responses and induction of germinal centers is considerably higher in FcγRIIB$^{-/-}$ than in wild-type animals after immunization with IgG-immune complexes (Getahun et al. 2004). Noteworthy is that although FcγRIIB inhibits responses to IgG-antigen complexes, the responses are far from completely suppressed (Fig. 3), showing that FcγRIIB downmodulates rather than completely turns off the response. This observation is in line with the finding that IgG-mediated suppression of responses to SRBC, which is often close to a 100 %, is not mediated by FcγRIIB (Karlsson et al. 1999, 2001).

4.3 Summary

The most likely mechanism explaining why IgG1, IgG2a, and IgG2b are able to enhance responses to protein antigens is that these immune complexes are captured by FcγR$^+$ dendritic cells, endocytosed and efficiently presented to CD4$^+$ T cells which in turn induce antibody production by B cells. This scenario is supported by the crucial role of activating FcγRs (Wernersson et al. 1999; Diaz de Ståhl 2001; Getahun et al. 2004; Hamano et al. 2000), which must be expressed on bone marrow-derived/CD11c$^+$ cells (Diaz de Ståhl 2001; de Jong et al. 2006), and by the strong upregulation of specific CD4$^+$ T cell responses (Getahun et al. 2004; de Jong et al. 2006).

5 IgG3-Mediated Enhancement of Antibody Responses

Murine IgG3 is the major subclass in responses against T cell-independent type 2 antigens but only constitutes a small fraction of the response against T cell-dependent protein antigens (Perlmutter et al. 1978; Rubinstein and Stein 1988). As the last amongst the IgG subclasses, IgG3 was found to be able to enhance antibody responses against small proteins like OVA and BSA (Diaz de Ståhl et al. 2003; Hjelm et al. 2005). In analogy with IgG1, IgG2a, and IgG2b, IgG3 has a dual effect and suppresses responses against SRBC (Heyman and Wigzell 1984; Brüggemann and Rajewsky 1982). Also the development of germinal centers is enhanced by IgG3 (Zhang et al. unpublished) whereas the effect on induction of memory and secondary responses has not yet been examined. IgG3-mediated enhancement of CD4$^+$ T cell proliferation is very modest in vivo and absent in vitro (Hjelm et al. 2005).

5.1 Complement and IgG3-Mediated Enhancement

IgG3-mediated enhancement is severely diminished in C3-depleted mice and in mice lacking CR1/2 (Diaz de Ståhl et al. 2003).

5.2 Fc-Receptors and IgG3-Mediated Enhancement

Enhancement by IgG3 works well in FcRγ$^{-/-}$ mice and in mice selectively lacking FcγRI (Diaz de Ståhl et al. 2003; Hjelm et al. 2005), the latter being the only Fc-receptor shown to bind IgG3 (Gavin et al. 1998). IgG3 enhances well in FcγRIIB$^{-/-}$ mice (Diaz de Ståhl et al. 2003) and the level of enhancement is not higher than in

wild-type mice (Diaz de Ståhl et al. 2003). Thus, IgG3-mediated enhancement is not dependent on Fc-receptors and is not negatively regulated by FcγRIIB.

5.3 Summary

It appears that enhancement by IgG3 resembles enhancement by IgM more than it resembles enhancement by IgG1, IgG2a, or IgG2b. Both IgM and IgG3 require complement and CR1/2 but do not seem to depend on FcRs whereas the situation is the reverse for IgG1, IgG2a, and IgG2b. Moreover, IgM and IgG3 have minor effects on $CD4^+$ T cell proliferation (Ding et al. 2013; Hjelm et al. 2005) whereas IgG2a is an efficient upregulator of $CD4^+$ T cells (Getahun et al. 2004). A possible explanation for the preferential use of complement by IgG3 but not by the other IgG subclasses, could be the different strategies used to activate complement. IgG1 is often unable to fix C1q whereas IgG2a and IgG2b requires that two IgG molecules aggregate to achieve sufficient avidity for binding C1q. For this to happen, high concentrations of specific IgG are required. IgG3 on the other hand has the capacity to self-aggregate and when one IgG3 molecule binds to a surface, other IgG3 molecules are attracted and bound via Fc-mediated cooperativity (Cooper et al. 1991; Greenspan and Cooper 1992). These different modes of C1q fixation indicate that lower concentrations of specific IgG3 than of IgG2a and IgG2b are required to activate the complement system.

The mechanism behind IgG3-mediated enhancement is probably the same as for IgM-mediated enhancement: immune complexes become opsonized with complement and bind to complement receptor 2 on MZ B cells which transport them from the marginal zone to B cell follicles and deposit the antigen onto $CR1^+$ FDC (Zhang et al. in press).

6 IgE-Mediated Enhancement of Antibody Responses

IgE was identified as a new antibody class by two laboratories in the 1960s (Ishizaka et al. 1966; Johansson and Bennich 1967). Studying reaginic antibodies from ragweed-sensitive patient sera, Ishizaka and colleagues found that these antibodies did not react with antibodies against any of the known immunoglobulin classes and named the new antibody γE-globulin (Ishizaka et al. 1966). At Uppsala University Hospital, a patient with a plasma cell tumor (multiple myeloma) secreting antibodies of a hitherto unknown antibody class was diagnosed and the myeloma protein was called myeloma-IgND (Johansson and Bennich 1967). Subsequently, the two research groups agreed to call the new antibody class IgE (Bennich et al. 1968). Whereas IgE is most well-known for causing allergic disease, its biological function is probably to defend us against parasites and possibly also against toxins (Palm et al. 2013; Marichal et al. 2013). A role for IgE in immune regulation was established when it was found that monoclonal TNP-specific IgE antibodies,

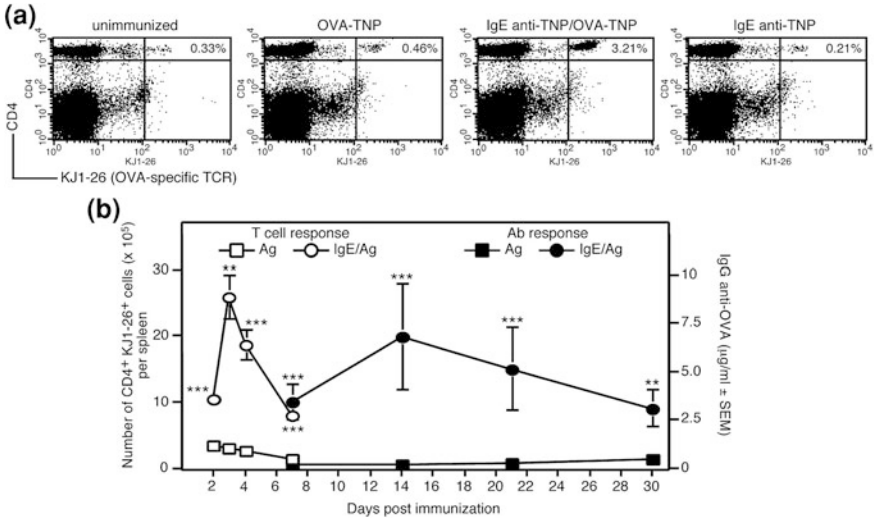


Fig. 4 IgE enhances antibody and T cell responses. Wild-type mice were adoptively transferred with spleen cells from DO11.10 mice containing 3×10^6 OVA-specific (KJ1-26$^+$) transgenic CD4$^+$ T cells. The next day, these mice were immunized intravenously with 20 μg OVA-TNP alone or with 50 μg IgE anti-TNP and 20 μg OVA-TNP. The number of OVA-specific T cells were determined in flow cytometry 3 days (**a**) or 2–7 (**b**) days after immunization. The IgG anti-OVA response was measured in ELISA (**b**) (*From* Getahun et al. 2005 (Copyright 2005. The American Association of Immunologists, Inc.))

administered to mice together with TNP-BSA, induced a much higher BSA-specific IgG response than did antigen alone (Heyman et al. 1993). The enhancement could be more than a 100-fold (Heyman et al. 1993; Gustavsson et al. 1994, 1998; Westman et al. 1997) and both primary antibody responses and priming for a memory response was affected (Gustavsson et al. 1994). IgE upregulates responses to BSA, OVA, and tetanus toxoid, but not to KLH or SRBC (Gustavsson et al. 1994; Fujiwara et al. 1994). Not only IgG responses, but also IgM, IgA, and IgE responses are enhanced (Gustavsson et al. 1994; Westman et al. 1997). There appears to be no skewing towards a Th1 or Th2 response since production of both IgG1 and IgG2a is upregulated (Gustavsson et al. 1994; Hjulström et al. 1995). In parallel with enhancing antibody responses, IgE causes an increase in proliferation and activation of specific CD4$^+$ T cells (Getahun et al. 2005; Hjelm et al. 2008; Carlsson et al. 2009; Henningsson et al. 2011) (Fig. 4).

6.1 Fc-Receptors and IgE-Mediated Enhancement

IgE does not activate complement, but binds to several Fc-receptors: FcεRI with high affinity (Metzger 1991), and FcεRII (or CD23) (Lawrence et al. 1975), FcγRIIB, FcγRIII, and FcγRIV (Takizawa et al. 1992; Mancardi et al. 2008) with

low to intermediate affinity. Somewhat surprisingly, the ability of IgE to enhance antibody and $CD4^+$ T cell responses depends only on CD23. This is evidenced by the severely impaired enhancement in $CD23^{-/-}$ mice (Fujiwara et al. 1994; Getahun et al. 2005; Gustavsson et al. 2000) and the unperturbed enhancement in $FcR\gamma^{-/-}$ (lacking FcεRI as well as activating FcγRs) (Wernersson et al. 1999; Hjelm et al. 2005) and $Fc\gamma IIB^{-/-}$ (Wernersson et al. 1999; Getahun et al. 2004) mice. IgE-mediated enhancement is intact in $IL\text{-}4^{-/-}$ mice (Hjulström et al. 1995). This indicates an important role of the CD23a isoform, which is constitutively expressed on B cells and FDC, rather than of the IL-4-dependent CD23b isoform (Rao et al. 1987; Maeda et al. 1992; Kondo et al. 1994; Yu et al. 2003). Experiments in $CD23^{-/-}$ mice after transfer of isolated B cells or in bone marrow chimeras, have established that expression of CD23 on B cells is sufficient for enhancement of both antibody and T cell responses (Getahun et al. 2005; Gustavsson et al. 2000).

In vitro, B cells can take up IgE-antigen complexes via CD23 and present antigenic peptides to $CD4^+$ T cells (Kehry and Yamashita 1989; Pirron et al. 1990; Carlsson et al. 2007). In vivo, IgE-antigen complexes induce a potent proliferation of T cells (Getahun et al. 2005; Carlsson et al. 2007). In addition, $CD23^+$ B cells in the blood capture IgE-antigen complexes and rapidly transport them to splenic B cell follicles (Hjelm et al. 2008). Based on these data, the requirement of $CD23^+$ B cells in IgE-mediated upregulation of immune responses in vivo could be explained either by enhanced presentation or enhanced transport of IgE-antigen complexes. When the antigen presenting capacity of B cells and $CD11c^+$ cells obtained from mice immunized with IgE-antigen complexes was tested, it was found that only $CD11c^+$ cells, and not B cells, could activate $CD4^+$ T cells ex vivo (Henningsson et al. 2011). Moreover, the ability of IgE to activate $CD4^+$ T cells in $CD23^{-/-}$ mice could be rescued by MHC-incompatible $CD23^+$ B cells which are unable to present antigen to T cells (Henningsson et al. 2011). This suggested that $CD23^+$ B cells transport and $CD11c^+$ cells present the antigen.

6.2 Summary

A likely scenario for how IgE upregulates antibody and T cell responses is that $CD23^+$ recirculating B cells capture IgE-antigen complexes in the blood and transport them to the follicles. The antigen is then delivered to $CD11c^+$ dendritic cells and presented to T cells which in turn help B cells to produce specific antibodies. Where and how the antigen transfer takes place is not known. Neither is it evident which biological role IgE-mediated enhancement plays. IgE is present in very low concentrations in normal serum but production of virus-specific IgE has been demonstrated during viral infections (Alexeyev et al. 1994; Welliver 2003). It is feasible that this takes place more often than is currently understood, and that immune complexes formed between IgE and virus are rapidly transported to splenic follicles, constituting a way for the immune system to enhance anti-viral responses.

Another speculative idea is that CD23 captures antigen independently of IgE. Unlike other FcRs, CD23 does not belong to the super-Ig family but is a type II integral membrane protein with a calcium-dependent lectin domain in the C-terminal end of the extracellular part (Bettler et al. 1989). Other members of the C-type lectin family interact with exogenous ligands, some of which are expressed on pathogens (McGreal et al. 2005). Should CD23 also be able to bind pathogens directly, rapid transport of antigen to splenic follicles could take place without the presence of specific IgE.

7 Concluding Remarks

The adjuvant effects of antibodies are likely to play an important role in normal antibody responses. IgG, produced in a primary response and still present at the time of a second antigen encounter, will act to induce a strong recall response and add to the effect of long-lived memory lymphocytes. Regulation by IgG is extremely complex with many subclasses binding to various FcγRs (co-expressed on different immune cells) and in addition often able to activate complement. IgG also has dual effects, suppressing responses to large antigens while enhancing responses to protein antigens. In order for IgG antibodies to be used as adjuvants in clinical settings, careful selection of well-defined monoclonals will therefore be required. In primary antibody responses, IgM seems to play a role as mice lacking secretory IgM have impaired responses. The role of specific IgM, acting via complement, in inducing primary responses is enigmatic given the normal antibody responses seen in knock-in mice with mutant IgM unable to activate complement. The last enhancing isotype, IgE, may play a biological role provided certain pathogens induce early IgE responses thereby starting the formation of an immune complex.

Acknowledgements I wish to thank all past and present members of my laboratory, who have shared my interest in revealing how antibodies feedback-regulate the production of themselves. The work has been supported by Uppsala University, the Swedish Research Council, Ellen, Walter and Lennart Hesselman's Foundation, Hans von Kantzow's Foundation, King Gustaf V:s 80 Years Foundation, Ollie and Elof Ericsson's Foundation, and Agnes and Mac Rudberg's Foundation.

References

Alexeyev OA, Ahlm C, Billheden J, Settergren B, Wadell G, Juto P (1994) Elevated levels of total and Puumala virus-specific immunoglobulin E in the Scandinavian type of hemorrhagic fever with renal syndrome. Clin Diagn Lab Immunol 1(3):269–272

Applequist SE, Dahlström J, Jiang N, Molina H, Heyman B (2000) Antibody production in mice deficient for complement receptors 1 and 2 can be induced by IgG/Ag and IgE/Ag, but not IgM/Ag complexes. J Immunol 165:2398–2403

Arnon TI, Horton RM, Grigorova IL, Cyster JG (2013) Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress. Nature 493(7434):684–688

Baumgarth N, Herman OC, Jager GC, Brown LE, Herzenberg LA, Chen J (2000) B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. J Exp Med 192(2):271–280

Bennich HH, Ishizaka K, Johansson SG, Rowe DS, Stanworth DR, Terry WD (1968) Immunoglobulin E: a new class of human immunoglobulin. Immunology 15(3):323–324

Bettler B, Hofstetter H, Rao M, Yokoyama WM, Kilchherr F, Conrad DH (1989) Molecular structure and expression of the murine lympocyte low affinity receptor for IgE ($Fc_{\varepsilon}RII$). Proc Natl Acad Sci USA 86:7566–7570

Brinc D, Lazarus AH (2009) Mechanisms of anti-D action in the prevention of hemolytic disease of the fetus and newborn. Hematol Am Soc Hematol Educ Program 185–191

Brinc D, Le-Tien H, Crow AR, Siragam V, Freedman J, Lazarus AH (2008) Transfusion of IgG-opsonized foreign red blood cells mediates reduction of antigen-specific B cell priming in a murine model. J Immunol 181(2):948–953

Brüggemann M, Rajewsky K (1982) Regulation of the antibody response against hapten-coupled erythrocytes by monoclonal anti-hapten antibodies of various isotypes. Cell Immunol 71:365–373

Carlsson F, Hjelm F, Conrad DH, Heyman B (2007) IgE enhances specific antibody and T cell responses in mice overexpressing CD23. Scand J Immunol 66:261–270

Carlsson F, Getahun A, Rutemark C, Heyman B (2009) Impaired antibody responses but normal proliferation of specific $CD4^+$ T cells in mice lacking complement receptors 1 and 2. Scand J Immunol 70(2):77–84

Cerottini JC, McConahey PJ, Dixon FJ (1969) The immunosuppressive effect of passively administered antibody IgG fragments. J Immunol 102:1008–1015

Chan PL, Sinclair NRSTC (1973) Regulation of the immune response. VI. Inability of F(ab′)2 antibody to terminate established immune responses and its ability to interfere with IgG antibody-mediated immunosuppression. Immunology 24:289–310

Cinamon G, Zachariah MA, Lam OM, Foss FW Jr, Cyster JG (2008) Follicular shuttling of marginal zone B cells facilitates antigen transport. Nat Immunol 9(1):54–62

Clarke CA, Donohoe WTA, Woodrow JC, Finn R, Krevans JR, Kulke W et al (1963) Further experimental studies on the prevention of Rh haemolytic disease. Br Med J 1:979–984

Cooper LJ, Schimenti JC, Glass DD, Greenspan NS (1991) H chain C domains influence the strength of binding of IgG for streptococcal group A carbohydrate. J Immunol 146(8):2659–2663

Coulie P, Van Snick J (1985) Enhancement of IgG anti-carrier responses by IgG2-anti-hapten antibodies in mice. Eur J Immunol 15:793–798

Daëron M, Lesourne R (2006) Negative signaling in Fc receptor complexes. Adv Immunol 89:39–86

de Jong JM, Schuurhuis DH, Ioan-Facsinay A, Welling MM, Camps MG, van der Voort EI et al (2006) Dendritic cells, but not macrophages or B cells, activate major histocompatibility complex class II-restricted CD4 + T cells upon immune-complex uptake in vivo. Immunology 119(4):499–506

de Ståhl Diaz (2001) T, Heyman B. IgG2a-mediated enhancement of antibody responses is dependent on FcRgamma + bone marrow-derived cells. Scand J Immunol 54(5):495–500

Dennert G (1971) The mechanism of antibody-induced stimulation and inhibition of the immune response. J Immunol 106:951–955

Diaz de Ståhl T, Dahlström J, Carroll MC, Heyman B (2003) A role for complement in feedback-enhancement of antibody responses by IgG3. J Exp Med 197:1183–1190

Ding Z, Bergman A, Rutemark C, Ouchida R, Ohno H, Wang JY et al (2013) Complement-activating IgM enhances the humoral but not the T cell immune response in mice. PLoS ONE 8(11):e81299

Donius LR, Handy JM, Weis JJ, Weis JH (2013) Optimal germinal center B cell activation and T-dependent antibody responses require expression of the mouse complement receptor Cr1. J Immunol 191(1):434–447

Ehrenstein MR, O'Keefe TL, Davies SL, Neuberger MS (1998) Targeted gene disruption reveals a role for natural secretory IgM in the maturation of the primary immune response. Proc Natl Acad Sci USA 95(17):10089–10093

Enriquez-Rincon F, Klaus GGB (1984) Differing effects of monoclonal anti-hapten antibodies on humoral responses to soluble or particulate antigens. Immunology 52:129–136

Ferguson AR, Youd ME, Corley RB (2004) Marginal zone B cells transport and deposit IgM-containing immune complexes onto follicular dendritic cells. Int Immunol 16(10):1411–1422

Fujiwara H, Kikutani H, Suematsu S, Naka T, Yoshida K, Yoshida K et al (1994) The absence of IgE antibody-mediated augmentation of immune responses in CD23-deficient mice. Proc Natl Acad Sci USA 91:6835–6839

Gavin AL, Barnes N, Dijstelbloem HM, Hogarth PM (1998) Identification of the mouse IgG3 receptor: Implications for antibody effector function at the interface between innate and adaptive immunity. J Immunol 160:20–23

Getahun A, Heyman B (2009) Studies on the mechanism by which antigen-specific IgG suppresses primary antibody responses: evidence for epitope masking and decreased localization of antigen in the spleen. Scand J Immunol 70(3):277–287

Getahun A, Dahlström J, Wernersson S, Heyman B (2004) IgG2a-mediated enhancement of Ab- and T-cell responses and its relation to inhibitory and activating $Fc_{\gamma}Rs$. J Immunol 172:5269–5276

Getahun A, Hjelm F, Heyman B (2005) IgE enhances antibody and T cell responses in vivo via $CD23^{+}$ B Cells. J Immunol 175(3):1473–1482

Greenspan NS, Cooper LJN (1992) Intermolecular cooperativity: a clue to why mice have IgG3? Immunol Today 13:164–168

Gustavsson S, Hjulström S, Liu T, Heyman B (1994) CD23/IgE-mediated regulation of the specific antibody response in vivo. J Immunol 152:4793–4800

Gustavsson S, Hjulström-Chomez S, Lidström B-M, Ahlborg N, Andersson R, Heyman B (1998) Impaired antibody responses in $H\text{-}2A^{b}$ mice. J Immunol 161:1765–1771

Gustavsson S, Wernersson S, Heyman B (2000) Restoration of the antibody response to IgE/antigen complexes in CD23-deficient mice by $CD23^{+}$ spleen or bone marrow cells. J Immunol 164:3990–3995

Hamano Y, Arase H, Saisho H, Saito T (2000) Immune complex and Fc receptor-mediated augmentation of antigen presentation for in vivo Th cell responses. J Immunol 164(12):6113–6119

Harte PG, Cooke A, Playfair JHL (1983) Specific monoclonal IgM is a potent adjuvant in murine malaria vaccination. Nature 302:256–258

Heesters BA, Chatterjee P, Kim YA, Gonzalez SF, Kuligowski MP, Kirchhausen T, et al (2013) Endocytosis and recycling of immune complexes by follicular dendritic cells enhances B cell antigen binding and activation. Immunity 38:1164–1175

Henningsson F, Ding Z, Dahlin JS, Linkevicius M, Carlsson F, Grönvik KO et al (2011) IgE-mediated enhancement of CD4+ T cell responses in mice requires antigen presentation by CD11c+ cells and not by B cells. PLoS ONE 6(7):e21760

Henry C, Jerne N (1968) Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. J Exp Med 128:133–152

Heyman B (2000) Regulation of antibody responses via antibodies, complement, and Fc receptors. Annu Rev Immunol 18:709–737

Heyman B (2003) Feedback regulation by IgG antibodies. Immunol Lett 88:157–161

Heyman B (2013) Antibody mediated regulation of humoral immunity. In: Nimmerjahn F (ed) Molecular and cellular mechanisms of antibody activity. Springer, New York, pp 221–249

Heyman B, Wigzell H (1984) Immunoregulation by monoclonal sheep erythrocyte specific IgG antibodies. Suppression is correlated to level of antigen binding and not to isotype. J Immunol 132:1136–1143

Heyman B, Wigzell H (1985) IgM enhances and IgG suppresses immunological memory in mice. Scand J Immunol 21:255–266

Heyman B, Andrighetto S, Wigzell H (1982) Antigen dependent IgM-mediated enhancement of the sheep erythrocyte response in mice. Evidence for induction of B cells with specificities other than that of the injected antibodies. J Exp Med 155:994–1009

Heyman B, Pilström L, Shulman MJ (1988a) Complement activation is required for IgM-mediated enhancement of the antibody response. J Exp Med 167:1999–2004

Heyman B, Wiersma E, Nose M (1988b) Complement activation is not required for IgG-mediated suppression of the antibody response. Eur J Immunol 18:1739–1743

Heyman B, Liu T, Gustavsson S (1993) In vivo enhancement of the specific antibody response via the low affinity receptor for IgE. Eur J Immunol 23:1739–1742

Hjelm F, Carlsson F, Verbeek S, Heyman B (2005) IgG3-mediated enhancement of the antibody response is normal in Fc gammaRI-deficient mice. Scand J Immunol 62(5):453–461

Hjelm F, Carlsson F, Getahun A, Heyman B (2006) Antibody-mediated regulation of the immune response. Scand J Immunol 64(3):177–184

Hjelm F, Karlsson MCI, Heyman B (2008) A novel B-cell mediated transport of IgE-immune complexes to the follicle of the spleen. J Immunol 180:6604–6610

Hjulström S, Landin A, Jansson L, Holmdahl R, Heyman B (1995) No role of interleukin-4 in CD23/IgE-mediated enhancement of the murine antibody response in vivo. Eur J Immunol 25:1469–1472

Honjo K, Kubagawa Y, Jones DM, Dizon B, Zhu Z, Ohno H et al (2012) Altered Ig levels and antibody responses in mice deficient for the Fc receptor for IgM (FcmuR). Proc Natl Acad Sci USA 109(39):15882–15887

Ishizaka K, Ishizaka T, Hornbrook MM (1966a) Physicochemical properties of reaginic antibody. V. Correlation of reaginic activity with gamma-E-globulin antibody. J Immunol 97(6):840–853

Ishizaka K, Ishizaka T, Hornbrook MM (1966b) Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J Immunol 97(1):75–85

Johansson SG, Bennich H (1967) Immunological studies of an atypical (myeloma) immunoglobulin. Immunology 13(4):381–394

Karlsson MCI, Wernersson S, Diaz de Ståhl T, Gustavsson S, Heyman B (1999) Efficient IgG-mediated suppression of primary antibody responses in Fc-gamma receptor-deficient mice. Proc Natl Acad Sci USA 96:2244–2249

Karlsson MCI, Getahun A, Heyman B (2001) Fc_{γ}RIIB in IgG-mediated suppression of antibody responses: different impact in vivo and in vitro. J Immunol 167:5558–5564

Kehry MR, Yamashita LC (1989) Low-affinity IgE receptor (CD23) function on mouse B cells: role in IgE-dependent antigen focusing. Proc Natl Acad Sci USA 86:7556–7560

Klaus GGB (1979) Generation of memory cells. III. Antibody class requirements for the generation of B-memory cells by antigen-antibody complexes. Immunology 37:345–351

Kondo H, Ichikawa Y, Nakamura K, Tsuchiya S (1994) Cloning of cDNAs for new subtypes of murine low-affinity Fc receptor for IgE (Fc_{ε}RII/CD23). Int Arch Allergy Immunol 105:38–48

Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW et al (2009) Identity of the elusive IgM Fc receptor (FcmuR) in humans. J Exp Med 206(12):2779–2793

Lawrence DA, Weigle WO, Spiegelberg HL (1975) Immunoglobulins cytophilic for human lymphocytes, monocytes, and neutrophils. J Clin Invest 55:268–275

Link A, Zabel F, Schnetzler Y, Titz A, Brombacher F, Bachmann MF (2012) Innate immunity mediates follicular transport of particulate but not soluble protein antigen. J Immunol 188(8):3724–3733

Maeda K, Burton GF, Padgett DA, Conrad DH, Huff TF, Masuda A et al (1992) Murine follicular dendritic cells and low affinity Fc receptors for IgE (Fc_{ε}RII). J Immunol 148:2340–2347

Mancardi DA, Iannascoli B, Hoos S, England P, Daeron M, Bruhns P (2008) FcgammaRIV is a mouse IgE receptor that resembles macrophage FcepsilonRI in humans and promotes IgE-induced lung inflammation. J Clin Invest 118(11):3738–3750

Marichal T, Starkl P, Reber LL, Kalesnikoff J, Oettgen HC, Tsai M et al (2013) A beneficial role for immunoglobulin E in host defense against honeybee venom. Immunity 39:963–975
McGreal EP, Miller JL, Gordon S (2005) Ligand recognition by antigen-presenting cell C-type lectin receptors. Curr Opin Immunol 17:18–24
Metzger H (1991) The high affinity receptor for IgE on mast cells. Clin Exp Allergy 21:269–279
Na D, Kim D, Lee D (2006) Mathematical modeling of humoral immune response suppression by passively administered antibodies in mice. J Theor Biol 241(4):830–851
Nie X, Basu S, Cerny J (1997) Immunization with immune complex alters the repertoire of antigen-reactive B cells in the germinal centers. Eur J Immunol 27(12):3517–3525
Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science 310(5753):1510–1512
Ouchida R, Mori H, Hase K, Takatsu H, Kurosaki T, Tokuhisa T et al (2012) Critical role of the IgM Fc receptor in IgM homeostasis, B-cell survival, and humoral immune responses. Proc Natl Acad Sci USA 109(40):E2699–E2706
Palm NW, Rosenstein RK, Yu S, Schenten DD, Florsheim E, Medzhitov R (2013) Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. Immunity 39:976–985
Perlmutter RM, Hansburg D, Briles DE, Nicolotti RA, Davie JM (1978) Subclass restriction of murine anti-carbohydrate antibodies. J Immunol 121:566–572
Pirron U, Schlunck T, Prinz JC, Rieber EP (1990) IgE-dependent antigen focusing by human B lymphocytes is mediated by low-affinity receptor for IgE. Eur J Immunol 20:1547–1551
Qin D, Wu J, Vora KA, Ravetch JV, Szakal AK, Manser T et al (2000) Fc gamma receptor IIB on follicular dendritic cells regulates the B cell recall response. J Immunol 164(12):6268–6275
Rao M, Lee WT, Conrad DH (1987) Characterization of a monoclonal antibody directed against the murine B lymphocyte receptor for IgE. J Immunol 138:1845–1851
Rubinstein LJ, Stein KE (1988) Murine immune response to the *N. meningitidis* group C capsular polysaccharide: ontogeny. J Immunol 141:4352–4356
Rutemark C, Alicot E, Bergman A, Ma M, Getahun A, Ellmerich S et al (2011) Requirement for complement in antibody responses is not explained by the classic pathway activator IgM. Proc Natl Acad Sci USA 108(43):E934–E942
Rutemark C, Bergman A, Getahun A, Hallgren J, Henningsson F, Heyman B (2012) Complement receptors 1 and 2 in murine antibody responses to IgM-complexed and uncomplexed sheep erythrocytes. PLoS ONE 7(7):e41968
Shima H, Takatsu H, Fukuda S, Ohmae M, Hase K, Kubagawa H et al (2010) Identification of TOSO/FAIM3 as an Fc receptor for IgM. Int Immunol 22(3):149–156
Sinclair NRSC (1969) Regulation of the immune response. I. Reduction in ability of specific antibody to inhibit longlasting IgG immunological priming after removal of the Fc fragment. J Exp Med 129:1183–1201
Takizawa F, Adamczewski M, Kinet J-P (1992) Identification of the low affinity receptor for immunoglobulin E on mouse mast cells and macrophages as $Fc_{\gamma}RII$ and $Fc_{\gamma}RIII$. J Exp Med 176:469–476
Tao TW, Uhr JW (1966) Capacity of pepsin-digested antibody to inhibit antibody formation. Nature 212:208–209
Terres G, Habicht GS, Stoner RD (1974) Carrier-specific enhancement of the immune response using antigen-antibody complexes. J Immunol 112:804–811
Uhr JW, Möller G (1968) Regulatory effect of antibody on the immune response. Adv Immunol 8:81–127
Urbaniak SJ, Greiss MA (2000) RhD haemolytic disease of the fetus and the newborn. Blood Rev 14(1):44–61
von Behring E, Wernicke E (1892) Über Immunisierung und Heilung von Versuchstieren bei der Diphterie. Z Hyg Infektionskrankheit 12:10–44
Welliver RC (2003) Respiratory syncytial virus and other respiratory viruses. Pediatr Infect Dis J 22(2 Suppl):S6–S10; discussion S-2

Wernersson S, Karlsson M, Dahlström J, Mattsson R, Verbeek JS, Heyman B (1999) IgG-mediated enhancement of Ab responses is low in FcR_{γ} chain deficient mice and increased in $Fc_{\gamma}RII$ deficient mice. J Immunol 163:618–622

Westman S, Gustavsson S, Heyman B (1997) Early expansion of secondary B cells after primary immunization with antigen complexed with IgE. Scand J Immunol 46:10–15

Whited Collisson E, Andersson B, Lamon EW (1984) Avidities of hapten-specific antibodies when the responses are modulated by anti-carrier antibodies. Immunology 53:443–449

Wiersma EJ, Coulie PG, Heyman B (1989) Dual immunoregulatory effects of monoclonal IgG-antibodies: suppression and enhancement of the antibody response. Scand J Immunol 29:439–448

Wiersma EJ, Nose M, Heyman B (1990) Evidence of IgG-mediated enhancement of the antibody response in mice without classical pathway complement activation. Eur J Immunol 20:2585–2589

Youd ME, Ferguson AR, Corley RB (2002) Synergistic roles of IgM and complement in antigen trapping and follicular localization. Eur J Immunol 32:2328–2337

Yu LCH, Montagnac G, Yang P-C, Conrad DH, Benmerah A, Perdue MH (2003) Intestinal epithelial CD23 mediates enhanced antigen transport in allergy: evidence for a novel splice form. Am J Physiol Gastrointes Liver Physiol 285:G223–G234

Zhang Y, Meyer-Hermann M, George LA, Figge MT, Khan M, Goodall M et al (2013) Germinal center B cells govern their own fate via antibody feedback. J Exp Med 210(3):457–464

Zhang L, Ding Z, Xu H, Heyman B Marginal zone B cells transport IgG3-immune complexes to splenic follicles. J Immunol (in press)

IgA, IgA Receptors, and Their Anti-inflammatory Properties

Sanae Ben Mkaddem, Ivy Christou, Elisabetta Rossato, Laureline Berthelot, Agnès Lehuen and Renato C. Monteiro

Abstract Immunoglobulin A (IgA) is the most abundantly produced antibody isotype in mammals. The primary function of IgA is to maintain homeostasis at mucosal surfaces and play a role in immune protection. IgA functions mainly through interaction with multiple receptors including IgA Fc receptor I (FcαRI), transferrin receptor 1 (CD71), asialoglycoprotein receptor (ASGPR), Fcα/μR, FcRL4, and DC-SIGN/SIGNR1. In this review we discuss recent data demonstrating anti-inflammatory functions of IgA through two receptors, the FcαRI and DC-SIGN/SIGNR1 interactions in the regulation of immunity. Serum monomeric IgA is able to mediate an inhibitory signal following the interaction with FcαRI. It results in partial phosphorylation of its FcRγ-ITAM and the recruitment of the tyrosine phosphatase SHP-1, which induces cell inhibition following the formation of intracellular clusters named inhibisomes. In contrast, cross-linking of FcαRI by multimeric ligands induces a full phosphorylation of the FcRγ-ITAM leading to

S. B. Mkaddem · I. Christou · E. Rossato · L. Berthelot · R. C. Monteiro
Inserm, U1149, Centre de Recherche sur l'Inflammation, Paris, France

S. B. Mkaddem · I. Christou · E. Rossato · L. Berthelot · R. C. Monteiro
CNRS ERL8252, Paris, France

S. B. Mkaddem · I. Christou · E. Rossato · L. Berthelot · R. C. Monteiro
Université Paris Diderot, Sorbonne Paris Cité, Site Xavier Bichat, Paris, France

S. B. Mkaddem · I. Christou · E. Rossato · L. Berthelot · A. Lehuen · R. C. Monteiro
Laboratoire d'Excellence Inflamex, Paris, France

A. Lehuen
Inserm, U1016, Institut Cochin, Paris, France

R. C. Monteiro
Laboratoire Dysfonctionnements Immunitaires, DHU Fire, Hôpital Bichat Claude Bernard, Paris, France

R. C. Monteiro (✉)
INSERM UMR1149 & CNRS ERL8252, Faculté de Médecine Xavier Bichat,
16 Rue Henri Huchard, Paris 75018, Cedex 18, France
e-mail: renato.monteiro@inserm.fr

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_10,

the recruitment of the tyrosine kinase Syk and cell activation. In addition, secretory IgA can mediate a potent anti-inflammatory function following the sugar-dependent interaction with SIGNR1 on dendritic cells which induces an immune tolerance via regulatory T cell expansion. Overall, the anti-inflammatory effect of serum and secretory IgA plays a crucial role in the physiology and in the prevention of tissue damage in multiple autoimmune and inflammatory diseases.

Keywords Immunoglobulin A · ITAMi · Inflammation · FcαRI · SIGNR1

Contents

1 Introduction 222
2 IgA Structure and Metabolism 223
3 Functional Role of IgA 224
4 FcαRI Expression and Structure 225
5 Janus-like Functions of the FcαRI Molecule 227
6 Tolerogenic Role of Secretory IgA Through SIGNR1 229
7 Pathological Role of IgA 230
8 IgA as Therapeutic Agent 231
9 Concluding Remarks 232
References 233

1 Introduction

Immunoglobulin A (IgA) is the most abundant antibody isotype produced in the body and the predominant antibody (Ab) class present in mucosal areas such as gastrointestinal tract, the respiratory tract, and the vaginal tract where it has a crucial role in the immune responses. It exists in multiple molecular forms and two subclasses (Pabst 2012). The antibody IgA can occur as a monomer, but also in dimeric or even polymeric forms through interactions with the joining chain (J-chain). All these different forms are mainly found in the circulation, while secretory IgA (SIgA) is mainly found at mucosal surfaces. SIgA is generated by the binding of dimeric IgA via the J-chain to the polymeric immunoglobulin receptor (pIgR) at the basolateral side of the epithelium, which is subsequently transported to the luminal side. IgA is then released at the mucosal surface (lumen) by cleavage from the pIgR. In this process part of the pIgR, called the secretory component (SC), remains attached to the IgA molecule, and together they form the molecule SIgA. SIgA promotes immune exclusion by entrapping dietary antigens and microorganisms in the mucus, downregulates the expression of pro-inflammatory bacterial epitopes on commensal bacteria, and, in general, promotes the maintenance of appropriate bacterial communities within specific intestinal segments

(Fagarasan et al. 2002; Peterson et al. 2007; Phalipon et al. 2002). In addition, SIgA blocks or sterically hinders microbial components involved in epithelial attachment, mediates intra-epithelial neutralization of incoming pathogens and microbial inflammatory products, and facilitates antigen sampling by binding to microfold (M) cells, an epithelial-like cell type specialized in antigen capturing (Norderhaug et al. 1999; Kadaoui and Corthesy 2007; Mantis et al. 2002; Huang et al. 2005).

Mouse and human IgA biology differ in several aspects. In human serum, IgA occurs mainly in a monomeric form, while in mice polymeric IgA is the main form in serum. Furthermore, human IgA, but not mouse IgA, is divided into closely related subclasses, IgA1 and IgA2, of which the later one is less susceptible to proteolytic degradation. In serum, the subclass IgA1 is dominant, while in secretions the main isoform found is IgA2, although both IgA1 and IgA2 can be detected as SIgA (Woof and Kerr 2006).

IgA has been described to interact with various host receptors, which are pIgR (Mantis et al. 2011), FcαRI (CD89) (Monteiro and van de Winkel 2003), transferrin receptor (CD71) (Moura et al. 2001), asialoglycoprotein receptor (ASGPR) (Kerr et al. 1995), Fcα/μR (Yang et al. 2013), FcRL4 (Wilson et al. 2012), and DC-SIGN/SIGNR1 (Diana et al. 2013) although cell signaling after ligand binding is not yet well elucidated for some of these receptors. However, the FcαRI (CD89), a receptor expressed by neutrophils, monocyte/macrophages, dendritic cells, eosinophils, and Kupffer cells, has recently been demonstrated to induce either inhibitory or activating signals (Monteiro and van de Winkel 2003). Balance of these signals is considered to be important for the role of IgA in preserving homeostasis and tolerance at systemic sites (Blank et al. 2009). In mucosal areas in steady-state conditions, only a few dendritic cells are positive for FcαRI, and macrophages are negative (Smith et al. 2001). Intriguingly, this receptor has not been identified in mice, but it is expressed in rats, cows, goats, and monkeys (Morton et al. 1999).

2 IgA Structure and Metabolism

In contrast to other human immunoglobulin classes, which are exclusively present in a monomeric (m) form (IgG, IgD, and IgE) or in a polymeric form (IgM), IgA is found in both forms with a characteristic distribution in various body fluids: mIgA dominates in blood, but IgA in almost all external secretions is polymeric (Woof and Mestecky 2005). The molecular mass of a mIgA is 160 kDa. Due to the flexibility of both Fab fragments, the molecule may assume the Y- or T-shaped configuration (Boehm et al. 1999). Human IgA heavy (H) α1 and α2 chains contain one variable (V) region domain and three constant (C) region domains. IgA α chains display several important structural differences, compared with H chains of other isotypes; such differences include a unique hinge region between the Cα1 and Cα2 domains and the extension of the α chain C terminus by 18

amino acids that is essential for the ability of IgA to bind J-chain and form polymers (Low et al. 1976). Dimeric IgA interact with the pIg receptor (pIgR) expressed on epithelial cells. This binding allows for selective transport of pIgA through the epithelial cells (Woof and Mestecky 2005). Comparative structural studies of the amino acid sequences of IgA1 and IgA2 H chains revealed major differences in their hinge regions. The IgA2 H-chain hinge region is shorter due to a deletion of 13 amino acids in the hinge region. The extended hinge region of IgA1 may add sequential flexibility of the Fab fragments and thereby increases the antigen-binding ability of IgA1 molecules. The amino acid sequence of the hinge region in IgA1 is reminiscent of that of mucins; serine (Ser) and threonine (Thr) residues provide up to six potential sites for the attachment of *O*-linked glycans. The hinge region of human IgA1 is the only known substrate that is susceptible to the selective proteolytic cleavage by numerous and heterogeneous IgA-specific proteases produced by pathogenic bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *N. meningitidis*, and others (Vidarsson et al. 2005). Although the hinge region is present in IgA of other vertebrate species (e.g., cows, pigs, dogs, mice, and rabbits), it exhibits a low degree of sequence homology, glycosylation, and susceptibility to bacterial IgA1-specific proteases, compared to human IgA1 (Mestecky et al. 2005). Notably, IgA molecules of the above-mentioned species structurally resemble human IgA2.

3 Functional Role of IgA

IgA is the class of antibodies that shows the highest daily new synthesis and, at a concentration of about 2–3 mg/ml, is the second most prevalent antibody in the serum after IgG (Woof and Kerr 2006). IgA are differentially distributed between the systemic and mucosal immune system and play a key role in immune protection (Mantis et al. 2011; Bakema and van Egmond 2011). SIgA plays an important role in different functions in the mucosal immune system. While high-affinity IgA antibodies (from T cell-dependent pathways) are thought to protect intestinal mucosal surfaces against invasion by pathogenic microorganisms, low-affinity IgA antibodies (from T cell-independent pathways) are important to confine commensal bacteria to the intestinal lumen (Pabst 2012). Serum monomeric IgA (mIgA) is thought to play a minor role in systemic immune responses. The major role of serum mIgA in physiology is to promote a powerful anti-inflammatory effect. It has been demonstrated by several groups more than 30 years ago that in the absence of antigen, serum IgA is capable to downregulate many cell responses (Blank et al. 2009; Bakema and van Egmond 2011). However, the molecular basis for such an action remained elusive until recently with the discovery of the ability of FcαRI to mediate inhibition through the ITAM of their associated FcRγ chain (see below). IgA is classically known for neutralizing toxins and bacteria (viruses) at mucosal surfaces (Williams and Gibbons 1972; Mazanec et al. 1993) by interfering with their motility, by competing for epithelial adhesion

sites, and by improving the viscoelastic properties of the airway secretions (Puchelle et al. 1980). The secretory component (SC) protects SIgA from proteolytic degradation and is involved in establishing local interactions with bronchial mucus, thereby contributing to the "trapping" and removal of the antigen ("immune exclusion") (Phalipon and Corthesy 2003).

4 FcαRI Expression and Structure

FcαRI expression begins as early as the promyelocytic stage in differentiation (Monteiro and van De Winkel 2003). FcαRI expression is restricted to cells of the myeloid lineage including neutrophils, eosinophils, most of monocytes/macrophages, interstitial dendritic cells, Kupffer cells, and cell lines corresponding to these cell types. Tonsilar, splenic and alveolar macrophages all express FcαRI, in contrast to the lack in intestinal mucosal macrophages (Smith et al. 2001). FcαRI expression is constitutive and independent of the presence of IgA ligand, since the receptor is expressed at similar levels on cells from IgA-deficient patients (Chevailler et al. 1989).

Several anti-FcαRI (CD89) mouse monoclonal antibodies (mAb) have been generated (Bakema and van Egmond 2011) (Fig. 1a). Most of them recognize nonpolymorphic determinants on FcαRI (Monteiro and van de Winkel 2003). The CD89 mAb epitopes on FcαRI have been characterized (52). Monoclonal Ab that bind in the EC1 domain of FcαRI (e.g., My43 and MIP8a) can block IgA binding, whereas those that bind in EC2 do not (Fig. 1a).

The level of FcαRI expression on cells varies between 57,000 molecules on monocytes and 66,000 on neutrophils (Kubagawa et al. 1997). A number of cytokines and other agents modulate FcαRI expression. FcαRI expression levels are upregulated on neutrophils in response to formyl-methionyl-leucyl-phenylalanine (FMLP), interleukin 8, and tumor necrosis factor α (TNF-α) (Monteiro and van de Winkel 2003). Expression of FcαRI on monocytes and monocyte-like cell lines can be upregulated by phorbol esters, calcitriol, lipopolysaccharide (LPS), TNF-α, granulocyte-macrophage colony stimulating factor (GM-CSF), and IL-1β (Monteiro and van de Winkel 2003). FcαRI is downregulated by transforming growth factor (TGF-β), interferon γ, suramin, and by its ligand (Monteiro and van de Winkel 2003).

The FcαRI gene is located in the distal part of the q-arm on chromosome 19, at 19q13.4 (Kremer et al. 1992). The molecular structure of FcαRI classifies this FcR as a member of the Ig gene superfamily. It is distantly related to other FcR genes (∼20 % homology) such as the FcγR and FcεRI genes, that are all located on chromosome 1 (Wines et al. 1999). Interestingly, FcαRI is more homologous (∼35 %) to another family of receptors, the so-called leukocyte receptor cluster, that includes the killer-inhibitory/activatory (KIR/KAR)-related immunoreceptors, the Ig-like transcripts (ILTs), the leukocyte and monocyte/macrophage Ig-like receptors (LIRs, MIRs) (Monteiro and van de Winkel 2003). FcαRI is also closely

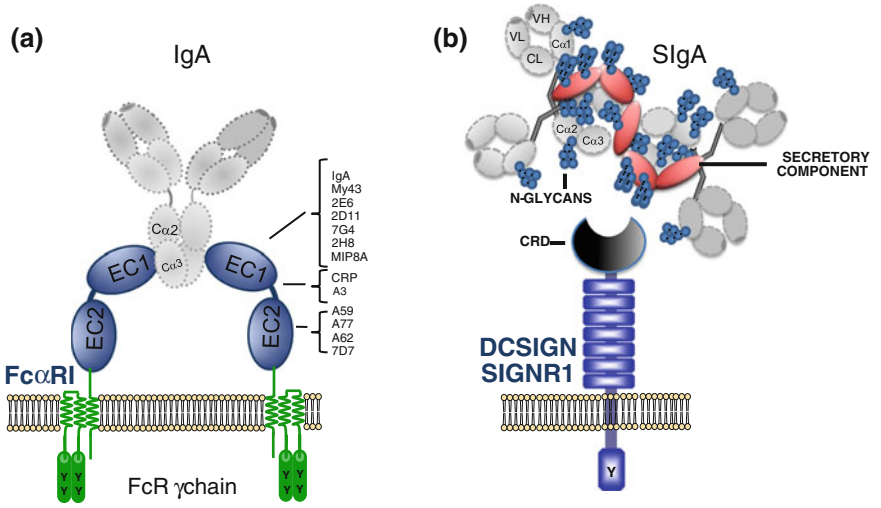


Fig. 1 **a** Schematic representation of immunoglobulin A (IgA) binding to the FcαRI–FcRγ-chain complex in a 2:1 stoichiometry. Two FcαRI bind each Fcα fragment at their Cα2 and Cα3 junctions via extracellular domain 1 (EC1). The FcRγ chain containing its intracellular immunoreceptor tyrosine-based activation motif (ITAM) is shown. Sites recognized by monoclonal antibodies and C-reactive protein recognizing different ECs of FcαRI are depicted. **b** DC-SIGN/SIGNR1 and secretory IgA (SIgA) representation. DC-SIGN/SIGNR1 is a type II transmembrane protein with a short aminoterminal cytoplasmic tail, a neck region, and a single carboxyl terminal carbohydrate recognition domain (CRD; or C-type lectin domain), and is expressed on immature monocyte-derived DCs. DC-SIGN recognizes both internal branched mannose residues as well as terminal di-mannoses, α1-3 and α1-4 fucosylated glycan structures and certain N-aceltylglucosamine containing molecules on self proteins and/or pathogens. SIgA–SIGNR1 interaction depends on mannose residues and the presence of secretory component. A tyrosine residue is found in its intracellular tail representing a potential site for ligand signaling, but its implication in SIgA response remains to be demonstrated

related to the bovine Fcγ2R and human and mouse platelet-specific collagen receptor (GPVI) (Jandrot-Perrus et al. 2000). It is noteworthy that no mouse homologue for FcαRI has been identified, in spite of intensive efforts to find one.

Two isoforms have been identified on human phagocytes, the FcαRI a.1, and a.2 (Patry et al. 1996). While the a.1 isoform is expressed by several blood cells of myeloid lineage, the a.2 isoform is exclusively expressed by alveolar macrophages. FcαRI a.1 represents a type I, 287-amino acid protein containing a 21 amino acid hydrophobic leader that is removed during processing to form the mature 266 amino acid FcαRI a.1 full-length glycoprotein (Maliszewski et al. 1990). FcαRI is composed of two extracellular Ig-like domains, a predicted transmembrane region and a cytoplasmic tail devoid of recognized signaling motifs. The protein core has a predicted Mr of 30 kDa and bears five potential N-linked glycosylation sites, and several putative O-glycosylation sites. Mature cell surface FcαRI display heterogeneous glycosylation with Mr ranging from 50 to 100 kDa, depending on the cell type (Monteiro et al. 1990). Deglycosylation

experiments using endoglycosydase F or O, indeed, confirmed a heterogeneous FcαRI glycosylation with two molecular species, one of 32 and a second of 34 kDa, possibly attributable to inaccessibility of some carbohydrates FcαRI a.2 has a deletion in the extracellular domain of 22 amino acids and a backbone of 28 kDa (Monteiro and van de Winkel 2003).

The FcαRI binding site for IgA has been located in the membrane-distal EC1 domain (Morton et al. 1999; Wines et al. 1999). This represents a unique feature among the "two-domain type" FcR since FcγR and FcεRI all bind their respective Ig ligands via the membrane-proximal EC2 domains. It is noteworthy that the closely related bovine Fcγ2R and p58 KIR molecules also bind their ligand (bovine IgG2 and HLA molecules, respectively) via their EC1 domain (Monteiro et al. 1990). The high degree of similarity between FcαRI and p58 KIR proteins allowed a three-dimensional model of CD89 to be proposed based on the solved structure of KIR.

FcαRI is a low-to-moderate affinity receptor for IgA (Ka approximately $5 \times 10^6\ M^{-1}$). Rapid dissociation of the FcαRI:IgA complex (t1/2 ~ 25 s) using recombinant soluble FcαRI suggests monomeric IgA binds transiently to cellular FcαRI, whereas IgA immune complexes bind avidly (Wines et al. 1999). FcαRI binds IgA1 and IgA2 molecules at the boundary between the Cα2 and Cα3 domains (Carayannopoulos et al. 1996).

Whether or not mouse IgA binds to human FcαRI has been controversial. Initial studies used erythrocytes coated with mouse IgA myeloma MOPC-315 to detect human IgA receptors (Lum et al. 1979). Although later studies by others failed to observe binding of mouse IgA to human FcαRI (Pleass et al. 1999), recent studies using plasmon resonance (Biacore) have shown that mouse IgA can bind to human recombinant FcαRI but with a very low avidity (Berthelot et al. 2012). This may explain data obtained from human CD89 transgenic (Tg) mice in which mouse IgA interacts with human CD89 on cells and in the circulation as soluble receptors (Launay et al. 2000).

5 Janus-like Functions of the FcαRI Molecule

The ligation of FcRs by Ig and antigen can trigger numerous cellular effector functions including phagocytosis, antibody-dependent cellular cytotoxicity, and the secretion of cytokines or other inflammatory mediators (Nimmerjahn and Ravetch 2008). Thus, FcRs provide a crucial link between the humoral and the cellular arms of the immune system. Some FcRs, such as FcαRI, when associated with the ITAM-bearing adaptor FcRγ, can act as bifunctional receptors. Its signaling can be activating as well as inhibitory. This depends on the ligand and subsequent configuration (involving Syk or SHP-1 phosphatase) of the ITAM, resulting in an activating or an inhibitory ITAM motif.

Cross-linking of FcαRI by IgA immune complexes or polymeric IgA requires the association of FcαRI with the FcRγ subunit and initiates ITAM-dependent cellular responses (Launay et al. 1999). The FcRγ-chain ITAM consists of a

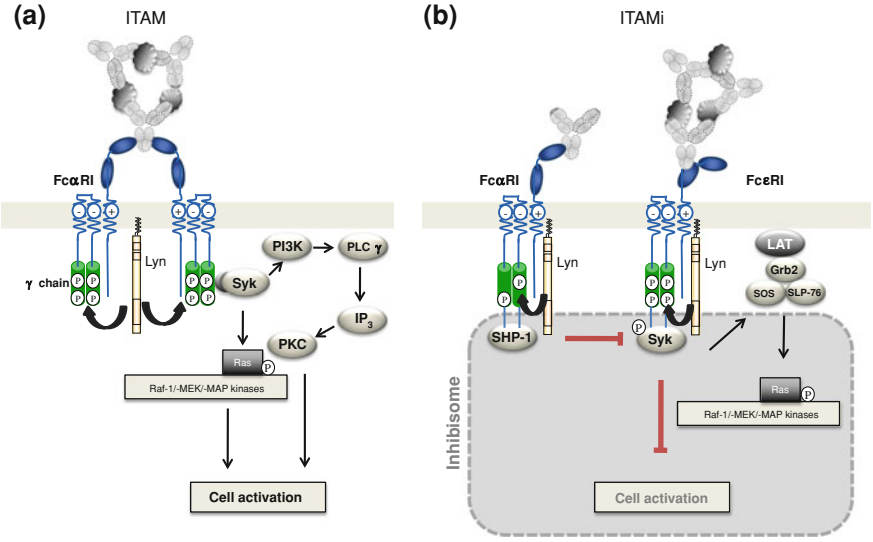


Fig. 2 Simplified scheme of FcαRI signaling pathways. **a** ITAM signaling pathway: Cross-linking of FcαRI by immunoglobulin A (IgA) containing immune complexes results in full phosphorylation of ITAM tyrosines within the FcRγ chain by *Src* kinase Lyn. These then serve as "docking" sites for recruitment of Syk, phospholipase (PLC)-γ, which facilitates activation of multiple (and subsequential) targets such as PI3K, PLC-γ, following the activation of multimolecular adapter protein complex. This results in cellular functions such as phagocytosis, Ab-dependent-cellular cytotoxicity, respiratory burst, degranulation, antigen-presentation, and release of cytokines and inflammatory mediators. **b** Inhibitory ITAM (ITAMi) signaling pathway: Triggering of FcαRI by monomeric serum IgA results in partial phosphorylation of FcαR-associated ITAM tyrosines by receptor-associated Src family kinases, resulting in a transient recruitment of Syk followed by a stable SHP-1 recruitment and formation of inhibisome clusters (*gray rectangle*), which impair phosphorylation of Syk, LAT, and ERK. These inhibitory signals allow the dephosphorylation of other signaling pathways activated by engagement of heterologous activating receptors, such as FcεRI

conserved stretch of paired tyrosines and leucines separated by seven amino acids in a consensus sequence (YxxLx_{6-7}YxxL). The *Src* kinase Lyn phosphorylates the tyrosines within the associated FcRγ ITAM. These then serve as "docking" sites for the recruitment of the tyrosine kinase Syk, which facilitates the activation of multiple targets, such as the PI3K, and induces the downstream release of IP3 and diacylglycerol to trigger calcium release, the activation of Raf-1–MEK–MAP kinases signaling pathways and subsequently cell activation (Monteiro and van de Winkel 2003). Of note, depending on the cell type or cell stimulation, FcαRI activation may trigger specific signaling and functional responses (Monteiro and van de Winkel 2003; Bakema and van Egmond 2011) (Fig. 2a).

It has been recently demonstrated that ITAMs can also propagate inhibitory signals when they are in a conformation that we have named inhibitory ITAM (ITAMi) (Blank et al. 2009; Pinheiro da Silva et al. 2008; Pasquier et al. 2005).

The inhibitory ITAM (ITAMi) pathway takes place in the absence of receptor coaggregation and without an immunoreceptor tyrosine-based inhibitory motif (ITIM), which is known for inhibiting immune responses. All forms of IgA can interact with the FcαRI, but they differ in their binding capacities. Monomeric IgA only binds with low affinity to the FcαRI and activates the ITAMi signaling, which does not lead to cell activation or degranulation/oxidative burst (in the case of granulocytes) (Pasquier et al. 2005) and in parallel, inhibits the activation of heterologous receptors (such as other Fc receptors, cytokine receptors, chemokine receptors, and TLRs) (Monteiro 2010) (Fig. 2b). Targeting of FcαRI by mIgA transduces an ITAMi signaling characterized by a transient Syk recruitment followed by the recruitment of tyrosine phosphatase-1 (SHP-1) to the FcRγ ITAM and the movement of FcαRI to lipid rafts (Pasquier et al. 2005; Kanamaru et al. 2008; Pfirsch-Maisonnas et al. 2011). After raft recruitment, both inhibitory and activating receptors and the inhibitory molecular effector (SHP-1) can be found in intracellular clusters that we have called "inhibisomes." These clusters play a crucial role in the inhibition of different signaling pathways induced by the heterologous receptors. Therefore, similar to ITIM-mediated signals, downregulation of the response of the heterologous activating receptor, that is also recruited into rafts, involves the association of inhibitory receptors with the tyrosine phosphatase SHP-1 (Pfirsch-Maisonnas et al. 2011). Thus, both IgA-induced activating and inhibiting signals depend on FcαRI–FcRγ-chain ITAM, but differ in the recruitment of tyrosine kinases *versus* tyrosine phosphatases, respectively. As such, it has been proposed that the cross-linking of FcαRI during infection with IgA-opsonized pathogens results in pro-inflammatory responses, whereas naturally occurring serum IgA (which is not complexed with an antigen) induces inhibitory signals through the FcαRI, in order to dampen excessive immune responses (Blank et al. 2009). Indeed, anti-FcαRI Fab treatment, by initiating ITAMi signaling suppressed manifestations of allergic asthma and glomerulonephritis in FcαRI transgenic mice immunized with anti-IgE immune complexes or anti-glomerular basement antibodies (Pasquier et al. 2005; Kanamaru et al. 2008).

6 Tolerogenic Role of Secretory IgA Through SIGNR1

In a similar manner to mIgA, SIgA has also a powerful anti-inflammatory effect due to its ability to interact with DC through SIGNR1 receptor. SIGNR1 is a mouse homolog of DC-SIGN, a C-type lectin receptor that was recently described as a receptor for human SIgA on the cell surface of DC (Baumann et al. 2010). Similar to its interaction with DC-SIGN (Baumann et al. 2010), SIgA–SIGNR1 interaction is dependent on sugars, notably on mannose residues, on Ca^{2+} and the presence of the secretory component (Fig. 1b). In our recent study (Diana et al. 2013), we showed that SIgA prevents the activation of the immune system by regulating the function of mouse bone-marrow-derived DC (BMDC) through SIGNR1. Preincubation with SIgA inhibits the maturation and the production of

pro-inflammatory cytokines by BMDC, which instead harbor a tolerogenic phenotype and produce large amounts of IL-10. Importantly, BMDC pretreated with SIgA promote the expansion of IL-10-secreting Foxp3+ Treg cells. Moreover, in vivo injection of SIgA-DC, loaded with self-peptides, prevents the development of autoimmune diseases, such as experimental autoimmune encephalomyelitis and type 1 diabetes. Although there are no data on SIgA–DC-SIGN interaction in inducing tolerance, it is interesting to note that triggering of DC-SIGN by anti-DC-SIGN fusion proteins can induce DC tolerogenic activity through IL-10 secretion (Caparros et al. 2006). Therefore, these data suggest that SIgA interaction with lectin-like receptors such as SIGNR1/DC-SIGN has a hitherto unknown regulatory function in the bloodstream, which opens new therapeutic avenues for the treatment of autoimmune and inflammatory diseases.

7 Pathological Role of IgA

Several inflammatory diseases are associated with increased serum IgA levels and IgA immune complexes (Bakema and van Egmond 2011; Monteiro 2010). These disorders include IgA nephropathy (IgAN), Henoch-Schönlein purpura (HSP), ankylosing spondylitis, Sjögren's syndrome, alcoholic liver cirrhosis, celiac disease, inflammatory bowel disease, dermatitis herpetiformis, and AIDS (Monteiro and van de Winkel 2003). IgAN is the most common IgA-associated disease, characterized by the deposition of polymeric IgA1 in the kidney. It has been recently shown (Berthelot et al. 2012) that mice expressing both human IgA1 and CD89 display features of IgAN with circulating and mesangial deposits of IgA1 and soluble CD89 (sCD89) complexes resulting in kidney inflammation, hematuria, and proteinuria. Mice expressing IgA1 alone have endothelial but not mesangial IgA1 deposits. Interestingly, transglutaminase 2 plays an essential role in mesangial IgA-sCD89 deposition in IgAN, possibly through their ability to cross-link the mesangial IgA1 receptor, the transferrin receptor or CD71 (Berthelot et al. 2012). Moreover, soluble CD89, which is only present in patients complexed with IgA, also binds CD71 directly and independently of IgA. Moreover, several groups suggest that the O-glycan side chains in the hinge of the glomerular IgA1 are highly under galactosylated in IgAN favoring interaction with CD89 and CD71 (Monteiro and van de Winkel 2003; Moura et al. 2004). Together, these findings indicate that multiple interactions between different actors are required for disease development.

At the other end of the spectrum, IgA deficiency, characterized by decreased or absent levels of serum IgA, has been shown to be the most common primary immunodeficiency with up to 1 in 200 individuals affected in some ethnic groups (Hammarstrom et al. 2000; al-Attas and Rahi 1998). In line with the previously mentioned inhibitory and anti-inflammatory functions of IgA, it is well documented that IgA-deficient individuals have recurrent mucosal infections and an increased incidence of allergy or autoimmune disease, especially idiopathic thrombocytopenic

purpura and arthritis. It is estimated that 50–77 % of IgA-deficient patients experience recurrent infections while around 30 % of the patients have an autoimmune disease or allergic symptoms (Jacob et al. 2008). Despite the deficiency of serum IgA FcαRI is expressed on the cells of IgA-deficient patients (Monteiro and van de Winkel 2003). This could be interpreted that in the absence of its natural ligand (serum monomeric IgA) FcαRI receptor may not be able to induce immune cell inhibition (Jacob et al. 2008), thus favoring autoimmunity development.

8 IgA as Therapeutic Agent

Overall, IgA and FcαRI play a significant double function in vivo by maintaining immune homeostasis in systemic and mucosal compartments. Naturally occurring serum IgA induces inhibitory signals to dampen excessive immune responses, whereas cross-linking of FcαRI during infection with IgA-associated pathogens results in pro-inflammatory responses. The manipulation of the FcαRI function may thus offer novel promising therapeutic strategies for the treatment of inflammation. The potential of IgA mAbs as immunoregulators has mainly been investigated in in vitro experiments due to the absence of FcαRI in mice. However, the increasing availability of transgenic mouse models will greatly facilitate the assessment of these antibodies in preclinical models prior to clinical trials. Currently, two human FcαRI transgenic mouse models are available. In the first mouse model the FcαRI is preferentially expressed on neutrophilic granulocytes (van Egmond et al. 1999), while in the second model the receptor is mainly expressed on monocytes/macrophages (Launay et al. 2000; van Egmond et al. 1999). For example, using the first model, dimeric IgA (dIgA), which is produced in the lamina propria, but neither SIgA nor IgG, was able to induce neutrophil recruitment via leukotriene B4 (van der Steen et al. 2009). Using these models with complementary FcαRI expression on the key players of innate immune responses will allow to study the specific role of the receptor in these cells during inflammatory responses. Moreover, the timing of therapeutic intervention can be decided depending on whether the receptor is shown to mainly participate in neutrophil-dependent inflammatory responses (acute phase) or monocytes/macrophages-dependent responses (chronic phase).

One of the main limitations of treating patients with IgA is that the therapeutic efficacy of the antibody lies in the concentration, thus high antibody quantities are needed to observe a therapeutic effect compared to monoclonal antibodies. Nowadays, with the development of human IgA knock-in mouse model, higher quantities can be produced using standard hybridoma technology (Duchez et al. 2010). Alternatively, optimized approaches in IgA purification, such as incorporation of glutamine synthetase selection vectors into the producer cells (Beyer et al. 2009) or identification of IgA-binding peptides using phage display (Hatanaka et al. 2012), will allow the isolation of serum human IgA in higher quantities and with no

interfering contaminations. Once these difficulties are surmounted, then IgA-specific therapy could be performed.

Alternative to IgA, FcαRI can be modulated to obtain anti-inflammatory treatment by harnessing ITAMi-induced inhibitory signaling as it was demonstrated to prevent disease development in several inflammatory disease mouse models. For example, we have previously tested whether FcαRI targeting using anti-FcαRI Fab fragments (ITAMi signaling due to monovalent targeting) could prevent the development of asthma. FcαRI transgenic mice (expressing human FcαRI on monocytes/macrophages) were sensitized with allergen, followed by a challenge with an allergen/methacholine leading to bronchial hyper-reactivity. Pretreatment of FcαRI Tg animals with anti-FcαRI Fab considerably reduced symptoms when compared to animals treated with isotype control Fab antibodies (Pasquier et al. 2005). Similarly, we demonstrated the therapeutic potential of anti-FcαRI Fab treatment for the inhibition of inflammatory responses in kidney inflammatory models such as nephrotoxic nephritis or the ureteral obstruction model of renal tubular interstitial fibrosis (Kanamaru et al. 2008). Treatment with anti-FcαRI Fab impaired inflammatory cell infiltration and fibrosis development (Kanamaru et al. 2008), whereas cross-linking of the receptor worsened these diseases (Blank et al. 2009). Interestingly, direct analysis of blood monocytes showed that treatment with anti-FcαRI Fab prevented the activation of monocytes from both healthy subjects and patients suffering from IgAN, induced by several stimuli including LPS, TNFα, and MCP-1 (Kanamaru et al. 2008). These findings demonstrated that anti-FcαRI Fab could be used as a new therapeutic tool to prevent the progression of renal inflammatory diseases. Further studies are now required to determine whether monomeric IgA treatment may also be beneficial to prevent or reverse an established inflammatory disease.

9 Concluding Remarks

Receptors for IgA play a significant role in vivo in maintaining the immune homeostasis in systemic and mucosal compartments. In this review, we summarized the current knowledge on two types of IgA receptors, focusing on FcαRI (CD89) and DC-SIGN/SIGNR1. These receptors appear to play an important role in immunity by linking the IgA to either anti-inflammatory or inflammatory responses. A role for specific IgA receptors has been implicated in a variety of pathological conditions. Recent studies support a role for IgA antibodies and FcαRI-directed molecules as therapeutics for human disease.

Acknowledgments This work was supported in part by grants from Agence Nationale de la Recherche (ANR grants MIEN-2009 and BLANC International-2012) and from the LabEx Inflamex. S.B.M. was supported by a grant from the French Foundation ARC (PDF20100601037).

References

al-Attas RA, Rahi AH (1998) Primary antibody deficiency in Arabs: first report from eastern Saudi Arabia. J Clin Immunol 18:368–371

Bakema JE, van Egmond M (2011) The human immunoglobulin A Fc receptor FcalphaRI: a multifaceted regulator of mucosal immunity. Mucosal Immunol 4:612–624

Baumann J, Park CG, Mantis NJ (2010) Recognition of secretory IgA by DC-SIGN: implications for immune surveillance in the intestine. Immunol Lett 131:59–66

Berthelot L, Papista C, Maciel TT, Biarnes-Pelicot M, Tissandie E, Wang PH, Tamouza H, Jamin A, Bex-Coudrat J, Gestin A et al (2012) Transglutaminase is essential for IgA nephropathy development acting through IgA receptors. J Exp Med 209:793–806

Beyer T, Lohse S, Berger S, Peipp M, Valerius T, Dechant M (2009) Serum-free production and purification of chimeric IgA antibodies. J Immunol Methods 346:26–37

Blank U, Launay P, Benhamou M, Monteiro RC (2009) Inhibitory ITAMs as novel regulators of immunity. Immunol Rev 232:59–71

Boehm MK, Woof JM, Kerr MA, Perkins SJ (1999) The Fab and Fc fragments of IgA1 exhibit a different arrangement from that in IgG: a study by X-ray and neutron solution scattering and homology modelling. J Mol Biol 286:1421–1447

Caparros E, Munoz P, Sierra-Filardi E, Serrano-Gomez D, Puig-Kroger A, Rodriguez-Fernandez JL, Mellado M, Sancho J, Zubiaur M, Corbi AL (2006) DC-SIGN ligation on dendritic cells results in ERK and PI3K activation and modulates cytokine production. Blood 107:3950–3958

Carayannopoulos L, Hexham JM, Capra JD (1996) Localization of the binding site for the monocyte immunoglobulin (Ig) A-Fc receptor (CD89) to the domain boundary between Calpha2 and Calpha3 in human IgA1. J Exp Med 183:1579–1586

Chevailler A, Monteiro RC, Kubagawa H, Cooper MD (1989) Immunofluorescence analysis of IgA binding by human mononuclear cells in blood and lymphoid tissue. J Immunol 142:2244–2249

Diana J, Moura IC, Vaugier C, Gestin A, Tissandie E, Beaudoin L, Corthesy B, Hocini H, Lehuen A, Monteiro RC (2013) Secretory IgA induces tolerogenic dendritic cells through SIGNR1 dampening autoimmunity in mice. J Immunol 191:2335–2343

Duchez S, Amin R, Cogne N, Delpy L, Sirac C, Pascal V, Corthesy B, Cogne M (2010) Premature replacement of mu with alpha immunoglobulin chains impairs lymphopoiesis and mucosal homing but promotes plasma cell maturation. Proc Natl Acad Sci USA 107:3064–3069

Fagarasan S, Muramatsu M, Suzuki K, Nagaoka H, Hiai H, Honjo T (2002) Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. Science 298:1424–1427

Hammarstrom L, Vorechovsky I, Webster D (2000) Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). Clin Exp Immunol 120:225–231

Hatanaka T, Ohzono S, Park M, Sakamoto K, Tsukamoto S, Sugita R, Ishitobi H, Mori T, Ito O, Sorajo K et al (2012) Human IgA-binding peptides selected from random peptide libraries: affinity maturation and application in IgA purification. J Biol Chem 287:43126–43136

Huang YT, Wright A, Gao X, Kulick L, Yan H, Lamm ME (2005) Intraepithelial cell neutralization of HIV-1 replication by IgA. J Immunol 174:4828–4835

Jacob CM, Pastorino AC, Fahl K, Carneiro-Sampaio M, Monteiro RC (2008) Autoimmunity in IgA deficiency: revisiting the role of IgA as a silent housekeeper. J Clin Immunol 28(Suppl 1):S56–S61

Jandrot-Perrus M, Busfield S, Lagrue AH, Xiong X, Debili N, Chickering T, Le Couedic JP, Goodearl A, Dussault B, Fraser C et al (2000) Cloning, characterization, and functional studies of human and mouse glycoprotein VI: a platelet-specific collagen receptor from the immunoglobulin superfamily. Blood 96:1798–1807

Kadaoui KA, Corthesy B (2007) Secretory IgA mediates bacterial translocation to dendritic cells in mouse Peyer's patches with restriction to mucosal compartment. J Immunol 179:7751–7757

Kanamaru Y, Pfirsch S, Aloulou M, Vrtovsnik F, Essig M, Loirat C, Deschenes G, Guerin-Marchand C, Blank U, Monteiro RC (2008) Inhibitory ITAM signaling by Fc alpha RI-FcR gamma chain controls multiple activating responses and prevents renal inflammation. J Immunol 180:2669–2678

Kerr MA, Stewart WW, Bonner BC, Greer MR, MacKenzie SJ, Steele MG (1995) The diversity of leucocyte IgA receptors. Contrib Nephrol 111:60–64 (Discussion 65)

Kremer EJ, Kalatzis V, Baker E, Callen DF, Sutherland GR, Maliszewski CR (1992) The gene for the human IgA Fc receptor maps to 19q13.4. Hum Genet 89:107–108

Kubagawa H, Shimada T, Shimo K, Lassoued K, Monteiro RC, Cooper MD (1997) CD89 Workshop Panel report. Leucocyte Typing VI, pp 1028–1029

Launay P, Patry C, Lehuen A, Pasquier B, Blank U, Monteiro RC (1999) Alternative endocytic pathway for immunoglobulin A Fc receptors (CD89) depends on the lack of FcRgamma association and protects against degradation of bound ligand. J Biol Chem 274:7216–7225

Launay P, Grossetete B, Arcos-Fajardo M, Gaudin E, Torres SP, Beaudoin L, Patey-Mariaud de Serre N, Lehuen A, Monteiro RC (2000) Fcalpha receptor (CD89) mediates the development of immunoglobulin A (IgA) nephropathy (Berger's disease). Evidence for pathogenic soluble receptor-Iga complexes in patients and CD89 transgenic mice. J Exp Med 191:1999–2009

Low TL, Liu YS, Putnam FW (1976) Structure, function, and evolutionary relationships of Fc domains of human immunoglobulins A, G, M, and E. Science 191:390–392

Lum LG, Muchmore AV, O'Connor N, Strober W, Blaese RM (1979) Fc receptors for IgA on human B, and human non-B, non-T lymphocytes. J Immunol 123:714–719

Maliszewski CR, March CJ, Schoenborn MA, Gimpel S, Shen L (1990) Expression cloning of a human Fc receptor for IgA. J Exp Med 172:1665–1672

Mantis NJ, Cheung MC, Chintalacharuvu KR, Rey J, Corthesy B, Neutra MR (2002) Selective adherence of IgA to murine Peyer's patch M cells: evidence for a novel IgA receptor. J Immunol 169:1844–1851

Mantis NJ, Rol N, Corthesy B (2011) Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. Mucosal Immunol 4:603–611

Mazanec MB, Nedrud JG, Kaetzel CS, Lamm ME (1993) A three-tiered view of the role of IgA in mucosal defense. Immunol Today 14:430–435

Mestecky J, Moldoveanu Z, Russell MW (2005) Immunologic uniqueness of the genital tract: challenge for vaccine development. Am J Reprod Immunol 53:208–214

Monteiro RC (2010) Role of IgA and IgA fc receptors in inflammation. J Clin Immunol 30:1–9

Monteiro RC, van de Winkel JG (2003) IgA Fc receptors. Annu Rev Immunol 21:177–204

Monteiro RC, Kubagawa H, Cooper MD (1990) Cellular distribution, regulation, and biochemical nature of an Fc alpha receptor in humans. J Exp Med 171:597–613

Morton HC, van Zandbergen G, van Kooten C, Howard CJ, van de Winkel JG, Brandtzaeg P (1999) Immunoglobulin-binding sites of human FcalphaRI (CD89) and bovine Fcgamma2R are located in their membrane-distal extracellular domains. J Exp Med 189:1715–1722

Moura IC, Centelles MN, Arcos-Fajardo M, Malheiros DM, Collawn JF, Cooper MD, Monteiro RC (2001) Identification of the transferrin receptor as a novel immunoglobulin (Ig)A1 receptor and its enhanced expression on mesangial cells in IgA nephropathy. J Exp Med 194:417–425

Moura IC, Arcos-Fajardo M, Sadaka C, Leroy V, Benhamou M, Novak J, Vrtovsnik F, Haddad E, Chintalacharuvu KR, Monteiro RC (2004) Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy. J Am Soc Nephrol 15:622–634

Nimmerjahn F, Ravetch JV (2008) Fcgamma receptors as regulators of immune responses. Nat Rev Immunol 8:34–47

Norderhaug IN, Johansen FE, Krajci P, Brandtzaeg P (1999) Domain deletions in the human polymeric Ig receptor disclose differences between its dimeric IgA and pentameric IgM interaction. Eur J Immunol 29:3401–3409

Pabst O (2012) New concepts in the generation and functions of IgA. Nat Rev Immunol 12:821–832

Pasquier B, Launay P, Kanamaru Y, Moura IC, Pfirsch S, Ruffie C, Henin D, Benhamou M, Pretolani M, Blank U et al (2005) Identification of FcalphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. Immunity 22:31–42

Patry C, Sibille Y, Lehuen A, Monteiro RC (1996) Identification of Fc alpha receptor (CD89) isoforms generated by alternative splicing that are differentially expressed between blood monocytes and alveolar macrophages. J Immunol 156:4442–4448

Peterson DA, McNulty NP, Guruge JL, Gordon JI (2007) IgA response to symbiotic bacteria as a mediator of gut homeostasis. Cell Host Microbe 2:328–339

Pfirsch-Maisonnas S, Aloulou M, Xu T, Claver J, Kanamaru Y, Tiwari M, Launay P, Monteiro RC, Blank U (2011) Inhibitory ITAM signaling traps activating receptors with the phosphatase SHP-1 to form polarized "inhibisome" clusters. Sci Signal 4:ra24

Phalipon A, Corthesy B (2003) Novel functions of the polymeric Ig receptor: well beyond transport of immunoglobulins. Trends Immunol 24:55–58

Phalipon A, Cardona A, Kraehenbuhl JP, Edelman L, Sansonetti PJ, Corthesy B (2002) Secretory component: a new role in secretory IgA-mediated immune exclusion in vivo. Immunity 17:107–115

Pinheiro da Silva F, Aloulou M, Benhamou M, Monteiro RC (2008) Inhibitory ITAMs: a matter of life and death. Trends Immunol 29:366–373

Pleass RJ, Dunlop JI, Anderson CM, Woof JM (1999) Identification of residues in the CH2/CH3 domain interface of IgA essential for interaction with the human fcalpha receptor (FcalphaR) CD89. J Biol Chem 274:23508–23514

Puchelle E, Zahm JM, Girard F, Bertrand A, Polu JM, Aug F, Sadoul P (1980) Mucociliary transport in vivo and in vitro. Relations to sputum properties in chronic bronchitis. Eur J Respir Dis 61:254–264

Smith PD, Smythies LE, Mosteller-Barnum M, Sibley DA, Russell MW, Merger M, Sellers MT, Orenstein JM, Shimada T, Graham MF et al (2001) Intestinal macrophages lack CD14 and CD89 and consequently are down-regulated for LPS- and IgA-mediated activities. J Immunol 167:2651–2656

van der Steen L, Tuk CW, Bakema JE, Kooij G, Reijerkerk A, Vidarsson G, Bouma G, Kraal G, de Vries HE, Beelen RH et al (2009) Immunoglobulin A: Fc(alpha)RI interactions induce neutrophil migration through release of leukotriene B4. Gastroenterology 137(2018–2029):e2011–e2013

van Egmond M, Hanneke van Vuuren AJ, van de Winkel JG (1999) The human Fc receptor for IgA (Fc alpha RI, CD89) on transgenic peritoneal macrophages triggers phagocytosis and tumor cell lysis. Immunol Lett 68:83–87

Vidarsson G, Overbeeke N, Stemerding AM, van den Dobbelsteen G, van Ulsen P, van der Ley P, Kilian M, van de Winkel JG (2005) Working mechanism of immunoglobulin A1 (IgA1) protease: cleavage of IgA1 antibody to Neisseria meningitidis PorA requires de novo synthesis of IgA1 Protease. Infect Immun 73:6721–6726

Williams RC, Gibbons RJ (1972) Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science 177:697–699

Wilson TJ, Fuchs A, Colonna M (2012) Cutting edge: human FcRL4 and FcRL5 are receptors for IgA and IgG. J Immunol 188:4741–4745

Wines BD, Hulett MD, Jamieson GP, Trist HM, Spratt JM, Hogarth PM (1999) Identification of residues in the first domain of human Fc alpha receptor essential for interaction with IgA. J Immunol 162:2146–2153

Woof JM, Kerr MA (2006) The function of immunoglobulin A in immunity. J Pathol 208:270–282

Woof JM, Mestecky J (2005) Mucosal immunoglobulins. Immunol Rev 206:64–82

Yang X, Zhao Q, Zhu L, Zhang W (2013) The three complementarity-determining region-like loops in the second extracellular domain of human Fc alpha/mu receptor contribute to its binding of IgA and IgM. Immunobiology 218:798–809

Humanized Mice to Study FcγR Function

Stylianos Bournazos, David J. DiLillo and Jeffrey V. Ravetch

Abstract Passive immunotherapy represents a promising therapeutic intervention for a number of neoplastic, chronic inflammatory, and infectious diseases, with several monoclonal antibodies currently under development or already in use in the clinic. While Fab–antigen interactions play a crucial role in the activity of an antibody, it has become clear that Fc-mediated effector functions are involved during antibody-mediated activities in vivo. A complete understanding of the contributions of effector activities mediated by an antibody during its in vivo function is required for the development of antibodies with improved therapeutic efficacies. Animal models that are commonly used for the preclinical evaluation of antibodies include murine and non-human primate species, whose FcγRs present substantial structural, functional, and genetic variation compared with their human counterparts. Therefore, the use of such animal models provides limited information on the role of human IgG Fc–FcγR interactions during the in vivo activities of antibodies intended for human therapeutics. In this chapter, we describe the development and evaluation of an FcγR-humanized mouse model for the study of human FcγR function in vivo. In this model, endogenous mouse FcγR genes have been deleted and human FcγRs are expressed as transgenes that faithfully recapitulate the unique pattern of human FcγR expression. Evaluation of the in vivo activities of a number of cytotoxic or therapeutic antibodies using FcγR-humanized mice provided useful insights into human IgG Fc effector function. This mouse model has become a vital preclinical model for testing therapeutic human antibodies to treat malignancies, autoimmunity, inflammation, and infectious disease.

S. Bournazos · D. J. DiLillo · J. V. Ravetch (✉)
Laboratory of Molecular Genetics and Immunology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
e-mail: ravetch@rockefeller.edu

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_11,

Contents

1 Introduction 238
2 Obstacles in Studying in Vivo Fc Effector Function 238
3 FcγR-Humanized Mice 240
3.1 Development of FcγR-Humanized Mice 240
3.2 Evaluation of Antibody-Mediated Effector Function in FcγR-Humanized Mice 241
4 Limitations and Conclusions 245
References 246

1 Introduction

Over the past several decades, significant technical advances have resulted in the development of highly specific, well-tolerated monoclonal antibodies with remarkable efficacy against a number of neoplastic, chronic inflammatory, and infectious diseases (Casadevall et al. 2004; Chan and Carter 2010; Nimmerjahn and Ravetch 2007, 2012). While the activity of an antibody had been previously thought to be the result solely of the interaction of its Fab domain with the respective antigen, substantial evidence from the clinical use of monoclonal antibodies as well as from animal models strongly support the conclusion that Fc effector activity is crucial for the in vivo activities mediated by antibodies. For example, diminished in vivo antibody activity has been reported in the absence of FcγR expression, as in the case of FcγR knock-out mice or when antibodies were expressed as IgG subclass variants, including human IgG4 or IgG2, that exhibit minimal capacity for FcγR interactions (Nimmerjahn and Ravetch 2005; Hessell et al. 2007; Corti et al. 2011). Similarly, modulation of the affinity of an antibody for particular classes of activating or inhibitory FcγRs through Fc domain engineering of the amino acid backbone or its associated *N*-linked glycan structure greatly influences the in vivo activity of an antibody, as evidenced in a number of animal disease models (Dilillo et al. 2014; Smith et al. 2012; Bournazos et al. 2014; Horton et al. 2010; Li and Ravetch 2011; Natsume et al. 2009a, b; Shields et al. 2002; Shinkawa et al. 2003). Furthermore, allelic variants of human FcγRIIa and FcγRIIIa that exhibit differential affinity for IgG have been previously shown to represent predictive factors of the therapeutic outcome for antibody-based therapeutics (Mellor et al. 2013; Zhang et al. 2007; Tamura et al. 2011).

2 Obstacles in Studying in Vivo Fc Effector Function

Given the substantial role of Fc–FcγR interactions during the in vivo activities of antibodies, the development of strategies to systematically study the Fc-mediated effector activities of antibodies intended for human therapeutics is of extreme

importance. The preclinical development of antibodies is typically performed in murine or non-human primate disease models, which may provide insights into the Fc-mediated effector function of antibodies. Indeed, the FcγR family is conserved among species, and the murine or non-human primate FcγRs are highly structurally and functionally homologous to their human counterparts. For example, among the various species, FcγRs are broadly classified as either activating and inhibitory, depending on their capacity to mediate immunoactivating or immunosuppressive signals following IgG engagement, and share highly conserved signaling components (ITAM and ITIM motifs for activating or inhibitory FcγRs, respectively) (Nimmerjahn and Ravetch 2006).

Despite such similarities, murine and non-human primate models present a number of fundamental differences that do not precisely mirror the structural diversity and unique expression patterns observed for human FcγRs on human cells. For example, FcγRIIc and FcγRIIIb are uniquely expressed in humans and are absent from other species, including non-human primates. This is due to the unique genomic organization of the human FcγR locus, which is the result of gene duplication of the ancestral locus through non-homologous recombination that gave rise to human *FCGR2C* and *FCGR3B* genes encoding for FcγRIIc and FcγRIIIb, respectively (Qiu et al. 1990). Additionally, expression patterns of FcγRs also differ between mice, non-human primates and humans (Table 1). For example, murine monocyte-derived dendritic cells express FcγRI, FcγRIIb, FcγRIII, and FcγRIV, whereas human monocyte-derived dendritic cells express only FcγRIIa and FcγRIIb. Likewise, neutrophils from non-human primates express FcγRI, FcγRIIa, and FcγRIIb whereas human neutrophils express FcγRIIa, FcγRIIb, and FcγRIIIb.

An additional determinant that further complicates this situation is the substantial genetic variation—either in the form of single nucleotide polymorphisms or copy number variants—that has been described for almost all human FcγR genes. Such variation greatly influences either FcγR expression or the affinity of the FcγR for human IgG, thereby affecting the in vivo Fc effector functions of antibodies (Bournazos et al. 2009). Indeed, FcγR genetic variants have been associated with susceptibility to infection or autoimmune disease, and the clinical outcome of therapeutic antibodies (Mellor et al. 2013; Bournazos et al. 2009). Although no polymorphisms have been reported for mouse FcγRs, several polymorphic variants have been described for non-human primate FcγR genes, particularly FcγRIIa and FcγRIII, and it is unknown how such variation affects the capacity of these receptors to interact with human IgG (Nguyen et al. 2011; Rogers et al. 2006). It is therefore clear that any results obtained from such animal models cannot be directly extrapolated between species or precisely predict human IgG Fc-mediated effector function.

Previous attempts to study human FcγR–IgG interactions utilized in vitro assays with human FcγR-expressing cells. As expected, such strategies completely fail to recapitulate the complexity of the in vivo IgG Fc-mediated effector activity and do not reflect the diversity of cellular populations that participate during an in vivo response. An alternative model that combines the presence of human

Table 1 Comparison of FcγR expression pattern among humans, murine, and non-human primate species

		FcγRI	FcγRIIa (mFcγRIII)	FcγRIIb	FcγRIIc (human only)	FcγRIIIa (mFcγRIV)	FcγRIIIb (human only)
B cells	Human	–	–	++++	–	–	–
	Rhesus	–	–	–/+		–	
	Mouse	–	–	++++		–	
Monocytes	Human	+++	++	+++	–	–/++	–
	Rhesus	++++	+	+		–/+	
	Mouse	+++	++	+++		++	
Neutrophils	Human	–	+++	+	–	–	+++++
	Rhesus	++++	++++	++		–	
	Mouse	++	++	+		+++	
NK cells	Human	–	–	–	++	+++	–
	Rhesus	–	–	–		+	
	Mouse	–	++	–		–	

FcγR-expressing cells and the complexity of the in vivo conditions is the use of murine models with reconstituted human leukocyte progenitor cells. Such models (i.e., mice reconstituted with human $CD34^+$ cells or PBMCs) have been recently developed and used mainly for the study of infectious diseases, like HIV and HCV, for which murine species are resistant (Dorner et al. 2011; Horwitz et al. 2013; Klein et al. 2012). Apart from the cost as well as the reconstitution variability they present, such reconstituted humanized murine models are severely immunocompromised (*Rag1/2*$^{-/-}$ or SCID and cytokine common γ chain$^{-/-}$), lack functional lymphoid cell compartments (T, B, and NK cells) and present defects in myeloid effector cell function and development. Additionally, the concurrent presence of murine- and human-derived FcγR-expressing effector cells do not allow for an accurate evaluation of human antibody interactions, given the capacity of human IgG to also engage certain classes of murine FcγRs (Pietzsch et al. 2012). For this reason, the development of novel in vivo strategies are required to precisely address the contribution of Fc-mediated effector function, especially given the ever-increasing number of antibody-based therapeutics being developed for the treatment of infectious, neoplastic, and chronic inflammatory diseases.

3 FcγR-Humanized Mice

3.1 Development of FcγR-Humanized Mice

FcγR engagement represents a crucial step in determining the in vivo activity of an antibody, as it mediates a number of diverse pro-inflammatory, immunomodulatory or other effector activities by the various FcγR-expressing leukocyte populations (Nimmerjahn and Ravetch 2006). Given the substantial interspecies

structural, functional, and genetic variation, the in vivo Fc effector activities of human antibodies cannot be precisely evaluated in vivo, as conventional animal models fail to reflect the unique pattern of human FcγR expression as well as FcγR structural and functional diversity.

To overcome this problem, several attempts have been made to introduce human FcγR as transgenes and study the in vivo effects of human antibodies in human FcγR transgenic mice (Li et al. 1996; Heijnen et al. 1996; McKenzie et al. 1999). More recently, an in vivo mouse model that faithfully recapitulates the unique pattern of human FcγR structural and functional diversity has been developed (Smith et al. 2012). This model involved the generation of a mouse line in which all mouse FcγR genes have been deleted and human FcγR genes have been introduced as BAC transgenes to be expressed under the control of their endogenous promoters and regulatory elements. Mice deficient in all classes of mouse FcγRs were generated by targeted deletion of the genes encoding the α chain of the FcγRs, which mediates IgG binding. In contrast, these mice retained the FcγR γ chain, which acts as the accessory signaling subunit for certain classes of FcγRs. The introduction of human FcγR genes as BAC transgenes ensured that the correct expression pattern and cell-type specificity. Indeed, as summarized in Table 2, FcγR-humanized mice demonstrate a cellular pattern of expression similar to that observed in human cells (Smith et al. 2012). Furthermore, as previously reported for human cells, the expression of particular classes of human FcγRs, such as FcγRI, has been shown to be modulated by cytokines (such as IFN-γ) in FcγR-humanized mice, suggesting that FcγR expression regulation is conserved between this humanized mouse strain and humans. Additionally, FcγR-humanized mice exhibit no functional or developmental defects, develop normally and exhibit physiological reproductive capacity. Lymphoid tissue development and architecture is normal and they are capable of mounting a normal immune response against immunization with various antigens (Smith et al. 2012).

3.2 Evaluation of Antibody-Mediated Effector Function in FcγR-Humanized Mice

The FcγR-humanized mouse model has now been used to assess passively administered antibodies in the context of inflammation, neoplastic disease, and infectious disease. Further, FcγR-humanized mice have become an important preclinical platform to test Fc modifications that modulate the FcγR-mediated effector functions of therapeutic human antibodies in vivo, such as point mutations in the human IgG1 Fc that allow for selective and enhanced engagement of specific human FcγRs. Examples of such Fc-modified human IgG1 antibodies that have been tested in FcγR-humanized mice include the S239D/I332E (SDIE) mutant (enhances the affinity of the Fc for all human FcγRs), the G236A/S239D/A330L/I332E (GASDALIE) mutant (which selectively enhances the affinity of the Fc for FcγRIIa and FcγRIIIa), the S267E (SE) and S267E/L328F (SELF) mutants

Table 2 Expression profile of human FcγRs in humans and FcγR-humanized mice

	FcγRI	FcγRIIa	FcγRIIb	FcγRIIIa	FcγRIIIb	
B cells	–	–	+	–	–	Human
	–	–	+	–	–	Humanized FcγR mice
T cells	–	–	–	–	–	Human
	–	–	–	–	–	Humanized FcγR mice
Monocytes	+	+	+	–	–	Human
	+	+	+	–	–	Humanized FcγR mice
Neutrophils	–	+	+	–	+	Human
	–	+	+	–	+	Humanized FcγR mice
NK cells	–	–	–	+	–	Human
	–	–	–	+	–	Humanized FcγR mice
Macrophages	–	+	+	+	–	Human
	–	+	+	+	–	Humanized FcγR mice
Dendritic cells	–	+	+	–	–	Human
	–	+	+	–	–	Humanized FcγR mice

(which selectively enhance the affinity of the Fc for the inhibitory FcγRIIb), and the N297A mutant (which abrogates all FcγR engagement) (Smith et al. 2012). Thus, FcγR-humanized mice have now been used to compare human IgG-mediated effector function mediated by a variety of Fc-engineered antibodies in multiple disease models (Table 3).

As an example of human FcγR-mediated inflammation, passive administration of mouse anti-platelet monoclonal antibody clone 6A6 is a model of idiopathic thrombocytopenic purpura (ITP) and results in the rapid and acute depletion of blood platelet numbers in wild-type mice in an FcγR-dependent manner. Whether a human IgG1 version of the 6A6 antibody was also capable of platelet depletion in the context of the full array of human FcγRs was tested in FcγR-humanized mice (Smith et al. 2012). Indeed, passive administration of huIgG1 6A6 antibody efficiently and rapidly depleted platelets in FcγR-humanized mice, but not in mice lacking FcγR expression. The administration of an equivalent dose of the GASDALIE Fc variant of the 6A6 antibody, which selectively increases Fc engagement to activating FcγRIIa and FcγRIIIa, increased platelet depletion by 1.5-fold. A second model of inflammation has also been tested in FcγR-humanized mice: type III hypersensitivity reactions mediated by immune complexes. Antibody immune complexes generated by heat-aggregation of IgG initiate an anaphylactic response when administered intravenously to mice (Finkelman et al. 2005). Thus, heat-aggregated human IgG administered to FcγR-humanized mice led to rapid anaphylaxis, as demonstrated by a dramatic decrease in core body temperature (Smith et al. 2012). Such anaphylaxis was not seen in mice lacking the expression of any FcγRs, and only a modest anaphylaxis was seen in wild-type mice. These studies demonstrate that FcγR-humanized mice serve as a reliable animal model for human antibody-mediated inflammation.

Table 3 Antibodies tested in the FcγR-humanized mouse model

Model	Antibody	Result	Reference
Platelet depletion (ITP)	Anti-platelet β-integrin	FcγR-dependent platelet depletion mediated by huIgG1 mAb and enhanced by GASDALIE mutant	Smith et al. (2012)
Type 3 hypersensitivity	Heat-aggregated IgG	Heat-aggregated huIgG administration led to severe anaphylaxis	Smith et al. (2012)
B16 melanoma	Anti-gp75 mAb	FcγR-dependent clearance of lung metastases enhanced by GASDALIE mutant	Smith et al. (2012)
B cell depletion	CD40 mAb	FcγR-dependent B cell depletion enhanced by SDIE mutant	Smith et al. (2012)
T cell depletion	CD4 mAb	FcγR-dependent T cell depletion enhanced by GASDALIE mutant	Smith et al. (2012)
Anti-tumor agonistic effect	CD40 mAb	FcγRIIb-dependent anti-tumor T cell responses enhanced by SE mutant	Li and Ravetch (2011), Smith et al. (2012)
Anti-tumor agonistic effect	Anti-DR5 mAb	FcγRIIb-dependent anti-tumor T cell effects enhanced by SE mutant	Li and Ravetch (2012)
Influenza	Anti-HA stalk	Anti-HA broadly neutralizing antibody-mediated protection from lethal virus infection enhanced by GASDALIE mutant	Dilillo et al. (2014)
Anthrax	Anti-PA	Anti-PA neutralizing antibody-mediated protection from lethal anthrax infection enhanced by GASDALIE mutant	Bournazos et al. (2014)

FcγR-humanized mice have also been used to assess the activities of immunotherapeutic human antibodies in the context of neoplastic disease. The first tumor studies to be carried out in FcγR-humanized mice utilized the B16 melanoma tumor model. Passive treatment with murine TA99 monoclonal antibody, specific for the gp75 (TRP75) tumor antigen expressed by B16 melanoma tumor cells, clears B16 lung metastases in an activating FcγR-dependent manner (Nimmerjahn and Ravetch 2005). When administered to FcγR-humanized mice bearing B16 tumors, human IgG1 TA99 antibody successfully cleared ∼40 % of tumor metastases, while the antibody had no effect in mice lacking any FcγRs (Smith et al. 2012). Further, no effect on metastases was seen when a human IgG1 mutant that does not engage FcγRs (N297A mutation) was used. However, an Fc-engineered GASDALIE mutant TA99 antibody (Fc modified for enhanced engagement of FcγRIIa and FcγRIIIa) augmented tumor clearance by twofold and cleared >80 % of metastases (Smith et al. 2012). Similarly, the FcγR-dependent depletion of B cells and $CD4^+$ T cells by human IgG1 versions of anti-CD40 and anti-CD4 antibodies, respectively, has been demonstrated in FcγR-humanized mice. Further, the activities of these human IgG1 antibodies have been augmented using Fc mutants that enhance interactions with activating FcγRs (SDIE and GASDALIE mutants, respectively) (Smith et al. 2012). Thus, these studies establish FcγR-humanized mice as an important model for testing cytotoxic tumor-specific human antibodies for FcγR-mediated effects in vivo.

Because the expression pattern of human FcγRs in FcγR-humanized mice recapitulates the pattern seen in humans (Table 2), this mouse model also represents an invaluable model to test human therapeutic immunomodulatory antibodies that engage immune receptors to regulate immune system function. Many such immunomodulatory antibodies have anti-tumor activities. For example, agonistic anti-CD40 monoclonal antibody is a potent anti-cancer immunotherapeutic that boosts the activation of cytotoxic CD8 T cells to increase anti-tumor activity. Recently, the mechanism of action of agonistic anti-CD40 antibody has been shown to be absolutely dependent on its engagement with the inhibitory FcγR, FcγRIIb (Li and Ravetch 2011). Co-administration of a human IgG1 anti-CD40 antibody with a dendritic cell-targeted anti-OVA immunization strategy showed a modest anti-OVA $CD8^+$ T cell response in mice humanized for FcγRIIa and FcγRIIb, but no anti-OVA $CD8^+$ response was seen when the FcγR-null binding N297A mutant of anti-CD40 antibody was used (Li and Ravetch 2011). However, the anti-OVA $CD8^+$ T cell response was boosted greater than 12-fold when the SE Fc variant (enhances Fc interactions with FcγRIIb) of anti-CD40 antibody was co-administered during immunization. These results were recapitulated in FcγR-humanized mice as well (Smith et al. 2012), and correlated with survival after challenge with OVA-expressing tumor cells (Li and Ravetch 2011). Similar results were seen when human IgG1 and SE mutant anti-CD40 antibodies were administered to FcγRIIa/FcγRIIb-humanized mice challenged with either $CD40^+$ or $CD40^-$ tumors cells in the absence of immunization. Parallel studies in FcγRIIb-humanized mice using anti-DR5 antibodies, which also require engagement of inhibitory FcγRIIb for their in vivo anti-tumor and hepatotoxic effects,

demonstrated enhanced tumor clearance using Fc-engineered anti-DR5 antibody with enhanced affinity for FcγRIIb (Li and Ravetch 2012). Thus, FcγR-humanized mice are an ideal platform for testing immunomodulatory antibodies for use as anti-tumor therapeutics.

FcγR-humanized mice have also been used to test antibody effector function in the context of infectious disease. Recent studies have demonstrated that broadly neutralizing anti-influenza hemagglutinin (HA) antibodies require interactions with activating FcγRs for their ability to mediate passive protection against lethal influenza challenge in vivo (Dilillo et al. 2014). In addition, a chimeric human IgG1 anti-HA monoclonal antibody (clone 6F12) protected FcγR-humanized mice from lethal influenza virus challenge in a dose-dependent manner. Since anti-HA antibody required interactions with activating FcγRs to mediate protection in vivo, whether Fc-engineering the anti-HA antibody Fc to enhance interactions with activating human FcγRs was assessed. Selectively engaging activating human FcγRIIa and FcγRIIIa with GASDALIE mutant anti-HA antibody augmented in vivo protection during viral challenge by approximately twofold (Dilillo et al. 2014). Similar studies using anti-anthrax toxin neutralizing antibodies in FcγR-humanized mice have also been performed, in which a chimeric human IgG1 version of an anti-anthrax protective antigen (PA) antibody (clone 19D9) was generated (Bournazos et al. 2014). The ability of this antibody to protect FcγR-humanized mice from lethal *B. anthracis* challenge was dependent on human FcγR expression, since protection was only seen in FcγR-humanized mice and not in mice lacking FcγRs. Further, Fc-engineering this anti-PA neutralizing antibody to selectively enhance interactions with human activating FcγRs (GASDALIE mutant) resulted in augmented protection against *B. anthracis* challenge. Thus, FcγR-humanized mice are an ideal model system for testing neutralizing antibodies for human FcγR effector-mediated function during protection against a variety of pathogens.

4 Limitations and Conclusions

While FcγR-humanized mice represent a powerful tool for assessing the contributions of human FcγRs during human antibody-mediated effector function in vivo, some limitations exist. For example, human target antigens to which therapeutic antibodies bind must be expressed as transgenes in mice and crossed onto the FcγR-humanized background in order to assess the therapeutic potential of antibodies directed at host-expressed targets. Examples of such therapeutic antibodies with anti-tumor activities include anti-CD20, anti-Her2, anti-EGFR, anti-CD40, anti-CTLA-4, and anti-PD-1 antibodies. Alternatively, since FcγR-humanized mice are fully immunocompetent, studies with antibodies targeting tumor antigens must utilize mouse tumor cell lines expressing the human tumor antigen. Another limitation to the FcγR-humanized mouse model is that repeated or long-term administration of human IgG will result in an anti-human IgG response that will confound experimental results. Thus, the introduction of a

human IgG1 knock-in transgene into FcγR-humanized mice would resolve this situation by tolerizing the mice to human IgG1 administration.

Currently, FcγR-humanized mice only express one class of human FcγRs, and therefore do not take into account the array of other receptors for human IgG. Future versions of FcγR-humanized mice will include deletions of these receptors and human transgenes to replace them. For example, the neonatal Fc receptor, FcRn, regulates antibody half-life in vivo, and the interaction between the Fc and FcRn can be manipulated to increase antibody half-life through Fc-engineering. Several IgA receptors, including FcαRI (CD89), the Fcα/μR, and the polymeric Ig receptor, as well as the high and low affinity IgE receptors, FcεRI and CD23 respectively, are alternative Fc receptors with important biological functions whose human counterparts may be expressed in mice. In addition, human DC-SIGN, which engages sialylated IgG molecules to mediate anti-inflammatory activities, is another candidate human Fc receptor for inclusion in the humanized mouse model. Thus, future iterations of FcγR-humanized mice will include other classes of FcγRs to even more fully recapitulate the variety of immunoglobulin-binding proteins expressed by humans. Regardless, FcγR-humanized mice are a powerful tool that will facilitate investigations into the consequences of human FcγR engagement by human antibodies that engage a variety of targets in vivo models of inflammation, malignancy, and infectious disease.

References

Bournazos S, Chow SK, Abboud N, Casadevall A, Ravetch JV (2014) Human IgG Fc domain engineering enhances antitoxin neutralizing antibody activity. J Clin Invest 124(2):725–729

Bournazos S, Woof JM, Hart SP, Dransfield I (2009) Functional and clinical consequences of Fc receptor polymorphic and copy number variants. Clin Exp Immunol 157(2):244–254

Casadevall A, Dadachova E, Pirofski LA (2004) Passive antibody therapy for infectious diseases. Nat Rev Microbiol 2(9):695–703

Chan AC, Carter PJ (2010) Therapeutic antibodies for autoimmunity and inflammation. Nat Rev Immunol 10(5):301–316

Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, Jarrossay D, Vachieri SG, Pinna D, Minola A, Vanzetta F, Silacci C, Fernandez-Rodriguez BM, Agatic G, Bianchi S, Giacchetto-Sasselli I, Calder L, Sallusto F, Collins P, Haire LF, Temperton N, Langedijk JP, Skehel JJ, Lanzavecchia A (2011) A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science 333(6044):850–856

Dilillo DJ, Tan GS, Palese P, Ravetch JV (2014) Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus in vivo, Nat Med 20(2):143–151

Dorner M, Horwitz JA, Robbins JB, Barry WT, Feng Q, Mu K, Jones CT, Schoggins JW, Catanese MT, Burton DR, Law M, Rice CM, Ploss A (2011) A genetically humanized mouse model for hepatitis C virus infection. Nature 474(7350):208–211

Finkelman FD, Rothenberg ME, Brandt EB, Morris SC, Strait RT (2005) Molecular mechanisms of anaphylaxis: lessons from studies with murine models. J Allergy Clin Immunol 115(3):449–457

Heijnen IA, van Vugt MJ, Fanger NA, Graziano RF, de Wit TP, Hofhuis FM, Guyre PM, Capel PJ, Verbeek JS, van de Winkel JG (1996) Antigen targeting to myeloid-specific human FcγRI/CD64 triggers enhanced antibody responses in transgenic mice. J Clin Invest 97(2):331–338

Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, Lanigan CM, Landucci G, Forthal DN, Parren PW, Marx PA, Burton DR (2007) Fc receptor but not complement binding is important in antibody protection against HIV. Nature 449(7158):101–104

Horton HM, Bernett MJ, Peipp M, Pong E, Karki S, Chu SY, Richards JO, Chen H, Repp R, Desjarlais JR, Zhukovsky EA (2010) Fc-engineered anti-CD40 antibody enhances multiple effector functions and exhibits potent *in vitro* and *in vivo* antitumor activity against hematologic malignancies. Blood 116(16):3004–3012

Horwitz JA, Halper-Stromberg A, Mouquet H, Gitlin AD, Tretiakova A, Eisenreich TR, Malbec M, Gravemann S, Billerbeck E, Dorner M, Büning H, Schwartz O, Knops E, Kaiser R, Seaman MS, Wilson JM, Rice CM, Ploss A, Bjorkman PJ, Klein F, Nussenzweig MC (2013) HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. Proc Natl Acad Sci USA 110(41):16538–16543

Klein F, Halper-Stromberg A, Horwitz JA, Gruell H, Scheid JF, Bournazos S, Mouquet H, Spatz LA, Diskin R, Abadir A, Zang T, Dorner M, Billerbeck E, Labitt RN, Gaebler C, Marcovecchio PM, Incesu RB, Eisenreich TR, Bieniasz PD, Seaman MS, Bjorkman PJ, Ravetch JV, Ploss A, Nussenzweig MC (2012) HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. Nature 492(7427):118–122

Li F, Ravetch JV (2011) Inhibitory Fcγ receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. Science 333(6045):1030–1034

Li F, Ravetch JV (2012) Apoptotic and antitumor activity of death receptor antibodies require inhibitory Fcγ receptor engagement. Proc Natl Acad Sci USA 109(27):10966–10971

Li M, Wirthmueller U, Ravetch JV (1996) Reconstitution of human FcγRIII cell type specificity in transgenic mice. J Exp Med 183(3):1259–1263

McKenzie SE, Taylor SM, Malladi P, Yuhan H, Cassel DL, Chien P, Schwartz E, Schreiber AD, Surrey S, Reilly MP (1999) The role of the human Fc receptor FcγRIIA in the immune clearance of platelets: a transgenic mouse model. J Immunol 162(7):4311–4318

Mellor JD, Brown MP, Irving HR, Zalcberg JR, Dobrovic A (2013) A critical review of the role of Fcγ receptor polymorphisms in the response to monoclonal antibodies in cancer. J Hematol Oncol 6:1

Natsume A, Niwa R, Satoh M (2009a) Improving effector functions of antibodies for cancer treatment: Enhancing ADCC and CDC. Drug Des Devel Ther 3:7–16

Natsume A, Shimizu-Yokoyama Y, Satoh M, Shitara K, Niwa R (2009b) Engineered anti-CD20 antibodies with enhanced complement-activating capacity mediate potent anti-lymphoma activity. Cancer Sci 100(12):2411–2418

Nguyen DC, Scinicariello F, Attanasio R (2011) Characterization and allelic polymorphisms of rhesus macaque (*Macaca mulatta*) IgG Fc receptor genes. Immunogenetics 63(6):351–362

Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science 310(5753):1510–1512

Nimmerjahn F, Ravetch JV (2006) Fcγ receptors: old friends and new family members. Immunity 24(1):19–28

Nimmerjahn F, Ravetch JV (2007) Antibodies Fc receptors and cancer. Curr Opin Immunol 19(2):239–245

Nimmerjahn F, Ravetch JV (2012) Translating basic mechanisms of IgG effector activity into next generation cancer therapies. Cancer Immun 12:13

Pietzsch J, Gruell H, Bournazos S, Donovan BM, Klein F, Diskin R, Seaman MS, Bjorkman PJ, Ravetch JV, Ploss A, Nussenzweig MC (2012) A mouse model for HIV-1 entry. Proc Natl Acad Sci USA 109(39):15859–15864

Qiu WQ, de Bruin D, Brownstein BH, Pearse R, Ravetch JV (1990) Organization of the human and mouse low-affinity FcγR genes: duplication and recombination. Science 248(4956):732–735

Rogers KA, Scinicariello F, Attanasio R (2006) IgG Fc receptor III homologues in nonhuman primate species: genetic characterization and ligand interactions. J Immunol 177(6):3848–3856

Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SH, Presta LG (2002) Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. J Biol Chem 277(30):26733–26740

Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, Uchida K, Anazawa H, Satoh M, Yamasaki M, Hanai N, Shitara K (2003) The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278(5):3466–3473

Smith P, DiLillo DJ, Bournazos S, Li F, Ravetch JV (2012) Mouse model recapitulating human Fcγ receptor structural and functional diversity. Proc Natl Acad Sci USA 109(16):6181–6186

Tamura K, Shimizu C, Hojo T, Akashi-Tanaka S, Kinoshita T, Yonemori K, Kouno T, Katsumata N, Ando M, Aogi K, Koizumi F, Nishio K, Fujiwara Y (2011) FcγR2A and 3A polymorphisms predict clinical outcome of trastuzumab in both neoadjuvant and metastatic settings in patients with HER2-positive breast cancer. Ann Oncol 22(6):1302–1307

Zhang W, Gordon M, Schultheis AM, Yang DY, Nagashima F, Azuma M, Chang HM, Borucka E, Lurje G, Sherrod AE, Iqbal S, Groshen S, Lenz HJ (2007) *FCGR2A* and *FCGR3A* polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. J Clin Oncol 25(24):3712–3718

FcRn: From Molecular Interactions to Regulation of IgG Pharmacokinetics and Functions

Dilip K. Challa, Ramraj Velmurugan, Raimund J. Ober and E. Sally Ward

Abstract The neonatal Fc receptor, FcRn, is related to MHC class I with respect to its structure and association with $\beta_2$microglobulin (β_2m). However, by contrast with MHC class I molecules, FcRn does not bind to peptides, but interacts with the Fc portion of IgGs and belongs to the Fc receptor family. Unlike the 'classical' Fc receptors, however, the primary functions of FcRn include salvage of IgG (and albumin) from lysosomal degradation through the recycling and transcytosis of IgG within cells. The characteristic feature of FcRn is pH-dependent binding to IgG, with relatively strong binding at acidic pH (<6.5) and negligible binding at physiological pH (7.3–7.4). FcRn is expressed in many different cell types, and endothelial and hematopoietic cells are the dominant cell types involved in IgG homeostasis in vivo. FcRn also delivers IgG across cellular barriers to sites of pathogen encounter and consequently plays a role in protection against infections, in addition to regulating renal filtration and immune complex-mediated antigen presentation. Further, FcRn has been targeted to develop both IgGs with extended half-lives and FcRn inhibitors that can lower endogenous antibody levels. These approaches have implications for the development of longer lived therapeutics and the removal of pathogenic or deleterious antibodies.

Abbreviations

APCs	Antigen presenting cells
BBB	Blood–brain barrier
CNS	Central nervous system
DCs	Dendritic cells

D. K. Challa · R. Velmurugan · R. J. Ober · E. Sally Ward (✉)
Department of Immunology, University of Texas Southwestern Medical Center, 6000 Harry Hines Blvd, Dallas, TX 75390, USA
e-mail: sally.ward@utsouthwestern.edu

R. J. Ober
Department of Electrical Engineering, University of Texas at Dallas, Richardson, TX 75080, USA

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_12,

ECs	Endothelial cells
FcRn	Neonatal Fc receptor
GBM	Glomerular basement membrane
HCs	Hematopoietic cells
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
ICs	Immune complexes
Ig	Immunoglobulin
IVIG	Intravenous immunoglobulin
KO	Knockout
LP	Lamina propria
mAbs	Monoclonal antibodies
MALT	Mucosa-associated lymphoid tissue
MHC	Major histocompatibility
MLNs	Mesenteric lymph nodes
Myo Vb	Motor myosin Vb
OVA	Ovalbumin
PCT	Proximal convoluted tubule
β_2m	$\beta_2$microglobulin
TC	Transport carrier
TLR	Toll-like receptor
WT	Wildtype

Contents

1 Introduction 250
2 FcRn Biology 251
3 Functions of FcRn 254
3.1 IgG Homeostasis 254
3.2 Transport of IgG Across Cellular Barriers 256
3.3 Maintenance and Regulation of Renal Filtration 258
3.4 Possible Role in Clearing IgG from Immune-Privileged Sites 261
3.5 Role in Antigen Presentation 262
4 FcRn-Targeted Therapies 263
5 Concluding Remarks 265
References 266

1 Introduction

The neonatal Fc receptor (FcRn), as the name indicates, was first described for its role in the transfer of IgG from mother's milk across the neonatal gut epithelial barrier into the neonatal bloodstream (Brambell 1970). It is also referred to as a

major histocompatibility (MHC) class I-related receptor since it shares structural similarity with MHC class I (Simister and Mostov 1989). FcRn belongs to the class of Fc receptors that bind to immunoglobulin G (IgG). However, FcRn differs from other members (collectively referred as FcγRs) of this class in multiple ways: (1) FcRn is expressed in hematopoietic cells (HCs) as well as non-HCs (Borvak et al. 1998; Zhu et al. 2001; Akilesh et al. 2007; Perez-Montoyo et al. 2009), whereas FcγR expression is primarily confined to cells of hematopoietic origin (Nimmerjahn and Ravetch 2008; Hogarth and Pietersz 2012); (2) the cytoplasmic domain of FcRn lacks the ability to signal intracellularly (Kuo et al. 2009), whereas FcγRs (except human FcγRIIIB) or their subunit (γ chain) have immunoreceptor tyrosine-based activatory or inhibitory motifs (ITAMs or ITIMs) in their cytoplasmic domains, which can mediate intracellular signaling (Nimmerjahn and Ravetch 2008; Hogarth and Pietersz 2012); (3) the key function of FcRn involves recycling and transcytosis of IgG (Roopenian and Akilesh 2007; Ward and Ober 2009; Kuo et al. 2010), while FcγRs regulate the immune complex-mediated effector functions of innate immune cells (Nimmerjahn and Ravetch 2008; Hogarth and Pietersz 2012).

Two primary and very well-studied functions of FcRn include the regulation of IgG homeostasis and IgG transport across cellular barriers (Ward and Ober 2009). FcRn is expressed in many different cell types, some of which can be found in all organs of the body (Akilesh et al. 2007; Perez-Montoyo et al. 2009). As a result, the functions of FcRn are not localized to a single organ or cell type, an attribute required for regulating the homeostasis and transport of the ubiquitous immune molecule, IgG. FcRn also regulates the homeostasis of albumin (Chaudhury et al. 2003), although the binding site on FcRn is different for the two molecules (Andersen et al. 2006; Oganesyan et al. 2014) and hence they do not compete with each other for FcRn binding. Recently, FcRn has been shown to also play an important role in the regulation of renal filtration (Akilesh et al. 2008; Sarav et al. 2009) and antigen presentation (Qiao et al. 2008; Baker et al. 2011). In this review, we discuss data that elucidate the mechanisms through which FcRn performs these multiple functions. The well-defined role of antibodies in autoimmunity (Naparstek and Plotz 1993) and the emergence of IgG-based therapeutics (Chan and Carter 2010; Scott et al. 2012) have motivated the development of many FcRn-targeting therapies that have shown promise in preclinical studies. These studies will also be reviewed.

2 FcRn Biology

FcRn exists as a heterodimer of the MHC class I-like heavy chain and $\beta_2$microglobulin (β_2m), which are noncovalently associated (Simister and Mostov 1989). Association with β_2m is required for the expression and normal functioning of FcRn (Claypool et al. 2002). The MHC class I-like heavy chain includes glycosylated α1–3 domains, a transmembrane domain and a ~42 amino acid

cytoplasmic tail (Kuo et al. 2009). Crystallographic studies of a rat FcRn-rat Fc (IgG2a) complex revealed that the α2 domain residues (Glu117, Glu118, Glu132, Trp133, Glu135, and Asp137) and Ile1 of β_2m combined with the carbohydrate of rat FcRn interacts with residues (Ile253, His310, His435, and minor role for His436) at the CH2–CH3 interface of rat Fc (Martin et al. 2001). The role of His433 of the Fc region in these interactions is contentious (Raghavan et al. 1995; Medesan et al. 1997; Kim et al. 1999; Shields et al. 2001). The stoichiometry of the interaction between FcRn and Fc or IgG is 2:1, as shown by equilibrium gel filtration or sedimentation equilibrium assays (Sanchez et al. 1999; Schuck et al. 1999). The FcRn:Fc (or IgG):FcRn interaction is asymmetric, with different dissociation constants for the two binding sites (Schuck et al. 1999). This, combined with a recent three-dimensional structure of human FcRn bound to an engineered human Fc fragment (Oganesyan et al. 2014), indicate that occupancy of the 'first' site on IgG results in conformational changes that reduce the affinity of FcRn for the second site. Further, the FcRn-IgG interaction is highly pH-dependent, with relatively high affinity binding at acidic pH (<6.5) and no detectable binding at physiological pH (7.4) (Raghavan et al. 1993; Popov et al. 1996). Site-directed mutagenesis studies have shown that the pH-dependence is imparted by His310 and His435 of human Fc (Raghavan et al. 1995) (or His310, His435, and His436 of rodent Fc (Medesan et al. 1997)), which get protonated at acidic pH. These positively charged histidines can then form a salt bridge with the corresponding residues of the FcRn heavy chain (Martin et al. 2001). However, the crystal structure of the complex of human FcRn bound to an engineered human Fc fragment (M252Y/S254T/T256E) was recently solved, which indicates that His310 of human Fc is the most important histidine residue for pH-dependent binding (Oganesyan et al. 2014).

Studies using mutated versions of FcRn have localized the endocytosis and transcytosis signals within the cytoplasmic tail of FcRn, which include the conserved motifs tryptophan (Trp311) and dileucine (Leu322, Leu323) (Newton et al. 2005). A calmodulin-binding site in the membrane proximal region of human FcRn has also been identified that controls the transcytosis and half-life of FcRn in epithelial cells in a calcium-dependent manner (Dickinson et al. 2008). Also, rodent FcRn has three extracellular N-glycan moieties that are absent in human FcRn, which has only one N-linked glycan common to both human and rodent FcRn (Kuo et al. 2009). Interestingly, when human FcRn is rodentized in terms of N-glycan moieties, its steady-state distribution changes (from basolateral) to the apical membrane and its predominant direction of transcytosis (basolateral to apical) is reversed, resulting in the transport of IgG from the apical to basolateral side (Kuo et al. 2009).

Although recent data suggests a slightly different picture (see Sect. 3.1), in the past it was hypothesized that FcRn in vascular endothelial cells (ECs) is most important for recycling of IgG, since these cells form a large surface area that is in contact with the bloodstream. Therefore, FcRn trafficking with respect to IgG recycling has been extensively studied in ECs (Ober et al. 2004a, b; Prabhat et al. 2007; Gan et al. 2009). The recycling process has been characterized in human

FcRn-Green Fluorescent Protein (GFP)-transfected human microvasculature ECs (HMEC-1), using live-cell fluorescence imaging (Ober et al. 2004b). In these studies, fluorescently labeled wildtype (WT) human IgG1 was used to trace the path of recycling IgG, and a mutated variant (H435A), which binds to FcRn with negligible affinity at both physiological and acidic pH, was used to track IgG that does not bind to FcRn. Based on the results from these and subsequent studies (Ober et al. 2004a; Prabhat et al. 2007; Gan et al. 2009, 2013), a model for FcRn recycling/transcytosis has been constructed, which can be summarized in three steps (Fig. 1): (1) Cells nonspecifically pinocytose extracellular fluid including IgG into adaptor protein containing pH domain, PTB domain, and leucine zipper motif 1 positive ($APPL1^{+}$) vesicular transport carriers (TCs), which then fuse with sorting endosomes. The acidic environment in these compartments facilitates IgG binding to FcRn. (2) FcRn-IgG complexes are sorted into recycling or transcytotic TCs. These TCs subsequently fuse with the plasma membrane, followed by the release of IgG into the serum or interstitial space due to the physiological (near-neutral) pH. (3) Meanwhile, the sorting endosomes mature to late endosomes, which deliver their luminal contents to lysosomes, resulting in the degradation of any IgG that failed to be recycled by FcRn.

FcRn-mediated transcytosis has also been extensively studied using Madin–Darby canine kidney (MDCK) cells (Claypool et al. 2004; Tesar et al. 2006), which form polarized monolayers when cultured in vitro, a property necessary for studying transcytosis. In human FcRn-transfected MDCK cells, FcRn localizes predominantly to apical intracellular compartments, with surface expression primarily on the basolateral side. Importantly, FcRn was demonstrated to transcytose IgG in both basolateral to apical and apical to basolateral directions, the latter being dominant (Claypool et al. 2004). What factors define whether IgG is recycled or transcytosed? Although this question has not been answered completely, studies have identified molecular effectors for these processes which include Rab *GTPases* and motor myosin Vb (Myo Vb). Rab *GTPases* are regulated by GTP-GDP exchange cycles, and in combination with soluble NSF attachment protein receptors (SNAREs) can regulate the merging of different organellar membranes (Somsel and Wandinger-Ness 2000; Miaczynska and Zerial 2002; Jahn et al. 2003). Also, when active, Rab *GTPases* can activate or recruit effector molecules such as kinases, phosphatases, motors, etc. Consequently, these proteins control multiple intracellular trafficking processes (Stenmark 2009; Agola et al. 2011). On the other hand, myosin motors are mechanical, enzymatic motors, which generate energy by hydrolyzing ATP to drive cargo along actin filaments (Hammer and Sellers 2012). Rab11 *GTPase* associates with FcRn during recycling in HMEC-1 cells (Ward et al. 2005), and regulates recycling in MDCK cells (Tzaban et al. 2009), whereas Myo Vb and Rab25 *GTPase* are involved in bidirectional transcytosis in MDCK cells (Tzaban et al. 2009).

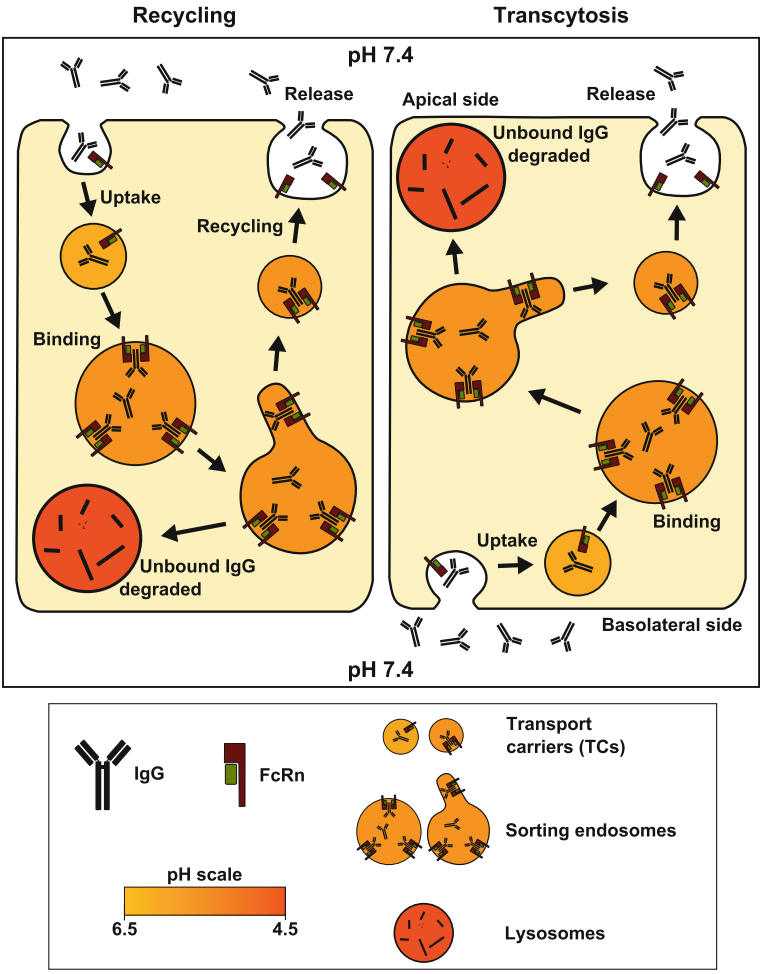


Fig. 1 FcRn-mediated recycling and transcytosis of IgG. Cells internalize IgG through fluid-phase pinocytosis into tubulovesicular TCs, which subsequently fuse with sorting endosomes. The acidic pH in these compartments favors the binding of IgG to FcRn. FcRn with bound IgG sorts into TCs, which either recycle or transcytose to the plasma membrane. The near-neutral pH on the plasma membrane results in the release of IgG from FcRn into the extracellular fluid

3 Functions of FcRn

3.1 IgG Homeostasis

IgG and albumin constitute ~80 % of total serum protein with mean concentrations as high as 10 and 40 mg/ml, respectively (Dati et al. 1996). The primary reason for the high abundance of these proteins is their extraordinarily long serum

half-life. IgG has a serum half-life of ~22 days in humans (Spiegelberg and Fishkin 1972) and ~8 days in mice (Vieira and Rajewsky 1988; Ghetie et al. 1996). Multiple studies have convincingly shown that the extended half-life of IgG (and albumin) is FcRn-mediated. The first in vivo evidence for this came from studies using β_2m-deficient knockout (KO) mice, which do not express functional FcRn in addition to having other defects such as $CD8^+$ T cell deficiency. In these mice, IgG has an extremely short half-life (Ghetie et al. 1996; Israel et al. 1996; Junghans and Anderson 1996). Later, similar conclusions were obtained using FcRn KO mice (Roopenian et al. 2003), which are more specific tools than β_2m KO mice for studying FcRn biology. In addition, based on archived blood samples a study has identified two deceased humans (with familial hypercatabolic hypoproteinemia), who were analogous to β_2m KO mice, i.e., β_2m expression was almost completely inhibited in these patients (soluble β_2m levels in their serum were <1 % of normal) due to a point mutation in the leader peptide of their β_2m gene (Wani et al. 2006). IgG and albumin levels were abnormally low in their serum, also indicating a role for FcRn in humans in protecting IgG and albumin from catabolism.

As mentioned earlier, FcRn is expressed in many different cell types across the body. In adult humans, FcRn expression can be found in skin microvasculature, retinal, and placental ECs (Antohe et al. 2001; Ober et al. 2004b; Powner et al. 2014), monocytes, macrophages, dendritic cells (DCs) (Zhu et al. 2001), T and B lymphocytes (van Bilsen et al. 2010), keratinocytes (Cauza et al. 2005), hepatocytes (Andersen et al. 2012), epithelial cells of intestine (Israel et al. 1997; Dickinson et al. 1999), mammary gland (Cianga et al. 2003), kidney (Haymann et al. 2000), lung (Spiekermann et al. 2002), eye (Powner et al. 2014) and the female genital tract (Li et al. 2011). In adult mice, FcRn has been localized to vascular ECs of some, but not all organs (Akilesh et al. 2007), macrophages, DCs (Akilesh et al. 2007; Perez-Montoyo et al. 2009), B cells (Perez-Montoyo et al. 2009) and epithelial cells of kidney (Akilesh et al. 2008), alveolus (Spiekermann et al. 2002), intestine (Akilesh et al. 2007), choroid plexus (Akilesh et al. 2007), eye (Kim et al. 2008), and the female genital tract (Li et al. 2011). It is not clear in which cell types/organ FcRn is crucial for persistence of IgG (and albumin). Experiments using bone marrow chimeras of WT and FcRn KO mice revealed that FcRn in HCs and non-HCs contribute almost equally to IgG homeostasis (Akilesh et al. 2007; Kobayashi et al. 2009). Subsequent studies using Cre-loxp technology-based cell type-specific FcRn KO mice demonstrated that FcRn-expressing ECs and HCs are the major sites of IgG homeostasis (Perez-Montoyo et al. 2009).

The relative contribution of different cell types to IgG recycling depends on many factors, including the number of FcRn-expressing cells within each group, FcRn expression levels, the rate of pinocytic/phagocytic activity and the concentration of IgG in the respective microenvironments. Also, the relative contribution of cells might change during inflammation, since toll-like receptor (TLR) ligands and proinflammatory cytokines have been shown to modulate FcRn expression. In particular, CpG oligodeoxynucleotide (TLR9 ligand), *lipopolysaccharide* (TLR4 ligand), tumor necrosis factor (TNF)-α and interleukin-1β have been shown to

upregulate FcRn expression in intestinal epithelial cells and/or monocytes (Liu et al. 2007b). By contrast, interferon-γ has been shown to downregulate FcRn expression in intestinal epithelial cells and monocytes (Liu et al. 2008). Determining the contribution of each cell type to IgG protection, and how this changes under inflammatory conditions, will aid in developing accurate pharmacokinetic-modeling tools required for optimizing the delivery of IgG-based therapeutics.

3.2 Transport of IgG Across Cellular Barriers

3.2.1 IgG Transfer from Mother to Fetus or Neonate

IgG is the only immunoglobulin subclass that is actively transported from mother to fetus/neonate. Although both mother-to-fetus and mother-to-neonate transfer of IgG can occur in rodents and humans, the former is dominant in humans while the latter plays a major role in rodents.

In mice, FcRn expression in the yolk sac mediates the materno fetal transfer of IgG (Medesan et al. 1996). However, at birth, the concentration of IgG in the serum of neonatal mice is only 20–30 % of that in adult mice (Appleby and Catty 1983) and hence, IgG transport during gestation in mice is considered to be of relatively low importance. In rodents, the transfer of passive immunity in the form of IgG primarily occurs postnatally (Appleby and Catty 1983). Upon ingestion of IgG-containing maternal milk, IgG, and other milk proteins reach the proximal small intestine (the stomach is less acidic in neonates). Acidic pH in the duodenum allows IgG to be selectively endocytosed by enterocytes in an FcRn-dependent fashion (Jones and Waldmann 1972; Rodewald and Abrahamson 1982; Rodewald and Kraehenbuhl 1984). Internalized IgG is then transcytosed across the cell to the basolateral membrane, where the physiological, near-neutral pH results in the release of IgG from FcRn into the intestinal tissue. IgG can subsequently transfer into the blood through the lymphatics. Coincidentally, in rodents, FcRn expression in enterocytes rapidly decreases at around weaning age (Martin et al. 1997; Akilesh et al. 2007).

In newborn infants, the concentration of IgG in the serum is at levels similar to those observed in mothers (Salimonu et al. 1978). This indicates that maternofetal transport of IgG (during the third trimester of pregnancy) is extremely efficient in humans. The transport is mediated by FcRn expressed in syncytiotrophoblasts (Leach et al. 1996; Simister et al. 1996; Firan et al. 2001), which constitute the continuous, multinucleate epithelium separating the mother from fetus. On the apical side, the brush border surface of syncytiotrophoblast is bathed in maternal blood, whilst the basolateral membrane faces fetal blood capillaries. In brief, the maternal serum containing IgG is pinocytosed into the endosomes of syncytiotrophoblasts, followed by IgG transcytosis to the fetal side (basolateral membrane), where the near-neutral pH enables IgG dissociation from FcRn.

3.2.2 Transport of IgG to Sites of Pathogen Encounter and Immune Activation

The mucosal surfaces of the airways, urogenital tract, and intestine are the primary sites where a multicellular organism such as a mammalian species interacts with the environment. These surfaces employ multiple mechanisms to protect against invasion of pathogens or harmful agents, which include (McGhee and Fujihashi 2012): (1) a polarized epithelial cell barrier, (2) secretions (containing antimicrobial substances including IgA, IgG and IgD) toward the apical (environment) side of the epithelial cell layer and (3) mucosa-associated lymphoid tissue (MALT), positioned on the basolateral side (beneath) of the epithelial barrier. MALT is primarily composed of innate (DCs, macrophages, etc.) and adaptive (T and B cells) immune cells, however, the composition of MALT varies significantly at each mucosal surface. Importantly, $CD103^+$ DCs in the lamina propria (LP, part of MALT in the gut) extend processes through the epithelial cell barrier into the intestinal lumen and capture antigens. The DCs then carry the captured antigen to the mesenteric lymph nodes (MLNs) where they present antigenic peptides to T cells (Schulz et al. 2009). An analogous function of antigen sampling has been shown to be performed by FcRn in intestinal epithelial cells in mice (Yoshida et al. 2004). In this study, transgenic mice expressing human FcRn (under the control of endogenous human promoter) and human β_2m in the absence of endogenous mouse FcRn expression were used because, as mentioned earlier, intestinal epithelial cells in WT mice downregulate FcRn expression at around weaning age (Akilesh et al. 2007), whereas intestinal epithelial cells in adult humans continue to express FcRn (Israel et al. 1997; Dickinson et al. 1999).

In these human FcRn transgenic mice, intravenously delivered anti-ovalbumin (OVA) IgG reached the luminal fluid of the small intestine within a few hours, but such transport of anti-OVA IgG into small intestinal fluid was substantially lower in FcRn KO mice. Further, intragastrically administered IgG-OVA complexes were transported into the LP in human FcRn transgenic mice (but not in FcRn KO mice) and subsequently, OVA^+ DCs were detected in the MLNs. Notably, intravenous delivery of anti-OVA IgG and oral delivery of OVA lead to the expansion of OVA-specific $CD4^+$ T cells in the MLNs of human FcRn transgenic mice. A similar FcRn-mediated phenomenon was seen to occur in the nasal mucosa (Yoshida et al. 2004). These observations clearly establish two FcRn-dependent immune functions: (1) FcRn contributes to the humoral immune response at mucosal surfaces by transporting IgG from the basolateral side of the epithelial cell barrier to mucosal secretions on the apical side (site of antigen or pathogen encounter). This can explain how IgG reaches mucosal fluids of the nasal cavity ($\sim$300 μg/ml (Hanson and Brandzaeg 1980) and rectum ($\sim$800 μg/ml; (Kozlowski et al. 1997). (2) FcRn can mediate the delivery of antigen (in the form of immune complexes) from the mucosal surface to the corresponding MALT where T cells can be stimulated. These mucosal immune functions of FcRn have also been demonstrated in a mouse model of colitis induced by *Citrobacter rodentium* infection (Yoshida et al. 2006). Importantly, this study highlighted the importance

of FcRn-mediated delivery of anti-pathogen IgG to the intestinal lumen, demonstrating that this antibody can prevent the attachment of *C. rodentium* to epithelial cells, an essential step in the initiation/progression of infection by this pathogen (Bry and Brenner 2004).

Unlike most mucosal surfaces where IgA is found in higher concentrations than other immunoglobulin subclasses (Woof and Mestecky 2005), in the human female genital tract IgG is the predominant immunoglobulin subclass (Johansson and Lycke 2003). With respect to this, a recent study has shown that bidirectional transcytosis of IgG can be carried out by FcRn expressed by female genital tract epithelial cells of humans (in vitro) and the female genital tract of mice (in vivo) (Li et al. 2011). Also, this study showed that intraperitoneal-delivery of anti-herpes simplex virus-2 (HSV-2) IgG conferred higher protection against vaginal infection of HSV-2 in WT mice than in FcRn KO mice. In order to account for the higher rate of IgG catabolism in FcRn KO mice, a 1.4 to 2.8-fold greater amount of anti-HSV-2 IgG was used in the KO mice. The lower level of protection observed in FcRn KO mice was attributed to an absence of FcRn-mediated transfer of IgG to the genital mucosal surface. However, improved mouse models lacking FcRn expression specifically in epithelial cells (such a model would be expected to have normal IgG catabolism) would be valuable tools to determine the role of FcRn-mediated IgG transcytosis in vaginal infections.

Interestingly, another recent study has indicated that FcRn can aid the transfer of human immunodeficiency virus (HIV)-1 across the epithelial cell barrier of genital mucosa (Gupta et al. 2013). In this in vitro study, the acidic pH on the apical side (as is the case for cervicovaginal secretions/fluid) enhanced FcRn-mediated transcytosis of HIV-1 (in complex with anti-virus IgG) across the epithelial cell barrier, releasing viable virus toward the basolateral side. Although this FcRn-mediated process can enhance viral entry into the genital tissue, IgG-coated viral particles will be primarily taken up by FcγR-expressing cells (primarily professional antigen presenting cells (APCs)) in the MALT, where they could induce subsequent T cell activation. However, it remains to be determined whether FcRn can contribute to viral dissemination or clearance during this process.

3.3 Maintenance and Regulation of Renal Filtration

Blood is filtered in nephrons, the functional units of kidneys, to form urine. Nephrons are made up of different kinds of tubules, each performing a different function (Fig. 2a). The head portion of the nephron, called the glomerular capsule, performs filtration, and the following proximal convoluted tubule (PCT) performs reabsorption of salt, water, glucose, albumin, etc. Blood, destined for filtration flows into glomerular capillaries (enclosed by the glomerular capsule), where filtration occurs, and the resultant filtrate flows into the lumen of the glomerular capsule. For filtration to occur, the plasma has to pass through three layers of filters (Fig. 2b) with increasing size selectivity (Fox 2011). The first filtration barrier is

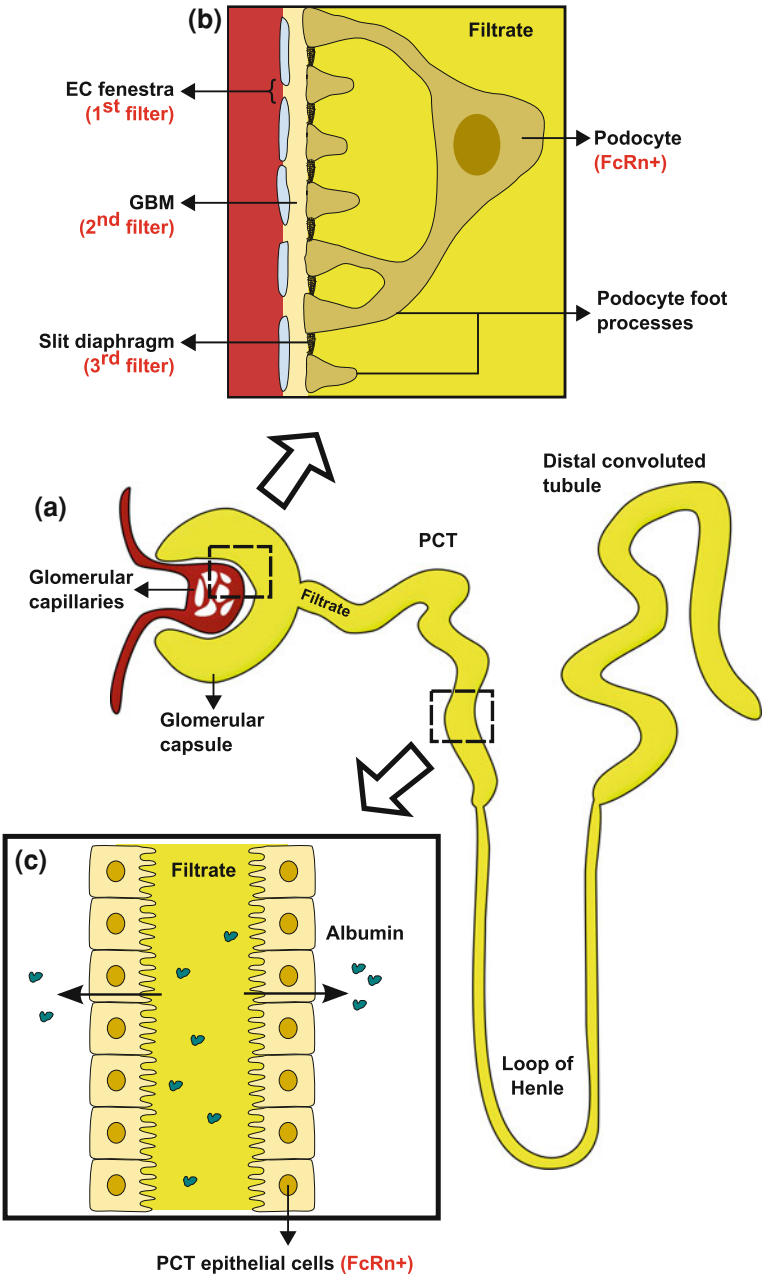


Fig. 2 FcRn-mediated functions in the kidney. **a** Schematic structure of nephron. **b** Plasma from glomerular capillaries passes through three different filters before flowing into the lumen of the glomerular capsule. During this process, IgG and albumin accumulate at the GBM or slit diaphragm and IgG (and possibly albumin) is cleared by FcRn in podocytes. **c** The filtrate that forms in the glomerular capsule contains significant amounts of albumin and flows into the lumen of the PCT, where FcRn in epithelial cells mediates transcytosis of albumin from the filtrate into the interstitial space in the kidney

formed by fenestrated ECs of glomerular capillaries. These fenestrae are large but charged, which may prevent bulky proteins from crossing the barrier. The second barrier is formed by the glomerular basement membrane (GBM), which has small and charged pores and lies immediately below the glomerular capillaries. Underneath the GBM lie specialized epithelial cells called podocytes, which have long extensions (foot processes) that wrap around the GBM. The foot processes interdigitate forming narrow slits, and are bridged by extracellular structures, referred to as slit diaphragms (Pavenstadt et al. 2003). The foot processes of podocytes along with associated slit diaphragms constitute the third filtration barrier. The pore size of the slit diaphragm is equal to or less than the size of albumin (Wartiovaara et al. 2004).

Considering the fact that ~180 L of glomerular filtrate is generated per day, it is very likely that albumin and IgG (which constitute ~80 % of serum proteins) accumulate at the GBM and/or slit diaphragm, resulting in the clogging of these biological filters. Hence, it has been hypothesized that a mechanism is in place to clear the filters of these accumulated proteins. In this context, a study has shown that FcRn in podocytes functions to remove accumulated IgG at the GBM (Akilesh et al. 2008). The role of renal FcRn in this process was confirmed primarily based on the observation that age-dependent glomerular accumulation of IgG is higher in FcRn KO mice by comparison with WT mice, despite the fact that serum IgG levels are significantly lower in FcRn KO mice. Based on the pattern of IgG accumulation observed in the glomerulus, podocytes were suggested to be the primary cells that clear the accumulated IgG. Also, the study shows that the protein-elimination function of podocytes is saturable. This finding might explain how immune complex deposition occurs in the kidneys of systemic lupus erythematosus (SLE) patients, which leads to nephritis.

The glomerular filtrate flowing into the PCT contains significant amounts of albumin, most of which is reclaimed by PCT epithelial cells (Russo et al. 2007). Importantly, these epithelial cells express high levels of FcRn (Akilesh et al. 2007). It has now become clear that FcRn in PCT cells is responsible for retrieval of albumin (Fig. 2c). The role of FcRn in this process is primarily based on two observations (Sarav et al. 2009). First, FcRn KO mice excrete more albumin in urine than WT mice. Second, in FcRn KO mice that were transplanted with one WT kidney (after nephrectomy of one native kidney) serum albumin levels increased, whereas WT mice transplanted with a KO kidney developed hypoalbuminemia. Also, based on the localization of exogenously added, labeled albumin in the kidneys of unmanipulated mice and transplant chimeras, it was suggested that albumin is reclaimed by the epithelial cells of the PCT. In this context, FcRn performs bidirectional transcytosis in human proximal tubular epithelial cells (Kobayashi et al. 2002). Hence, it is logical to assume that albumin reclaimed by the cells of the PCT would be transcytosed into the interstitium of kidneys, followed by drainage of albumin into the lymphatics and entry into the circulation. In addition, in the same study (Sarav et al. 2009), experiments using kidney transplant chimeras showed that renal FcRn aids elimination of IgG from plasma into urine. However, the mechanism through which IgG elimination occurs is unclear.

3.4 Possible Role in Clearing IgG from Immune-Privileged Sites

Some sites in the body are considered immune-privileged because immune surveillance at these sites is limited or absent. These sites include the central nervous system (CNS), eye, fetus/placenta, and testis. Complex blood–tissue barriers exist at these sites that limit or restrict the entry of immune cells and molecules from the blood into the tissue. In the CNS, one such barrier is the blood–brain barrier (BBB), which is formed by ECs that line the cerebral microvessels, basal lamina, and astrocytic endfeet (Abbott et al. 2006). Adjacent ECs of the BBB are connected through tight junctions, which only allow the passage of small hydrophobic molecules. IgG is large and hydrophilic in nature and hence its entry through the BBB is highly restricted. The concentration of IgG in a tissue relative to plasma is 1:500 for brain and 1:10 for most nonleaky tissues (Wang et al. 2008).

FcRn is expressed by BBB ECs in both mice (Akilesh et al. 2007) and rats (Schlachetzki et al. 2002). The presence or absence of FcRn in human BBB ECs has not been reported. However, we have observed FcRn expression in the human BBB endothelial cell line hCMEC/D3 (Sripad Ram, Raimund Ober, E. Sally Ward, unpublished). In rats, one study has shown that intracerebrally injected IgG is rapidly effluxed out of the CNS into the blood (Zhang and Pardridge 2001). It was also shown that this efflux or reverse transcytosis of labeled IgG can be blocked by intracerebral injection of excess unlabeled IgG, indicating a role for an Fc receptor in this process. Another recent study in rats has confirmed that FcRn mediates efflux of IgG from brain to blood (Cooper et al. 2013). In this study, 24 h following intracranial injection of two mutant IgGs, N434A (similar to WT IgG except that it has increased binding to FcRn at pH 6) and H435A (has negligible binding to FcRn at pH 6 and 7.4), N434A levels in the brain decreased, whereas H435A levels remained almost unchanged in comparison to their levels at 5 min postinjection.

In mice, data exist to both support (Deane et al. 2005) and refute (Garg and Balthasar 2009; Abuqayyas and Balthasar 2013) the role of FcRn in mediating IgG efflux from brain. In one study that supports such a role, centrally delivered anti-Aβ IgG and anti-Aβ IgG-Aβ complexes were transported out of the brain, and this was blocked by simultaneous delivery of anti-FcRn IgG or the use of FcRn KO mice (Deane et al. 2005). By contrast, a study has shown that the brain to blood exposure ratio of intravenously delivered IgG is similar in WT and FcRn KO mice (Abuqayyas and Balthasar 2013). Additional work is required to unambiguously determine the role of FcRn in IgG transport across the BBB. Further, FcRn is expressed by (ECs) of retinal vasculature, and may play a role in excluding IgG from the eye across the blood–retinal barrier (Powner et al. 2014).

3.5 Role in Antigen Presentation

Professional APCs (DCs, macrophages, and B cells) can present antigens to $CD8^+$ and $CD4^+$ T cells in the context of MHC class I and MHC class II, respectively. In general, intracellular antigens are proteasomally processed and presented on MHC class I molecules, and extracellularly derived antigens are processed in lysosomes and presented on MHC class II molecules (Neefjes et al. 2011). Under some circumstances, extracellular antigens are processed by proteasomes or within phagosomes and presented on MHC class I molecules. This type of antigen presentation can only be carried out by DCs (Kurts et al. 2010) and possibly macrophages (Houde et al. 2003; Asano et al. 2011) and is referred to as cross-presentation.

Importantly, all professional APCs in both mice and humans express FcRn (Zhu et al. 2001; Perez-Montoyo et al. 2009; van Bilsen et al. 2010). Professional APCs, except B cells, also express activating FcγRs, which in the presence of IgG-based immune complexes (ICs) mediate activation of APCs (Nimmerjahn and Ravetch 2008; Hogarth and Pietersz 2012; Guilliams et al. 2014). Further, antigens in the form of ICs are more efficiently internalized (through activating FcγRs) by APCs than soluble antigens and hence lead to more efficient T cell activation. With respect to this, a role similar to that played by FcγRs has been shown to be performed by FcRn (Qiao et al. 2008; Kobayashi et al. 2009). In one such study (Qiao et al. 2008), multimeric OVA ICs containing either WT IgG or a mutated IgG (IHH, no binding to FcRn at physiological and acidic pH, but no change in binding to FcγRs) were used in mouse $CD4^+$ T cell proliferation assays in the presence of either WT or FcRn KO DCs. In these assays, the proliferation of OVA-specific $CD4^+$ T cells decreased when DCs lacked FcRn or when ICs comprising IHH antibodies were used by comparison with that observed using WT DCs or ICs containing WT antibodies, respectively. These observations indicate a role for FcRn in IC-mediated antigen presentation. Similar observations were made using human cells, and also when in vitro-loaded (with ICs containing WT or IHH antibodies) WT or FcRn KO DCs were injected into WT mice. Based on the observed trafficking patterns of ICs and FcRn, it was demonstrated that FcRn rapidly transports WT ICs to lysosomes, leading to enhanced antigen presentation and T cell proliferation. In the assays described above, it is possible that some ICs would presumably cross-link FcγRs, leading to DC activation and cytokine secretion, which in turn would upregulate MHC class II and the associated invariant chain (Simmons et al. 2012; Guilliams et al. 2014). Invariant chain has been shown to also associate with FcRn and target it to late endosomes or lysosomal compartments (Ye et al. 2008). Hence, the invariant chain might have a role to play in diverting FcRn-bound ICs to lysosomes in APCs.

Recently, FcRn has also been shown to play a role in the cross-presentation of IC-derived antigens (Baker et al. 2011). In this study, mouse DCs pulsed with ICs comprising WT or IHH antibodies complexed with OVA (similar to those described above) were injected into WT mice that had also received labeled

OVA-specific $CD8^+$ T cells. The antigen in this case is exogenous and hence $CD8^+$ T cells will only be stimulated if the antigen is cross-presented. The proliferation of $CD8^+$ T cells was found to be many fold higher when WT IgG ICs were used in comparison to the proliferation observed with IHH IgG ICs, highlighting the importance of FcRn in IC-mediated cross-presentation. Interestingly, only $CD8^-CD11b^+$ DCs, but not $CD8^+CD11b^-$ DCs (shown to be the major mediators of cross-presentation of soluble and tumor antigens (Hildner et al. 2008)) were able to efficiently cross-present IC-derived antigen to $CD8^+$ T cells. Using IgG-opsonized, OVA-containing beads (IC-beads), it was also shown that the $FcRn^+$ phagosomes formed upon WT IgG IC-bead internalization by DCs had many features that facilitated cross-presentation by comparison with phagosomes formed by IHH IgG IC-beads. The features included lower pH, persistence of antigen in the phagosomes and enrichment of components of the cross-presentation machinery such as the transporter associated with antigen processing 1 (TAP1) and MHC class I. Finally, the authors suggest that ICs are internalized by DCs in an FcγR-dependent fashion, followed by the transfer of ICs from FcγRs to FcRn in acidic, endosomal compartments followed by cross-presentation. Taken together, FcRn is indicated to be important for the presentation of IC-derived antigen to both $CD4^+$ and $CD8^+$ T cells.

4 FcRn-Targeted Therapies

Monoclonal antibodies (mAbs), due to their specificity and long half-lives, are considered to be one of the most effective and safe therapies for many diseases. Currently, there are almost 350 mAbs that are either in early development or Food and Drug Administration (FDA)-approved for the treatment of inflammatory disorders, cancers, infectious diseases, and solid organ transplant rejection (Mahmud et al. 2010; Reichert 2013). As mentioned in the previous sections, FcRn functions to regulate the levels and many functional activities of IgGs. As a result, many therapies (mostly IgG-based) have been developed that target FcRn, and have shown promise in treating animal models of autoimmune diseases and cancer. FcRn-targeting therapies can be broadly classified into two distinct categories: (1) mAbs with extended half-life, which will have applications in any disease where mAbs can be used therapeutically and (2) agents that deplete endogenous antibodies, which will have applications in antibody-mediated pathologies and other situations in which antibody clearance is indicated.

During the last decade or so, a significant component of Fc-engineering efforts has focused on developing IgG mutants that vary in their binding to FcRn and have enhanced in vivo half-life, with an aim to boost the efficacy and/or reduce the dosing frequency of IgG-based therapies. The first report demonstrating that Fc engineering can be used to generate IgGs with increased in vivo persistence came from a study in which a mutated mouse IgG1 Fc (T252L/T254S/T256F) was produced using random mutagenesis and phage display. This mutated Fc fragment

has increased binding to mouse FcRn at acidic pH, but negligible binding at physiological pH, resulting in an extended half-life in mice by comparison with WT mouse IgG1-derived Fc (Ghetie et al. 1997). Subsequently, many engineered human IgGs have been developed with increased in vivo half-life, as validated in nonhuman primates (Hinton et al. 2004, 2006; Dall'Acqua et al. 2006; Yeung et al. 2009). Among these mutants, YTE (human IgG1—M252Y/S254T/T256E), exhibits ~4 fold increase in half-life relative to WT human IgG1 in nonhuman primates, which is the longest half-life extension reported to date (Dall'Acqua et al. 2006). Another mutant, HN (human IgG1—H433K/N434F), with increased pH-dependent binding to (human) FcRn has been shown to be more active than WT human IgG1 in FcRn-mediated transcytosis across the ex vivo human placenta (Vaccaro et al. 2006). Also, a recent study has shown that IgG with enhanced half-life has increased antitumor activity than WT IgG in tumor xenograft studies in mice (Zalevsky et al. 2010). Finally, based on the in vivo half-lives of various IgG mutants that were Fc-engineered with respect to their FcRn binding, it is clear that while an increase in IgG affinity toward FcRn at acidic pH is important, retention of low affinity at physiological pH is equally important to allow exocytic release from cells (Prabhat et al. 2007) and consequent persistence of an IgG (Dall'Acqua et al. 2002; Vaccaro et al. 2006; Yeung et al. 2009).

Autoantibodies lead to pathology in autoimmune diseases such as SLE, neuromyelitis optica, myasthenia gravis, and multiple sclerosis (Sherer et al. 2004; Conti-Fine et al. 2006; Jarius and Wildemann 2010; Popescu and Lucchinetti 2012). Also, antibodies can mediate rejection of organ allografts (Colvin and Smith 2005). Currently, approved treatments for depleting antibodies in such diseases, in a nonspecific manner, include plasmapheresis and high dose intravenous immunoglobulin (IVIG) (Orange et al. 2006; Winters 2012). Both these treatment modalities may lead to side effects or complications, but more importantly, the cost of these treatments is high (Heatwole et al. 2011; Winters et al. 2011). Hence, efforts have been undertaken to develop alternatives. IVIG lowers endogenous or pathogenic antibody levels only when used in high doses, which is essential for saturating FcRn (Hansen and Balthasar 2002; Li et al. 2005). Alternatively, FcRn can be saturated or blocked using low doses of agents that bind to FcRn with very high affinity. In the case of half-life extension, retention of low affinity towards FcRn at physiological pH limits the extent to which the affinity at acidic pH can be increased (Ward and Ober 2009; Yeung et al. 2009). Such a limitation is not relevant to the generation of effective FcRn blockers, and in fact, high affinity binding to FcRn at physiological pH is desirable in this case since it will enable the engineered antibody to be efficiently endocytosed by FcRn-mediated uptake into cells (Vaccaro et al. 2005; Prabhat et al. 2007). This in turn will result in increased competition with endogenous antibodies with respect to FcRn binding. One such Fc-engineered antibody is MST-HN (M252Y/S254T/T256E/H433K/N434F). Antibodies of this class have been shown to rapidly decrease endogenous antibody levels in mice and are called Abdegs (for antibodies that enhance IgG degradation) (Vaccaro et al. 2005). In a serum transfer model of arthritis in mice, Abdegs were able to reduce swelling and inflammation in the

joints in both therapeutic and prophylactic disease settings (Patel et al. 2011). Importantly, by comparison with Abdegs, 25–50 times higher amounts of IVIG were required to achieve similar therapeutic effects. Recently, Abdegs were also shown to ameliorate disease in a passive model of antibody-mediated experimental autoimmune encephalomyelitis by mediating both the rapid clearance and reducing the accumulation of encephalitogenic antibodies in the CNS (Challa et al. 2013).

Antibodies that bind to FcRn through their variable domains have also been developed that can block FcRn-mediated recycling of IgGs. Anti-rat (4C9) and anti-human (DVN24) antibodies specific for FcRn were shown to reduce the levels of exogenously administered tracer antibody in rats and human FcRn transgenic mice, respectively (Getman and Balthasar 2005; Christianson et al. 2012). Similarly, another anti-rat FcRn IgG, 1G3, was shown to reduce pathogenic antibody levels and disease symptoms in both passive and active models of myasthenia gravis in rats (Liu et al. 2007a). On the downside, antibody-based, FcRn blockers have short in vivo half-lives due to strong binding to FcRn at physiological pH, which results in increased accumulation in FcRn-expressing cells and reduced exocytic release (Dall'Acqua et al. 2002; Vaccaro et al. 2006; Liu et al. 2007a; Perez-Montoyo et al. 2009). Peptide-based FcRn blockers have also been developed. In particular, SYN1436, a dimer of an FcRn-binding peptide was able to significantly reduce the levels of exogenously added human IgG in human FcRn transgenic mice and endogenous antibody in nonhuman primates (Mezo et al. 2008). These peptide-based agents would be expected to exhibit an in vivo half-life that is lower than that of antibody-based FcRn blockers, primarily due to renal-mediated clearance. As a result, PEGylation has been employed to improve the in vivo pharmacokinetics and efficacy of such peptide-based FcRn blockers (Mezo et al. 2011).

5 Concluding Remarks

It is clear that in addition to playing a role in the homeostasis of IgG and albumin, FcRn mediates IgG transport to inaccessible sites (fetus, neonate, or mucosal surfaces) and possibly excludes IgG from immune-privileged sites. This knowledge offers opportunities for engineering antibodies for modulation of the intrinsic half-life and transport of the antibody itself or, through FcRn inhibition, altering the dynamics and levels of endogenous antibodies. Further, FcRn regulates kidney filtration of its ligands and contributes to antigen presentation to both $CD4^+$ and $CD8^+$ T cells. Although functions for FcRn at multiple different sites have been identified, the role of FcRn in other specialized cells such as hepatocytes and keratinocytes remains poorly defined.

References

Abbott NJ, Ronnback L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 7(1):41–53

Abuqayyas L, Balthasar JP (2013) Investigation of the role of FcγR and FcRn in mAb distribution to the brain. Mol Pharm 10(5):1505–1513

Agola JO, Jim PA, Ward HH et al (2011) Rab GTPases as regulators of endocytosis, targets of disease and therapeutic opportunities. Clin Genet 80(4):305–318

Akilesh S, Christianson GJ, Roopenian DC et al (2007) Neonatal FcR expression in bone marrow-derived cells functions to protect serum IgG from catabolism. J Immunol 179(7):4580–4588

Akilesh S, Huber TB, Wu H et al (2008) Podocytes use FcRn to clear IgG from the glomerular basement membrane. Proc Natl Acad Sci USA 105(3):967–972

Andersen JT, Dee Qian J, Sandlie I (2006) The conserved histidine 166 residue of the human neonatal Fc receptor heavy chain is critical for the pH-dependent binding to albumin. Eur J Immunol 36(11):3044–3051

Andersen JT, Foss S, Kenanova VE et al (2012) Anti-carcinoembryonic antigen single-chain variable fragment antibody variants bind mouse and human neonatal Fc receptor with different affinities that reveal distinct cross-species differences in serum half-life. J Biol Chem 287(27):22927–22937

Antohe F, Radulescu L, Gafencu A et al (2001) Expression of functionally active FcRn and the differentiated bidirectional transport of IgG in human placental endothelial cells. Hum Immunol 62(2):93–105

Appleby P, Catty D (1983) Transmission of immunoglobulin to foetal and neonatal mice. J Reprod Immunol 5(4):203–213

Asano K, Nabeyama A, Miyake Y et al (2011) CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. Immunity 34(1):85–95

Baker K, Qiao SW, Kuo TT et al (2011) Neonatal Fc receptor for IgG (FcRn) regulates cross-presentation of IgG immune complexes by CD8-CD11b+ dendritic cells. Proc Natl Acad Sci USA 108(24):9927–9932

Borvak J, Richardson J, Medesan C et al (1998) Functional expression of the MHC class I-related receptor, FcRn, in endothelial cells of mice. Int Immunol 10(9):1289–1298

Brambell FWR (1970) The transmission of passive immunity from mother to young. North Holland Publ Corp, Amsterdam

Bry L, Brenner MB (2004) Critical role of T cell-dependent serum antibody, but not the gut-associated lymphoid tissue, for surviving acute mucosal infection with Citrobacter rodentium, an attaching and effacing pathogen. J Immunol 172(1):433–441

Cauza K, Hinterhuber G, Ngelmaier-Hovorka R et al (2005) Expression of FcRn, the MHC class I-related receptor for IgG, in human keratinocytes. J Invest Dermatol 124(1):132–139

Challa DK, Bussmeyer U, Khan T et al (2013) Autoantibody depletion ameliorates disease in murine experimental autoimmune encephalomyelitis. MAbs 5(5):655–659

Chan AC, Carter PJ (2010) Therapeutic antibodies for autoimmunity and inflammation. Nat Rev Immunol 10(5):301–316

Chaudhury C, Mehnaz S, Robinson JM et al (2003) The major histocompatibility complex-related Fc receptor for IgG (FcRn) binds albumin and prolongs its lifespan. J Exp Med 197(3):315–322

Christianson GJ, Sun VZ, Akilesh S et al (2012) Monoclonal antibodies directed against human FcRn and their applications. MAbs 4(2)

Cianga P, Cianga C, Cozma L et al (2003) The MHC class I related Fc receptor, FcRn, is expressed in the epithelial cells of the human mammary gland. Hum Immunol 64(12):1152–1159

Claypool SM, Dickinson BL, Wagner JS et al (2004) Bidirectional transepithelial IgG transport by a strongly polarized basolateral membrane Fc-γ receptor. Mol Biol Cell 15:1746–1759

Claypool SM, Dickinson BL, Yoshida M et al (2002) Functional reconstitution of human FcRn in Madin-Darby canine kidney cells requires co-expressed human beta 2-microglobulin. J Biol Chem 277(31):28038–28050
Colvin RB, Smith RN (2005) Antibody-mediated organ-allograft rejection. Nat Rev Immunol 5(10):807–817
Conti-Fine BM, Milani M, Kaminski HJ (2006) Myasthenia gravis: past, present, and future. J Clin Invest 116(11):2843–2854
Cooper PR, Ciambrone GJ, Kliwinski CM et al (2013) Efflux of monoclonal antibodies from rat brain by neonatal Fc receptor, FcRn. Brain Res 1534:13–21
Dall'Acqua W, Woods RM, Ward ES et al (2002) Increasing the affinity of a human IgG1 to the neonatal Fc receptor: biological consequences. J Immunol 169(9):5171–5180
Dall'Acqua WF, Kiener PA, Wu H (2006) Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). J Biol Chem 281(33):23514–23524
Dati F, Schumann G, Thomas L et al (1996) Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP Reference Material (CRM 470). International federation of clinical chemistry. Community bureau of reference of the commission of the European communities. College of American pathologists. Eur J Clin Chem Clin Biochem 34(6):517–520
Deane R, Sagare A, Hamm K et al (2005) IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor. J Neurosci 25(50):11495–11503
Dickinson BL, Badizadegan K, Wu Z et al (1999) Bidirectional FcRn-dependent IgG transport in a polarized human intestinal epithelial cell line. J Clin Invest 104(7):903–911
Dickinson BL, Claypool SM, D'Angelo JA et al (2008) Ca2 + -dependent calmodulin binding to FcRn affects immunoglobulin G transport in the transcytotic pathway. Mol Biol Cell 19(1):414–423
Firan M, Bawdon R, Radu C et al (2001) The MHC class I related receptor, FcRn, plays an essential role in the maternofetal transfer of gammaglobulin in humans. Int Immunol 13:993–1002
Fox SI (2011) Human physiology, 12th edn. McGraw-Hill, New York, pp 577–585
Gan Z, Ram S, Ober RJ et al (2013) Using multifocal plane microscopy to reveal novel trafficking processes in the recycling pathway. J Cell Sci 126(Pt 5):1176–1188
Gan Z, Ram S, Vaccaro C et al (2009) Analyses of the recycling receptor, FcRn, in live cells reveal novel pathways for lysosomal delivery. Traffic 10(5):600–614
Garg A, Balthasar JP (2009) Investigation of the influence of FcRn on the distribution of IgG to the brain. AAPS J 11(3):553–557
Getman KE, Balthasar JP (2005) Pharmacokinetic effects of 4C9, an anti-FcRn antibody, in rats: implications for the use of FcRn inhibitors for the treatment of humoral autoimmune and alloimmune conditions. J Pharm Sci 94(4):718–729
Ghetie V, Hubbard JG, Kim JK et al (1996) Abnormally short serum half-lives of IgG in beta 2-microglobulin-deficient mice. Eur J Immunol 26(3):690–696
Ghetie V, Popov S, Borvak J et al (1997) Increasing the serum persistence of an IgG fragment by random mutagenesis. Nat Biotechnol 15(7):637–640
Guilliams M, Bruhns P, Saeys Y et al (2014) The function of Fcγ receptors in dendritic cells and macrophages. Nat Rev Immunol 14(2):94–108
Gupta S, Gach JS, Becerra JC et al (2013) The neonatal Fc receptor (FcRn) enhances human immunodeficiency virus type 1 (HIV-1) transcytosis across epithelial cells. PLoS Pathog 9(11):e1003776
Hammer JA 3rd, Sellers JR (2012) Walking to work: roles for class V myosins as cargo transporters. Nat Rev Mol Cell Biol 13(1):13–26
Hansen RJ, Balthasar JP (2002) Effects of intravenous immunoglobulin on platelet count and antiplatelet antibody disposition in a rat model of immune thrombocytopenia. Blood 100(6):2087–2093

Hanson LA, Brandzaeg P (1980) The mucosal defense system. In: Stiehm ER, Fulginity VA (eds) Immunologic disorders in infants and children. 2nd edn. Saunder WB, Philadelphia, pp 137–164

Haymann JP, Levraud JP, Bouet S et al (2000) Characterization and localization of the neonatal Fc receptor in adult human kidney. J Am Soc Nephrol 11(4):632–639

Heatwole C, Johnson N, Holloway R et al (2011) Plasma exchange versus intravenous immunoglobulin for myasthenia gravis crisis: an acute hospital cost comparison study. J Clin Neuromuscul Dis 13(2):85–94

Hildner K, Edelson BT, Purtha WE et al (2008) Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. Science 322(5904):1097–1100

Hinton PR, Johlfs MG, Xiong JM et al (2004) Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem 279(8):6213–6216

Hinton PR, Xiong JM, Johlfs MG et al (2006) An engineered human IgG1 antibody with longer serum half-life. J Immunol 176(1):346–356

Hogarth PM, Pietersz GA (2012) Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. Nat Rev Drug Discov 11(4):311–331

Houde M, Bertholet S, Gagnon E et al (2003) Phagosomes are competent organelles for antigen cross-presentation. Nature 425(6956):402–406

Israel EJ, Taylor S, Wu Z et al (1997) Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. Immunology 92(1):69–74

Israel EJ, Wilsker DF, Hayes KC et al (1996) Increased clearance of IgG in mice that lack beta 2-microglobulin: possible protective role of FcRn. Immunology 89(4):573–578

Jahn R, Lang T, Sudhof TC (2003) Membrane fusion. Cell 112(4):519–533

Jarius S, Wildemann B (2010) AQP4 antibodies in neuromyelitis optica: diagnostic and pathogenetic relevance. Nat Rev Neurol 6(7):383–392

Johansson M, Lycke NY (2003) Immunology of the human genital tract. Curr Opin Infect Dis 16(1):43–49

Jones EA, Waldmann TA (1972) The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. J Clin Invest 51(11):2916–2927

Junghans RP, Anderson CL (1996) The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. Proc Natl Acad Sci USA 93(11):5512–5516

Kim H, Fariss RN, Zhang C et al (2008) Mapping of the neonatal Fc receptor in the rodent eye. Invest Ophthalmol Vis Sci 49(5):2025–2029

Kim JK, Firan M, Radu CG et al (1999) Mapping the site on human IgG for binding of the MHC class I-related receptor. FcRn. Eur J Immunol 29(9):2819–2825

Kobayashi K, Qiao SW, Yoshida M et al (2009) An FcRn-dependent role for anti-flagellin immunoglobulin G in pathogenesis of colitis in mice. Gastroenterology 137(5):1746–1756 e1741

Kobayashi N, Suzuki Y, Tsuge T et al (2002) FcRn-mediated transcytosis of immunoglobulin G in human renal proximal tubular epithelial cells. Am J Physiol Renal Physiol 282(2):F358–F365

Kozlowski PA, Cu-Uvin S, Neutra MR et al (1997) Comparison of the oral, rectal, and vaginal immunization routes for induction of antibodies in rectal and genital tract secretions of women. Infect Immun 65(4):1387–1394

Kuo TT, Baker K, Yoshida M et al (2010) Neonatal Fc receptor: from immunity to therapeutics. J Clin Immunol 30(6):777–789

Kuo TT, de Muinck EJ, Claypool SM et al (2009) N-Glycan moieties in neonatal Fc receptor determine steady-state membrane distribution and directional transport of IgG. J Biol Chem 284(13):8292–8300

Kurts C, Robinson BW, Knolle PA (2010) Cross-priming in health and disease. Nat Rev Immunol 10(6):403–414

Leach JL, Sedmak DD, Osborne JM et al (1996) Isolation from human placenta of the IgG transporter, FcRn, and localization to the syncytiotrophoblast: implications for maternal- fetal antibody transport. J Immunol 157(8):3317–3322
Li N, Zhao M, Hilario-Vargas J et al (2005) Complete FcRn dependence for intravenous Ig therapy in autoimmune skin blistering diseases. J Clin Invest 115(12):3440–3450
Li Z, Palaniyandi S, Zeng R et al (2011) Transfer of IgG in the female genital tract by MHC class I-related neonatal Fc receptor (FcRn) confers protective immunity to vaginal infection. Proc Natl Acad Sci USA 108(11):4388–4393
Liu L, Garcia AM, Santoro H et al (2007a) Amelioration of experimental autoimmune myasthenia gravis in rats by neonatal FcR blockade. J Immunol 178(8):5390–5398
Liu X, Ye L, Bai Y et al (2008) Activation of the JAK/STAT-1 signaling pathway by IFN-γ can down-regulate functional expression of the MHC class I-related neonatal Fc receptor for IgG. J Immunol 181(1):449–463
Liu X, Ye L, Christianson GJ et al (2007b) NF-kappaB signaling regulates functional expression of the MHC class I-related neonatal Fc receptor for IgG via intronic binding sequences. J Immunol 179(5):2999–3011
Mahmud N, Klipa D, Ahsan N (2010) Antibody immunosuppressive therapy in solid-organ transplant: Part I. MAbs 2(2):148–156
Martin MG, Wu SV, Walsh JH (1997) Ontogenetic development and distribution of antibody transport and Fc receptor mRNA expression in rat intestine. Dig Dis Sci 42(5):1062–1069
Martin WL, West AP Jr, Gan L et al (2001) Crystal structure at 2.8 Å of an FcRn/heterodimeric Fc complex: mechanism of pH dependent binding. Mol Cell 7(4):867–877
McGhee JR, Fujihashi K (2012) Inside the mucosal immune system. PLoS Biol 10(9):e1001397
Medesan C, Matesoi D, Radu C et al (1997) Delineation of the amino acid residues involved in transcytosis and catabolism of mouse IgG1. J Immunol 158(5):2211–2217
Medesan C, Radu C, Kim JK et al (1996) Localization of the site of the IgG molecule that regulates maternofetal transmission in mice. Eur J Immunol 26(10):2533–2536
Mezo AR, Low SC, Hoehn T et al (2011) PEGylation enhances the therapeutic potential of peptide antagonists of the neonatal Fc receptor FcRn. Bioorg Med Chem Lett 21(21): 6332–6335
Mezo AR, McDonnell KA, Hehir CA et al (2008) Reduction of IgG in nonhuman primates by a peptide antagonist of the neonatal Fc receptor FcRn. Proc Natl Acad Sci USA 105(7):2337–2342
Miaczynska M, Zerial M (2002) Mosaic organization of the endocytic pathway. Exp Cell Res 272(1):8–14
Naparstek Y, Plotz PH (1993) The role of autoantibodies in autoimmune disease. Annu Rev Immunol 11:79–104
Neefjes J, Jongsma ML, Paul P et al (2011) Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol 11(12):823–836
Newton EE, Wu Z, Simister NE (2005) Characterization of basolateral-targeting signals in the neonatal Fc receptor. J Cell Sci 118(Pt 11):2461–2469
Nimmerjahn F, Ravetch JV (2008) Fcγ receptors as regulators of immune responses. Nat Rev Immunol 8(1):34–47
Ober RJ, Martinez C, Lai X et al (2004a) Exocytosis of IgG as mediated by the receptor, FcRn: An analysis at the single-molecule level. Proc Natl Acad Sci USA 101:11076–11081
Ober RJ, Martinez C, Vaccaro C et al (2004b) Visualizing the site and dynamics of IgG salvage by the MHC class I-related receptor. FcRn. J Immunol 172(4):2021–2029
Oganesyan V, Damschroder MM, Cook KE et al (2014) Structural insights Into neonatal Fc receptor-based recycling mechanisms. J Biol Chem 289(11):7812–7824
Orange JS, Hossny EM, Weiler CR et al (2006) Use of intravenous immunoglobulin in human disease: a review of evidence by members of the primary immunodeficiency committee of the American academy of allergy, asthma and immunology. J Allergy Clin Immunol 117(4 Suppl):S525–S553

Patel DA, Puig-Canto A, Challa DK et al (2011) Neonatal Fc receptor blockade by Fc engineering ameliorates arthritis in a murine model. J Immunol 187(2):1015–1022
Pavenstadt H, Kriz W, Kretzler M (2003) Cell biology of the glomerular podocyte. Physiol Rev 83(1):253–307
Perez-Montoyo H, Vaccaro C, Hafner M et al (2009) Conditional deletion of the MHC Class I-related receptor, FcRn, reveals the sites of IgG homeostasis in mice. Proc Natl Acad Sci USA 106(8):2788–2793
Popescu BF, Lucchinetti CF (2012) Pathology of demyelinating diseases. Annu Rev Pathol 7:185–217
Popov S, Hubbard JG, Kim J et al (1996) The stoichiometry and affinity of the interaction of murine Fc fragments with the MHC class I-related receptor. FcRn. Mol Immunol 33(6):521–530
Powner MB, McKenzie JA, Christianson GJ et al (2014) Expression of neonatal Fc receptor in the eye. Invest Ophthalmol Vis Sci 55(3):1607–1615
Prabhat P, Gan Z, Chao J et al (2007) Elucidation of intracellular recycling pathways leading to exocytosis of the Fc receptor, FcRn, by using multifocal plane microscopy. Proc Natl Acad Sci USA 104(14):5889–5894
Qiao SW, Kobayashi K, Johansen FE et al (2008) Dependence of antibody-mediated presentation of antigen on FcRn. Proc Natl Acad Sci USA 105(27):9337–9342
Raghavan M, Bonagura VR, Morrison SL et al (1995) Analysis of the pH dependence of the neonatal Fc receptor/immunoglobulin G interaction using antibody and receptor variants. Biochemistry 34(45):14649–14657
Raghavan M, Gastinel LN, Bjorkman PJ (1993) The class I major histocompatibility complex-related Fc receptor shows pH-dependent stability differences correlating with immunoglobulin binding and release. Biochemistry 32(33):8654–8660
Reichert JM (2013) Which are the antibodies to watch in 2013? MAbs 5(1):1–4
Rodewald R, Abrahamson DR (1982) Receptor-mediated transport of IgG across the intestinal epithelium of the neonatal rat. Ciba Found Symp 92:209–232
Rodewald R, Kraehenbuhl JP (1984) Receptor-mediated transport of IgG. J Cell Biol 99(1 Pt 2):159s–164s
Roopenian DC, Akilesh S (2007) FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 7(9):715–725
Roopenian DC, Christianson GJ, Sproule TJ et al (2003) The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. J Immunol 170(7):3528–3533
Russo LM, Sandoval RM, McKee M et al (2007) The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells: retrieval is disrupted in nephrotic states. Kidney Int 71(6):504–513
Salimonu LS, Ladipo OA, Adeniran SO et al (1978) Serum immunoglobulin levels in normal, premature and postmature newborns and their mothers. Int J Gynaecol Obstet 16(2):119–123
Sanchez LM, Penny DM, Bjorkman PJ (1999) Stoichiometry of the interaction between the major histocompatibility complex-related Fc receptor and its Fc ligand. Biochemistry 38(29):9471–9476
Sarav M, Wang Y, Hack BK et al (2009) Renal FcRn reclaims albumin but facilitates elimination of IgG. J Am Soc Nephrol 20(9):1941–1952
Schlachetzki F, Zhu C, Pardridge WM (2002) Expression of the neonatal Fc receptor (FcRn) at the blood-brain barrier. J Neurochem 81(1):203–206
Schuck P, Radu CG, Ward ES (1999) Sedimentation equilibrium analysis of recombinant mouse FcRn with murine IgG1. Mol Immunol 36(15–16):1117–1125
Schulz O, Jaensson E, Persson EK et al (2009) Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. J Exp Med 206(13):3101–3114
Scott AM, Wolchok JD, Old LJ (2012) Antibody therapy of cancer. Nat Rev Cancer 12(4):278–287

Sherer Y, Gorstein A, Fritzler MJ et al (2004) Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients. Semin Arthritis Rheum 34(2):501–537

Shields RL, Namenuk AK, Hong K et al (2001) High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. J Biol Chem 276(9):6591–6604

Simister NE, Mostov KE (1989) An Fc receptor structurally related to MHC class I antigens. Nature 337(6203):184–187

Simister NE, Story CM, Chen HL et al (1996) An IgG-transporting Fc receptor expressed in the syncytiotrophoblast of human placenta. Eur J Immunol 26(7):1527–1531

Simmons DP, Wearsch PA, Canaday DH et al (2012) Type I IFN drives a distinctive dendritic cell maturation phenotype that allows continued class II MHC synthesis and antigen processing. J Immunol 188(7):3116–3126

Somsel RJ, Wandinger-Ness A (2000) Rab GTPases coordinate endocytosis. J Cell Sci 113(Pt 2):183–192

Spiegelberg HL, Fishkin BG (1972) The catabolism of human G immunoglobulins of different heavy chain subclasses. 3. The catabolism of heavy chain disease proteins and of Fc fragments of myeloma proteins. Clin Exp Immunol 10(4):599–607

Spiekermann GM, Finn PW, Ward ES et al (2002) Receptor-mediated immunoglobulin G transport across mucosal barriers in adult life: functional expression of FcRn in the mammalian lung. J Exp Med 196(3):303–310

Stenmark H (2009) Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol 10(8):513–525

Tesar DB, Tiangco NE, Bjorkman PJ (2006) Ligand valency affects transcytosis, recycling and intracellular trafficking mediated by the neonatal Fc receptor. Traffic 7(9):1127–1142

Tzaban S, Massol RH, Yen E et al (2009) The recycling and transcytotic pathways for IgG transport by FcRn are distinct and display an inherent polarity. J Cell Biol 185(4):673–684

Vaccaro C, Bawdon R, Wanjie S et al (2006) Divergent activities of an engineered antibody in murine and human systems have implications for therapeutic antibodies. Proc Natl Acad Sci USA 103(49):18709–18714

Vaccaro C, Zhou J, Ober RJ et al (2005) Engineering the Fc region of immunoglobulin G to modulate in vivo antibody levels. Nat Biotechnol 23(10):1283–1288

van Bilsen K, Bastiaans J, Dik WA et al (2010) The neonatal Fc receptor is expressed by human lymphocytes. J Transl Med 8(Suppl 1):P1

Vieira P, Rajewsky K (1988) The half-lives of serum immunoglobulins in adult mice. Eur J Immunol 18(2):313–316

Wang W, Wang EQ, Balthasar JP (2008) Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 84(5):548–558

Wani MA, Haynes LD, Kim J et al (2006) Familial hypercatabolic hypoproteinemia caused by deficiency of the neonatal Fc receptor, FcRn, due to a mutant beta2-microglobulin gene. Proc Natl Acad Sci USA 103(13):5084–5089

Ward ES, Martinez C, Vaccaro C et al (2005) From sorting endosomes to exocytosis: association of Rab4 and Rab11 GTPases with the Fc receptor, FcRn, during recycling. Mol Biol Cell 16(4):2028–2038

Ward ES, Ober RJ (2009) Multitasking by exploitation of intracellular transport functions: the many faces of FcRn. Adv Immunol 103:77–115

Wartiovaara J, Ofverstedt LG, Khoshnoodi J et al (2004) Nephrin strands contribute to a porous slit diaphragm scaffold as revealed by electron tomography. J Clin Invest 114(10):1475–1483

Winters JL (2012) Plasma exchange: concepts, mechanisms, and an overview of the American Society for Apheresis guidelines. Hematol Am Soc Hematol Educ Program 2012:7–12

Winters JL, Brown D, Hazard E et al (2011) Cost-minimization analysis of the direct costs of TPE and IVIg in the treatment of Guillain-Barre syndrome. BMC Health Serv Res 11:101

Woof JM, Mestecky J (2005) Mucosal immunoglobulins. Immunol Rev 206:64–82

Ye L, Liu X, Rout SN et al (2008) The MHC class II-associated invariant chain interacts with the neonatal Fcγ receptor and modulates its trafficking to endosomal/lysosomal compartments. J Immunol 181(4):2572–2585

Yeung YA, Leabman MK, Marvin JS et al (2009) Engineering human IgG1 affinity to human neonatal Fc receptor: impact of affinity improvement on pharmacokinetics in primates. J Immunol 182(12):7663–7671

Yoshida M, Claypool SM, Wagner JS et al (2004) Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. Immunity 20(6):769–783

Yoshida M, Kobayashi K, Kuo TT et al (2006) Neonatal Fc receptor for IgG regulates mucosal immune responses to luminal bacteria. J Clin Invest 116(8):2142–2151

Zalevsky J, Chamberlain AK, Horton HM et al (2010) Enhanced antibody half-life improves in vivo activity. Nat Biotechnol 28(2):157–159

Zhang Y, Pardridge WM (2001) Mediated efflux of IgG molecules from brain to blood across the blood-brain barrier. J Neuroimmunol 114(1–2):168–172

Zhu X, Meng G, Dickinson BL et al (2001) MHC class I-related neonatal Fc receptor for IgG is functionally expressed in monocytes, intestinal macrophages, and dendritic cells. J Immunol 166(5):3266–3276

Part IV
FcRs and Disease

Human FcR Polymorphism and Disease

Xinrui Li, Andrew W. Gibson and Robert P. Kimberly

Abstract Fc receptors play a central role in maintaining the homeostatic balance in the immune system. Our knowledge of the structure and function of these receptors and their naturally occurring polymorphisms, including single nucleotide polymorphisms and/or copy number variations, continues to expand. Through studies of their impact on human biology and clinical phenotype, the contributions of these variants to the pathogenesis, progression, and/or treatment outcome of many diseases that involve immunoglobulin have become evident. They affect susceptibility to bacterial and viral pathogens, constitute as risk factors for IgG or IgE mediated inflammatory diseases, and impact the development of many autoimmune conditions. In this chapter, we will provide an overview of these genetic variations in classical FcγRs, FcRLs, and other Fc receptors, as well as challenges in achieving an accurate and comprehensive understanding of the FcR polymorphisms and genomic architecture.

Contents

1 Introduction 275
2 Human FcR Polymorphisms: Location and Functional Implications 276
2.1 Single Nucleotide Polymorphisms 276
2.2 Copy Number Variations (CNVs) 284
3 Human FcR Polymorphisms: Association with diseases 285
3.1 Infectious Diseases 285
3.2 Inflammatory and Autoimmune Diseases 287
3.3 Allergic Diseases 291
4 Association with Response to Antibody Therapy 292
5 Conclusions 293
References 293

X. Li · A. W. Gibson · R. P. Kimberly (✉)
Department of Medicine, University of Alabama at Birmingham,
Birmingham, AL 35294, USA
e-mail: rpk@uab.edu

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_13,

1 Introduction

Highly homologous in their extracellular sequences, members of the Fc receptor family have both structural differences as well as allelic variations which impact biological properties and their respective roles in pathophysiology. Investigation over the last two decades has demonstrated regulatory and/or coding single nucleotide polymorphisms (SNP) that change receptor biology through one of three mechanisms: quantitative receptor expression, ligand affinity, or signaling capacity. Emerging data have also demonstrated copy number variation (CNV) in the classical low affinity Fc receptors for IgG. Many of the SNPs and CNVs are associated with pathogenesis, severity, and/or treatment outcome in a range of immune-mediated diseases. Signaling and biology of Fc receptors are discussed in Chapter X and Y. In this chapter, we discuss the germ line variations in the genes encoding Fc receptors and how these variations impact receptor function and association with disease.

2 Human FcR Polymorphisms: Location and Functional Implications

2.1 Single Nucleotide Polymorphisms

Numerous single-nucleotide polymorphisms have been identified through Fc receptor sequence analysis, particularly within the classical low-affinity FcγR cluster located on the long arm of chromosome 1. The allele frequencies of these genetic variants, many of which have not been characterized for function, may differ across different ancestry groups. The more thoroughly studied SNPs with known functional relevance and disease association are presented in Tables 1 and 2.

2.1.1 FcγRIIa (*FCGR2A*)

A nonsynonymous polymorphism (519G > A, rs1801274) in exon 4 encoding the membrane proximal Ig-like domain of *FCGR2A* leads to an arginine (R) to histidine (H) change at position 131 and alters receptor affinity for ligand. The R131 and H131 alleles are co-dominantly expressed. The FcγRIIa-H131 allele readily binds human IgG2 while the R131 allele does not effectively bind IgG2 (Salmon et al. 1992; Parren et al. 1992). Studies with IgG3 suggest that the H131 allele may bind IgG3 with moderately greater affinity than the R131 allele (Parren et al. 1992; Bredius et al. 1994). Crystallographic analysis and molecular modeling studies suggest that the H131R position is on the contact interface between receptor-IgG (Maxwell et al. 1999). As the most broadly expressed FcγR across a range of cell types in humans, the variation in ligand affinity has functional relevance in

Table 1 Genetic variations of classical FcγRs

Receptor	Genetic variation	Functional property	Disease/trait
FcγRIIa	R131H (rs1801274)	H131: higher affinity, can bind IgG2	Infections (Bredius et al. 1993; Endeman et al. 2009; Platonov et al. 1998; Jansen et al. 1999; Yee et al. 2000; Sanders et al. 1994; Bredius et al. 1994; Yuan et al. 2005; Loke et al. 2002; Garcia et al. 2010; Diamantopoulos et al. 2013; Forthal et al. 2007), Autoimmune inflammation (International Consortium for Systemic Lupus Erythematosus et al. 2008; Shrestha et al. 2012; Onouchi et al. 2012; Karassa et al. 2002; Magnusson et al. 2004; Kyogoku et al. 2004; Weersma et al. 2010; Dijstelbloem et al. 1999; Norsworthy et al. 1999; Song et al. 1998; Dijstelbloem et al. 2000; Morgan et al. 2006; van der Pol et al. 2000, 2003; Khor et al. 2011), atopy (Wu et al. 2014)
	rs10919543	increasing mRNA expression	TA (Saruhan-Direskeneli et al. 2013)
	27Q > W (rs9427397) (rs9427398)	unknown	KD (Breunis et al. 2013)
	rs58055840	unknown	Immunocyte levels (Orru et al. 2013)
	rs10800309	unknown	UC (McGovern et al. 2010; Asano et al. 2009), SS (Lessard et al. 2013)
	rs12746613	unknown	RA (Raychaudhuri et al. 2009)
	rs6658353	unknown	SS (Lessard et al. 2013)
FcγRIIb	I232T (rs1050501)	T232: altered partition to lipid rafts; altered signaling capability	SLE (Kono et al. 2005; Chu et al. 2004; Chen et al. 2006), atopy (Wu et al. 2014)
	2B.1/2B.4[a] (rs3219018)	2B.4: higher promoter activity/ expression	SLE (Su et al. 2004; Blank et al. 2005; Olferiev et al. 2007), KD (Breunis et al. 2013)
	rs2125685	unknown	Periodontitis (Sugita et al. 2012)
FcγRIIc	STP/Q13 (rs10917661)	STP: pseudogene; Q13:expression	ITP (Breunis et al. 2008), SLE (Li et al. 2013), KD (Breunis et al. 2013)
	CNV	altered protein expression level	ITP (Breunis et al. 2008), SLE (Li et al. 2013)

(continued)

Table 1 (continued)

Receptor	Genetic variation	Functional property	Disease/trait
FcγRIIIa	V158F rs396991	V158: higher affinity for IgG1, IgG3	SLE (Wu et al. 1997; Edberg et al. 2002; Koene et al. 1998), RA (Morgan et al. 2006), GPA (Dijstelbloem et al. 1999), Lupus nephritis (Jonsen et al. 2007)
	CNV	Altered protein expression level	anti-GBM disease (Zhou et al. 2010)
	rs445509	unknown	Periodontitis (Chai et al. 2010)
FcγRIIIb	NA1/NA2[b]	NA1: higher affinity	SLE (Hatta et al. 1999), ITP (Foster et al. 2001)
	SH	unknown	unknown
	CNV	Altered protein expression level	SLE (Willcocks et al. 2008), ANCA vasculitis (Tse et al. 2000; Niederer et al. 2010), SS (Nossent et al. 2012)

[a] Promoter haplotype. 2B.1: -120G-386T; 2B.4: -120C-386A

[b] Coding haplotype. NA1: 141G 147C 227A 349G; NA2: 141C 147T 227G 349A

TA: Takayasu's arteritis; KD: Kawasaki disease; UC: ulcerative colitis; SS: Sjögren's Syndrome; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; ITP: idiopathic thrombocytopenia purpura; GPA: Granulomatosis with polyangitis (Wegener's granulomatosis); anti-GBM disease: Anti–glomerular basement membrane (anti-GBM) antibody disease; ANCA: anti neutrophil cytoplasmic antibodies

Table 2 Genetic variations of FcεR, FcαR, FCRLs and FcRn

Receptor	Genetic variation	Functional property	Disease/trait
FcεRI-α	−66T/C (rs2251746)	−66T:higher promoter activity/expression	AD (Hasegawa et al. 2003), asthma (Zhou et al. 2012), high IgE (Weidinger et al. 2008; Granada et al. 2012)
	−315C/T (rs2427827)	−315T:higher promoter activity/expression	Chronic urticarial (Kim et al. 2006; Bae et al. 2007), asthma (Shikanai et al. 1985; Potaczek et al. 2006; Zhou et al. 2012)
FcεRI-β	E237G	unknown	Atopy, asthma (Zhang et al. 2004; Yang et al. 2014), nasal allergy (Kim et al. 2007; Laprise et al. 2000; Nagata et al. 2001)
	I181L	unknown	Atopy (Li and Hopkin 1997)
	−109C/T	unknown	High IgE (Hizawa et al. 2000), asthma (Kim et al. 2006; Yang et al. 2014)
	−426C/T −654T/C	−426C and −654T:higher promoter activity/expression	atopy (Nishiyama et al. 2004)
FcεRII	R62 W (rs2228137)	W62:resistance to proteolytic cleavage	Asthma (Laitinen et al. 2000)
	rs3760687	unknown	High IgE (Sharma et al. 2014)
FcαRI	S248G	G248: higher IgA-mediated activation	SLE (Wu et al. 2007)
FCRL1	rs4971154	unknown	T1D (Plagnol et al. 2011)
FCRL3	rs7528684	Altered gene expression	SLE, RA, AITD (Osuga et al. 2011; Teles et al. 2011; Szczepanska et al. 2013); (Osuga et al. 2011; Teles et al. 2011; Szczepanska et al. 2013); T1D (Osuga et al. 2011; Teles et al. 2011; Szczepanska et al. 2013); MS (Osuga et al. 2011; Teles et al. 2011; Szczepanska et al. 2013); Endometriosis (Osuga et al. 2011; Teles et al. 2011; Szczepanska et al. 2013)
	rs7522061	unknown	MS (Osuga et al. 2011; Teles et al. 2011; Szczepanska et al. 2013)
	rs2282288	unknown	GD (Osuga et al. 2011; Teles et al. 2011; Szczepanska et al. 2013)
	rs11264798	unknown	T1D (Osuga et al. 2011; Teles et al. 2011; Szczepanska et al. 2013)
FCRL4	rs2777963	unknown	AS (Zeng et al. 2012)

(continued)

Table 2 (continued)

Receptor	Genetic variation	Functional property	Disease/trait
	rs14335	unknown	AS (Zeng et al. 2012)
	rs10489674	unknown	AS (Zeng et al. 2012)
FCRL5	rs12036228	unknown	AS (Tang et al. 2009)
	rs6427384	unknown	AS (Tang et al. 2009)
FcRn	VNTR[a]	altered promoter activity/expression	unknown

[a] VNTR: variable number of tandem repeats

AD: atopic dermatitis; T1D: type-1 diabetes; AITD: autoimmune thyroid disease; MS: multiple sclerosis; GD: Graves' disease; AS: ankylosing spondylitis

determining cellular interactions with IgG antibodies, including the clearance of IgG2 immune complexes. For example, neutrophils from FcγRIIa-H131 homozygous donors are much more effective than neutrophils from R131 homozygous donors in phagocytosing IgG2-opsonized particles (Bredius et al. 1993).

Several *FCGR2A* SNPs, including rs1801274 encoding R131H (International Consortium for Systemic Lupus Erythematosus 2008), as well as several variants in non-coding regions, including rs10919543 (Saruhan-Direskeneli et al. 2013), rs12746613 (Raychaudhuri et al. 2009), rs10800309 (McGovern et al. 2010; Asano et al. 2009), rs6658353 (Lessard et al. 2013), and rs6427609 (Kettunen et al. 2012), have been associated with disease phenotypes in various genome-wide association studies (GWAS). These disease association studies, based on high through put genotyping technologies, suggest that variation in FcγRIIa biology may contribute to a number of human disease phenotypes. However, not all variants identified through such studies have an obvious function or relationship to biological processes, and direct inference of pathophysiology requires further study. In some cases, SNP-based associations may be tagging linkage disequilibrium (LD) blocks. Given the segmental duplication in the classical low affinity *FCGR* cluster and the consequent high degree of genomic sequence homology, this region is not technically amenable to efficient genotyping with array-based strategies. Thus, genotyping coverage in genome-wide association studies is not optimal because of difficulty in accurate probe design and position assignment.

2.1.2 FcγRIIb (FCGR2B)

Some nonsynonymous coding SNPs in the FcR cluster affect the signaling capacity of the expressed receptor. In the *FCGR2B* gene locus, a nonsynonymous T > C SNP (rs1050501) encodes an isoleucine (I) to threonine (T) substitution at position 187 in the transmembrane domain; this variant is also known as I/T232 when the signal peptide is included in the numbering (Kyogoku et al. 2002; Li et al. 2003). The FcγRIIb-187threonine allele, which is less efficient in trans-locating into lipid rafts in the plane of the cell membrane, may result in decreased quantitative participation of FcγRIIb in the assembly of lipid raft-based signaling complexes with a resultant decreased inhibitory potential (Kono et al. 2005; Floto et al. 2005).

Su et al. identified a promoter haplotype (rs3219018) in FcγRIIb that alters receptor expression (Su et al. 2004). The less common promoter haplotype (–386C-120A) showed increased binding of transcription factors GATA4 and Yin-Yang 1, leading to higher receptor expression than found with the more frequent haplotype (–386G-120T) (Su et al. 2004a, b; Blank et al. 2005). Of note, sequence analysis of these promoter variants has revealed nearly identical sequence in the proximal promoter region of *FCGR2C*, thus underscoring the important consideration of the potential for expression of both receptors.

2.1.3 FcγRIIc (FCGR2C)

FCGR2C, often considered a pseudogene, has received less attention than other Fc receptors. The nonsynonymous SNP (202T > C, rs10917661) in its first extracellular domain changes the common allele (202T), which encodes a translation termination codon at residue position 13, to 202C, which encodes an open reading frame (ORF) for glutamine. The FcγRIIc-ORF allele produces an ITAM-containing activating receptor that has been detected on NK cells (Metes et al. 1998, 1999; Stewart-Akers et al. 2004) and B cells (Li et al. 2013). Functionally, NK cells bearing the ORF allele are capable of clearing anti-FcγRII coated particles through reverse antibody-mediated cellular cytotoxicity (ADCC) (Ernst et al. 2002; Breunis et al. 2008). On B cells, the FcγRIIc-ORF allele counterbalances the negative feedback of FcγRIIb on BCR signaling, resulting in enhanced B cell responsiveness including upstream signaling events such as tyrosine kinase phosphorylation and calcium transients, and integrated cell programs such as antibody production (Li et al. 2013).

2.1.4 FcγRIIIa (FCGR3A)

Similar to FcγRIIa, FcγRIIIa also has co-dominantly expressed alleles that affect receptor affinity for ligand. In the second extracellular domain of *FCGR3A*, a point substitution of T to G at nucleotide 559 (rs396991) changes the phenylalanine (F) at amino acid position 158 to valine (V). The FcγRIIIa-158V allele (also known as 176 V when the leader sequence is included) displays higher affinity for IgG1 and IgG3 relative to the 158F (176F) allele. The 158 V form is also capable of binding IgG4, while the 158F allele is not (Wu et al. 1997; Koene et al. 1997). NK cells from FcγRIIIa-158 V (high binder) homozygous donors exhibit increased calcium influx, greater CD25 expression, and faster apoptosis than those cells from FcγRIIIa-158F (low binder) homozygous donors (Wu et al. 1997).

2.1.5 FcγRIIIb (FCGR3B)

The GPI-anchored FcγRIIIb, mainly expressed on neutrophils, has three different allotypic variants, known as NA1, NA2, and SH. The neutrophil antigen (NA) variants NA1 and NA2 are a product of five nonsynonymous SNPs in the first Ig-like domain, with an asparagine to serine switch at amino acid position 65 resulting in altered glycosylation and reduced affinity in the NA2 allele (Ravetch and Perussia 1989; Salmon et al. 1990). FcγRIIIb-NA1 exhibits higher affinity and more efficient phagocytosis of IgG1 and IgG3 opsonized particles compared to the NA2 allele (Salmon et al. 1990). The SH allele results from an alanine to aspartic acid substitution at position 78 and is observed in the context of the NA2 allele (Bux et al. 1997). The exact function of the SH allele is not yet known.

2.1.6 FcαRI (FCAR)

FCAR (CD89) encodes the human IgA receptor FcαRI. A common SNP (844A > G) was identified through direct sequencing of the coding region of *FCAR* (CD89) (Jasek et al. 2004; Wu et al. 2007). This transition changes amino acid codon 248 in the cytoplasmic domain from serine to glycine, resulting in enhanced cellular functions. For example, when equivalently stimulated with human IgA, neutrophils homozygous for the FcαR-G248 allele produce significantly higher levels of IL-6 compared to neutrophils from homozygous FcαR-S248 individuals. In the absence of FcR γ-chain pairing, FcαR-S248 allele fails to induce pro-inflammatory cytokines. In contrast, FcαR-G248 maintains signaling capacity even without the FcRγ, producing both IL-6 and TNFα. The increased activity of the G248 form may reflect, at least in part, its enhanced association with the Src family kinase, Lyn (Wu et al. 2007).

2.1.7 FcεRI (FCER1A/B/G)

The high affinity Fc receptor for IgE, FcεRI, has SNPs in the promoter region of the receptor α-chain (*FCER1A*). Through mutational screening of the proximal promoter, −95T > C (also referred to as −66) and −344C > T (also referred as –335) SNPs have been identified in several ethnicities (Shikanai et al. 1985; Hasegawa et al. 2003; Potaczek et al. 2006). Functionally, the −95T allele has greater GATA-1 binding, increased transcription of *FCER1A* message, and enhanced FcεRI protein expression on mast cells compared to the −95C allele (Hasegawa et al. 2003; Nishiyama 2006). Similarly, the −344C to T transition increases the binding of Myc-associated zinc finger (MAZ) transcription factors, resulting in increased protein expression (Kim et al. 2006; Bae et al. 2007). Furthermore, these two SNPs affect proximal promoter activity in an additive manner, with the highest activity attributed to the −95T-344T haplotype (Kanada et al. 2008).

The other two subunits of the IgE receptor, the FcεRIγ and FcεRIβ, have also been screened for genetic variations. Although the *FCER1G* gene is highly conserved (Wu et al. 2002), the *FCER1B* gene (also named *MS4A2*) contains several SNPs in the promoter region. The −426C-654T haplotype has higher binding of Yin-Yang 1 and higher transcription activity relative to the −426T-654C haplotype (Nishiyama et al. 2004).

The low affinity receptor for IgE, FcεRII (CD23), carries a functional SNP at position 62 in exon 4, resulting in an arginine (R) to tryptophan (W) substitution. The less common W62 allele is resistant to proteolytic shedding while the common R62 allele is known to be cleaved by a wide range of proteases and shed from cell surface (Meng et al. 2007). Soluble FcεRII has mitogenic properties, promoting the survival and differentiation of germinal center B cells (Liu et al. 1991). In vitro experiments have also suggested that the R62 W SNP affects IgE production through affecting Erk phosphorylation, which results in altered B cell responsiveness to IL-4 (Chan et al. 2014).

2.1.8 FcRLs

The *FCRL* genes encoded at the autoimmunity-linked 1q23 locus are highly polymorphic with SNPs and many mRNA splice isoforms identified for each gene locus. However, proteins corresponding to most of the splice isoforms have not been identified (Davis et al. 2002). Numerous SNPs have been identified within the FCRL coding regions, introns, the upstream promoter and the downstream non-coding regions. With the exception of the *FCRL3* −T169C promoter SNP (rs7528684), which alters an NF-kB binding site and results in increased expression of the *FCRL3* mRNA and protein in PBMC, CD19 + B cells and CD8 + T cells subsets (Kochi et al. 2005; Gibson et al. 2009; Chu et al. 2011), little is known about functional correlates in *FCRL* family SNPs. Nevertheless, many studies have identified association between autoimmune disease and genetic variation in *FCRL* genes suggesting an important role in disease. Several case-control studies of *FCRL3* polymorphisms in autoimmunity are summarized in a recent review (Chistiakov and Chistiakov 2007).

2.1.9 FcRn (FCGRT)

Although no common functional SNPs have been identified to date in *FCGRT*, the gene that encodes the neonatal Fc receptor, FcRn, a variable number of tandem repeats (VNTR) region in the promoter region consists of one to five repeats of a 37-bp motif (VNTR1-VNTR5) (Sachs et al. 2006). VNTR3 is the most common allele in Caucasian and Asian populations, followed by VNTR2. In vitro experiments have shown that VNTR3 has stronger transcriptional activity compared to VNTR2, resulting in more FcRn expression. Under acidic conditions, monocytes homozygous for VNTR3 showed increased IgG binding capacity compared to monocytes derived from VNTR2/VNTR3 heterozygous individuals (Sachs et al. 2006).

2.2 Copy Number Variations (CNVs)

Allotyping individual for the NA1 and NA2 alleles of *FCGR3B* led to the earliest observed copy number variation (CNV) in the classical low affinity *FCGR* cluster. Lack of both alleles identified *FCGR3B* deficiency (Clark et al. 1990; Huizinga et al. 1990), and duplication of the gene was inferred when all three alleles of *FCGR3B* (NA1, NA2 and SH) were simultaneously detected in the same individual (Koene et al. 1998). Copy number variation of *FCGR3B* correlates with the expression level of FcγRIIIb and with the capacity of neutrophils to phagocytose immune complexes (Willcocks et al. 2008).

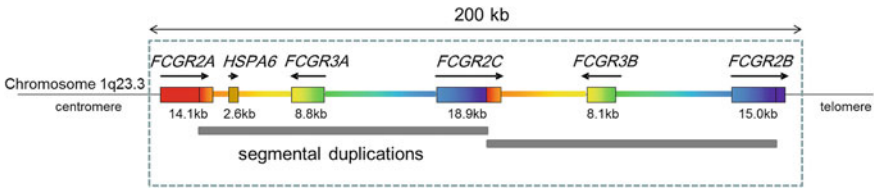


Fig. 1 Genomic structure of the classical low-affinity FCGR cluster. Identical colors represent sequence homology. Figure adapted from Li et al. (2009)

CNV has also been reported for *FCGR2C* and *FCGR3A*. Because *FCGR2C* and *FCGR3B* are adjacent in the genome (Fig. 1), CNV of both genes is highly correlated (de Haas et al. 1995; Reilly et al. 1994). Copy number of the *FCGR2C*-ORF allele correlates with FcγRIIc expression levels and consequently, activation status of NK cells (Breunis et al. 2008) and B cells (Li et al. 2013). Similarly, CNV of *FCGR3A* correlates with FcγRIIIa expression on NK cells (Breunis et al. 2009).

3 Human FcR Polymorphisms: Association with diseases

The central role of Fc receptors in supporting an appropriate humoral immune system has been demonstrated by numerous ex vivo and in vivo studies, in both human and model animals. Often one allele enhances activation and/or net immune system activity while the second allele tends to be less effective in eliciting responses, such as clearance and processing of immune complexes or antibody opsonized particles. Thus, functional FcR polymorphisms may significantly influence effector cell functions, thus providing diversity in host responses pertinent to many infectious, inflammatory and autoimmune diseases. For many SNPs, however, especially when they are in noncoding regions, the direct impact on biological function is not known and the potential influence on pathophysiology is ambiguous. An understanding of these associations and their implications for disease processes awaits further insight into the pertinent genomic architecture of the overall immune response.

3.1 Infectious Diseases

3.1.1 Infection with Encapsulated Bacteria

Often working in synergy with the complement system, FcγR-mediated clearance of antibody-coated microbes and FcγR-triggered inflammatory cytokine release are important mechanisms in eliminating infectious agents. Since human IgG2 is

relatively inefficient in initiating the complement cascade, the FcγRIIa-131H allele is the primary leukocyte receptor capable of effectively clearing IgG2-coated microbes, which is important in host defense against encapsulated bacteria such as *Streptococcus pneumonia*, *Hemophilus influenza,* and *Neisseria meningitidis* (Bredius et al. 1993; Jefferis and Kumararatne 1990; Endeman et al. 2009; Platonov et al. 1998; Jansen et al. 1999). In the context of *Strep pneumonia* pneumonia, the FcγRIIa-131R allele, which fails to bind IgG2, may be overrepresented in bacteremic patients, and in one study, the most severely infected bacteremic patients, who died within 1 week of hospitalization, were all homozygous for the R131 allele (Yee et al. 2000). Similarly, the FcγRIIa-131R allele is associated with increased infection by *Hemophilus influenza* and *Neisseria meningitidis* in multiple bacterial respiratory diseases and sepsis (Endeman et al. 2009; Platonov et al. 1998; Sanders et al. 1994; Bredius et al. 1994; Yuan et al. 2005). Of note, FcγRIIa also binds C-reactive protein with allele sensitivity reciprocal to IgG2 (Stein et al. 2000). High levels of CRP during infection may contribute to the clearance of IgG2-coated microbes by the R131 allele by opsonizing encapsulated bacteria and subsequently activating the complement mediated clearance (Weiser et al. 1998), which may compensate, at least in part, for the lack of FcγR-IgG2 mediated clearance in patients with the R131 allele.

3.1.2 Periodontitis

Periodontitis, an infectious disease caused by pathogenic anaerobic bacteria in the periodontium and the corresponding host response, is influenced by a combination of behavioral, environmental and genetic factors. Several types of FcγR-bearing cells are found in periodontal tissues, including neutrophils, lymphocytes and dendritic cells (Yuan et al. 1999). Functional studies largely focused on neutrophils have demonstrated that neutrophils homozygous for the FcγRIIa-131H allele were more efficient in bacterial phagocytosis, degranulation and elastase release (Nicu et al. 2007). In the same study the homozygous FcγRIIa-H131 patients also showed more bone loss than those with the H/R or R/R allotypes. Kobayashi et al. has also reported that neutrophils carrying the FcγRIIIb-NA2 allele showed lower reactivity to IgG1/IgG3 coated periodontopathic bacteria and induced weaker oxidative burst (Kobayashi et al. 2000).

Association studies calculating the clinical relevance of FcγR polymorphisms in periodontitis have reported mixed results, complicated by the difference in size and ethnicity of the population studied and the inconsistent definitions of disease stage and progression. A recent meta-analysis aggregating 17 studies reported modest association of FcγRIIa-131R with aggressive periodontitis in Asians, relatively strong association of the FcγRIIIb-NA1/NA2 polymorphism with both aggressive and chronic periodontitis, and a statistically insignificant relationship between the FcγRIIIa-F158 V and periodontitis (Song and Lee 2013). In studies of the distribution of the inhibitory FcγRIIb variants, significant enrichment of the FcγRIIb-232T allele in patients with aggressive periodontitis compared to both

chronic periodontitis patient and healthy control groups occurs in Japanese periodontitis patients (Yasuda et al. 2003). Furthermore, the composite genotype of FcγRIIb-232T plus FcγRIIIb-NA2 was strongly associated with aggressive periodontitis. The large number of B cells (Yuan et al. 1999) and the elevated antibody level (Horino et al. 1989) in periodontal lesions, as well as our understanding of the biology of the FcγRIIb-232T allele make the link between FcγRIIb-232T and periodontitis biologically plausible.

Besides the well-known polymorphisms, several other SNPs in the FcγR cluster have been identified in association with periodontitis. For example, the *FCGR2B*-nt645 + 25A/G (rs2125685) SNP in intron 4 was reported in Japanese patients and was related to changes in receptor expression level and severity of periodontitis (Sugita et al. 2012). A little studied SNP in *FCGR3A* (rs445509) was associated with chronic periodontitis in a Chinese population (Chai et al. 2010). Further study of these variants may elucidate their function and contribution to disease.

3.1.3 Virus Infection

Variants influencing Fc receptor function are also relevant in host defense mechanisms for virus infections. Dengue virus may co-opt Fcγ receptors for cell entry when the antibody-opsonized virus particles are phagocytized by FcγR-bearing myeloid cells, establishing infection in the phagocytes (Moi et al. 2010; Littaua et al. 1990; Garcia et al. 2011). Several studies have suggested the FcγRIIa-R131 allele may have a protective effect in Dengue virus infection (Loke et al. 2002; Garcia et al. 2010). The FcγRIIa-R131H SNP is one important factor in host defense, as it is also reported to be relevant in infections with A/H1N1 influenza (Zuniga et al. 2012), severe acute respiratory syndrome (SARS)- coronavirus (Yuan et al. 2005), and Epstein–Barr virus (Diamantopoulos et al. 2013). In human immunodeficiency virus (HIV) infection, patients with homozygous low affinity R131 allele showed the highest rate of disease progress (Forthal et al. 2007). The FcγRIIIa-V158F genotype also correlates with the development of Kaposi's sarcoma in HIV-infected patients (Forthal et al. 2007; Lehrnbecher et al. 2000).

3.2 Inflammatory and Autoimmune Diseases

3.2.1 Vasculitides

The vasculitides are a group of disorders that involve inflammation of the blood vessels. Although the etiology of vasculitis is often not clear, vascular inflammation can be immunologically mediated, triggered by immune complexes, anti-neutrophil cytoplasmic antibodies, anti-endothelial cell autoantibodies as well as by cell-mediated processes. The classification of the vasculitides is typically based

on the size of the affected vessel. Granulomatosis with polyangiitis (GPA), formerly known as Wegener's granulomatosis, is a type of neutrophil mediated vasculitis affecting small and medium sized vessels. GPA is often characterized by the presence of anti-neutrophil cytoplasmic antibodies (ANCA) (Nolle et al. 1989). Engagement of both ANCA target and Fc receptors on myeloid cells by ANCA elicits production of interleukin-8, a neutrophil chemotactic factor, and a series of effector programs such as oxidative burst, degranulation and release of neutrophil extracellular traps (NETs) (Ralston et al. 1997; Porges et al. 1994; Kessenbrock et al. 2009; Sangaletti et al. 2012). No clear association between GPA susceptibility and the FcγRIIa allotype has been demonstrated although some evidence suggests a relationship to the likelihood of relapsing disease (Edberg et al. 1997; Tse et al. 1999, 2000). FcγRIIIb, the numerically predominant FcγR on neutrophils, is the major receptor interacting with anti-PR3 IgG ANCA (Kocher et al. 1998), and *FCGR3B* CNV has been associated with GPA (Fanciulli et al. 2007). The FcγRIIIb-NA1 allele, known to induce stronger neutrophil activation than the NA2 allele (Salmon et al. 1990), has similar allele frequencies in GPA and healthy populations, suggesting no role in overall disease risk. However, the presence of the NA1 allele is associated with the development of severe renal damage in GPA patients (Neira et al. 1996; Kelley et al. 2011).

The recent identification of IgA ANCA in GPA, in addition to IgG ANCA, led to the investigation of the involvement of FcαRI in GPA pathogenesis. Indeed, the FcαRI-248G variant, which induces an augmented inflammatory response to IgA, was associated with overall susceptibility to GPA, as well as predisposition to severe renal disease (Kelley et al. 2011).

Kawasaki disease affects medium-sized blood vessels most commonly in children under 5 years of age. Genome wide association studies have identified an association between Kawasaki disease and the *FCGR2A* locus with the 131H variant conferring elevated disease risk (Shrestha et al. 2012; Onouchi et al. 2012). It is reasonable to speculate the FcγRIIa-131H bearing leukocytes are more pro-inflammatory in the setting of Kawasaki disease, although direct experimental evidence waits to be established. One might also anticipate an association between IgG receptor variants and intravenous immunoglobulin (IVIG), the only proven therapy for Kawasaki disease. Indeed, in Japanese patients, those with the FcγRIIa-131H allele responded more efficiently to IVIG administration. Patients with the 131R allele were more likely to develop coronary lesions even after treatment (Taniuchi et al. 2005). Consistent with the notion that tilting the immune system towards inflammation might be associated with disease expression, the *FCGR2C*-ORF SNP was recently reported to be enriched in Kawasaki disease patients (Breunis et al. 2013).

Takayasu's arteritis is a rare form of large vessel vasculitis. A recent GWAS in Turkish and North American Takayasu's arteritis patients identified a noncoding SNP in the *FCGR2A/FCGR3A* locus (rs10919543) as a susceptibility marker, which appeared to have a regulatory effect on *FCGR2A* transcript expression (Saruhan-Direskeneli et al. 2013).

Several other forms of chronic inflammatory diseases have been reported to have associations with the *FCGR* cluster. The *FCGR2A/2C* region has been related to susceptibility to ulcerative colitis, one sub-phenotype of inflammatory bowel disease, in two GWA studies (McGovern et al. 2010; Asano et al. 2013). In addition to the well-known FcγR-R131H variant, the rs10800309 variant in this locus awaits further work to determine potential functional relevance.

3.2.2 Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by autoantibodies and immune complexes. Although the etiology of SLE is unknown, many genes play a role in the susceptibility to and severity of the disease, and GWAS and candidate genes studies have identified the FCGRs as important contributors to the SLE diathesis (Harley et al. 2009).

A GWAS study of Europeans confirmed the association of *FCGR2A* (rs1801274; 519G > A encoding R131H) with SLE (International Consortium for Systemic Lupus Erythematosus 2008). This nonsynonymous SNP is a risk factor for lupus nephritis and systemic lupus erythematosus in African Americans (Salmon et al. 1996; Edberg et al. 2002), Caucasians (Manger et al. 2002; Karassa et al. 2002; Magnusson et al. 2004; Kyogoku et al. 2004) and Asians (Siriboonrit et al. 2003; Lee et al. 2002; Chu et al. 2004), as well as for myasthenia gravis in Caucasians (Weersma et al. 2010; van der Pol et al. 2003). Homozygosity for the transmembrane 187T variant of FcγRIIb is also associated with SLE susceptibility in Japanese (Kyogoku et al. 2002), Chinese (Chu et al. 2004) and Thais (Siriboonrit et al. 2003). Interestingly, the 187T allele has a lower frequency in European Americans and is not associated with SLE in either this ancestry group or in African Americans where the frequency of 187T is similar to that of Asians (Li et al. 2003; Magnusson et al. 2004). Whether this difference represents, less statistical power for detection of association in these groups or an epistatic effect is not certain. The FcγRIIb-187T allele may be a risk factor for anti-GBM disease in Chinese (Zhou et al. 2010) while a promoter haplotype, 2B.4 (−386C −120A), which alters *FCGR2B* gene expression is associated with SLE (Su et al. 2004). In a second patient population, homozygosity of the −386C allele alone (also referred to as −343C) affirmed an association of promoter variants with SLE (Blank et al. 2005). CNV in this receptor cluster, including the *FCGR2C*-ORF allele, may be associated with SLE in patients of European and African ancestry (Li et al. 2013).

The low IgG binding FcγRIIIa-158F is associated with SLE and with lupus nephritis (Wu et al. 1997; Karassa et al. 2002; Jonsen et al. 2007; Dong et al. 2013) in multiple ancestry groups including Europeans, African Americans (Edberg et al. 2002; Koene et al. 1998), Chinese (Chu et al. 2004), and Japanese (Kyogoku et al. 2002). Interestingly, homozygosity for the high IgG binding –158 V allele is a significant predictor of end-stage renal disease in a multiethnic group of SLE patients (Alarcon et al. 2006). Both FcγRIIIb CNV and NA1/NA2 alleles may be associated

with SLE in UK Caucasians (Willcocks et al. 2008), Thais (Siriboonrit et al. 2003), Japanese (Hatta et al. 1999), and Spanish (Gonzalez-Escribano et al. 2002).

The −169C > T SNP (rs7528684) in *FCRL3*, which alters an NFκB binding site and is associated with *FCRL3* mRNA and surface protein expression, is associated with autoimmunity in some ethnic groups. Associated with SLE, RA, and AITD in Japanese (Kochi et al. 2005; Gibson et al. 2009), this variant is not associated with these conditions in other ethnicities suggesting that it is not a general autoimmunity risk factor (Chistiakov and Chistiakov 2007). The −169C > T SNP is not associated with SLE in Chinese (You et al. 2008), Koreans (Choi et al. 2006), or Mexican patients with childhood-onset SLE (Ramirez-Bello et al. 2013), but the association with the presence of autoantibodies in Polish SLE patients suggests a possible role in production of autoantibodies (Piotrowski et al. 2013). Results of meta-analyses differ on whether the −169C > T is associated with SLE in different ethnicities (Breunis et al. 2013; Mao et al. 2010; Song et al. 2013), and the mechanism(s) through which this variant may contribute to SLE remains unclear.

3.2.3 Rheumatoid Arthritis and Juvenile Idiopathic Arthritis

Evidence for the contributions of the classical low-affinity Fcγ receptors to Rheumatoid Arthritis suggests that several polymorphisms may be associated with RA manifestations in different ethnic groups, although associations are not always consistent. While GWAS indicated that *FCGR2A* is associated with RA (Raychaudhuri et al. 2009), candidate gene studies suggest the *FCGR3A* is associated with RA (Morgan et al. 2000; Morgan et al. 2003) and a role for *FCGR2C* is unclear.

The −169C > T promoter SNP in FCRL3 is associated with RA in Caucasians and Chinese (Thabet et al. 2007; Eike et al. 2008; Maehlen et al. 2011; Wu et al. 2010), with JIA in Mexicans (Ramirez-Bello et al. 2013), and with JIA in Norwegian patients (Eike et al. 2008). This SNP has been correlated with increased FCRL3 surface expression on Tregs of patients with erosive RA (Bajpai et al. 2012), and the −169CC genotype may be correlated with radiographic severity in Korean RA patients (Han et al. 2012). A more detailed review of Fc receptor associations and reheumatoid arthritis is discussed in Chapter XX.

3.2.4 Spondyloarthropathies

The rs2777963T > C, rs14335A > G and rs10489674C > T polymorphisms in *FCRL4* have been associated with susceptibility and severity of ankylosing spondylitis (AS) in Han Chinese (Zeng et al. 2012). Similarly, in *FCRL5* two nonsynonymous SNPs, rs12036228C > T and rs6427384T > C in exon 5 and exon 7, respectively, and their C-T haplotype were found to be associated with

ankylosing spondylitis in HLA-B27 positive Han Chinese, suggesting a role in AS (Tang et al. 2009). However, the role, if any, of these SNPs in FCRL4 and 5 expression or function is unclear.

3.2.5 Diabetes Mellitus and Autoimmune Endocrinopathies

Several studies have found association between autoimmune endocrinopathies and SNPs in FCRL family members, although potential underlying mechanisms remain elusive. In a recent study of Type 1 Diabetes (T1D) the C-allele of *FCRL1* rs4971154 was strongly associated with the presence of the IA-2A autoantibody in serum suggesting a role in production of autoantibodies (Mao et al. 2010). Although the *FCRL3* −169C > T SNP was not associated with T1D in several studies of Caucasians (Eike et al. 2008; Owen et al. 2007; Duchatelet et al. 2008), a recent study of 8,506 T1D patients in the United Kingdom found a strong negative association between the C allele and anti-IA-2A autoantibody- positive T1D (Mao et al. 2010). The mechanism of association remains unclear.

In autoimmune thyroid disease, Owen et al. found modest association of the 3'UTR C > A SNP rs2282288 with Grave's Disease in Europeans (Owen et al. 2007). The −169TT promoter genotype of rs7528684 was associated with remission in Japanese AITD patients (Inoue et al. 2012), and with protection against Grave's Disease in Chinese (Gu et al. 2010). A potential role for FCRL3 in production of autoantibodies is supported by the observations that the rs11264798C > G and rs7528684C > T SNPs are associated with thyroid peroxidase autoantibody (TPOA) positivity in GD and anti- IA-2A positivity in T1D (Plagnol et al. 2011), while the rs7522061T > C SNP is associated with anti-876 ZnT8A positivity (autoantibody to the zinc transporter 8 in islet cells) in T1D patients (Howson et al. 2012).

3.2.6 Multiple Sclerosis

The *FCRL3* −169C > T SNP (rs7528684) has been associated with multiple sclerosis in a Spanish cohort (Martinez et al. 2007; Matesanz et al. 2008). While the T allele of the nonsynonymous coding SNP (rs7522061), which results in the N28D change, was found to be protective in Spanish, the G allele was a risk factor for MS in patients in the United Kingdom (Matesanz et al. 2008).

3.2.7 Inflammatory Bowel Disease

Despite its association with many autoimmune disorders in different ethnicities, the −169C > T SNP appears not to be associated with risk for ulcerative colitis, Crohn's disease or primary sclerosing cholangitis (Eike et al. 2008), or with Inflammatory Bowel Disease (Martinez et al. 2007).

3.3 Allergic Diseases

Allergic diseases are a type of hypersensitivity characterized by mast cell activation and IgE-mediated inflammation. The high-affinity IgE receptor expressed on mast cells, FcεRI, has long been considered a candidate gene in allergic diseases. Multiple studies have established a consistent genetic association between allergies and the promoter variants of FcεRI α-chain. The −66T > C and/or the −315C > T SNPs are associated with atopic dermatitis, chronic urticaria, asthma, and high serum IgE levels (Hasegawa et al. 2003; Potaczek et al. 2006; Kim et al. 2006; Bae et al. 2007; Zhou et al. 2012; Niwa et al. 2010). The −66T > C SNP was highlighted as the strongest hit in two GWA studies with high IgE levels (Weidinger et al. 2008; Granada et al. 2012). These genetic findings may be explained by functional studies that have demonstrated that both SNPs amplify transcription activity, increasing FcεRI expression on mast cells and basophils (Hasegawa et al. 2003; Kanada et al. 2008), and the well-established observation that surface FcεRI expression correlates positively with circulating IgE levels (MacGlashan 2005). Similarly, several SNPs in the FcεRI β-chain are associated with allergic inflammatory diseases such as atopy, asthma, and nasal allergy (Nishiyama et al. 2004; Zhang et al. 2004; Laprise et al. 2000; Nagata et al. 2001; Li and Hopkin 1997; Hizawa et al. 2000; Kim et al. 2006, 2007; Yang et al. 2014). Functional properties of these SNPs are not known.

The low-affinity IgE receptor on B cells, FcεRII (CD23), is important in regulating IgE production and B cell differentiation. The R62W alteration in the *FCER2* gene, that yields increased IgE binding and augmented ERK signaling (Chan et al. 2014), is associated with elevated serum IgE levels and an increased risk of severe asthma exacerbation in children (Laitinen et al. 2000; Koster et al. 2011; Tantisira et al. 2007). A promoter SNP in the *FCER2* gene, rs3760687, associated with increased total serum IgE (Sharma et al. 2014), may alter the activity of the transcription factors Sp1 and Sp3, leading to modulation of FcεRII expression (Potaczek et al. 2009).

Even though IgE and IgE receptors have been known to be the major players in allergic inflammation, allergen-specific IgG and FcγRs also play a role (Kaneko et al. 1995; Jonsson et al. 2012; Williams et al. 2012; Lau et al. 2005; Bruhns et al. 2005). In a candidate gene study, both the FcγRIIa-R131H and the FcγRIIb-I187T SNPs have been associated with atopy (Wu et al 2014). In this context, it is conceivable that FcγRIIa-H131 allele may clear allergen-IgG2 immune complexes more efficiently, preventing inflammation and tissue damage. Whether allergen-specific IgG2 levels vary in accordance with FcγRIIa polymorphisms is unknown. Furthermore, the FcγRIIb-187T allele may not be as effective in negatively regulating BCR function, resulting in increased B cell IgE production. Crosstalk between FcγRIIb and FcεRI on mast cells is also a possibility.

4 Association with Response to Antibody Therapy

The efficacy of therapeutic monoclonal antibodies used in autoimmune diseases to induce ADCC and deplete autoreactive B lymphocytes from circulation depends, at least in part, on the strength of the interaction of activating FcγRs with the therapeutic antibody on the opsonized target cells. The FcγRIIIa -158F/V polymorphism influences the efficacy of rituximab treatment, which targets the CD20 surface protein on B cells, with patients homozygous for the high binding -158 V allele showing the best response (Robledo et al. 2012; Cooper et al. 2012). The precedent that alleles which alter binding and function of FcγRIIa and FcγRIIIa may affect the efficacy of antibody therapy is an important principle in antibody-based therapeutics. A more extensive discussion of the role of Fc receptors in the use of therapeutic antibodies is presented in Chapter XX, "FcR and therapeutic antibodies".

5 Conclusions

Genetic variations in human Fc receptors, through their impact on antibody-mediated mechanisms, contribute to individual and population-based host defense and susceptibility to a range of human diseases. Fc receptor polymorphisms modulate the effectiveness of immune system in defense against invading pathogens by regulating immune cell activities. They also impact the handling of immune reactants and the threshold of immune tolerance. Complex clinical phenotypes, such as autoimmunity or allergy, involve multiple genetic and environmental factors, and the subtle regulatory effects of various naturally occurring polymorphisms are compounded in their impact over time. Accurate assessment of the contributions of Fc receptor polymorphisms to immune system function and clinical phenotype requires a careful understanding of the genomic structure, sequence homology, and known physiological responses of Fc receptors in addition to well phenotyped study populations for adequately powered association studies. Such studies have provided important insights into pathogenetic mechanisms and potential novel therapeutic approaches.

References

Salmon JE et al (1992) Allelic polymorphisms of human Fc gamma receptor IIA and Fc gamma receptor IIIB. Independent mechanisms for differences in human phagocyte function. J Clin Invest 89(4):1274–1281

Parren PW et al (1992) On the interaction of IgG subclasses with the low affinity Fc gamma RIIa (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. J Clin Invest 90(4):1537–1546

Bredius RG et al (1994a) Role of neutrophil Fc gamma RIIa (CD32) and Fc gamma RIIIb (CD16) polymorphic forms in phagocytosis of human IgG1- and IgG3-opsonized bacteria and erythrocytes. Immunology 83(4):624–630

Maxwell KF et al (1999) Crystal structure of the human leukocyte Fc receptor, Fc gammaRIIa. Nat Struct Biol 6(5):437–442

Bredius RG et al (1993) Phagocytosis of Staphylococcus aureus and Haemophilus influenzae type B opsonized with polyclonal human IgG1 and IgG2 antibodies. Functional hFc gamma RIIa polymorphism to IgG2. J Immunol 151(3):1463–1472

International Consortium for Systemic Lupus Erythematosus, G., et al (2008) Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. Nat Genet . 40(2):204–10

Saruhan-Direskeneli G et al (2013) Identification of multiple genetic susceptibility loci in Takayasu arteritis. Am J Hum Genet 93(2):298–305

Raychaudhuri S et al (2009) Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk. Nat Genet 41(12):1313–1318

McGovern DP et al (2010) Genome-wide association identifies multiple ulcerative colitis susceptibility loci. Nat Genet 42(4):332–337

Asano K et al (2009) A genome-wide association study identifies three new susceptibility loci for ulcerative colitis in the Japanese population. Nat Genet 41(12):1325–1329

Lessard CJ et al (2013) Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. Nat Genet 45(11):1284–1292

Kettunen J et al (2012) Genome-wide association study identifies multiple loci influencing human serum metabolite levels. Nat Genet 44(3):269–276

Kyogoku C et al (2002a) Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. Arthritis Rheum 46(5):1242–1254

Li X et al (2003) A novel polymorphism in the Fcgamma receptor IIB (CD32B) transmembrane region alters receptor signaling. Arthritis Rheum 48(11):3242–3252

Kono H et al (2005) FcgammaRIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. Hum Mol Genet 14(19):2881–2892

Floto RA et al (2005) Loss of function of a lupus-associated FcgammaRIIb polymorphism through exclusion from lipid rafts. Nat Med 11(10):1056–1058

Su K et al (2004a) A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. I. Regulatory FCGR2B polymorphisms and their association with systemic lupus erythematosus. J Immunol 172(11):7186–7191

Su K et al (2004b) A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. II. Differential binding of GATA4 and Yin-Yang1 transcription factors and correlated receptor expression and function. J Immunol 172(11):7192–7199

Blank MC et al (2005) Decreased transcription of the human FCGR2B gene mediated by the -343 G/C promoter polymorphism and association with systemic lupus erythematosus. Hum Genet 117(2–3):220–227

Metes D et al (1999) Ligand binding specificities and signal transduction pathways of Fc gamma receptor IIc isoforms: the CD32 isoforms expressed by human NK cells. Eur J Immunol 29(9):2842–2852

Stewart-Akers AM et al (2004) Fc gamma R expression on NK cells influences disease severity in rheumatoid arthritis. Genes Immun 5(7):521–529

Metes D et al (1998) Expression of functional CD32 molecules on human NK cells is determined by an allelic polymorphism of the FcgammaRIIC gene. Blood 91(7):2369–2380

Li X et al (2013) Allelic-dependent expression of an activating fc receptor on B cells enhances humoral immune responses. Sci Transl Med 5(216):216ra175

Ernst LK et al (2002) Allelic polymorphisms in the FcgammaRIIC gene can influence its function on normal human natural killer cells. J Mol Med (Berl) 80(4):248–257
Breunis WB et al (2008) Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura. Blood 111(3):1029–1038
Wu J et al (1997) A novel polymorphism of FcgammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. J Clin Invest 100(5):1059–1070
Koene HR et al (1997) Fc gammaRIIIa-158 V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood 90(3):1109–1114
Ravetch JV, Perussia B (1989) Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. J Exp Med 170(2):481–497
Salmon JE, Edberg JC, Kimberly RP (1990) Fc gamma receptor III on human neutrophils. Allelic variants have functionally distinct capacities. J Clin Invest 85(4):1287–1295
Bux J et al (1997) Characterization of a new alloantigen (SH) on the human neutrophil Fc gamma receptor IIIb. Blood 89(3):1027–1034
Jasek M et al (2004) A novel polymorphism in the cytoplasmic region of the human immunoglobulin A Fc receptor gene. Eur J Immunogenet 31(2):59–62
Wu J et al (2007) FcalphaRI (CD89) alleles determine the proinflammatory potential of serum IgA. J Immunol 178(6):3973–3982
Shikanai T et al (1985, 2002) Sequence variants in the FcepsilonRI alpha chain gene. J Appl Physiol 93(1):37–41
Hasegawa M et al (2003) A novel -66T/C polymorphism in Fc epsilon RI alpha-chain promoter affecting the transcription activity: possible relationship to allergic diseases. J Immunol 171(4):1927–1933
Potaczek DP et al (2006) The alpha-chain of high-affinity receptor for IgE (FcepsilonRIalpha) gene polymorphisms and serum IgE levels. Allergy 61(10):1230–1233
Nishiyama C (2006) Molecular mechanism of allergy-related gene regulation and hematopoietic cell development by transcription factors. Biosci Biotechnol Biochem 70(1):1–9
Kim SH et al (2006a) Genetic mechanism of aspirin-induced urticaria/angioedema. Curr Opin Allergy Clin Immunol 6(4):266–270
Bae JS et al (2007) Significant association of FcepsilonRIalpha promoter polymorphisms with aspirin-intolerant chronic urticaria. J Allergy Clin Immunol 119(2):449–456
Kanada S et al (2008) Two different transcription factors discriminate the -315C > T polymorphism of the Fc epsilon RI alpha gene: binding of Sp1 to -315C and of a high mobility group-related molecule to -315T. J Immunol 180(12):8204–8210
Wu J et al (2002) Conservation of FcepsilonRI gamma chain coding region in normals and in SLE patients. Lupus 11(1):42–45
Nishiyama C et al (2004) Polymorphisms in the Fc epsilon RI beta promoter region affecting transcription activity: a possible promoter-dependent mechanism for association between Fc epsilon RI beta and atopy. J Immunol 173(10):6458–6464
Meng JF, McFall C, Rosenwasser LJ (2007) Polymorphism R62 W results in resistance of CD23 to enzymatic cleavage in cultured cells. Genes Immun 8(3):215–223
Liu YJ et al (1991) Recombinant 25-kDa CD23 and interleukin 1 alpha promote the survival of germinal center B cells: evidence for bifurcation in the development of centrocytes rescued from apoptosis. Eur J Immunol 21(5):1107–1114
Chan MA et al (2014) FCER2 (CD23) Asthma-Related Single Nucleotide Polymorphisms Yields Increased IgE Binding and Egr-1 Expression in Human B Cells. Am J Respir Cell Mol Biol 50(2):263–269
Davis RS et al (2002) Fc receptor homologs: newest members of a remarkably diverse Fc receptor gene family. Immunol Rev 190:123–136
Kochi Y et al (2005) A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. Nat Genet 37(5):478–485

Gibson AW et al (2009) The FCRL3-169CT promoter single-nucleotide polymorphism, which is associated with systemic lupus erythematosus in a Japanese population, predicts expression of receptor protein on CD19 + B cells. Arthritis Rheum 60(11):3510–3512
Chu X et al (2011) A genome-wide association study identifies two new risk loci for Graves' disease. Nat Genet 43(9):897–901
Chistiakov DA, Chistiakov AP (2007) Is FCRL3 a new general autoimmunity gene? Hum Immunol 68(5):375–383
Sachs UJ et al (2006) A variable number of tandem repeats polymorphism influences the transcriptional activity of the neonatal Fc receptor alpha-chain promoter. Immunology 119(1):83–89
Clark MR et al (1990) An abnormality of the gene that encodes neutrophil Fc receptor III in a patient with systemic lupus erythematosus. J Clin Invest 86(1):341–346
Huizinga TW et al (1990) Maternal genomic neutrophil FcRIII deficiency leading to neonatal isoimmune neutropenia. Blood 76(10):1927–1932
Koene HR et al (1998a) Fc gamma RIIIB gene duplication: evidence for presence and expression of three distinct Fc gamma RIIIB genes in NA(1 + ,2 +)SH(+) individuals. Blood 91(2): 673–679
Willcocks LC et al (2008) Copy number of *FCGR3B*, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. J Exp Med 205(7):1573–1582
de Haas M et al (1995) Neutrophil Fc gamma RIIIb deficiency, nature, and clinical consequences: a study of 21 individuals from 14 families. Blood 86(6):2403–2413
Reilly AF et al (1994) Variation in human FCGR2C gene copy number. Immunogenetics 40(6):456
Breunis WB et al (2009) Copy number variation at the FCGR locus includes FCGR3A, FCGR2C and FCGR3B but not FCGR2A and FCGR2B. Hum Mutat 30(5):E640–E650
Li X et al (2009) Fcgamma receptors: structure, function and role as genetic risk factors in SLE. Genes Immun 10(5):380–389
Jefferis R, Kumararatne DS (1990) Selective IgG subclass deficiency: quantification and clinical relevance. Clin Exp Immunol 81(3):357–367
Endeman H et al (2009) The Fcgamma receptor IIA-R/R131 genotype is associated with severe sepsis in community-acquired pneumonia. Clin Vaccine Immunol 16(7):1087–1090
Platonov AE et al (1998) Association of human Fc gamma RIIa (CD32) polymorphism with susceptibility to and severity of meningococcal disease. Clin Infect Dis 27(4):746–750
Jansen WT et al (1999) Fcgamma receptor polymorphisms determine the magnitude of in vitro phagocytosis of Streptococcus pneumoniae mediated by pneumococcal conjugate sera. J Infect Dis 180(3):888–891
Yee AM et al (2000) Association between FcgammaRIIa-R131 allotype and bacteremic pneumococcal pneumonia. Clin Infect Dis 30(1):25–28
Sanders LA et al (1994) Fc gamma receptor IIa (CD32) heterogeneity in patients with recurrent bacterial respiratory tract infections. J Infect Dis 170(4):854–861
Bredius RG et al (1994b) Fc gamma receptor IIa (CD32) polymorphism in fulminant meningococcal septic shock in children. J Infect Dis 170(4):848–853
Yuan FF et al (2005) Influence of FcgammaRIIA and MBL polymorphisms on severe acute respiratory syndrome. Tissue Antigens 66(4):291–296
Stein MP et al (2000) C-reactive protein binding to FcgammaRIIa on human monocytes and neutrophils is allele-specific. J Clin Invest 105(3):369–376
Weiser JN et al (1998) Phosphorylcholine on the lipopolysaccharide of Haemophilus influenzae contributes to persistence in the respiratory tract and sensitivity to serum killing mediated by C-reactive protein. J Exp Med 187(4):631–640
Yuan ZN et al (1999) Topical distribution of Fc gammaRI, Fc gammaRII and Fc gammaRIII in inflamed human gingiva. J Clin Periodontol 26(7):441–447
Nicu EA et al (2007) Hyper-reactive PMNs in FcgammaRIIa 131 H/H genotype periodontitis patients. J Clin Periodontol 34(11):938–945

Kobayashi T et al (2000) Relevance of IgG receptor IIIb (CD16) polymorphism to handling of Porphyromonas gingivalis: implications for the pathogenesis of adult periodontitis. J Periodontal Res 35(2):65–73

Song GG, Lee YH (2013) Associations between FCGR2A rs1801274, FCGR3A rs396991, FCGR3B NA1/NA2 polymorphisms and periodontitis: a meta-analysis. Mol Biol Rep 40(8):4985–4993

Yasuda K et al (2003) FcgammaRIIB gene polymorphisms in Japanese periodontitis patients. Genes Immun 4(8):541–546

Horino K et al (1989) Effects of anti-Bacteroides gingivalis (Bg), and anti-Actinobacillus actinomycetemcomitans (Aa) antibodies on Bg and Aa. Nihon Shishubyo Gakkai Kaishi 31(1):1–12

Sugita N et al (2012) Association of the FcgammaRIIB-nt645 + 25A/G polymorphism with the expression level of the FcgammaRIIb receptor, the antibody response to Porphyromonas gingivalis and the severity of periodontitis. J Periodontal Res 47(1):105–113

Chai L et al (2010) SNPs of Fc-gamma receptor genes and chronic periodontitis. J Dent Res 89(7):705–710

Moi ML et al (2010) Involvement of the Fc gamma receptor IIA cytoplasmic domain in antibody-dependent enhancement of dengue virus infection. J Gen Virol 91(Pt 1):103–111

Littaua R, Kurane I, Ennis FA (1990) Human IgG Fc receptor II mediates antibody-dependent enhancement of dengue virus infection. J Immunol 144(8):3183–3186

Garcia G et al (2011) Long-term persistence of clinical symptoms in dengue-infected persons and its association with immunological disorders. Int J Infect Dis 15(1):e38–e43

Loke H et al (2002) Susceptibility to dengue hemorrhagic fever in vietnam: evidence of an association with variation in the vitamin d receptor and Fc gamma receptor IIa genes. Am J Trop Med Hyg 67(1):102–106

Garcia G et al (2010) Asymptomatic dengue infection in a Cuban population confirms the protective role of the RR variant of the FcgammaRIIa polymorphism. Am J Trop Med Hyg 82(6):1153–1156

Zuniga J et al (2012) Genetic variants associated with severe pneumonia in A/H1N1 influenza infection. Eur Respir J 39(3):604–610

Diamantopoulos PT et al (2013) Correlation of Fc-gamma RIIA polymorphisms with latent Epstein-Barr virus infection and latent membrane protein 1 expression in patients with low grade B-cell lymphomas. Leuk Lymphoma 54(9):2030–2034

Forthal DN et al (2007) FcgammaRIIa genotype predicts progression of HIV infection. J Immunol 179(11):7916–7923

Lehrnbecher TL et al (2000) Variant genotypes of FcgammaRIIIA influence the development of Kaposi's sarcoma in HIV-infected men. Blood 95(7):2386–2390

Nolle B et al (1989) Anticytoplasmic autoantibodies: their immunodiagnostic value in Wegener granulomatosis. Ann Intern Med 111(1):28–40

Ralston DR et al (1997) Antineutrophil cytoplasmic antibodies induce monocyte IL-8 release. Role of surface proteinase-3, alpha1-antitrypsin, and Fcgamma receptors. J Clin Invest 100(6):1416–1424

Porges AJ et al (1994) Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via Fc gamma RIIa. J Immunol 153(3):1271–1280

Kessenbrock K et al (2009) Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med 15(6):623–625

Sangaletti S et al (2012) Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. Blood 120(15):3007–3018

Edberg JC et al (1997) Analysis of FcgammaRII gene polymorphisms in Wegener's granulomatosis. Exp Clin Immunogenet 14(3):183–195

Tse WY et al (2000) Neutrophil FcgammaRIIIb allelic polymorphism in anti-neutrophil cytoplasmic antibody (ANCA)-positive systemic vasculitis. Clin Exp Immunol 119(3):574–577

Tse WY et al (1999) No association between neutrophil FcgammaRIIa allelic polymorphism and anti-neutrophil cytoplasmic antibody (ANCA)-positive systemic vasculitis. Clin Exp Immunol 117(1):198–205

Kocher M et al (1998) Antineutrophil cytoplasmic antibodies preferentially engage Fc gammaRIIIb on human neutrophils. J Immunol 161(12):6909–6914

Fanciulli M et al (2007) FCGR3B copy number variation is associated with susceptibility to systemic, but not organ-specific, autoimmunity. Nat Genet 39(6):721–723

Neira O et al (1996) Lyme disease in Chile. Prevalence study in selected groups. Rev Med Chil 124(5):537–544

Kelley JM et al (2011) IgA and IgG antineutrophil cytoplasmic antibody engagement of Fc receptor genetic variants influences granulomatosis with polyangiitis. Proc Natl Acad Sci U S A 108(51):20736–20741

Shrestha S et al (2012) Role of activating FcgammaR gene polymorphisms in Kawasaki disease susceptibility and intravenous immunoglobulin response. Circ Cardiovasc Genet 5(3):309–316

Onouchi Y et al (2012) A genome-wide association study identifies three new risk loci for Kawasaki disease. Nat Genet 44(5):517–521

Taniuchi S et al (2005) Polymorphism of Fc gamma RIIa may affect the efficacy of gamma-globulin therapy in Kawasaki disease. J Clin Immunol 25(4):309–313

Breunis WBH, Long T, Eileen Png, Geissler J, Nagelkerke S, Ellis J, Davila S, Chiea Chuen K, Levin M, Burgner D, Shimizu C, Burns JC.; Hibberd ML, Kuijpers TW (2013) Fc-Gamma Receptor Genetic Variation In Kawasaki Disease. In: American college of rheumatology2013, San Diego

Asano K et al (2013) Impact of allele copy number of polymorphisms in FCGR3A and FCGR3B genes on susceptibility to ulcerative colitis. Inflamm Bowel Dis 19(10):2061–2068

Harley IT et al (2009) Genetic susceptibility to SLE: new insights from fine mapping and genome-wide association studies. Nat Rev Genet 10(5):285–290

Salmon JE et al (1996) Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. J Clin Invest 97(5):1348–1354

Edberg JC et al (2002) Genetic linkage and association of Fcgamma receptor IIIA (CD16A) on chromosome 1q23 with human systemic lupus erythematosus. Arthritis Rheum 46(8): 2132–2140

Manger K et al (2002) Fcgamma receptor IIa, IIIa, and IIIb polymorphisms in German patients with systemic lupus erythematosus: association with clinical symptoms. Ann Rheum Dis 61(9):786–792

Karassa FB et al (2002) Role of the Fcgamma receptor IIa polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. Arthritis Rheum 46(6):1563–1571

Magnusson V et al (2004) Polymorphisms of the Fc gamma receptor type IIB gene are not associated with systemic lupus erythematosus in the Swedish population. Arthritis Rheum 50(4):1348–1350

Kyogoku C et al (2004) Association of Fcgamma receptor IIA, but not IIB and IIIA, polymorphisms with systemic lupus erythematosus: A family-based association study in Caucasians. Arthritis Rheum 50(2):671–673

Siriboonrit U et al (2003) Association of Fcgamma receptor IIb and IIIb polymorphisms with susceptibility to systemic lupus erythematosus in Thais. Tissue Antigens 61(5):374–383

Lee EB et al (2002) Fcgamma receptor IIIA polymorphism in Korean patients with systemic lupus erythematosus. Rheumatol Int 21(6):222–226

Chu ZT et al (2004) Association of Fcgamma receptor IIb polymorphism with susceptibility to systemic lupus erythematosus in Chinese: a common susceptibility gene in the Asian populations. Tissue Antigens 63(1):21–27

Weersma RK et al (2010) Association of FcgR2a, but not FcgR3a, with inflammatory bowel diseases across three Caucasian populations. Inflamm Bowel Dis 16(12):2080–2089

van der Pol WL et al (2003) Association of the Fc gamma receptor IIA-R/R131 genotype with myasthenia gravis in Dutch patients. J Neuroimmunol 144(1–2):143–147
Zhou XJ et al (2010a) FCGR2B gene polymorphism rather than FCGR2A, FCGR3A and FCGR3B is associated with anti-GBM disease in Chinese. Nephrol Dial Transplant 25(1): 97–101
Jonsen A et al (2007) Association between SLE nephritis and polymorphic variants of the CRP and FcgammaRIIIa genes. Rheumatology (Oxford) 46(9):1417–1421
Dong C et al (2013) FcgammaRIIIa SNPs and haplotypes affect human IgG binding and association with lupus nephritis in African Americans. Arthritis Rheum, doi: 10.1002/art.38337
Koene HR et al (1998b) The Fc gammaRIIIA-158F allele is a risk factor for systemic lupus erythematosus. Arthritis Rheum 41(10):1813–1818
Kyogoku C et al (2002b) Studies on the association of Fc gamma receptor IIA, IIB, IIIA and IIIB polymorphisms with rheumatoid arthritis in the Japanese: evidence for a genetic interaction between HLA-DRB1 and FCGR3A. Genes Immun 3(8):488–493
Alarcon GS et al (2006) Time to renal disease and end-stage renal disease in PROFILE: a multiethnic lupus cohort. PLoS Med 3(10):e396
Hatta Y et al (1999) Association of Fc gamma receptor IIIB, but not of Fc gamma receptor IIA and IIIA polymorphisms with systemic lupus erythematosus in Japanese. Genes Immun 1(1):53–60
Gonzalez-Escribano MF et al (2002) FcgammaRIIA, FcgammaRIIIA and FcgammaRIIIB polymorphisms in Spanish patients with systemic lupus erythematosus. Eur J Immunogenet 29(4):301–306
You Y et al (2008) Lack of association between Fc receptor-like 3 gene polymorphisms and systemic lupus erythematosus in Chinese population. J Dermatol Sci 52(2):118–122
Choi CB et al (2006) The -169C/T polymorphism in FCRL3 is not associated with susceptibility to rheumatoid arthritis or systemic lupus erythematosus in a case-control study of Koreans. Arthritis Rheum 54(12):3838–3841
Ramirez-Bello J et al (2013) Juvenile rheumatoid arthritis and asthma, but not childhood-onset systemic lupus erythematosus are associated with FCRL3 polymorphisms in Mexicans. Mol Immunol 53(4):374–378
Piotrowski P et al (2013) The FCRL3 -169T > C polymorphism might be associated with some autoantibody presence in patients with SLE in a Polish population. Mod Rheumatol 24(2):296–299
Mao C et al (2010) Association between Fc receptor-like 3 C169T polymorphism and risk of systemic lupus erythematosus: a meta-analysis. Mol Biol Rep 37(1):191–196
Song GG et al (2013) Fc receptor-like 3 (FCRL3) -169 C/T polymorphism and systemic lupus erythematosus: a meta-analysis. Rheumatol Int 33(9):2323–2329
Morgan AW et al (2000) Fcgamma receptor type IIIA is associated with rheumatoid arthritis in two distinct ethnic groups. Arthritis Rheum 43(10):2328–2334
Morgan AW et al (2003) FcgammaRIIIA-158 V and rheumatoid arthritis: a confirmation study. Rheumatology (Oxford) 42(4):528–533
Thabet MM et al (2007) FCRL3 promoter 169 CC homozygosity is associated with susceptibility to rheumatoid arthritis in Dutch Caucasians. Ann Rheum Dis 66(6):803–806
Eike MC et al (2008) The FCRL3 -169T > C polymorphism is associated with rheumatoid arthritis and shows suggestive evidence of involvement with juvenile idiopathic arthritis in a Scandinavian panel of autoimmune diseases. Ann Rheum Dis 67(9):1287–1291
Maehlen MT et al (2011) FCRL3 -169C/C genotype is associated with anti-citrullinated protein antibody-positive rheumatoid arthritis and with radiographic progression. J Rheumatol 38(11):2329–2335
Wu H et al (2010) Fc receptor-like 3 gene polymorphisms confer susceptibility to rheumatoid arthritis in a Chinese population. Hum Immunol 71(12):1203–1208

Bajpai UD et al (2012) A functional variant in FCRL3 is associated with higher Fc receptor-like 3 expression on T cell subsets and rheumatoid arthritis disease activity. Arthritis Rheum 64(8):2451–2459
Han SW et al (2012) FCRL3 gene polymorphisms contribute to the radiographic severity rather than susceptibility of rheumatoid arthritis. Hum Immunol 73(5):537–542
Zeng Z et al (2012) Association of FCRL4 polymorphisms on disease susceptibility and severity of ankylosing spondylitis in Chinese Han population. Clin Rheumatol 31(10):1449–1454
Tang X et al (2009) A single-nucleotide polymorphism marker within the FCRL5 gene and HLA-B27 positive Han Chinese ankylosing spondylitis patients. Tissue Antigens 74(4):314–316
Owen CJ et al (2007) Analysis of the Fc receptor-like-3 (FCRL3) locus in Caucasians with autoimmune disorders suggests a complex pattern of disease association. J Clin Endocrinol Metab 92(3):1106–1111
Duchatelet S et al (2008) FCRL3 -169CT functional polymorphism in type 1 diabetes and autoimmunity traits. Biomed Pharmacother 62(3):153–157
Inoue N et al (2012) Associations between autoimmune thyroid disease prognosis and functional polymorphisms of susceptibility genes, CTLA4, PTPN22, CD40, FCRL3, and ZFAT, previously revealed in genome-wide association studies. J Clin Immunol 32(6):1243–1252
Gu LQ et al (2010) Clinical associations of the genetic variants of CTLA-4, Tg, TSHR, PTPN22, PTPN12 and FCRL3 in patients with Graves' disease. Clin Endocrinol (Oxf) 72(2):248–255
Plagnol V et al (2011) Genome-wide association analysis of autoantibody positivity in type 1 diabetes cases. PLoS Genet 7(8):e1002216
Howson JM et al (2012) Genetic association of zinc transporter 8 (ZnT8) autoantibodies in type 1 diabetes cases. Diabetologia 55(7):1978–1984
Martinez A et al (2007a) FcRL3 and multiple sclerosis pathogenesis: role in autoimmunity? J Neuroimmunol 189(1–2):132–136
Matesanz F et al (2008) The high producer variant of the Fc-receptor like-3 (FCRL3) gene is involved in protection against multiple sclerosis. J Neuroimmunol 195(1–2):146–150
Martinez A et al (2007b) Epistatic interaction between FCRL3 and MHC in Spanish patients with IBD. Tissue Antigens 69(4):313–317
Zhou J et al (2012) Association of polymorphisms in the promoter region of FCER1A gene with atopic dermatitis, chronic uticaria, asthma, and serum immunoglobulin E levels in a Han Chinese population. Hum Immunol 73(3):301–305
Niwa Y et al (2010) FcepsilonRIalpha gene (FCER1A) promoter polymorphisms and total serum IgE levels in Japanese atopic dermatitis patients. Int J Immunogenet 37(2):139–141
Weidinger S et al (2008) Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. PLoS Genet 4(8):e1000166
Granada M et al (2012) A genome-wide association study of plasma total IgE concentrations in the Framingham Heart Study. J Allergy Clin Immunol 129(3):840–845 e21
MacGlashan D Jr (2005) IgE and FcepsilonRI regulation. Clin Rev Allergy Immunol 29(1):49–60
Kim YK et al (2007) Association and functional relevance of E237G, a polymorphism of the high-affinity immunoglobulin E-receptor beta chain gene, to airway hyper-responsiveness. Clin Exp Allergy 37(4):592–598
Zhang X et al (2004) The E237G polymorphism of the high-affinity IgE receptor beta chain and asthma. Ann Allergy Asthma Immunol 93(5):499–503
Laprise C et al (2000) Evidence for association and linkage between atopy, airway hyper-responsiveness, and the beta subunit Glu237Gly variant of the high-affinity receptor for immunoglobulin E in the French-Canadian population. Immunogenetics 51(8–9):695–702
Nagata H et al (2001) Association between nasal allergy and a coding variant of the Fc epsilon RI beta gene Glu237Gly in a Japanese population. Hum Genet 109(3):262–266
Li A, Hopkin JM (1997) Atopy phenotype in subjects with variants of the beta subunit of the high affinity IgE receptor. Thorax 52(7):654–655
Hizawa N et al (2000) A common FCER1B gene promoter polymorphism influences total serum IgE levels in a Japanese population. Am J Respir Crit Care Med 161(3 Pt 1):906–909

Kim SH et al (2006b) A polymorphism of MS4A2 (- 109T > C) encoding the beta-chain of the high-affinity immunoglobulin E receptor (FcepsilonR1beta) is associated with a susceptibility to aspirin-intolerant asthma. Clin Exp Allergy 36(7):877–883
Yang HJ et al (2014) Association of the MS4A2 gene promoter C-109T or the 7th exon E237G polymorphisms with asthma risk: A meta-analysis. Clin Biochem 47(7-8):605–611
Laitinen T et al (2000) Association study of the chromosomal region containing the FCER2 gene suggests it has a regulatory role in atopic disorders. Am J Respir Crit Care Med 161(3 Pt 1):700–706
Koster ES et al (2011) FCER2 T2206C variant associated with chronic symptoms and exacerbations in steroid-treated asthmatic children. Allergy 66(12):1546–1552
Tantisira KG et al (2007) FCER2: a pharmacogenetic basis for severe exacerbations in children with asthma. J Allergy Clin Immunol 120(6):1285–1291
Sharma V et al (2014) A role of FCER1A and FCER2 polymorphisms in IgE regulation. Allergy 69(2):231–236
Potaczek DP et al (2009) Interaction of functional FCER2 promoter polymorphism and phenotype-associated haplotypes. Tissue Antigens 74(6):534–538
Kaneko M et al (1995) Allergen-specific IgG1 and IgG3 through Fc gamma RII induce eosinophil degranulation. J Clin Invest 95(6):2813–2821
Jonsson F et al (2012) Human FcgammaRIIA induces anaphylactic and allergic reactions. Blood 119(11):2533–2544
Williams JW, Tjota MY, Sperling AI (2012) The contribution of allergen-specific IgG to the development of th2-mediated airway inflammation. J Allergy (Cairo) 2012:236075
Lau S et al (2005) Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood–report of the German Multicentre Allergy Study (MAS 90). Allergy 60(6):766–773
Bruhns P, Fremont S, Daeron M (2005) Regulation of allergy by Fc receptors. Curr Opin Immunol 17(6):662–669
Wu et al (2014) Functional Fc gamma receptor polymorphisms are associated with human allergy. PLOS one 9(2):e89196
Robledo G et al (2012) Association of the FCGR3A-158F/V gene polymorphism with the response to rituximab treatment in Spanish systemic autoimmune disease patients. DNA Cell Biol 31(12):1671–1677
Cooper N et al (2012) Platelet-associated antibodies, cellular immunity and FCGR3a genotype influence the response to rituximab in immune thrombocytopenia. Br J Haematol 158(4): 539–547
Dijstelbloem HM et al (1999) Fcgamma receptor polymorphisms in Wegener's granulomatosis: risk factors for disease relapse. Arthritis Rheum 42(9):1823–1827
Norsworthy P et al (1999) Overrepresentation of the Fcgamma receptor type IIA R131/R131 genotype in caucasoid systemic lupus erythematosus patients with autoantibodies to C1q and glomerulonephritis. Arthritis Rheum 42(9):1828–1832
Song YW et al (1998) Abnormal distribution of Fc gamma receptor type IIa polymorphisms in Korean patients with systemic lupus erythematosus. Arthritis Rheum 41(3):421–426
Dijstelbloem HM et al (2000) Fcgamma receptor polymorphisms in systemic lupus erythematosus: association with disease and in vivo clearance of immune complexes. Arthritis Rheum 43(12):2793–2800
Morgan AW et al (2006a) Association of FCGR2A and FCGR2A-FCGR3A haplotypes with susceptibility to giant cell arteritis. Arthritis Res Ther 8(4):R109
van der Pol WL et al (2000) IgG receptor IIa alleles determine susceptibility and severity of Guillain-Barre syndrome. Neurology 54(8):1661–1665
Khor CC et al (2011) Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. Nat Genet 43(12):1241–1246
Orru V et al (2013) Genetic variants regulating immune cell levels in health and disease. Cell 155(1):242–256

Chen JY et al (2006) Association of a transmembrane polymorphism of Fcgamma receptor IIb (FCGR2B) with systemic lupus erythematosus in Taiwanese patients. Arthritis Rheum 54(12):3908–3917

Olferiev M et al (2007) The role of activating protein 1 in the transcriptional regulation of the human FCGR2B promoter mediated by the -343 G - > C polymorphism associated with systemic lupus erythematosus. J Biol Chem 282(3):1738–1746

Morgan AW et al (2006b) Analysis of Fcgamma receptor haplotypes in rheumatoid arthritis: FCGR3A remains a major susceptibility gene at this locus, with an additional contribution from FCGR3B. Arthritis Res Ther 8(1):R5

Zhou XJ et al (2010b) Copy number variation of FCGR3A rather than FCGR3B and FCGR2B is associated with susceptibility to anti-GBM disease. Int Immunol 22(1):45–51

Foster CB et al (2001) Polymorphisms in inflammatory cytokines and Fcgamma receptors in childhood chronic immune thrombocytopenic purpura: a pilot study. Br J Haematol 113(3):596–599

Niederer HA et al (2010) Copy number, linkage disequilibrium and disease association in the FCGR locus. Hum Mol Genet 19(16):3282–3294

Nossent JC, Rischmueller M, Lester S (2012) Low copy number of the Fc-gamma receptor 3B gene FCGR3B is a risk factor for primary Sjogren's syndrome. J Rheumatol 39(11):2142–2147

Osuga Y et al (2011) Lymphocytes in endometriosis. Am J Reprod Immunol 65(1):1–10

Teles JS et al (2011) Association of FCRL3 C-169T promoter single-nucleotide polymorphism with idiopathic infertility and infertility-related endometriosis. J Reprod Immunol 89(2):212–215

Szczepanska M et al (2013) The FCRL3 -169T > C polymorphism and the risk of endometriosis-related infertility in a Polish population. Arch Gynecol Obstet 288(4):799–804

Bridging Autoantibodies and Arthritis: The Role of Fc Receptors

Hanane el Bannoudi, Andreea Ioan-Facsinay and René E. M. Toes

Abstract Autoantibodies represent a hallmark of Rheumatoid arthritis (RA), which is a chronic inflammatory autoimmune disease characterized by inflammation and damage in the joints. Anti-Citrullinated Protein Antibodies (ACPA) are the most prominent autoantibodies present in RA patients. These autoantibodies have been intensively investigated during the last 20 years due to their diagnostic and predictive value. Furthermore, they are believed to be involved in mediating the damage associated with RA. Antibodies of the IgG isotype interact with the immune system via Fcγ receptors expressed on immune cells as well as nonimmune cells. These receptors, therefore, form the bridge between Fcγ receptor-positive cells and antibodies complexed to antigen allowing the modulation and activation of cellular immune responses that are involved in immune defense against invading microorganisms. However, in case triggered by antibodies against self-antigens, they can also play a pivotal role in the induction and perpetuation of autoimmune diseases such as RA. Mouse models have been indispensably important for understanding the role of Fcγ receptors in the development of arthritis. Here we discuss the contribution of autoantibodies to the pathogenesis of arthritis in preclinical animal models, as well as RA, in relation to their interaction with the different (immune inhibitory and activating) Fcγ receptors.

Contents

1 Introduction 304
2 Autoantibodies in Rheumatoid Arthritis 305
2.1 Acpa 305
2.2 Anti-Carbamylated Protein Antibodies 306

H. el Bannoudi · A. Ioan-Facsinay · R. E. M. Toes (✉)
Department of Rheumatology, Leiden University Medical Center, C1-R, Albinusdreef 2, 2333 Leiden, ZA, The Netherlands
e-mail: R.E.M.Toes@lumc.nl

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_14,

3 Fcγ Receptors 306
3.1 Activating Fcγ Receptors in Mouse Models of Arthritis 308
3.2 Inhibitory Fcγ Receptor in Mouse Models for Arthritis 310
3.3 Human Fcγ Receptors 311
4 Association Between Fcγ Receptor Genes and RA 312
5 Conclusion 313
References 314

1 Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation in the joint, leading to the damage of cartilage and bone. It affects approximately 1 % of the general population (Gabriel 2001).

The pathogenesis of RA is not fully understood. Infiltration of immune cells (B cells, T cells, macrophages, and neutrophils) into the synovial tissue, together with a systemic presence of autoantibodies constitute the hallmark of RA. The infiltrating immune cells are considered to be an important source of cytokines, such as Interleukin-6 (IL-6), Tumor Necrosis factor α (TNF-α), and other pro-inflammatory cytokines. The produced cytokines will provide an inflammatory loop, by activating immune cells, stromal cells, or osteoclasts and/or by attracting additional immune cells, which will ultimately result in severe inflammation in the joints.

Development of RA is associated with the presence of certain human leukocyte antigen (HLA) alleles, pointing to a contribution of T cells to disease susceptibility (Gregersen et al. 1987). In addition, the presence of autoantibodies in most RA patients and the efficacy of B cell-depleting therapies points to a prominent role of B cells in the pathogenesis of RA.

Rheumatoid factor (RF) and Anti-Citrullinated Protein Antibodies (ACPA) are the most well-described autoantibodies in RA. RF autoantibodies are directed against the Fc region of IgG molecules, whereas ACPA are antibodies directed against proteins containing the posttranslationally acquired amino acid citrulline. The presence of these autoantibodies, in particular ACPA is associated with severe disease (Nishimura et al. 2007; Schellekens et al. 2000; Visser et al. 1996).

Autoantibodies have long been believed to be involved in the pathogenesis of RA. Their main effector functions are mediated by the Fc region, which can interact with the complement system and Fc receptors. Fc receptors are expressed on a variety of immune cells and represent the link between humoral responses and cellular effector functions. For each class of antibody, a distinct class of cell-bound Fc receptor has been described: Fcγ receptors for IgG antibodies, Fcα receptors for IgA, Fcε receptors for IgE, and Fcα/μ receptor for IgA/IgM.

Several studies have elegantly shown the importance of antibodies in the development of arthritis in various preclinical arthritis models. By using genetically modified mice, it was shown that both the complement system and Fcγ receptors (FcγR) play a role in mediating the pathogenic function of antibodies in

arthritis. In this review, we will not address the role of the complement system to arthritis development and progression, but focus on several aspects related to the autoantibody systems present in RA and their (potential) impact to contribute to disease pathogenesis through interaction with Fcγ receptors.

2 Autoantibodies in Rheumatoid Arthritis

2.1 Acpa

ACPA are present in a high percentage of RA patients and display a high disease specificity. ACPA have been shown to be a collection of antibodies directed against different citrulline-containing proteins. Some of these antibodies exhibit a significant level of cross-reactivity toward a variety of citrullinated proteins, while others seem to be rather specific. Analyses of sera from individuals at different stages of the disease (pre-clinical, pre-RA, RA) suggested that the fine-specificity of ACPA changes and broadens especially in the pre-RA stage, leading to a response against a large number of proteins in established disease (Ioan-Facsinay et al. 2011; van der Woude et al. 2010). Despite several studies extensively characterizing the ACPA response and the citrullinated antigens present in the joints of RA patients (e.g., vimentin, fibrinogen, etc.) (van et al. 2010; Wegner et al. 2010) the antigen(s) responsible for the induction of the citrullinated protein-specific B cell response are yet unknown.

ACPA, like RF, can be present years before the manifestation of the clinical symptoms associated with RA (Nielen et al. 2004) and can be detected systemically, as well as locally in synovial fluid (Kunkel et al. 1961a, b; Winchester et al. 1970). However, their presence is relatively more abundant in synovial fluid compared to serum. Furthermore, ACPA-producing B cells have been found to be present in peripheral blood as well as synovial fluid of RA patients (Amara et al. 2013; Bellatin et al. 2012; Kerkman et al. 2013), pointing to the local production of these antibodies. Interestingly, the presence of ACPA is predictive for a more aggressive disease course, resulting in a more severe joint damage (Meyer et al. 2006; van der Helm-van Mil AH et al. 2005). Together, these observations have led to the hypothesis that ACPA may have a pathogenic role in RA (Kuhn et al. 2006; Seeling et al. 2013). The beneficial effects observed with B cell depleting (anti-CD20) therapy and the effects of ACPA on osteoclastogenesis and bone loss support this hypothesis (Cohen et al. 2006).

Citrullination is a posttranslational modification whereby the amino acid arginine is modified into the amino acid citrulline. This process is mediated by Peptidyl Arginine Deiminases, enzymes that are encoded by genes harboring genetic variations that have been identified as risk factor for RA-development (Suzuki et al. 2003). Both the specificity of the ACPA-response for RA and the genetic associations in the genes encoding the enzymes creating the antigens recognized by ACPA, implicate a contribution of the "anti-citrulline" response to RA pathogenesis.

2.2 Anti-Carbamylated Protein Antibodies

Recently, a new category of antibodies in RA patients, anti-Carbamylated Protein (anti-CarP-antibodies) has been identified. Anti-CarP antibodies recognize carbamylated proteins containing a posttranslationally acquired homocitrulline-residue that is generated from a lysine residue upon exposition to cyanate.

Anti-CarP antibodies represent an autoantibody-family that is partially cross-reactive to citrullinated proteins. Forty five percent of RA patients harbor IgG autoantibodies recognizing carbamylated antigens in serum. Interestingly, anti-CarP autoantibodies are present in approximately 20 % of ACPA-negative RA patients. The presence of Anti-Carp autoantibodies is, like the presence of ACPA, predictive for disease development in subjects at risk to develop RA, as well as for a more severe disease course in patients suffering from established arthritis (Shi et al. 2011).

3 Fcγ Receptors

Fcγ receptors are cell surface glycoproteins that belong to the immunoglobulin superfamily. They interact with the Fc-part of IgG antibodies. In mice, four different classes of Fcγ receptors have been described: FcγRI, FcγRII, FcγRIII, and FcγRIV. In humans, 6 different Fc gamma receptors have been identified: FcγRI, FcγRIIA, FcγRIIB, FCγRIIC, FcγRIIIA, and FcγRIIIB. FcγRI is a high affinity receptor being able to bind monomeric IgG. All FcγRs can bind IgG complexed to antigen, so-called immune-complexes. FcγR are very homologous and share structural similarities. Likewise, a high molecular homology has been described for murine and human FcγRs, such as for FcγRI and FcγRIIB. The remaining members of the low affinity receptors share some similarities between mouse and human: mouse FcγRIII is related to human FcγRIIA and mouse FcγRIV is highly homologous to human FcγRIIIA (Nimmerjahn et al. 2005; Ravetch et al. 2001).

With the exception of FcγRII (mouse and human) and human FcγRIIIB, all Fcγ receptors are composed of a ligand-binding α chain associated with a dimer of covalently linked FcRγ chains. The latter is responsible for the intracellular signalling of FcγR, as well as for their surface expression. The intracellular domain of the FcRγ chain contains an immunoreceptor tyrosine-based activation motif (ITAM), which mediates cellular activation. In contrast, the intracellular domain of FcγRIIB contains an immunoreceptor tyrosine-based inhibition (ITIM) motif rendering it capable of inhibiting cellular activation induced via cell-activating receptors (Daeron et al. 1997). Human FcγRIIIB is an exception as this receptor is anchored in the membrane via a GPI-link and thus lacks an intracellular domain.

Animal models have been indispensable for the study of the role of FcγR in regulating immune responses. Different animal models for arthritis have been developed. In some of the models, disease development is dependent on the

presence of antibodies against an antigen present in the joint. This antigen is either an autoantigen (murine collagen in collagen-induced arthritis or collagen antibody-induced arthritis (CAIA), glucose-6-phosphate isomerase (GPI) in K/BxN model or K/BxN serum transfer model, murine proteoglycan in proteoglycan-induced arthritis (PGIA)) or a foreign antigen injected in the joint, against which antibodies were elicited previously through immunization (mBSA in antigen-induced arthritis) or passive antiserum transfer (lysozyme in immune complex-mediated arthritis). Not surprisingly, Fcγ receptors have been found to be relevant for disease development in these models, albeit their relative contribution can be different in different models. Establishing the individual role of each FcγR for disease development has been challenging due to their redundant function and cellular expression. Moreover, the effector pathways initiated by FcγR and the complement system upon binding of immune complexes (ICs) are highly redundant and a cross-talk between these two systems often occurs. Therefore, the development of mice deficient for individual or well-defined combinations of FcγR has been instrumental in investigating the role of these molecules in arthritis.

Collagen-Induced Arthritis (CIA) is a frequently used animal model for RA. CIA can be readily induced in mice with MHC haplotype H-2^q and H-2^r. Immunization of these mice with bovine collagen type II (CII) results in the induction of an antibody response cross-reactive with murine collagen. These antibodies are sufficient for the induction of arthritis and transfer of these antibodies into a naïve mice is able to transfer disease, independently of B and T cells (Stuart et al. 1983). The CAIA, in which one or a combination of a few collagen-specific antibodies is injected into naïve mice, is a passive model for arthritis, in which the effector phase of the disease can be investigated (Terato et al. 1992). Similar to CIA, PGIA is an arthritis model in which immunization with human proteoglycan leads to generation of antibodies cross-reactive to murine proteoglycan. These antibodies will induce disease by binding to their target in the joint and initiate inflammation (Mikecz et al. 1996).

Another widely used model of arthritis is the K/BxN model in which a self-antigen, GPI presented on the NOD MHC molecule H2-A^{g7} is recognized by the KRN TCR transgenic T cells, leading to activation of GPI-specific B cells and subsequent arthritis. Similar to the CIA model, serum transfer from diseased K/BxN mice can transfer disease to naïve mice, even in the absence of T and B cells (Korganow et al. 1999).

Antigen-induced arthritis (AIA) is a model in which mice are immunized systemically with methylated BSA (mBSA) leading to generation of BSA-specific antibodies and are subsequently injected with BSA in the knee. Formation of ICs in the knee will lead to arthritis (Hunneyball et al. 1986). Immune complex-induced arthritis (ICA) is similar to AIA, except that the mice are passively immunized against lysozyme by being injected with lysozyme-specific antiserum, followed by injection of the target antigen, lysozyme, in the knee (van Lent et al. 1992).

The findings regarding the role of FcγR-deficient mice in these most frequently used models of arthritis will be further discussed.

3.1 Activating Fcγ Receptors in Mouse Models of Arthritis

Activating FcγR are expressed on a diversity of immune cells such as monocytes, macrophages, neutrophils, mast cells, basophils, platelets, and NK cells (Nimmerjahn et al. 2006a, b).

These FcγR can induce, upon binding of ICs, a plethora of cellular effector functions such as antibody-dependent cellular cytotoxicity, degranulation, phagocytosis as well as the release of inflammatory cytokines. Interestingly, it has been shown that antigens, when complexed in antigen-antibody complexes promote a stronger T-cell-mediated immune response as compared to the antigen alone. This is explained by an enhanced DCs maturation and antigen presentation following triggering of FcγR by ICs (Regnault et al. 1999; Schuurhuis et al. 2002).

Intriguingly, FcγR are not only involved in the activation of "conventional" immune cells, but have also been shown to have a direct effect on osteoclast precursors, by affecting osteoclastogenesis. Remodeling of bone can result in bone erosions. This is directly relevant to RA as bone erosions are one of the hallmarks of RA. Bone remodeling can be modulated through the FcγR-mediated release of cytokines by classical immune cells that can subsequently modulate osteoclast development and activity. However, more recently, it was shown that FcγR expressed on osteoclasts enhance bone destruction by direct binding of autoantibodies resulting in enhanced osteoclasts generation and bone resorption (Seeling et al. 2013).

In most mouse models of arthritis, the absence of the FcRγ chain results in strongly diminished or absent disease, indicating that the FcRγ chain associated activating receptors, FcγRI, III and IV are involved in the development of the disease. In the CIA model, disruption of the common gamma chain (FcRγ) in arthritis-susceptible DBA1 mice results in the protection from CIA. Although both knockout and wildtype mice develop similar anti-CII-antibody titers and collagen-specific proliferative responses of splenocytes, they show a clear difference in the development of CIA, indicating that FcγR are crucially involved in the effector phase of the disease (Kleinau et al. 2000). Likewise, FcRγ chain KO mice are protected against arthritis in the CAIA model (Kagari et al. 2003) and PGIA (Kaplan et al. 2002), the K/BxN serum transfer models (Ji et al. 2002) as well as immune-complex-mediated-arthritis (ICA) (Blom et al. 2000). In the AIA model, expression of the FcRγ chain is crucial for the chronic phase of cartilage destruction, although it does not affect the early phase of cartilage degradation (initial 7 days of disease) (van Lent et al. 2000).

These findings obtained underlie the significance of the activating Fcγ receptors in arthritis development in those models that crucially depend on the contribution of antibodies. The relative contribution of the individual activating FcγR has been addressed using well-defined KO mice lacking one or more activating Fcγ receptors. These studies showed a differential involvement, depending on the models used (Boross et al. 2008; Diaz de et al. 2002; Ioan-Facsinay et al. 2002;

Kagari et al. 2003; Kaplan et al. 2005; Kleinau et al. 2000; Nabbe et al. 2003; Nandakumar et al. 2003; van Lent et al. 2001, 2006).

FcγRIII is generally considered to be the most prominent FcγR in mediating inflammation and is critically required for (early) onset of arthritis, whereas FcγRI is predominantly involved in cartilage destruction in some models.

FcγRIII plays a prominent role in most models described above, except the AIA model. Elegant studies using cell-specific FcγR deficient mice have indicated that FcγR are engaged in different stages of the disease and on different immune cells. In the K/BxN model, FcγRIII is required for access of pathogenic antibodies to the joints (Binstadt et al. 2006), while expression of a FcRγ chain-dependent receptor on neutrophils (other than FcγRIII) is required for migration of neutrophils into the joint (Monach et al. 2010). As FcγRIII-deficient mice were largely protected against arthritis in this model, while mice lacking FcγRIII specifically on neutrophils developed the disease, it is likely that FcγRIII on other cells in the joint, possibly mast cells, essentially contributes to the disease. Also, a contribution of other FcRγ chain-dependent receptors should be considered (discussed below).

In the CIA model, arthritis has been shown to be crucially dependent on neutrophils and macrophages and FcγRIII. Interestingly, adoptive transfer of FcγRIII+ macrophages into FcγRIII-deficient mice restores the susceptibility to arthritis (Andren et al. 2006), indicating that FcγRIII on macrophages is essential for disease development.

Similar to the previous models, in mice lacking FcγRIII, the influx and activation of inflammatory cells was reduced as was the cartilage destruction in the ICA model. FcγRIII ko mice were also resistant to PGIA-induced arthritis (Kaplan et al. 2005).

In the passive K/BxN model, arthritis is abrogated in mice lacking the FcRγ chain (Kyburz et al. 2000), but is not totally abolished in mice lacking FcγRIII, suggesting that FcγRI and FγRIV are also involved in the induction of arthritis (Ji et al. 2002). Their contribution was investigated in two elegant studies using mice deficient for several Fc receptors (Boross et al. 2008; Mancardi et al. 2011). These studies indicated that FcγRIV was sufficient for arthritis induction in K/BxN mice, in the absence of all other FcγR (Mancardi et al. 2011). Moreover, neutrophils and monocytes/macrophages were required for arthritis in these ko mice (Mancardi et al. 2011). While these studies suggested no role of FcγRI in the K/BxN model of arthritis, one publication showed that FcγRI can play a role in the CIA model, in the absence of FcγRIII and FcγRII (Boross et al. 2008). This indicates that the involvement of FcγRI is probably secondary to that of FcγRIII and becomes evident only at high titer collagen-specific antibodies, such as are found in the FcγRII ko mice with CIA (Boross et al. 2008).

FcγRI is the predominant Fcγ receptor involved in the induction of arthritis in the AIA model. Interestingly, inflammatory cell influx is unchanged in the absence of FcγRI/II/III in these mice compared with FcγRII deficient mice, indicating an involvement of either FcγRIV or complement in this process (van Lent et al. 2006). However, cartilage destruction was greatly reduced in the FcγRI-deficient

mice (Ioan-Facsinay et al. 2002) and unchanged in FcγRIII ko mice (van Lent et al. 2000), suggesting a major role of FcγRI in this model.

FcγRIV is not only expressed on macrophages/monocytes and neutrophils but also on osteoclasts. These cells differentiate from monocytes following stimulation via receptor activator of nuclear factor-kappa B ligand (RANKL) and are responsible for the bone destruction. It is becoming apparent that FcγRIV can have a role in the regulation of osteoclasts maturation. FcγRIV expressed on immature osteoclasts is upregulated during maturation and upon autoantibody triggering results in further enhancement of the osteoclast differentiation. Noteworthy, targeted depletion of FcγRIV on osteoclasts protects mice from antibody-dependent bone destruction and reduces the number of osteoclasts in the inflamed joints. Together indicating the potency of FcγRIV to directly contribute to bone-erosions during arthritis (Seeling et al. 2013).

FcγRIIA is the most widespread human activating FcγR but is not expressed on murine cells. In order to understand its potential role in the development of RA, transgenic mice bearing the human *FcγRIIA* gene have been generated. Introduction of the *FcγRIIA* gene confers higher susceptibility to CIA and CAIA (Tan et al. 2005). In CAIA, FcγRIIA promotes a severe and a rapid arthritis development possibly by enhancing the production of TNF-α by macrophages, which is a prototypic pro-inflammatory cytokine indisputably implicated in the pathogenesis of RA. This could indicate that FcγRIIA contributes to the development of arthritis by decreasing the activation threshold of, for example macrophages, leading to overproduction of pro-inflammatory cytokines such TNF-α (Tan et al. 2005). More recently, another study has shown that expression of FcγRIIA on neutrophils is sufficient to restore susceptibility to arthritis in the K/BxN serum transfer model, in mice lacking all endogenous activating FcγR (Tsuboi et al. 2011), indicating a crucial role for neutrophils in this model.

3.2 Inhibitory Fcγ Receptor in Mouse Models for Arthritis

The inhibitory receptor FcγRIIB is the most broadly distributed FcγR. It is expressed, among others, by monocytes, macrophages, B cells, neutrophils, basophils, mast cells, and dendritic cells (DCs) (Daeron et al. 1997; Malbec et al. 2002; Ravetch et al. 2001). The ligand binding site located on the extracellular portion is similar to that of activating receptors, however, the cytoplasmic domain is different. The intracellular portion contains an ITIM motif to which the function of the receptor is attributed. FcγRIIB has a central role in maintaining tolerance by regulating the humoral response, which is remarkably enhanced in FcγRIIB-deficient mice upon antigen challenge. Due to its inhibitory function, FcγRIIB has gained considerable attention in the study of autoimmune diseases such as RA. Its role in the development of CIA is well established. Deletion of FcγRIIB renders mice with either a permissive or a nonpermissive background susceptible to CIA (Kleinau

et al. 2000; Yuasa et al. 1999). The importance of FcγRIIB in arthritis inhibition is further confirmed in the PGIA, K/BxN, AIA, and CAIA arthritis models (Corr et al. 2002; Kaplan et al. 2002; Nandakumar et al. 2003; van Lent et al. 2001).

FcγRIIB is believed to maintain an appropriate immune response and to prevent arthritis development by regulating antibody production and excessive cell mediated immune responses. When this regulation is disturbed, mice become susceptible for the development of arthritis. Abrogation of the FcγRIIB leads to a heightened production of pathogenic antibodies in most models of arthritis (Kaplan et al. 2002; van Lent et al. 2001; Yuasa et al. 1999). Indeed, the expression of the FcγRIIB on B cells was found to be crucial for the protection against arthritis indicating a role for FcγRIIB in maintaining B cell tolerance/responsiveness (Brownlie et al. 2008). Likewise, FcγRII has a major role at the level of effector cell function, as macrophages of FcγRIIB knockout mice show a hyper-responsiveness to IgG ICs stimulation resulting in an augmented production of pro-inflammatory cytokines (Yuasa et al. 1999). This dual function of FcγRII is probably the underlying mechanism for the increased susceptibility of FcγRIIB deficient mice for arthritis in most models studied (Corr et al. 2002; Kaplan et al. 2002; Nandakumar et al. 2003; van Lent, Nabbe et al. 2001)

3.3 Human Fcγ Receptors

A hallmark feature of RA is the presence of autoantibodies directed against different antigens (Ioan-Facsinay et al. 2011). The high prevalence of autoantibodies can give rise to the formation of antibody antigen complexes. Indeed, ICs can be found in the circulation as well as in the affected joints of RA patients (Kunkel et al. 1961a, b; Winchester et al. 1970; Zhao et al. 2008). Their presence is associated with a more severe disease suggesting a possible contribution of ICs in the pathogenesis of RA (Meyer et al. 2006; van der Helm-van Mil et al. 2005). ICs can elicit effector functions through interaction with FcγRs. In vitro formed ICs containing ACPA directed against citrullinated fibrinogen as well as ICs obtained from synovial fluid of RA patients have an inflammatory potential via their ability to activate macrophages to produce the pro-inflammatory cytokine TNF-α in a FcγRII dependent manner. The IgG content of the ICs correlates with TNF-α levels pointing to a contribution of FcγRs (Clavel et al. 2008; Laurent et al. 2011; Mathsson et al. 2006). Interestingly, neutrophils present in the synovial fluid of RA patients exhibit an activated phenotype and ICs have been considered to be their major activating factor (Robinson et al. 1994).

The balance of activating and inhibitory FcγR is proposed to be disturbed in RA patients. The expression level of FcγRI, FcγRIIA, and FcγRIII is reported to be increased in synovial macrophages, neutrophils, monocytes, and monocyte-derived macrophages of RA patients. This altered expression could lead to an enhanced production of TNF-α following stimulation with ICs (Blom et al. 2003; Quayle et al. 1997; Wijngaarden et al. 2004).

Considering its major role in regulating humoral and cellular immune responses in mice, one could predict that the expression of the inhibitory receptor FcγRIIB is decreased on immune cells of RA patients. Indeed, the expression of FcγRIIB on B cells as well as the percentage of B cells expressing FcγRIIB are reported to be lower in RA patients compared to healthy controls (Prokopec et al. 2010). Although the expression of FcγRIIB in RA patients was low, it was still able to inhibit the proliferation of B cells following a suboptimal stimulation with IgG anti-μ. However, the extent of the inhibition was reduced compared with B cells from control individuals expressing higher levels of FcγRIIB (Prokopec et al. 2010). Furthermore, in contrast to the expression of the activating Fcγ receptors, the expression of the inhibitory FcγRIIB on DCs is reported to be increased in RA patients having lower disease activity. This high expression of FcγRIIB was proposed to enable DCs to inhibit TLR mediated pro-inflammatory response, to suppress T cells proliferation and to promote a Th2 response and regulatory T cell development following stimulation with ICs (Wenink et al. 2009).

4 Association Between Fcγ Receptor Genes and RA

Fcγ receptors are encoded by several genes on chromosome 1 (Nimmerjahn et al. 2006a, b; Su et al. 2002). Variations in genes encoding Fcγ receptors are considered as modifying factors for disease-susceptibility or outcome in various autoimmune diseases (Myhr et al. 1999; Tan et al. 2000). Genetic variations like single nucleotide polymorphism (SNP) and copy number variation (CNV) have been reported to have biological consequences on the function of FcγR-complex. An SNP is a variation in a DNA sequence occurring as a result of a single nucleotide difference between paired chromosomes. CNV is defined as an alteration in the number of a certain sequence of DNA (1 kb) when compared with a reference genome. Normally each gene has two copies, however, it is becoming apparent that genes can have more or less than two copies.

Several studies have reported conflicting results on the potential contribution of CNV in the Fcγ-region, most likely as a consequence of the high-sequence homology in this region, making it challenging to perform genetic studies in FcγR-region (Thabet et al. 2009). Therefore, there is currently no consensus on the possible contribution of FcγR-CNV on disease susceptibility or severity.

Likewise, also most studies with respect to other genetic variations in the FcγR-region in relation to RA-susceptibility and severity should be taken with caution as opposing findings have been reported.

FcγRIIA and *FcγRIIIA* have been considered as possible disease modifying genes in RA (Alizadeh et al. 2007; Milicic et al. 2002; Morgan et al. 2000, 2003; Thabet et al. 2009). These genes harbor SNPs in the region encoding IgG binding site of the FcγRIIA and FcγRIIIA receptors leading to the rise of two receptors with different binding capabilities to IgG ICs (Koene et al. 1997; Parren et al. 1992; Warmerdam et al. 1991). The higher affinity binding variant of FcγRIIA has

a histidine at position 131, whereas the low affinity binding receptor has an arginine at position 131(Salmon et al. 1992). FcγRIIIA containing a valine (V) at position 158 is a high binding receptor whereas FcγRIIIA with a phenylalanine (F) at position 158 is a low binding receptor (Ravetch et al. 1989).

Patients homozygous or heterozygous for *FcγRIIA* arginine allele have been reported to suffer from a more severe disease RA as compared to individuals homozygous for *FcγRIIA* histidine allele. The presence of the low binding receptors on immune cells might contribute to the development of a severe disease via their impaired ability to clear ICs (Brun et al. 2002). Likewise, the risk for developing RA is reported to be increased especially in individuals homozygous for *FcγRIIIA*-158 V allele (Morgan et al. 2000). However, these findings are not consistent as other studies have found no association between *FcγRIIA*, *FcγRIIIA* genes and RA (Kyogoku et al. 2002a, b; Morgan et al. 2006; Nieto et al. 2000).

By analogy, *FcγRIIIB* gene has two common polymorphic forms, Neutrophils antigen 1(NA1) and NA2, which differ in five nucleotides that result in four amino acid differences. The presence of *FcγRIIIB*-NA2 is reported to increase the level of association between *FcγRIIIA*-V158 and RA compared to *FcγRIIIA*-158F where RA patients with nodules showed the strongest association (Morgan et al. 2006).

An SNP in the gene region encoding for the inhibitory receptor FcγRIIB (Ile232Thr) has been characterized as well (Kyogoku et al. 2002a, b). The presence of this variant is associated with an increased radiological joint damage and was found not to be linked to the susceptibility to RA. Therefore, it is mainly considered as a marker for RA disease severity (Radstake et al. 2006).

Most importantly, a recent large, meta-analyses incorporating many genome wide association studies involving more than 100,000 subjects probably provides the best evidence for an association between the FcγR-region and RA. In this study, no association between RA and, for example, the common polymorphic forms of FcγRIIB and *FcγRIIIB* has been reported, indicating that former studies indicating a potential association between *FcγRIII* and RA should be taken with great caution. However, this study did reveal an association between an SNP near the *FcγRIIA* gene (OR 1.13; 95 % CI: 1.08–1.19), providing relevant evidence for a contribution of the *FcγRIIA* gene in RA susceptibility (Okada et al. 2014).

5 Conclusion

Since the discovery of the Fcγ receptors, considerable efforts have been made to understand their role in the development of RA. Using mouse models we have gained great knowledge about the role of these receptors in the maintenance of tolerance and the induction of arthritis and bone damage in preclinical animal models. Although we are still far from fully understanding of the relative contribution of different FcγR to development and progression of RA, current evidence clearly points to a contribution of FcγR in the pathogenesis of autoantibody-positive RA.

References

Alizadeh BZ, Valdigem G, Coenen MJ, Zhernakova A, Franke B, Monsuur A, van Riel PL, Barrera P, Radstake TR, Roep BO, Wijmenga C, Koeleman BP (2007) Association analysis of functional variants of the FcgRIIa and FcgRIIIa genes with type 1 diabetes, celiac disease and rheumatoid arthritis. Hum Mol Genet 16(21):2552–2559

Amara K, Steen J, Murray F, Morbach H, Fernandez-Rodriguez BM, Joshua V, Engstrom M, Snir O, Israelsson L, Catrina AI, Wardemann H, Corti D, Meffre E, Klareskog L, Malmstrom V (2013) Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition. J Exp Med 210(3):445–455

Andren M, Xiang Z, Nilsson G, Kleinau S (2006) FcgammaRIII-expressing macrophages are essential for development of collagen-induced arthritis. Scand J Immunol 63(4):282–289

Bellatin MF, Han M, Fallena M, Fan L, Xia D, Olsen N, Branch V, Karp D, Stastny P (2012) Production of autoantibodies against citrullinated antigens/peptides by human B cells. J Immunol 188(7):3542–3550

Binstadt BA, Patel PR, Alencar H, Nigrovic PA, Lee DM, Mahmood U, Weissleder R, Mathis D, Benoist C (2006) Particularities of the vasculature can promote the organ specificity of autoimmune attack. Nat Immunol 7(3):284–292

Blom AB, Radstake TR, Holthuysen AE, Sloetjes AW, Pesman GJ, Sweep FG, van de Loo FA, Joosten LA, Barrera P, van Lent PL, van den Berg WB (2003) Increased expression of Fcgamma receptors II and III on macrophages of rheumatoid arthritis patients results in higher production of tumor necrosis factor alpha and matrix metalloproteinase. Arthritis Rheum 48(4):1002–1014

Blom AB, van Lent PL, van VH, Holthuysen AE, Jacobs C, van de Putte, LB, van de Winkel JG, van den Berg WB (2000) Fc gamma R expression on macrophages is related to severity and chronicity of synovial inflammation and cartilage destruction during experimental immune-complex-mediated arthritis (ICA). Arthritis Res 2(6):489–503

Boross P, van Lent PL, Martin-Ramirez J, van der Kaa J, Mulder MH, Claassens JW, van den Berg WB, Arandhara VL, Verbeek JS (2008) Destructive arthritis in the absence of both FcgammaRI and FcgammaRIII. J Immunol 180(7):5083–5091

Brownlie RJ, Lawlor KE, Niederer HA, Cutler AJ, Xiang Z, Clatworthy MR, Floto RA, Greaves DR, Lyons PA, Smith KG (2008) Distinct cell-specific control of autoimmunity and infection by FcgammaRIIb. J Exp Med 205(4):883–895

Brun JG, Madland TM, Vedeler CA (2002) Immunoglobulin G fc-receptor (FcgammaR) IIA, IIIA, and IIIB polymorphisms related to disease severity in rheumatoid arthritis. J Rheumatol 29(6):1135–1140

Clavel C, Nogueira L, Laurent L, Iobagiu C, Vincent C, Sebbag M, Serre G (2008) Induction of macrophage secretion of tumor necrosis factor alpha through Fcgamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. Arthritis Rheum 58(3):678–688

Cohen SB, Emery P, Greenwald MW, Dougados M, Furie RA, Genovese MC, Keystone EC, Loveless JE, Burmester GR, Cravets MW, Hessey EW, Shaw T, Totoritis MC (2006) Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum 54(9):2793–2806

Corr M, Crain B (2002) The role of FcgammaR signaling in the K/B x N serum transfer model of arthritis. J Immunol 169(11):6604–6609

Daeron M (1997) Negative regulation of mast cell activation by receptors for IgG. Int Arch Allergy Immunol 113(1–3):138–141

de Diaz ST, Andren M, Martinsson P, Verbeek JS, Kleinau S (2002) Expression of FcgammaRIII is required for development of collagen-induced arthritis. Eur J Immunol 32(10):2915–2922

Gabriel SE (2001) The epidemiology of rheumatoid arthritis. Rheum Dis Clin North America 27(2):269–281

Gregersen PK, Silver J, Winchester RJ (1987) The shared epitope hypothesis—an approach to understanding the molecular-genetics of susceptibility to rheumatoid-arthritis. Arthritis Rheum 30(11):1205–1213

Hunneyball IM, Crossley MJ, Spowage M (1986) Pharmacological studies of antigen-induced arthritis in BALB/c mice. I. characterization of the arthritis and the effects of steroidal and non-steroidal anti-inflammatory agents. Agents Actions 18(3–4):384–393

Ioan-Facsinay A, de Kimpe SJ, Hellwig SM, van Lent PL, Hofhuis FM, van Ojik HH, Sedlik C, da Silveira SA, Gerber J, de Jong YF, Roozendaal R, Aarden LA, van den Berg WB, Saito T, Mosser D, Amigorena S, Izui S, van Ommen GJ, van VM, van de Winkel JG, Verbeek JS (2002) FcgammaRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. Immunity 16(3):391–402

Ioan-Facsinay A, El-Bannoudi H, Scherer HU, van der Woude D, Menard HA, Lora M, Trouw LA, Huizinga TWJ, Toes REM (2011) Anti-cyclic citrullinated peptide antibodies are a collection of anti-citrullinated protein antibodies and contain overlapping and non-overlapping reactivities. Ann Rheum Dis 70(1):188–193

Ji H, Ohmura K, Mahmood U, Lee DM, Hofhuis FM, Boackle SA, Takahashi K, Holers VM, Walport M, Gerard C, Ezekowitz A, Carroll MC, Brenner M, Weissleder R, Verbeek JS, Duchatelle V, Degott C, Benoist C, Mathis D (2002) Arthritis critically dependent on innate immune system players. Immunity 16(2):157–168

Kagari T, Tanaka D, Doi H, Shimozato T (2003) Essential role of Fc gamma receptors in anti-type II collagen antibody-induced arthritis. J Immunol 170(8):4318–4324

Kaplan CD, Cao Y, Verbeek JS, Tunyogi-Csapo M, Finnegan A (2005) Development of proteoglycan-induced arthritis is critically dependent on Fcgamma receptor type III expression. Arthritis Rheum 52(5):1612–1619

Kaplan CD, O'Neill SK, Koreny T, Czipri M, Finnegan A (2002) Development of inflammation in proteoglycan-induced arthritis is dependent on Fc gamma R regulation of the cytokine/chemokine environment. J Immunol 169(10):5851–5859

Kerkman PF, Rombouts Y, van der Voort EI, Trouw LA, Huizinga TW, Toes RE, Scherer HU (2013) Circulating plasmablasts/plasmacells as a source of anticitrullinated protein antibodies in patients with rheumatoid arthritis. Ann Rheum Dis 72(7):1259–1263

Kleinau S, Martinsson P, Heyman B (2000) Induction and suppression of collagen-induced arthritis is dependent on distinct fcgamma receptors. J Exp Med 191(9):1611–1616

Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de HM (1997) Fc gammaRIIIa-158 V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood 90(3):1109–1114 (PM:9242542)

Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, Degott C, Kikutani H, Rajewsky K, Pasquali JL, Benoist C, Mathis D (1999) From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. Immunity 10(4):451–461

Kuhn KA, Kulik L, Tomooka B, Braschler KJ, Arend WP, Robinson WH, Holers VM (2006) Antibodies against citrullinated proteins enhance tissue injury in experimental autoimmune arthritis. J Clin Invest 116(4):961–973

Kunkel HG, Muller-Eberhard HJ, Fudenberg HH, Tomasi TB (1961a) Gamma globulin complexes in rheumatoid arthritis and certain other conditions. J Clin Invest 40:117–129

Kunkel HG, Muller-Eberhard HJ, Fudenberg HH, Tomasi TB (1961b) Gamma globulin complexes in rheumatoid arthritis and certain other conditions. J Clin Invest 40:117–129

Kyburz D, Carson DA, Corr M (2000) The role of CD40 ligand and tumor necrosis factor alpha signaling in the transgenic K/BxN mouse model of rheumatoid arthritis. Arthritis Rheum 43(11):2571–2577

Kyogoku C, Dijstelbloem HM, Tsuchiya N, Hatta Y, Kato H, Yamaguchi A, Fukazawa T, Jansen MD, Hashimoto H, van de Winkel JG, Kallenberg CG, Tokunaga K (2002a) Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. Arthritis Rheum 46(5):1242–1254

Kyogoku C, Tsuchiya N, Matsuta K, Tokunaga K (2002b) Studies on the association of Fc gamma receptor IIA, IIB, IIIA and IIIB polymorphisms with rheumatoid arthritis in the Japanese: evidence for a genetic interaction between HLA-DRB1 and FCGR3A. Genes Immun 3(8):488–493

Laurent L, Clavel C, Lemaire O, Anquetil F, Cornillet M, Zabraniecki L, Nogueira L, Fournie B, Serre G, Sebbag M (2011) Fcgamma receptor profile of monocytes and macrophages from rheumatoid arthritis patients and their response to immune complexes formed with autoantibodies to citrullinated proteins. Ann Rheum Dis 70(6):1052–1059

Malbec O, Attal JP, Fridman WH, Daeron M (2002) Negative regulation of mast cell proliferation by FcgammaRIIB. Mol Immunol 38(16–18):1295–1299

Mancardi DA, Jonsson F, Iannascoli B, Khun H, van RN, Huerre M, Daeron M, Bruhns P (2011) Cutting Edge: The murine high-affinity IgG receptor FcgammaRIV is sufficient for autoantibody-induced arthritis. J Immunol 186(4):1899–1903

Mathsson L, Lampa J, Mullazehi M, Ronnelid J (2006) Immune complexes from rheumatoid arthritis synovial fluid induce FcgammaRIIa dependent and rheumatoid factor correlated production of tumour necrosis factor-alpha by peripheral blood mononuclear cells. Arthritis Res Ther 8(3):R64

Meyer O, Nicaise-Roland P, Santos MD, Labarre C, Dougados M, Goupille P, Cantagrel A, Sibilia J, Combe B (2006) Serial determination of cyclic citrullinated peptide autoantibodies predicted five-year radiological outcomes in a prospective cohort of patients with early rheumatoid arthritis. Arthritis Res Ther 8(2):R40

Mikecz K, Glant TT (1996) Immunoregulation of proteoglycan-induced arthritis in Balb/c mice. Am J Ther 3(1):42–51

Milicic A, Misra R, Agrawal S, Aggarwal A, Brown MA, Wordsworth BP (2002) The F158 V polymorphism in FcgammaRIIIA shows disparate associations with rheumatoid arthritis in two genetically distinct populations. Ann Rheum Dis 61(11):1021–1023

Monach PA, Nigrovic PA, Chen M, Hock H, Lee DM, Benoist C, Mathis D (2010) Neutrophils in a mouse model of autoantibody-mediated arthritis: critical producers of Fc receptor gamma, the receptor for C5a, and lymphocyte function-associated antigen 1. Arthritis Rheum 62(3):753–764

Morgan AW, Barrett JH, Griffiths B, Subramanian D, Robinson JI, Keyte VH, Ali M, Jones EA, Old RW, Ponchel F, Boylston AW, Situnayake RD, Markham AF, Emery P, Isaacs JD (2006) Analysis of Fcgamma receptor haplotypes in rheumatoid arthritis: FCGR3A remains a major susceptibility gene at this locus, with an additional contribution from FCGR3B. Arthritis Res Ther 8(1):R5

Morgan AW, Griffiths B, Ponchel F, Montague BM, Ali M, Gardner PP, Gooi HC, Situnayake RD, Markham AF, Emery P, Isaacs JD (2000) Fcgamma receptor type IIIA is associated with rheumatoid arthritis in two distinct ethnic groups. Arthritis Rheum 43(10):2328–2334

Morgan AW, Keyte VH, Babbage SJ, Robinson JI, Ponchel F, Barrett JH, Bhakta BB, Bingham SJ, Buch MH, Conaghan PG, Gough A, Green M, Lawson CA, Pease CT, Markham AF, Ollier WE, Emery P, Worthington J, Isaacs JD (2003) FcgammaRIIIA-158 V and rheumatoid arthritis: a confirmation study. Rheumatology (Oxford) 42(4):528–533

Myhr KM, Raknes G, Nyland H, Vedeler C (1999) Immunoglobulin G Fc-receptor (FcgammaR) IIA and IIIB polymorphisms related to disability in MS. Neurology 52(9):1771–1776

Nabbe KC, Blom AB, Holthuysen AE, Boross P, Roth J, Verbeek S, van Lent PL, van den Berg WB (2003) Coordinate expression of activating Fc gamma receptors I and III and inhibiting Fc gamma receptor type II in the determination of joint inflammation and cartilage destruction during immune complex-mediated arthritis. Arthritis Rheum 48(1):255–265

Nandakumar KS, Andren M, Martinsson P, Bajtner E, Hellstrom S, Holmdahl R, Kleinau S (2003) Induction of arthritis by single monoclonal IgG anti-collagen type II antibodies and enhancement of arthritis in mice lacking inhibitory FcgammaRIIB. Eur J Immunol 33(8):2269–2277

Nielen MM, van SD, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, Habibuw MR, Vandenbroucke JP, Dijkmans BA (2004) Specific autoantibodies precede the

symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 50(2):380–386

Nieto A, Caliz R, Pascual M, Mataran L, Garcia S, Martin J (2000) Involvement of Fcgamma receptor IIIA genotypes in susceptibility to rheumatoid arthritis. Arthritis Rheum 43(4):735–739

Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV (2005) FcgammaRIV: a novel FcR with distinct IgG subclass specificity. Immunity 23(1):41–51

Nimmerjahn F, Ravetch JV (2006a) Fcgamma receptors: old friends and new family members. Immunity 24(1):19–28

Nimmerjahn F, Ravetch JV (2006b) Fcgamma receptors: old friends and new family members. Immunity 24(1):19–28

Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, Saigo K, Morinobu A, Koshiba M, Kuntz KM, Kamae I, Kumagai S (2007) Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med 146(11):797–808

Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, Yoshida S, Graham RR, Manoharan A, Ortmann W, Bhangale T, Denny JC, Carroll RJ, Eyler AE, Greenberg JD, Kremer JM, Pappas DA, Jiang L, Yin J, Ye L, Su DF, Yang J, Xie G, Keystone E, Westra HJ, Esko T, Metspalu A, Zhou X, Gupta N, Mirel D, Stahl EA, Diogo D, Cui J, Liao K, Guo MH, Myouzen K, Kawaguchi T, Coenen MJ, van Riel PL, van de Laar MA, Guchelaar HJ, Huizinga TW, Dieude P, Mariette X, Bridges SL Jr, Zhernakova A, Toes RE, Tak PP, Miceli-Richard C, Bang SY, Lee HS, Martin J, Gonzalez-Gay MA, Rodriguez-Rodriguez L, Rantapaa-Dahlqvist S, Arlestig L, Choi HK, Kamatani Y, Galan P, Lathrop M, Eyre S, Bowes J, Barton A, de VN, Moreland LW, Criswell LA, Karlson EW, Taniguchi A, Yamada R, Kubo M, Liu JS, Bae SC, Worthington J, Padyukov L, Klareskog L, Gregersen PK, Raychaudhuri S, Stranger BE, De Jager PL, Franke L, Visscher PM, Brown MA, Yamanaka H, Mimori T, Takahashi A, Xu H, Behrens TW, Siminovitch KA, Momohara S, Matsuda F, Yamamoto K, Plenge RM (2014) Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 506(7488):376–381

Parren PW, Warmerdam PA, Boeije LC, Arts J, Westerdaal NA, Vlug A, Capel PJ, Aarden LA, van de Winkel JG (1992) On the interaction of IgG subclasses with the low affinity Fc gamma RIIa (CD32) on human monocytes, neutrophils, and platelets. Analysis of a functional polymorphism to human IgG2. J Clin Invest 90(4):1537–1546

Prokopec KE, Rhodiner M, Matt P, Lindqvist U, Kleinau S (2010) Down regulation of Fc and complement receptors on B cells in rheumatoid arthritis. Clin Immunol 137(3):322–329

Quayle JA, Watson F, Bucknall RC, Edwards SW (1997) Neutrophils from the synovial fluid of patients with rheumatoid arthritis express the high affinity immunoglobulin G receptor, Fc gamma RI (CD64): role of immune complexes and cytokines in induction of receptor expression. Immunology 91(2):266–273

Radstake TR, Franke B, Wenink MH, Nabbe KC, Coenen MJ, Welsing P, Bonvini E, Koenig S, van den Berg WB, Barrera P, van Riel PL (2006) The functional variant of the inhibitory Fcgamma receptor IIb (CD32B) is associated with the rate of radiologic joint damage and dendritic cell function in rheumatoid arthritis. Arthritis Rheum 54(12):3828–3837

Ravetch JV, Bolland S (2001) IgG Fc receptors. Annu Rev Immunol 19:275–290

Ravetch JV, Perussia B (1989) Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions. J Exp Med 170(2):481–497

Regnault A, Lankar D, Lacabanne V, Rodriguez A, Thery C, Rescigno M, Saito T, Verbeek S, Bonnerot C, Ricciardi-Castagnoli P, Amigorena S (1999) Fcgamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. J Exp Med 189(2):371–380

Robinson JJ, Watson F, Bucknall RC, Edwards SW (1994) Role of Fc gamma receptors in the activation of neutrophils by soluble and insoluble immunoglobulin aggregates isolated from the synovial fluid of patients with rheumatoid arthritis. Ann Rheum Dis 53(8):515–520

Salmon JE, Edberg JC, Brogle NL, Kimberly RP (1992) Allelic polymorphisms of human Fc gamma receptor IIA and Fc gamma receptor IIIB. Independent mechanisms for differences in human phagocyte function. J Clin Invest 89(4):1274–1281

Schellekens GA, Visser H, de Jong BAW, Van den Hoogen FHJ, Hazes JMW, Breedveld FC, van Venrooij WJ (2000) The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 43(1):155–163

Schuurhuis DH, Ioan-Facsinay A, Nagelkerken B, van Schip JJ, Sedlik C, Melief CJ, Verbeek JS, Ossendorp F (2002) Antigen-antibody immune complexes empower dendritic cells to efficiently prime specific CD8 + CTL responses in vivo. J Immunol 168(5):2240–2246

Seeling M, Hillenhoff U, David JP, Schett G, Tuckermann J, Lux A, Nimmerjahn F (2013) Inflammatory monocytes and Fcgamma receptor IV on osteoclasts are critical for bone destruction during inflammatory arthritis in mice. Proc Natl Acad Sci USA 110(26):10729–10734

Shi J, Knevel R, Suwannalai P, van der Linden MP, Janssen GM, van Veelen PA, Levarht NE, van der Helm-van Mil AH, Cerami A, Huizinga TW, Toes RE, Trouw LA (2011) Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. Proc Natl Acad Sci USA 108(42):17372–17377

Stuart JM, Dixon FJ (1983) Serum transfer of collagen-induced arthritis in mice. J Exp Med 158(2):378–392

Su K, Wu J, Edberg JC, McKenzie SE, Kimberly RP (2002) Genomic organization of classical human low-affinity Fcgamma receptor genes. Genes Immun 3(Suppl 1):S51–S56

Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, Nagasaki M, Nakayama-Hamada M, Kawaida R, Ono M, Ohtsuki M, Furukawa H, Yoshino S, Yukioka M, Tohma S, Matsubara T, Wakitani S, Teshima R, Nishioka Y, Sekine A, Iida A, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K (2003) Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 34(4):395–402

Tan SY (2000) FcgammaRIIa polymorphism in systemic lupus erythematosus. Kidney Blood Press Res 23(2):138–142

Tan SC, Mottram PL, van de Velde NC, Powell MS, Power D, Slocombe RF, Wicks IP, Campbell IK, McKenzie SE, Brooks M, Stevenson AW, Hogarth PM (2005) Development of spontaneous multisystem autoimmune disease and hypersensitivity to antibody-induced inflammation in Fcgamma receptor IIa-transgenic mice. Arthritis Rheum 52(10):3220–3229

Terato K, Hasty KA, Reife RA, Cremer MA, Kang AH, Stuart JM (1992) Induction of arthritis with monoclonal antibodies to collagen. J Immunol 148(7):2103–2108

Thabet MM, Huizinga TW, Marques RB, Stoeken-Rijsbergen G, Bakker AM, Kurreeman FA, White SJ, Toes RE, van der Helm-van Mil AH (2009) Contribution of Fcgamma receptor IIIA gene 158 V/F polymorphism and copy number variation to the risk of ACPA-positive rheumatoid arthritis. Ann Rheum Dis 68(11):1775–1780

Tsuboi N, Ernandez T, Li X, Nishi H, Cullere X, Mekala D, Hazen M, Kohl J, Lee DM, Mayadas TN (2011) Regulation of human neutrophil Fcgamma receptor IIa by C5a receptor promotes inflammatory arthritis in mice. Arthritis Rheum 63(2):467–478

van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Toes RE, Huizinga TW (2005) Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. Arthritis Res Ther 7(5):R949–R958

van der Woude D, Rantapaa-Dahlqvist S, Ioan-Facsinay A, Onnekink C, Schwarte CM, Verpoort KN, Drijfhout JW, Huizinga TW, Toes RE, Pruijn GJ (2010) Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. Ann Rheum Dis 69(8):1554–1561

van Lent PL, Grevers L, Lubberts E, de Vries TJ, Nabbe KC, Verbeek S, Oppers B, Sloetjes A, Blom AB, van den Berg WB (2006) Fcgamma receptors directly mediate cartilage, but not bone, destruction in murine antigen-induced arthritis: uncoupling of cartilage damage from bone erosion and joint inflammation. Arthritis Rheum 54(12):3868–3877

van Lent PL, Nabbe K, Blom AB, Holthuysen AE, Sloetjes A, van de Putte LB, Verbeek S, van den Berg WB (2001) Role of activatory Fc gamma RI and Fc gamma RIII and inhibitory Fc gamma RII in inflammation and cartilage destruction during experimental antigen-induced arthritis. Am J Pathol 159(6):2309–2320

van Lent PL, van den Bersselaar LA, van den Hoek AE, van de Loo AA, van den Berg WB (1992) Cationic immune complex arthritis in mice–a new model. Synergistic effect of complement and interleukin-1. Am J Pathol 140(6):1451–1461

van Lent PL, van Vuuren AJ, Blom AB, Holthuysen AE, van de Putte LB, van de Winkel JG, van den Berg WB (2000) Role of Fc receptor gamma chain in inflammation and cartilage damage during experimental antigen-induced arthritis. Arthritis Rheum 43(4):740–752

Van SK, Tilleman K, De CM, De KF, Elewaut D, Deforce D (2010) Citrullinated vimentin as an important antigen in immune complexes from synovial fluid of rheumatoid arthritis patients with antibodies against citrullinated proteins. Arthritis Res Ther 12(4):R132

Visser H, Gelinck LBS, Kampfraath AH, Breedveld FC, Hazes JMW (1996) Diagnostic and prognostic characteristics of the enzyme linked immunosorbent rheumatoid factor assays in rheumatoid arthritis. Ann Rheum Dis 55(3):157–161

Warmerdam PA, van de Winkel JG, Vlug A, Westerdaal NA, Capel PJ (1991) A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. J Immunol 147(4):1338–1343

Wegner N, Lundberg K, Kinloch A, Fisher B, Malmstrom V, Feldmann M, Venables PJ (2010) Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. Immunol Rev 233(1):34–54

Wenink MH, Santegoets KC, Roelofs MF, Huijbens R, Koenen HJ, van BR, Joosten I, Meyer-Wentrup F, Mathsson L, Ronnelid J, Adema GJ, Bonvini E, Koenig S, van den Berg WB, van Riel PL, Radstake TR (2009) The inhibitory Fc gamma IIb receptor dampens TLR4-mediated immune responses and is selectively up-regulated on dendritic cells from rheumatoid arthritis patients with quiescent disease. J Immunol 183(7):4509–4520

Wijngaarden S, van de Winkel JG, Jacobs KM, Bijlsma JW, Lafeber FP, van Roon JA (2004) A shift in the balance of inhibitory and activating Fcgamma receptors on monocytes toward the inhibitory Fcgamma receptor IIb is associated with prevention of monocyte activation in rheumatoid arthritis. Arthritis Rheum 50(12):3878–3887

Winchester RJ, Agnello V, Kunkel HG (1970) Gamma globulin complexes in synovial fluids of patients with rheumatoid arthritis. Partial characterization and relationship to lowered complement levels. Clin Exp Immunol 6(5):689–706

Yuasa T, Kubo S, Yoshino T, Ujike A, Matsumura K, Ono M, Ravetch JV, Takai T (1999) Deletion of fcgamma receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. J Exp Med 189(1):187–194

Zhao X, Okeke NL, Sharpe O, Batliwalla FM, Lee AT, Ho PP, Tomooka BH, Gregersen PK, Robinson WH (2008) Circulating immune complexes contain citrullinated fibrinogen in rheumatoid arthritis. Arthritis Res Ther 10(4):R94

The FcγR of Humans and Non-human Primates and Their Interaction with IgG: Implications for Induction of Inflammation, Resistance to Infection and the Use of Therapeutic Monoclonal Antibodies

P. Mark Hogarth, Jessica C. Anania and Bruce D. Wines

Abstract Considerable effort has focused on the roles of the individual members of the FcγR receptor (FcγR) family in inflammatory diseases and humoral immunity. Recent work has revealed major roles in infection and in particular HIV pathogenesis and immunity. In addition, FcγR functions underpin the action of many of the successful therapeutic monoclonal antibodies. This emphasises the need for a greater understanding of FcγR function in humans and in the NHP which provides a key model for human immunity and preclinical testing of antibodies. We discuss recent key aspects of the human FcγR receptor biology and structure to define differences and similarities in activity between the human and macaque Fc receptors. These differences and similarities nuance the interpretation of infection and vaccine studies in the macaque. Indeed passive IgG antibody protection in lentivirus infection models in the macaque provided early evidence for the role of Fc receptors in anti-HIV immunity that have subsequently gained support from human vaccine trials. None-the-less the diverse functions and cellular contexts of FcγR receptor expression ensure there is much still to understand of the protective and deleterious effects of FcγRs in HIV infection. Careful comparative studies of human and non–human primate FcγRs will facilitate our appreciation of what attributes of HIV specific IgG antibodies, either acquired naturally or via vaccination, are most important for protection.

P. M. Hogarth (✉)
Burnet Institute, 85 Commercial Road, Melbourne, VIC 3004, Australia
e-mail: pmhogarth@burnet.edu.au

J. C. Anania · B. D. Wines
Burnet Institute, GPO Box 2284, Melbourne, VIC 3001, Australia

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_15,

Contents

1 Introduction 322
2 The Human FcγR Family 324
2.1 FcγRI 324
2.2 FcγRII 325
2.3 FcγRIII 326
3 Interaction of Human IgG with Human Fc Receptors 327
3.1 The Structural Basis of FcγR: IgG Interactions 327
3.2 Polymorphism Influences FcγR Binding of IgG and Susceptibility to Disease 331
4 FcγR and Resistance or Susceptibility to HIV 333
4.1 FcγRI 335
4.2 FcγRII 335
4.3 FcγRIIIa 336
5 Non-human Primate (Macaque) FcγRs 338
5.1 Sequence Comparison of Human and Macaque IgG and FcγRs. Specificity of Human FcγR are Not Equivalent in Macaque Species 338
5.2 Polymorphisms in Macaque FcγR 340
5.3 Comparison of Macaque and Human IgG 340
5.4 Divergent Tissue Expression Profiles of Human and Macaque FcγR 342
6 Conclusions 342
References 343

1 Introduction

In its broadest context inflammation is a homoeostatic process that involves tissue damage and, under normal circumstances, resolution or repair. In the context of the humoral immune system inflammation induced by antibodies is frequently used to resist and resolve infection.

One of the major mechanisms by which antibodies induce inflammation and control the response is through antibody engagement of specific cell-surface receptors—the Fc receptors (FcR). These receptors are essentially receptors for immune complexes and are primarily, though not exclusively, expressed on innate immune cells, thereby linking the humoral immune system with a cell based effector arm. Since antibodies have a large number of significantly different biological roles in vivo, the interaction between FcR and the different antibody classes is one of the most fundamentally important in immunity and initiates a wide range of immunological and inflammatory responses (Daeron and Lesourne 2006; Hogarth 2002; Hogarth and Pietersz 2012; Hulett and Hogarth 1994; Ravetch 2010).

Under normal circumstances antibody engagement of activating type Fc receptors, FcγRI (also known as CD64), FcγRIIa, IIb, IIc (CD32a, b, c), FcγRIIIa, IIIb, (CD16a, b) induce a cell-based inflammatory response manifest by different biological phenomena. These include phagocytosis of opsonised particles (bacteria), antibody dependent killing (ADCC) of large opsonised targets, including opsonised

virus-infected normal cells and perhaps transformed cells. Additional responses include the activation of platelets in situ, mast cell and neutrophil degranulation and the release of inflammatory mediators (eicosanoids, histamines or superoxide) effecting the local tissue environment and the signalling to other leukocytes via cytokine and chemokine release.

These Fc receptor dependent pro-inflammatory reactions, mediated by so-called activating FcγR, are also modulated by immune complex co-engagement with the inhibitory FcγR, FcγRIIb. As locally or systemically potent as these inflammatory responses can be, under normal circumstances they are well regulated with minimal sequelae.

However, if insufficiently regulated the immune complex: Fc receptor interaction can induce serious pathological inflammation leading to morbidity and even death. This is most spectacularly evident in life-threatening IgE dependent allergic reactions, mediated through the high affinity IgE receptor, FcεRI. The IgG Fc receptors, FcγRs, are also major contributors to chronic inflammation caused by IgG immune complexes in systemic lupus erythematous (SLE) and related diseases. Genome wide association studies (GWAS) as well as analysis of individual Fc receptor polymorphisms frequently indicates significant disease association in inflammatory bowel disease, rheumatoid arthritis, a number of vasculitides and systemic lupus erythematous (SLE) (Harley et al. 2008; Lessard et al. 2013; McGovern et al. 2010; Meziani et al. 2012; Willcocks et al. 2008).

The FcγRs are key components of both immune complex induced inflammatory processes and the regulation of immune responses (Daeron and Lesourne 2006; Hogarth 2002; Hogarth and Pietersz 2012; Hulett and Hogarth 1994; Ravetch 2010). FcγR dependant responses induced by immune complex binding not only occur at the effector level, for example ADCC, phagocytosis or mediator release, but also have major roles in shaping B and T cell adaptive immune responses. This role of the inhibitory FcγR in the regulation of B cell responses is well characterised (Cambier 2013), but FcR working cooperatively with pattern recognition receptor uptake of immune complexes by antigen presenting cells also drives T cell responses (Means et al. 2005; Amigorena 2002).

In an practical context, the successful harnessing of these same inflammatory or regulatory processes has underpinned the spectacular success of the monoclonal antibody (mAb) therapeutics industry. The harnessing of pro-inflammatory FcγR dependent effectors forms the basis for some of the successful targeting of cancer cells. In vitro studies largely implicate ADCC or phagocytosis in the protective pro-inflammatory effect reviewed in Hogarth and Pietersz (2012). Conversely anti-inflammatory therapeutics include the harnessing of the inhibitory FcγRIIb by antibodies targeting activating type immunoreceptors but whose Fc portions have been engineered to selectively or preferentially engage FcγRIIb. This results in a stable co-clustering of an activating type immunoreceptor with the inhibitory FcγRIIb (Chu et al. 2012, 2013; Horton et al. 2011).

The roles of the FcγR in autoimmune inflammation especially in mouse models of inflammation have been extensively reviewed elsewhere (Hogarth 2002; Hogarth and Pietersz 2012; Mihai and Nimmerjahn 2013; Ravetch 2010). In this

review we will focus on the new aspects of the role of human Fc receptors in inflammation, especially their emerging role in infection, particularly HIV. We will also compare the human and non-human primates (NHP), in particular macaque species, Fc receptors and their interaction with IgG. This is especially significant in the light of the use of NHP as models of human immunity and in particular as preclinical models in the testing of immunogenicity and immunity of vaccines and human therapeutic monoclonal antibodies (mAbs).

2 The Human FcγR Family

The receptors for IgG, FcγR are the most genetically diverse FcR. Six major IgG FcγRs are known, these include FcγRI, FcγRIIa, IIb, IIc, FcγRIIIa, and FcγRIIIb. Additional functionally relevant splice variants have also been identified. These receptors have been well characterised in human, and mice, but surprising little is known of the FcγR of NHP. Many aspects of the human and mouse receptors have extensively reviewed, in particular the genetics and biochemistry (Hulett and Hogarth 1994; Hogarth 2002), models of inflammation (Hogarth 2002; Takai 2005; Ravetch 2010; Mihai and Nimmerjahn 2013) and the signalling using Immunoreceptor Tyrosine-based Activation Motif (ITAM) and Immunoreceptor Tyrosine-based Inhibitory motif (ITIM) (Waterman and Cambier 2010; Daeron and Lesourne 2006; Cady et al. 2008). However it is important to emphasise certain unique aspects as follows.

2.1 FcγRI

FcγRI is the high affinity receptor for IgG which efficiently binds monomeric IgG and small immune complexes. This function could be important for binding antigen opsonised with limiting amounts of antibody, for example early in a developing immune response where antibody levels are low (Barnes et al. 2002; Gavin et al. 1998a, b). Whilst its high affinity for IgG ensures it can interact with monomeric IgG for extended periods. The readily measurable off rate (Gavin et al. 1998b; Bruhns et al. 2009) suggests that in vivo it is likely to be freely exchanging with other circulating monomeric IgG. FcγRI is induced on monocytes, macrophages, neutrophils, dendritic cells and masts cells in pro-inflammatory environments (Table 1) and reviewed in (Hogarth and Pietersz 2012). Mouse studies suggest this provides an important link between antibody responses and antigen presentation for T cell mediated immunity (Gavin et al. 1998a; Barnes et al. 2002) which are supported by human in vitro studies of immune complexes (Sallusto and Lanzavecchia 1994).

Table 1 FcγR distribution and expression in human and NHP leukocytes[a]

		FcγRI	FcγRIIa	FcγRIIb	FcγRIIc	FcγRIIIa	FcγRIIIb
T cells	Human	–	–	+	–	+[b]	–
	Macaque	•[c]	–	•	•	•	–[d]
B cells	Human	–	–	++	–	–	–
	Macaque	•	–	+	•	–	–[d]
NK cells	Human	–	–	–	+[e]	++	–
	Macaque	•	–	•	•	++	–[d]
Macrophages	Human	+[f]	+++	++	•	+	–
	Macaque	•	•	•	•	+	–[d]
Monocytes	Human	+[f]	+++	+	–	++	–
	Macaque	•	+++	•	•	+	–[d]
Neutrophils	Human	+[f]	+++	+[g]	•	+	++[h]
	Macaque	•	+++	•	•	–	–[d]
Eosinophils	Human	+[f]	++	•	•	–	•
	Macaque	•	•	•	•	•	–[d]
Basophils	Human	–	++	++	–	–	+
	Macaque	•	•	•	•	•	–[d]
Mast cells	Human	+[f]	++[i]	+	–	++	•
	Macaque	•	•	•	•	•	–[d]
Platelets	Human	–	++	–	–	–	–
	Macaque	•	++	•	•	•	–[d]

+++ Strong, ++ Moderate, + Weak, − No expression

[a] Macaque data comprised from rhesus, cynomolgus and pigtail, *See text* for detail

[b] γδ T cells

[c] • = Not tested

[d] No gene expressed

[e] Expressed in only 30 % of humans

[f] Induced upon exposure to cytokines

[g] Conflicting results

[h] GPI-linked

[i] Unstimulated cord blood—derived mast cells

2.2 *FcγRII*

FcγRIIa, FcγRIIb and FcγRIIc are encoded by three related genes. The extracellular domains of FcγRIIa and FcγRIIb are highly related (95 % identity) but the cytoplasmic tails are distinct, containing an activating ITAM or inhibitory ITIM respectively. FcγRIIc is expressed in only 20 % of the population and appears to be the product of a crossover between FcγRIIb and FcγRIIa. As a result its extracellular domains are most highly related to FcγRIIb and its ITAM containing cytoplasmic tail to FcγRIIa (van der Heijden et al. 2012).

FcγRIIa is unique to primates (Hibbs et al. 1988). It is the most widespread and abundant of the activating IgG receptors, being present on all leukocytes and platelets with the exception of T, B and NK cells (Table 1) (Hogarth and Pietersz 2012; Hogarth 2002).

FcγRIIa has several unique properties. It is the only FcR, and indeed one of the few immunoreceptors, that contains the activating ITAM in its cytoplasmic tail, i.e. ligand binding domains and ITAM are in the one polypeptide. Despite its clearly defined role in ITAM dependant signalling, additional studies have revealed its capacity to also participate in inhibitory function (Cady et al. 2008, 2010; Waterman and Cambier 2010).

The role of this receptor in the development and regulation of inflammation emerged initially from the use of recombinant ectodomains of FcγRIIa in acute models of immune complex Type-III hypersensitivity (Ierino et al. 1993). These initial studies were subsequently expanded in human FcγRIIa transgenic mice (Ierino et al. 1993; Tan Sardjono et al. 2003, 2005; Pietersz et al. 2009).

Furthermore, crystallographic and mutagenesis evidence also suggests that this receptor exists in dimeric states (Maxwell et al. 1999; Powell et al. 2006; Ramsland et al. 2011) that are necessary for optimal signalling function. This property appears to be unique among Fc receptors, as similar organisation of other FcγR is lacking. Indeed immune complex induced signalling by other FcR does not appear to require assembly of an organised quaternary state and aggregation alone is sufficient (Metzger 1992).

FcγRIIb is the archetypal inhibitory receptor whose ITIM based inhibitory function exerts a powerful effect on ITAM signalling pathways. It is expressed in most leukocytes including B cells, monocytes, macrophages, basophils, mast cells and controversially on neutrophils (Hogarth and Pietersz 2012) see also (Cassard et al. 2012) and (Jonsson and Daeron 2012) (Table 1). It is absent from platelets and most T cells but expression on CD8 memory T cells has been recently reported (Starbeck-Miller et al. 2014).

The inhibitory function of FcγRIIb requires co-recruitment or aggregation with an activating receptor. This leads to the subsequent recruitment of the inositol phosphatase *SHIP*, as well as the protein tyrosine phosphatases *SHP1* and *SHP2*, which ensures potent modulation of ITAM generated signals from FcεRI, FcγRIIIa, FcγRIIa and also the B cell antigen receptor (Daeron et al. 1995; Daeron and Lesourne 2006; Cady et al. 2008).

2.3 FcγRIII

FcγRIIIa is the most abundant FcγR on NK cells and also γδ Tcells, which are major mediators of ADCC (Table 1). On myeloid cells it is readily detected on macrophages and mast cells. It is also largely absent from circulating $CD14^+$ blood monocytes, however a small subset is defined by the presence of FcγRIIIa where it has a major role in phagocytosis as it does in tissue macrophages (Hogarth and Pietersz 2012).

FcγRIIIb is an enigmatic lipid anchored FcγR, which is it is closely related to FcγRIIIa. Despite a lack of cytoplasmic peptide tail its cell activating signalling function is dependent on src kinases. Additional evidence suggests that it also

requires FcγRIIa for effective signal transduction (Naziruddin et al. 1992). FcγRIIIb is expressed primarily and abundantly on neutrophils and possibly basophils (Jonsson and Daeron 2012; Cassard et al. 2012) and despite its abundant presence on human cells on human neutrophils and is almost certainly absent from the macaque genome see below (Table 1).

3 Interaction of Human IgG with Human Fc Receptors

3.1 The Structural Basis of FcγR: IgG Interactions

All human FcγR bind human IgG1 and human IgG3 albeit with different affinities ranging from nanomolar for the high affinity FcγRI, to low micromolar for the low affinity receptors, FcγRII and FcγRIII. By contrast the binding of IgG2 or IgG4 is more selective. Complexes of IgG2 are bound only by FcγRIIa, specifically by the "low responder" FcγRIIa allelic polymorphism form containing histidine at position 131 (FcγRIIa-His^{131}). The presence of arginine in the corresponding allele (FcγRIIa-Arg^{131}) impairs IgG2 binding. Interestingly, IgG4 complexes are bound poorly by the low affinity FcγRs, but monomeric IgG4 binds readily to the FcγRI (Table 4) (Hogarth and Pietersz 2012; Bruhns et al. 2009).

Our understanding of human FcγR interactions with IgG is largely derived from the analysis of interaction with human IgG1. Mutagenesis studies of FcγR (Maxwell et al. 1999; Hulett et al. 1995, 1999) or of IgG1 (Burton and Woof 1992; Duncan et al. 1988; Shields et al. 2001, 2002) together with more recent crystallographic studies of FcγRIIa and FcγRIII (Radaev et al. 2001; Ramsland et al. 2011; Sondermann et al. 2000; Mizushima et al. 2011; Ferrara et al. 2011) have revealed how IgG1 interacts with the FcγR.

The crystallographic analysis indicates that the low affinity FcγRs interact asymmetrically with the IgG1. The receptor "inserts" between the heavy chains of one IgG1 molecule adjacent to the hinge (Fig. 1). The major contact surface with IgG occurs in the second extracellular domain and is formed primarily by the BC loop, C'E loop and the FG loop as well is the "Trp sandwich" formed from two tryptophan residues of the inter-domain linker and BC loop of the FcγR (Table 2).

In the IgG, the five principal sites of interaction with FcγRs (Fig. 1) are: the lower hinges (Leu^{234}-Leu^{235}-Gly^{236}-Gly^{237}) of both heavy chains; the hinge proximal FG loop (Ala-Leu-Pro^{329} Ala-Pro) in heavy chain A and hinge proximal (Asp^{265}) B/C loop (Ser-Val-Asp^{265}-Val-Ser) in heavy chain B. The fifth site is the glycan of Chain A linked to Asn^{297}. These sequences are identical in human IgG3 and also remarkably in all macaque IgGs, (Table 3) but are critically different in human IgG2 and IgG4 (Hogarth and Pietersz 2012).

Whilst the contacts between IgG1 and FcγRIIa or FcγRIII are similar, the principal difference in FcγRIII is the more extensive interactions with the *N*-glycan of the heavy chain Asn^{297}. The greatly enhanced binding of defucosylated IgG1 to

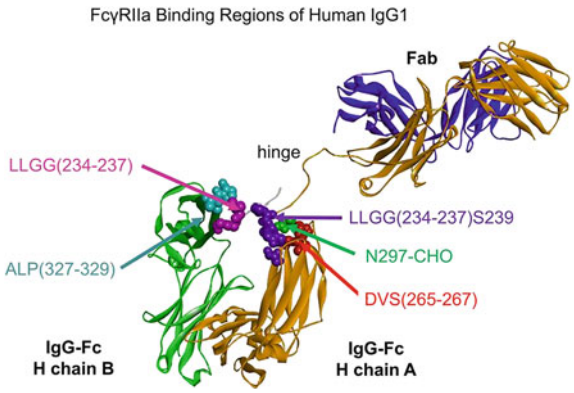


Fig. 1 Fc receptor interaction sites in human IgG1. The residues shown are key contacts in the Fc of heavy chain A and heavy chain B. *Note* that the Fab fragment in chain B has been removed for clarity and the structure shown is based on the FcγRIIa structure of (Ramsland et al. 2011) and similar interactions are apparent for Fc: FcγRIII (Sondermann et al. 2000; Radaev et al. 2001)

FcγRIII, which greatly improves ADCC and possibly therapy with mAbs, results from a local effect involving the adjacent Tyr^{296} (Ferrara et al. 2011; Mizushima et al. 2011) and/or the generation of unique interactions between the N-linked glycans of the receptor and defucosylated IgG1.

Structural knowledge of the interaction of IgG with the high affinity FcγRI is lacking. Mutagenesis studies of IgG and FcγRI, taken together with the structure of an unliganded FcγRI (Lu et al. 2011), indicated that many of the interactions of IgG and the high affinity FcγR are likely to be similar to the low affinity FcγRs. But clearly differences exist to account for the higher affinity particularly of IgG4 which binds very poorly to the low affinity receptors (Bruhns et al. 2009; Powell et al. 1999).

The extent to which the interaction of IgG2 and IgG4 with FcγR follows the "canonical rules" of IgG1 binding is not clear (Table 3). Sequence differences in the lower hinge of IgG2 (Pro^{234}-Val^{235}-Ala^{236}-Gly^{237}) or IgG4 the lower hinge (Phe^{234}-Leu^{235}-Gly^{236}-Gly^{237}) are clearly distinct from the Leu^{234}-Leu^{235} -Gly^{236} -Gly^{237} of IgG1 and IgG3. However, mutagenesis of the Leu^{235} of IgG4 ablates FcγR binding, suggesting that like IgG1, the lower hinge in IgG4 is essential for FcγR binding. However, other major structural changes to the CH2 FG loop (Gly-Leu-Pro^{329}-Ser-Ser) of IgG4 (Fig. 1 and Table 3) suggests it cannot interact with the critical tryptophans of the "Trp sandwich" in FcγRII or FcγRIIIb, which is absolutely essential for the binding of IgG1 and IgG3 (Davies et al. 2014; Rispens et al. 2014).

Table 2 Comparison of the IgG binding region of human and macaque FcγR[a]

Receptor[b]	Tryptophan sandwich		BC loop							C'E loop								F strand	FG loop				G strand	
FcγRI[c]	**84**	**107**	**110**	**111**	**112**	**113**	**114**	**115**	**116**	**122**	**123**	**124**	**125**	**126**	**127**	**128**	**129**	**149**	**151**	**152**	**153**	**154**	**155**	**156**
Human	W	W	K	L	V	Y	N	V	L	K	A	F	K	F	F	H	W	S	M	G	K	H	R	Y
M. nemestrina	W	W	K	L	V	Y	N	V	L	K	A	F	K	F	F	Y	R	S	M	G	K	H	R	Y
M. mulatta	W	W	K	L	V	Y	N	V	L	K	A	F	K	F	F	Y	R	S	M	G	K	H	Y	T
M. fascicularis	W	W	K	L	V	Y	N	V	L	K	A	F	K	F	F	Y	R	S	M	G	K	H	R	Y
FcγRIIa	**87**	**110**	**113**	**114**	**115**	**116**	**117**	**118**	**119**	**125**	**126**	**127**	**128**	**129**	**130**	**131**	**132**	**152**	**155**	**156**	**157**	**158**	**159**	**160**
Human	*W*	*W*	K	*P*	L	*V*	*K*	V	T	*K*	*S*	Q	K	*F*	S	*H/R*[d]	*L*	T	I	G	*Y*	T	L	F
M. nemestrina	W	W	K	P	L	V	K	V	T	K	S	Q	K	F	S	H/P[d]	M	T	I	G	Y	T	P	Y
M. mulatta	W	W	K	P	L	V	K	V	A	K	S	Q	K	F	S	H	M	T	I	G	Y	T	P	Y
M. fascicularis	W	W	K	P	L	V	K	V	T	K	S	Q	K	F	S	H	M	T	I	G	Y	T	P	Y
FcγRIIb	**87**	**110**	**113**	**114**	**115**	**116**	**117**	**118**	**119**	**125**	**126**	**127**	**128**	**129**	**130**	**131**	**132**	**152**	**155**	**156**	**157**	**158**	**159**	**160**
Human	W	W	K	P	L	V	K	V	T	K	S	K	K	F	S	R	S	T	I	G	Y	T	L	Y
M. nemestrina	W	W	K	P	L	I	K	V	T	I	S	K	K	F	S	H	M	T	I	G	Y	T	P	Y
M. mulatta	W	W	K	P	L	I	K	V	T	I	S	K	K	F	S	H	M	T	I	G	Y	T	P	Y
M. fascicularis	W	W	K	P	L	I	K	V	T	I	S	K	K	F	S	H	M	T	I	G	Y	T	P	Y

(continued)

Table 2 (continued)

Receptor[b]	Tryptophan sandwich		BC loop							C'E loop							F strand		FG loop				G strand	
FcγRIIIa	**90**	**113**	**116**	**117**	**118**	**119**	**120**	**121**	**122**	**128**	**129**	**130**	**131**	**132**	**133**	**134**	**135**	**155**	**158**	**159**	**160**	**161**	**162**	**163**
Human	W	W	T	A	L	H	K	V	T	K	G	R	K	Y	F	H	H	R	V	G	S	K	N	V
M. nemestrina	W	W	T	L	L	H	K	V	T	K	G	R	K	Y	F	H	H	R	I	G	S	K	N	V
M. mulatta	W	W	T	L	L	H	K	V	T	K	G	R	K	Y	F	H	Q	R	I	G	S	K	N	V
M. fascicularis	W	W	T	L	L	H	K	V	T	K	G	R	K	Y	F	H	Q	R	I	G	S	K	N	V
FcγRIIIb[e]	**90**	**113**	**116**	**117**	**118**	**119**	**120**	**121**	**122**	**128**	**129**	**130**	**131**	**132**	**133**	**134**	**135**	**155**	**158**	**159**	**160**	**161**	**162**	**163**
Human	*W*	*W*	*T*	*A*	L	**H**	**K**	V	**T**	K	**D**	**R**	**K**	**Y**	F	**H**	**H**	**R**	*V/F*[d]	*G*	S	*K*	N	V

[a] Residues of human FcγRIIa and human FcγRIIIb in contact with IgG1 H chain A Fc are underlined; or those in contact with IgG1 H chain B Fc are italicized underlined. *Note* Lys^{125} and Ser^{126} of hFcγRIIa make weak contact with glycan of Asn^{297} and in hFcγRIIIb Lys^{117}, Trp^{119}, Tyr^{129}, and Arg^{152} make critical contact with this glycan. Data for hFcγRIIa from (Ramsland et al. 2011) and hFcγRIIIb (Radaev et al. 2001) and (Sondermann et al. 2000). Aminio acid sequence of pigtail FcγRI and FcγRIII are taken H. Trist and P.M. Hogarth (unpublished); see also (Rogers et al. 2006; Nguyen et al. 2011; Lu et al. 2011; Trist et al. 2014)

[b] *M. fascicularis*– cynomolgus macaque; *M. mulatta*– rhesus macaque; *M. nemestrina* –pigtail macaque

[c] FcγRI, is based on the sequence of (Lu et al. 2011), with the predicted N-terminal residue of the mature protein as position +1 after the removal of the 20 amino acid signal sequence. FcγRIIa/FcγRIIb and FcγRIII residues are numbered based on (Trist et al. 2014) and (Radaev et al. 2001), respectively

[d] Clinically significant polymorphic residues

[e] No apparent equivalent for FcγRIIIb, CD16b, in macaques – see text for detail

3.2 Polymorphism Influences FcγR Binding of IgG and Susceptibility to Disease

The binding of human IgG by FcγR, subsequent signal transduction and their roles in human inflammation is confounded by genetic polymorphism which affects IgG binding and/or alter effector functions, e.g. the balance of activation over inhibition. Such polymorphisms are highly significant in human inflammatory and infectious diseases.

By comparison to NHP (see below) the human FcγR show relatively limited polymorphism. However, several clinically significant polymorphisms are known. In human FcγRIIa, the so-called "low/high responder" polymorphism is determined by the presence of histidine or arginine at position 131, respectively (Tate et al. 1992; Warnerdanm et al. 1990). It is associated with susceptibility to autoimmune diseases including SLE (Karassa et al. 2002) rheumatoid arthritis (Alizadeh et al. 2007) Guillian-Barre syndrome (van Sorge et al. 2005), susceptibility to gram negative infection (Sole-Violan et al. 2011; Domingo et al. 2002), susceptibility to HIV (Brouwer et al. 2004; Forthal et al. 2007), the clinical outcome of antibody therapy in some malignancies (Bibeau et al. 2009; Cartron et al. 2002; Weng and Levy 2003) and also controversially with resistance to malaria (Giha et al. 2012; Maiga et al. 2014).

How these polymorphisms manifest themselves in disease processes is not entirely clear. Certainly, the FcR based effector function of IgG2 is dependent on this polymorphism, as the avid binding and specificity for IgG2 is determined by FcγRIIa -His^{131}. Clearly in situation dominated by an IgG2 response altered effector function as a consequence of altered FcγRIIa interaction may account for lack of responsiveness. Such a situation is plausible in responses to encapsulated gram negative infections where IgG2 can be the dominant immunoglobulin in the humoral response. Indeed homozygozity for the FcγRIIa-Arg^{131} is a risk factor in poor response to meningococcal disease (Domingo et al. 2002). The structural studies of the high and low responder FcγRIIa suggest that the IgG:FcγR interaction is more readily accommodated by the smaller histidine side chain compared to the longer arginine side chain (Ramsland et al. 2011).

Clinically significant polymorphisms have also been identified in FcγRIIIa and FcγRIIIb. FcγRIIIa-Val/Phe^{158} and the FcγRIIIb-NA1/NA2 polymorphisms affects IgG binding and are associated with disease susceptibility in inflammatory diseases. FcγRIII Val^{158} (FcγRIIIa-158 Val^{158}) confers a higher affinity than Phe^{158} (FcγRIIIa-Phe^{158}) (Tables 2 and 4). Allele distribution is skewed in SLE patients (Koene et al. 1998), rheumatoid arthritis (Alizadeh et al. 2007), Wegeners Granulomatosis (Dijstelbloem et al. 1999) and can affect the outcome of mAb therapy (Weng and Levy 2003). Alleles of FcγRIIIb are also associated with autoimmune disease, including Guillain Barre Syndrome (van Sorge et al. 2005), $ANCA^{+}$ vasculitis (Tse et al. 2000) and in infectious disease especially when co-inherited with risk alleles of FcγRII (Fijen et al. 1993).

Table 3 Alignment of FcγR binding sites of human and macaque IgG sub classes[a]

Domain		Lower hinge						CH_2										
		Chain A and Chain B						B/C—Chain A						F/G—Chain B				
		234[b]	235	236	237	238	239	264	265	266	267	268	297	327	328	329	330	331
Human	IgG1	L	L	G	G	P	S	V	D	V	S	H	N	A	L	P	A	P
	IgG2	***P***	***V***	***A***	G	P	S	V	D	V	S	H	N	***G***	L	P	A	P
	IgG3	L	L	G	G	P	S	V	D	V	S	H	N	A	L	P	A	P
	IgG4	***F***	L	G	G	P	S	V	D	V	S	***Q***	N	***G***	L	P	**S**	**S**
Rhesus macaque *M. mulatta*	IgG1	L	L	G	G	P	S	V	D	V	S	***Q***	N	A	L	P	A	P
	IgG2	L	L	G	G	P	S	V	D	V	S	***Q***	N	A	L	P	A	P
	IgG3	L	L	G	G	P	S	V	D	V	S	***Q***	N	***G***	L	P	A	P
	IgG4	L	L	G	G	P	S	V	D	V	S	***Q***	N	***G***	L	P	A	P
Cynomolgus macaque *M. fascicularis*	IgG1	L	L	G	G	P	S	V	D	V	S	***Q***	N	A	L	P	A	P
	IgG2	L	L	G	G	P	S	V	D	V	S	***Q***	N	A	L	P	A	P
	IgG3	L	L	G	G	P	S	V	D	V	S	***Q***	N	A	L	P	A	P
	IgG4	L	L	G	G	P	S	V	D	V	S	***Q***	N	***G***	L	P	A	P
Pigtail macaque *M. nemestina*	IgG1	L	L	G	G	P	S	V	D	V	S	***Q***	N	A	L	P	A	P
	IgG2	L	L	G	G	P	S	V	D	V	S	***Q***	N	***G***	L	P	A	P
	IgG3	L	L	G	G	P	S	V	D	V	S	***Q***	N	***G***	L	P	A	P
	IgG4	***V***	L	G	G	P	S	V	D	V	S	***Q***	N	***G***	L	P	A	P

[a] Residues that differ from the archetypal FcγR binding regions of human IgG1 are bold italicized. See reference (Jacobsen et al. 2011; Nguyen et al. 2011; Scinicariello et al. 2004; Warncke et al. 2012) for details

[b] Kabat residue number

Table 4 Relative binding avidity of complexes of human IgG subclasses to Fcγ receptors in human and macaque[a]

Receptor	Species	IgG1	IgG2	IgG3	IgG4
FcγRI	Human	++++	−	++++	+++
	Macaque	+++	+++	+++	+++
FcγRIIa	Human-R131	++	±	++++	±
	Human-H131	++	++	++++	±
	Macaque	++	++	++	++
FcγRIIb	Human	+±	−	+	±
	Macaque	+	++	+	±
FcγRIIIa	Human-V158	++	±	++	±
	Human-F158	+	−	−	±
	Macaque	++	+	++	±
FcγRIIIb[b]	Human	+	−	++	−

+++ Strong, ++ Moderate, + Weak or — No avidity of Fc receptor

[a] Binding values for FcγRI are based on are monoclonal IgG binding to cells or SPR (BIAcore) analysis. Binding of FcγRII and FcγRIII is based on binding of immune complexes to cell expressed receptor or SPR (BIAcore) analysis of monoclonal IgG. See references (Warncke et al. 2012; Trist et al. 2014; Powell et al. 1999; Maenaka et al. 2001; Bruhns et al. 2009) for details

[b] Macaques do not express FcγRIIIb

Whether a simple alteration to affinity and/or specificity of an activating FcγR for IgG is alone sufficient to account for disease susceptibility is unclear. However, polymorphism in FcγRIIb has a profound effect on signal transduction and the capacity of this inhibitory receptor to modulate responses (Li et al. 2003). Substitution of threonine at position 232 for isoleucine in the membrane spanning region alters the receptors capacity to localise to lipid micro-domains in the membrane, which in turn affects the extent of its inhibitory activity. This polymorphism is associated with autoimmune disease and also with protection from severe malaria which is reflected in differences in allele frequency in European versus Africans (Clatworthy et al. 2007). Relatively small alterations to the capacity of this receptor to modulate activating pro-inflammatory signals can have significant effects on activating receptor function. It is likely that such differences reflect a possible imbalance in the regulation of inflammation, which is pro-survival in environments of intense infectious disease pressure but becomes an autoimmunity risk in the absence of such pressure.

4 FcγR and Resistance or Susceptibility to HIV

Many of the principles of Fc receptor function that have emerged from experimental systems in vivo and in vitro as well as genetic analysis of immune complex induced inflammatory disease may also inform as to the role of FcRs in human infection. Here we will focus on the resurgent interest in the role of antibodies and Fc receptor function in HIV immunity.

More than 30 million people are infected with HIV. Currently what constitutes immunity to HIV and how this is generated is unknown but occurs by *experimentum naturae* in elite controllers and some highly exposed but unaffected individuals (Autran et al. 2011; International et al. 2010; Lichterfeld and Yu 2012). Although antiretroviral treatments have improved the outlook for many HIV patients, understanding the antibody response is now a focus of understanding the acquisition of controlling immunity and is key to developing improved vaccination strategies to confront the HIV pandemic (Doria-Rose et al. 2014).

Recent in human studies in vitro and in vivo in human clinical trials and in macaques, provide increasing evidence for a critical role of antibody in effective immunity and resistance to HIV (Barouch et al. 2013a, b; Gomez-Roman et al. 2005; Hessell et al. 2007, 2009; Moog et al. 2014). There is clear evidence to the effectiveness of HIV antibodies, including broadly neutralising antibodies (bNAbs), in preventing transmission and in control of viral replication being dependant on the engagement of appropriate FcR dependant effector mechanisms. Indeed the most promising vaccine trail in humans, the Thai RV144 trial, resulted in modest protection of the vaccinated group in which only weak CTL and neutralising antibody responses were elicited (Haynes et al. 2012; Rerks-Ngarm et al. 2009). Rather the FcR mediated antibody activity correlated with protection sparking interest in understanding how these receptors mediate antibody protection against HIV (Waltz 2012).

The use of bNAbs and mutants that ablate FcγR binding have also implicated FcγR effector function is important in HIV immunity. Indeed bNAbs at concentrations insufficient for complete neutralisation are none-the-less active in triggering antibody dependent cell-mediated viral inhibition (ADCVI) by macrophages or other cell types and ADCC primarily by NK cells (Hessell et al. 2009). Ablation of the 'general' FcγR binding activity of the IgG1 NAb b12 by mutation of the lower hinge residues of the Leucine 234 and Leucine 235 to alanine (Leu^{234}-Leu^{235} to Ala^{234}-Ala^{235} mutant) greatly reduced its protection of macaques against SHIV in (Hessell et al. 2007) demonstrating a dominance of FcR mediated cellular functions over neutralisation for protection in this in vivo model.

However antibodies act in concert with different Fc receptors on different cells and so may be protective or deleterious in different contexts. While IgG opsonisation has been shown to protect macaques from SIV/SHIV challenge (Gomez-Roman et al. 2005; Hessell et al. 2007, 2009), IgG can also facilitate the transcytosis of antigens across the mucosal epithelium of the genital tract by engaging the transport receptor FcRn. In this setting the non-neutralising IgG HIV antibodies may be undesirable, and sub-neutralising titres of NAbs may be similar, in binding FcRn in the low pH of the human female genital tract and so transporting infective virus across the epithelium and promoting transmission of infection (Gupta et al. 2013).

The poor fidelity of HIV reverse transcription gives rise to the rapid generation of virus variants and immune selection on these includes CTL responses and neutralising antibodies. However some mutations do not fit either criteria, suggesting they do affect non-neutralising antibody binding and so implicates other

antibody effector functions in the control of HIV infection (Chung et al. 2011; Isitman et al. 2012).

Despite this awareness of the importance of FcRs in antibody immunity to HIV there is a lack of definition of the FcR dependent mechanisms underlying antibody resistance to HIV. There is emerging knowledge of how protection involves particular FcRs on particular leukocytes and by what of several possible processes such as, phagocytosis, ADCC, ADCVI (Peressin et al. 2014) protection is mediated and how these are all affected by FcR polymorphisms. A number of studies have shown that HIV infection both alters FcγR expression and functionality on monocytes, macrophages, dendritic cells and NK cells (Kedzierska et al. 2003; Lichtfuss et al. 2012; Ludlow et al. 2012; Dugast et al. 2011; Leeansyah et al. 2007). To date the interaction of broadly effective HIV antibodies with human FcRs is relatively focussed on ADCC via NK cell FcγRIIIa but the role of other FcγR and leukocytes becoming more apparent (Kramski et al. 2012). Importantly, the evidence to date suggests that each activating FcγR has plays some role, protective and interestingly in some instances deleterious, in HIV infection.

4.1 FcγRI

FcγRI is induced on in pro-inflammatory environments, including the acute phase of HIV infection (Dugast 2011). Interestingly macrophage mediated inactivation of HIV treated with infected patient antibodies is inhibited by blocking FcγRI (Holl et al. 2004). Furthermore, the expression of FcγRI in an HIV infectable Hela cell ($CD4^+$, $CCR5^+$ and $CXCR4^+$) showed a potent inhibition of infection by anti-gp41 antibodies. In contrast there was no effect by the expression of FcγRIIa or FcγRIIIa (Perez et al. 2009). FcγRI has a specialised function in endocytosis (Barnes et al. 2002) and inactivation of toxins (Abboud et al. 2010) which may relate to its mechanism of HIV inactivation. It has been proposed that the expression of FcγRI by infected macrophages may result in the increased local concentration of NAb which can then bind virus and inhibit cell to cell spread (Peressin et al. 2014).

4.2 FcγRII

The activities of the Fc receptor pair FcγRIIa and FcγRIIb determine cell activation by antibody. These two key Fc receptors are reported to be a determinate of antibody mediated protection in slow progressors (Ackerman et al. 2013b). These two forms have opposing functions, FcγRIIa activates myeloid immune cells that engage protective antibodies. In contrast, FcγRIIb inhibits cell activation by antibodies and so regulates normal antibody responses. However in infectious

disease and vaccination this regulation can negatively limit the cell mediated protection by anti-pathogen antibodies.

The efficacy of protection afforded by anti-HIV antibodies is thus likely to be determined by the breadth of the response in covering virus variants and the engagement of activating Fc receptors.

Just as genetic polymorphisms of the two most abundant human IgG Fc receptors FcγRIIa and FcγRIIIa profoundly affect interaction with IgG and the induction of effector responses in inflammation or in therapeutic anti-cancer antibodies in patients so it is also clear that polymorphisms in FcRs are known to contribute to degree of anti-HIV immunity individual humans (Forthal et al. 2007; French et al. 2010). Indeed the histidine/arginine131 FcγRIIa polymorphism is reported to affect AIDS progression as Arg^{131} homozygous HIV infected individuals more rapidly decline to CD4 counts $<200/mm^3$ (Forthal et al. 2007) and in a vaccine study the His^{131} homozygous patients showed better control of virus replication (French et al. 2010). In both studies anti-gp120 IgG2 antibodies were observed and proposed to confer some protective activity in the FcγRIIa His^{131} homozygous patients.

In contrast to these studies indicating protective roles for IgG2, IgG2 replete and depleted sera from the Vax004 trial (Forthal et al. 2011) demonstrated a role for IgG2 in inhibiting the internalisation of HIV-VLPs by monocytes. A differing functional importance FcγRIIa on monocytes and antigen presenting cells may underlie the differing roles of IgG2 in these studies. A study of controller and non-controller HIV patient cohorts showed controller patients, particularly those lacking protective HLA-B57 developed IgG2 responses to HIV gag proteins. One proposed mechanism is that FcγRIIa mediates antigen presentation of IgG2 opsonized gag proteins (French et al. 2013).

Dysfunction of the gut barrier in HIV infection leads to the translocation of microbial products and systemic immune activation. A recent study has shown that CD16+ monocytes from HIV infected patients have increased capacity to produce IL-23 in response to LPS/TLR4 stimulation (Manuzak et al. 2013). Fc receptors are likely to play a role in this systemic immune activation since cross talk between FcRs and pattern recognition receptors can shape immune responses by together setting the nature of co-stimulation and the cytokine milieu under which T cell immunity develops. Specifically FcγRIIa and TLR4 co-recruitment by IgG opsonised bacteria stimulates dendritic cell secretion of IL-23 p19 essential for increased Th17 development (den Dunnen et al. 2012).

4.3 FcγRIIIa

Although protection in the RV144 trial correlated with NK cell mediated ADCC activity, it is yet unclear by which mechanisms and in what settings FcγRIIIa is important for protection. A recent study in macaques using a bNAb (b12) optimised for FcγRIIIa binding and so ADCC showed no increased protection to a

mucosal SHIV challenge (Moldt et al. 2012). Indeed vaccinees in the non-protective VAX004 trial with the Val^{158} polymorphism of FcγRIIIa were at greater risk of infection than the placebo group (Forthal et al. 2012), thus FcγRIIIa may not play a protective role against transmission. Indeed although FcγRIIIa is coupled to an ITAM containing FcR gamma subunit and is generally considered an activating receptor, low stoichiometric ligand binding can result in partial phosphorylation of the ITAM tyrosines and phosphatase *SHP1* recruitment and inhibitory signalling (Aloulou et al. 2012). This may adversely influence some cellular antiviral mechanisms particularly as HIV particles express few envelope spikes ($\sim$14) and so can only be opsonised at low valency (Klein and Bjorkman 2010). Hence ADCC by NK cells may be a mechanism of FcγRIIIa mediated protection (Forthal et al. 2005; Forthal and Moog 2009) while in other settings this FcR may exacerbate infection. Indeed the CD16+ population of monocytes is highly susceptible to HIV infection which is attributed to high levels of CCR5 and low APOBEC3G activity (Ellery et al. 2007) but may also be affected by uptake of IgG opsonised virus.

A number of studies suggest FcγRIIIa mediated ADCC by NK cells may be a major defense against HIV by the destruction of IgG opsonised infected cells. Lambotte et al. (2013) showed significantly higher ADCC capable antibody responses in controller patients than viremic patients and this difference was clearer when the controller patients were stratified by HLA-B57 expression. Controller patients lacking the protective HLA-B57 presumably have a lower CD8+ cytotoxic T cell mediated immunity to HIV gag and these also had the highest level of ADCC active antibodies. A study of antibodies from HIV+ subjects in an ADCVI assay found antibodies from controller individuals more potently activated donor NK cells for multiple effector functions, including degranulation and secretion of both IFNγ and TNFα, compared to antibodies from all other HIV+ subjects. The serum IgG from HIV infected subjects, and more particularly controllers, showed increased proportion of agalactosyl IgG. Most importantly, purified gp120 specific IgG from HIV+ individuals compared to their bulk serum IgG showed a marked increase in the proportion proportion of agalactosyl and non-fucosylated IgGs, but without clear differences between the different HIV+ groups (Ackerman et al. 2013a). Non-fucosylated IgG is of particular interest as it has 100-fold enhanced affinity for FcγRIIIa that translates to a correspondingly increased activity in ADCC (Ferrara et al. 2011; Shields et al. 2002).

Antibodies active in ADCC in an NK assay will also be potentially active in a number of cellular effector functions (Moog et al. 2014) but the virus itself testifies to the potential importance of ADCC in controlling infection as it evades ADCC by the action of its accessory protein Vpu. Vpu is proposed to target the interferon induced protein tetherin, which otherwise inhibits viral particle release, so infected cells are more easily opsonised and targeted for ADCC (Alvarez et al. 2014). A study examining the activity of sera from a cohort of long term slow progressors (LTSP) and non-LTSP HIV infected patients in a NK/FcγRIIIa mediated ADCC assay mapped activity to linear epitopes using HIV peptides. Similar numbers from both cohorts reacted with envelope derived peptides but unexpectedly the

TLSP cohort showed significantly higher number of responses to accessory proteins including Vpu (Wren et al. 2012). Thus Vpu itself may be a target for ADCC but this study did not examine if these antibodies further enhance ADCC by inhibiting Vpu activity and increasing target expression on the infected cell by tetherin-mediated surface trapping of nascent virus.

5 Non-human Primate (Macaque) FcγRs

NHP particularly macaque species, serve as experimental and preclinical safety models of human immunity, antibody responses and effector functions in infection, notably HIV. Additionally, they are utilised as models of vaccine responses and in particular for preclinical evaluation of complex mechanisms of action and pharmacokinetics of mAb. However, the design and interpretation of experimental data is often based on the assumption that NHP Fc receptors and their antibody interactions faithfully mimic those of humans. Yet, despite an abundance of studies of human FcR and human IgG structure and function, there is a remarkable paucity of information on NHP FcR IgG interaction. This lack of systematic and comprehensive investigation of NHP Fc receptors is surprising given prominence of NHPs as models of human immunity, their use in evaluation of vaccines and preclinical testing models of therapeutic biologicals particularly mAb.

Analyses to date indicate that, as a first approximation, macaque and human FcγR share sequence similarity and many similar properties, as do the macaque IgG subclasses. However there are very significant differences in specificity and affinity for human IgG subclasses and surprising differences in tissue expression. These differences may also be confounded by the use of different model systems in vitro; different ligand formats and the use of different species and even in different populations of a single species, for example the Indian and Chinese rhesus macaques for which differences are known (Scinicariello et al. 2004).

This section focusses on analyses of FcR of the three commonly used species of macaque: *M. mulatta* the rhesus macaque, *M. fasicularis* the cynomolgous macaque and *M. nemestrina* the pigtail macaque.

5.1 Sequence Comparison of Human and Macaque IgG and FcγRs. Specificity of Human FcγR are Not Equivalent in Macaque Species

As in humans, all three macaque species express high and low affinity IgG Fc receptors—FcγRI (high affinity receptor), FcγRIIa and FcγRIIb, as well as FcγRIII (low affinity receptors). Considerable sequence identity is apparent between these receptors and their human orthologues. It is greatest in the high affinity receptor

(95 identity % for FcγRI) (Table 2) (Nguyen et al. 2011) (Hogarth and Trist unpublished) whereas sequence diversity is greatest in the low affinity receptors, especially FcγRIIa (85 %) (Nguyen et al. 2011; Trist et al. 2014).

Cynomolgus macaque FcγRI binds human IgG1, IgG3, IgG4, with high affinity, as does human FcγRI, though the affinity of IgG4 appears lower in the cynomolgus FcγRI relative to the other subclasses (Tables 2 and 4) (Warncke et al. 2012). Meanwhile, the human IgG binding profile of cynomolgus FcγRIIIa is also similar to human FcγRIII-Val^{158} allele, showing considerably greater binding of human IgG1 and IgG3 but not IgG2 or IgG4 (Tables 2 and 4).

However, the greatest differences in the binding of human IgG between human and macaque FcγR occur in interactions with FcγRIIa and FcγRIIb. The human IgG binding profile of the inhibitory FcγRIIb is most dramatically different from human in cynomolgus and pig-tail macaque (Warncke et al. 2012; Trist et al. 2014). The macaque FcγRIIb has a broader IgG specificity than human FcγRIIb, as macaque FcγRIIb avidly binds human IgG2. This surprising difference in specificity is primarily due to the presence of histidine at position 131 in macaque FcγRIIb. By contrast, arginine is present in this position in human FcγRIIb which precludes interaction with the human IgG2. This difference in specificity is also manifest in the "high and low responder" allelic forms of FcγRIIa (see above and Tables 2 and 4). Interestingly many non-human and human primates, including rhesus macaques, also have histidine in this position and presumably can bind human IgG2 (Trist et al. 2014).

Macaque FcγRIIa binds human IgG1 IgG2 and IgG3. The presence of histidine at position 131 in the macaque receptor is responsible for the specific binding of IgG2 (Trist et al. 2014). However, the binding of human IgG1 and IgG2 is impaired by comparison to the binding of their human counterparts but IgG3 binding is unaffected (Tables 2 and 4) (Trist et al. 2014).

The impaired binding of human IgG1 and IgG2 to FcγRIIa results from the replacement of the Leu^{159} and Phe^{160} in human FcγRIIa, with Pro^{259} Tyr^{150} in macaque FcγRIIa. These residues of the G-strand do not contact IgG, but impinge directly on the "Trp sandwich" and Tyr^{157} of the FG loop, resulting in reduced contact with the Pro^{329} and the lower hinge of IgG respectively (Trist et al. 2014). These amino acids are conserved in FcγRIIa of all three macaque species (Jacobsen et al. 2011; Nguyen et al. 2011; Trist et al. 2014; Warncke et al. 2012) but are not in other NHP. Pro^{159} Tyr^{160} are also present in all macaque inhibitory FcγRIIb, however any effect on IgG binding is unknown (Table 2).

The impaired binding of human IgG1 and IgG2 by macaque FcγRIIa, but avid binding of IgG2 by macaque FcγRIIb, shows there is a distinct hierarchy of binding of IgG2 and IgG1 in macaque FcγRIIa and FcγRIIb compared to humans (Warncke et al. 2012; Trist et al. 2014). As a consequence of these differences, particularly the relatively greater binding to the inhibitory FcγRIIb, antibody effector functions in macaques in vivo, especially of IgG2, may not entirely recapitulate human effector responses (Trist et al. 2014).

5.2 Polymorphisms in Macaque FcγR

Polymorphism in human Fc receptors can be functionally important, affecting IgG binding and/or signal transduction as discussed above (Tate et al. 1992; Warner-danm et al. 1990). Despite the relatively limited sequence data available from pigtail macaque (Trist et al. 2014), rhesus macaque (Nguyen et al. 2011) and cynomolgus macaques (Jacobsen et al. 2011) it is clear that macaque Fc receptors exhibit extensive polymorphism. This high level of polymorphism, results in individual variation in as much as 5 % of the amino acid content of the receptors. FcγRIIa appears to be the most polymorphic with at least eight alleles defined in 10 pigtail macaques (Trist et al. 2014) and seven alleles in rhesus macaque (Nguyen et al. 2011); allelic forms of FcγRI, FcγRIII are also known (Nguyen et al. 2011) and (Trist and Hogarth unpublished). Interestingly, FcγRIIb appears to be the least polymorphic receptor (Nguyen et al. 2011; Scinicariello et al. 2004).

However, the functional significance of these polymorphisms has only been investigated in pigtail macaque where hypo-functional receptors encoded by FcγRIIa alleles have been identified (Trist et al. 2014). Thus polymorphism results in the substitution of proline for the critical histidine at position 131 of the IgG binding site and effectively ablates human IgG1 and IgG2 binding and profoundly reduces the binding of IgG3 (Table 2). Interestingly the binding of macaque IgG is also largely ablated.

In other macaque species and NHPs, polymorphic residues occur in, and around, the IgG binding sites or structurally significant regions of FcγRIIa (Table 3) (Trist et al. 2014). These include altered glycosylation sites in rhesus macaques and baboons at Asp/Asn^{128} adjacent to Phe^{129}, which in human FcγRIIa is a critical to IgG binding (Ramsland et al. 2011). Similarly, in marmoset a possible N-glycosylation is present at position 133. Whist as yet uncharacterised, previous experience with the human FcγR shows that alterations in N-glycosylation affect FcγR structure and ligand binding properties (van Sorge et al. 2003) and thereby affect FcγR function.

Polymorphism also results in variation in the cytoplasmic tail particularly of FcγRIIa in rhesus and pigtail macaque (Nguyen et al. 2011; Trist et al. 2014). These include sequence variation in the ITAM around the canonical tyrosine residues of the motif but whether this is any functional impact is presently unknown.

5.3 Comparison of Macaque and Human IgG

Like humans, rhesus and cynomolgus macaques, and presumably pigtail macaque, have four IgG subclasses; IgG1 IgG2 IgG3 IgG4. However, unlike human IgG subclasses the macaque IgG lack the sequence diversity in regions that in human IgG1 are necessary for the interaction with FcγR. Thus the lower hinge (Leu^{234}

-Leu^{235}-Gly^{236}-Gly^{237}) and the adjacent segments of Cγ2, BC loop containing Asp^{265} as well as the FG loop containing the Pro^{329} (Table 3) (Jacobsen et al. 2011; Scinicariello et al. 2004; Warncke et al. 2012) of the macaque IgG subclasses are identical to each other and to human IgG1 in these regions with the exception of a Gly^{327} of macaque IgG3 and/or IgG4 (Table 3).

The observation that the lower hinge sequence of all macaque IgG classes is identical to human IgG1 is surprising given the variation in this segment in the human IgG and its influence on binding to human FcγR (Bruhns et al. 2009; Powell et al. 1999). It is perhaps not surprising then that the macaque IgG subclasses all showed similar receptor binding characteristics, as determined by SPR using biacore analysis (Warncke et al. 2012). What is surprising however is that despite these modest differences in receptor binding characteristics, the subclasses behave differently in experimental models using whole cells expressing FcγR which shows that IgG1 and IgG3 have potent activity but IgG2 and IgG4 do not (Jacobsen et al. 2011).

Apart from the lack of sequence diversity in the FcγR binding regions, there are several other significant differences between the macaque and human IgG subclasses. The hinge of macaque IgG3 is encoded by a single exon which in human IgG3 is encoded by multiple homologous exons. This results in a shorter hinge region in the macaque which potentially renders it less flexible than its human counterpart. Importantly, this increased flexibility in human hinge region is thought to confer enhanced HIV-neutralizing ability (Scinicariello et al. 2004; Scharf et al. 2001).

Macaque IgG4 also exhibits significant structural difference from human which are likely to impart a significantly different effects on function. The half molecule exchange, commonly referred to as Fab arm exchange, is a unique property of human IgG4 (van der Neut Kolfschoten et al. 2007) but is unlikely to occur in macaques. Human IgG4 possess a serine at position 228 in the core hinge (Cys^{226}-Pro^{227}-Ser^{228}-Cys^{229}) which destabilises the adjacent inter heavy chain disulphide bonds. This results in the exchange of heavy chains and their attached light chain between individual IgG4 molecules to produce bi-specific antibodies (van der Neut Kolfschoten et al. 2007). However both cynomolgus and rhesus IgG4 (and all IgG subclasses) have proline in this position (Jacobsen et al. 2011; Scinicariello et al. 2004) which stabilises the disulphide bonds preventing the exchange. Thus IgG4 in macaques is likely to retain a classical FcR activating function, rather than the "anti-inflammatory" action of the half molecule exchanged human IgG4 (van der Neut Kolfschoten et al. 2007; Warncke et al. 2012). Whether such binding differences are indeed reflected in differences in biological responses remains to be determined, particularly as substantial sequence differences are apparent in the cytoplasmic tails of NHP FcγR. Thus, it is a formal possibility that differences in the IgG binding to Fc receptors are "compensated" by differences in signal transduction or receptor expression on cells.

5.4 Divergent Tissue Expression Profiles of Human and Macaque FcγR

The tissue distribution of FcγR in macaques has not been as comprehensively studied as humans. Analysis to date indicates that although macaque FcγR expression is similar to human, key differences have been identified in pigtail macaque (Trist et al. 2014) (Hogarth unpublished), in rhesus macaque (Rogers et al. 2006; Choi et al. 2008) and cynomolgus macaque (Table 1) (Warncke et al. 2012).

Flow cytometry shows FcγRIIIa is present on macaque NK cells and monocytes (Webster and Johnson 2005; Choi et al. 2008). FcγRIIa is present on monocytes; macrophages, neutrophils and platelets; (Mahan et al. 1993; Trist et al. 2014; Warncke et al. 2012) by PCR FcγRIIb is present in B cells and classical $CD14^+$ monocytes (Hogarth unpublished).

The major difference between human and macaque receptor expression is observed for FcγRIII which is absent from macaque neutrophils (Warncke et al. 2012; Rogers et al. 2006; Trist et al. 2014). Only one FcγRIII gene is observed in the rhesus macaque genome which likely encodes an ortholog of human FcγRIIIa, rather than the GPI-anchored FcγRIIIb. The lack of FcγRIII expression may be off-set by increased expression of FcγRIIa on neutrophils (Trist et al. 2014). FcγRIIIb appears to expressed on other NHP neutrophils (Rogers et al. 2006).

6 Conclusions

In the three decades since their molecular cloning the human Fc receptors have emerged as one of the key receptor families in human immunity initiating a wide range of immunological and inflammatory responses which is reflective of the large and diverse roles of antibodies in vivo.

Lessons learned from the considerable investigations of FcγR roles in the activation or regulation of antibody induced autoimmune inflammation are likely to be equally applicable in understanding newly emerging importance of antibodies in other diseases including HIV. A growing body of evidence suggests antibodies and FcγR functions may be keys to the development of effective immunity which suggests also that successful vaccines may well depend on the thorough understanding of Fc receptor functions.

Similarly this knowledge of pro- and anti-inflammatory effects of antibodies mediated through FcγR has not only provided mechanistic explanations for antibody function but has also provided the basis for the deliberate manipulation of effector responses by appropriately engineered mAbs.

There is a considerable reliance on outbred populations of NHP, particularly macaques, as an animal model of human immunity, viral pathogenesis as well as the preclinical development of vaccines and therapeutic antibodies. Despite this there is a surprising lack of systematic analysis of NHP FcR functions and until recently, little understanding of the interaction of human IgG with NHP FcγR.

Whilst NHP are a robust model of human immunity there are clear differences in interaction of human IgG subclasses with human FcγR compared to those of the macaque FcγR. Furthermore the existence of a non-functional or hypo-functional receptors as a result of the surprising polymorphism in macaque FcγR adds complexity to the modelling of human IgG behaviour in vivo. The possibility that FcγR effector responses, normally attributable to human IgG may not be faithfully reflected in macaques suggests that prudent design of experiments or testing and data interpretation is required.

A mechanistic understanding of immune process induced or modified by the interaction of IgG and FcγR in normal and pathological immunity will assist in the understanding the natural history of effective immune responses in infection and greatly assist the development of active, vaccine based protection, and passive antibody therapies.

References

Abboud N, Chow SK, Saylor C, Janda A, Ravetch JV, Scharff MD, Casadevall A (2010) A requirement for FcgammaR in antibody-mediated bacterial toxin neutralization. J Exp Med 207(11):2395–2405

Ackerman ME, Crispin M, Yu X, Baruah K, Boesch AW, Harvey DJ, Dugast AS, Heizen EL, Ercan A, Choi I, Streeck H, Nigrovic PA, Bailey-Kellogg C, Scanlan C, Alter G (2013a) Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. J Clin Investig 123(5):2183–2192. doi:10.1172/JCI65708

Ackerman ME, Dugast AS, McAndrew EG, Tsoukas S, Licht AF, Irvine DJ, Alter G (2013b) Enhanced phagocytic activity of HIV-specific antibodies correlates with natural production of immunoglobulins with skewed affinity for FcgammaR2a and FcgammaR2b. J Virol 87(10):5468–5476. doi:10.1128/JVI.03403-12

Alizadeh BZ, Valdigem G, Coenen MJH, Zhernakova A, Franke B, Monsuur A, van Riel PLCM, Barrera P, Radstake TRDJ, Roep BO, Wijmenga C, Koeleman BPC (2007) Association analysis of functional variants of the FcgRIIa and FcgRIIIa genes with type 1 diabetes, celiac disease and rheumatoid arthritis. Hum Mol Genet 16(21):2552–2559. doi:10.1093/hmg/ddm194

Aloulou M, Ben Mkaddem S, Biarnes-Pelicot M, Boussetta T, Souchet H, Rossato E, Benhamou M, Crestani B, Zhu Z, Blank U, Launay P, Monteiro RC (2012) IgG1 and IVIg induce inhibitory ITAM signaling through FcgammaRIII controlling inflammatory responses. Blood 119(13):3084–3096. doi:10.1182/blood-2011-08-376046

Alvarez RA, Hamlin RE, Monroe A, Moldt B, Hotta MT, Rodriguez Caprio G, Fierer DS, Simon V, Chen BK (2014) HIV-1 Vpu antagonism of tetherin inhibits antibody-dependent cellular cytotoxic responses by natural killer cells. J Virol. doi:10.1128/JVI.00449-14

Amigorena S (2002) Fc gamma receptors and cross-presentation in dendritic cells. J Exp Med 195(1):F1–F3

Autran B, Descours B, Avettand-Fenoel V, Rouzioux C (2011) Elite controllers as a model of functional cure. Current opinion in HIV and AIDS 6(3):181–187. doi:10.1097/COH.0b013e328345a328

Barnes N, Gavin AL, Tan PS, Mottram P, Koentgen F, Hogarth PM (2002) FcgammaRI-deficient mice show multiple alterations to inflammatory and immune responses. Immunity 16(3):379–389

Barouch DH, Liu J, Peter L, Abbink P, Iampietro MJ, Cheung A, Alter G, Chung A, Dugast AS, Frahm N, McElrath MJ, Wenschuh H, Reimer U, Seaman MS, Pau MG, Weijtens M,

Goudsmit J, Walsh SR, Dolin R, Baden LR (2013a) Characterization of humoral and cellular immune responses elicited by a recombinant adenovirus serotype 26 HIV-1 Env vaccine in healthy adults (IPCAVD 001). J Infect Dis 207(2):248–256. doi:10.1093/infdis/jis671

Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, Liu J, Abbink P, Maxfield LF, Seaman MS, Dugast AS, Alter G, Ferguson M, Li W, Earl PL, Moss B, Giorgi EE, Szinger JJ, Eller LA, Billings EA, Rao M, Tovanabutra S, Sanders-Buell E, Weijtens M, Pau MG, Schuitemaker H, Robb ML, Kim JH, Korber BT, Michael NL (2013b) Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell 155(3):531–539. doi:10.1016/j.cell.2013.09.061

Bibeau F, Lopez-Crapez E, Di Fiore F, Thezenas S, Ychou M, Blanchard F, Lamy A, Penault-Llorca F, Frébourg T, Michel P, Sabourin J-C, Boissière-Michot F (2009) Impact of FcγRIIa-FcγRIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. J Clin Oncol 27(7):1122–1129. doi:10.1200/jco.2008.18.0463

Brouwer KC, Lal RB, Mirel LB, Yang C, Eijk AM, Ayisi J, Otieno J, Nahlen BL, Steketee R, Lal AA, Shi YP (2004) Polymorphism of Fc receptor IIa for IgG in infants is associated with susceptibility to perinatal HIV-1 infection. AIDS 18(8):1187–1194

Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, Daeron M (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113(16):3716–3725

Burton DR, Woof JM (1992) Human antibody effector function. In: Frank JD (ed) Advances in immunology, vol 51. Academic Press, pp 1–84. doi:http://dx.doi.org/10.1016/S0065-2776(08)60486-1

Cady CT, Powell MS, Harbeck RJ, Giclas PC, Murphy JR, Katial RK, Weber RW, Hogarth PM, Johnson S, Bonvini E, Koenig S, Cambier JC (2010) IgG antibodies produced during subcutaneous allergen immunotherapy mediate inhibition of basophil activation via a mechanism involving both FcgammaRIIA and FcgammaRIIB. Immunol Lett 130(1–2):57–65

Cady CT, Rice JS, Ott VL, Cambier JC (2008) Regulation of hematopoietic cell function by inhibitory immunoglobulin G receptors and their inositol lipid phosphatase effectors. Immunol Rev 224:44–57. doi:10.1111/j.1600-065X.2008.00663.x

Cambier JC (2013) Autoimmunity risk alleles: hotspots in B cell regulatory signaling pathways. J Clin Investig 123(5):1928–1931. doi:10.1172/JCI69289

Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, Watier H (2002) Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. Blood 99(3):754–758. doi:10.1182/blood.V99.3.754

Cassard L, Jonsson F, Arnaud S, Daeron M (2012) Fcgamma receptors inhibit mouse and human basophil activation. J Immunol 189(6):2995–3006. doi:10.4049/jimmunol.1200968

Choi EI, Wang R, Peterson L, Letvin NL, Reimann KA (2008) Use of an anti-CD16 antibody for in vivo depletion of natural killer cells in rhesus macaques. Immunology 124(2):215–222. doi:10.1111/j.1365-2567.2007.02757.x

Chu SY, Horton HM, Pong E, Leung IW, Chen H, Nguyen DH, Bautista C, Muchhal US, Bernett MJ, Moore GL, Szymkowski DE, Desjarlais JR (2012) Reduction of total IgE by targeted coengagement of IgE B-cell receptor and FcgammaRIIb with Fc-engineered antibody. J Allergy Clin Immunol 129(4):1102–1115. doi:10.1016/j.jaci.2011.11.029

Chu SY, Yeter K, Kotha R, Pong E, Miranda Y, Phung S, Chen H, Lee SH, Leung I, Bonzon C, Desjarlais JR, Stohl W, Szymkowski DE (2013) Suppression of rheumatoid arthritis B cells by XmAb5871, an anti-CD19 antibody that coengages B cell antigen receptor and FcgammaRIIb inhibitory receptor. Arthritis Rheum. doi:10.1002/art.38334

Chung AW, Isitman G, Navis M, Kramski M, Center RJ, Kent SJ, Stratov I (2011) Immune escape from HIV-specific antibody-dependent cellular cytotoxicity (ADCC) pressure. Proc Natl Acad Sci USA 108(18):7505–7510. doi:10.1073/pnas.1016048108

Clatworthy MR, Willcocks L, Urban B, Langhorne J, Williams TN, Peshu N, Watkins NA, Floto RA, Smith KG (2007) Systemic lupus erythematosus-associated defects in the inhibitory

receptor FcgammaRIIb reduce susceptibility to malaria. Proc Natl Acad Sci USA 104(17):7169–7174. doi:10.1073/pnas.0608889104

Daeron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S, Fridman WH (1995) The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. Immunity 3(5):635–646

Daeron M, Lesourne R (2006) Negative signaling in Fc receptor complexes. Adv Immunol 89:39–86

Davies AM, Rispens T, Ooijevaar-de Heer P, Gould HJ, Jefferis R, Aalberse RC, Sutton BJ (2014) Structural determinants of unique properties of human IgG4-Fc. J Mol Biol 426(3):630–644. doi:10.1016/j.jmb.2013.10.039

den Dunnen J, Vogelpoel LT, Wypych T, Muller FJ, de Boer L, Kuijpers TW, Zaat SA, Kapsenberg ML, de Jong EC (2012) IgG opsonization of bacteria promotes Th17 responses via synergy between TLRs and FcgammaRIIa in human dendritic cells. Blood 120(1):112–121. doi:10.1182/blood-2011-12-399931

Dijstelbloem HM, Scheepers RHM, Oost WW, Stegeman CA, Van Der Pol WL, Sluiter WJ, Kallenberg CGM, Van De Winkel JGJ, Tervaert JWC (1999) Fcγ receptor polymorphisms in Wegener's granulomatosis: risk factors for disease relapse. Arthritis Rheum 42(9):1823–1827. doi:10.1002/1529-0131(199909)42:9<1823:AID-ANR5>3.0.CO;2-X

Domingo P, Muñiz-Diaz E, Baraldès MA, Arilla M, Barquet N, Pericas R, Juárez C, Madoz P, Vázquez G (2002) Associations between Fc gamma receptor IIA polymorphisms and the risk and prognosis of meningococcal disease. Am J Med 112 (1):19–25. doi:http://dx.doi.org/10.1016/S0002-9343(01)01047-6

Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, Dekosky BJ, Ernandes MJ, Georgiev IS, Kim HJ, Pancera M, Staupe RP, Altae-Tran HR, Bailer RT, Crooks ET, Cupo A, Druz A, Garrett NJ, Hoi KH, Kong R, Louder MK, Longo NS, McKee K, Nonyane M, O'Dell S, Roark RS, Rudicell RS, Schmidt SD, Sheward DJ, Soto C, Wibmer CK, Yang Y, Zhang Z, Nisc Comparative S, Mullikin JC, Binley JM, Sanders RW, Wilson IA, Moore JP, Ward AB, Georgiou G, Williamson C, Abdool Karim SS, Morris L, Kwong PD, Shapiro L, Mascola JR (2014) Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature. doi:10.1038/nature13036

Dugast AS, Tonelli A, Berger CT, Ackerman ME, Sciaranghella G, Liu Q, Sips M, Toth I, Piechocka-Trocha A, Ghebremichael M, Alter G (2011) Decreased Fc receptor expression on innate immune cells is associated with impaired antibody-mediated cellular phagocytic activity in chronically HIV-1 infected individuals. Virology 415(2):160–167. doi:10.1016/j.virol.2011.03.012

Duncan AR, Woof JM, Partridge LJ, Burton DR, Winter G (1988) Localization of the binding site for the human high-affinity Fc receptor on IgG. Nature 332(6164):563–564. doi:10.1038/332563a0

Ellery PJ, Tippett E, Chiu YL, Paukovics G, Cameron PU, Solomon A, Lewin SR, Gorry PR, Jaworowski A, Greene WC, Sonza S, Crowe SM (2007) The CD16 + monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. J Immunol 178(10):6581–6589

Ferrara C, Grau S, Jager C, Sondermann P, Brunker P, Waldhauer I, Hennig M, Ruf A, Rufer AC, Stihle M, Umana P, Benz J (2011) Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcgammaRIII and antibodies lacking core fucose. Proc Natl Acad Sci USA 108(31):12669–12674. doi:10.1073/pnas.1108455108

Fijen CA, Bredius RG, Kuijper EJ (1993) Polymorphism of IgG Fc receptors in meningococcal disease. Ann Intern Med 119(7 Pt 1):636

Forthal DN, Gabriel EE, Wang A, Landucci G, Phan TB (2012) Association of Fcgamma receptor IIIa genotype with the rate of HIV infection after gp120 vaccination. Blood 120(14):2836–2842. doi:10.1182/blood-2012-05-431361

Forthal DN, Landucci G, Bream J, Jacobson LP, Phan TB, Montoya B (2007) FcgammaRIIa genotype predicts progression of HIV infection. J Immunol 179(11):7916–7923

Forthal DN, Landucci G, Ding H, Kappes JC, Wang A, Thung I, Phan T (2011) IgG2 inhibits HIV-1 internalization by monocytes, and IgG subclass binding is affected by gp120 glycosylation. AIDS 25(17):2099–2104. doi:10.1097/QAD.0b013e32834b64bd

Forthal DN, Landucci G, Phan TB, Becerra J (2005) Interactions between natural killer cells and antibody Fc result in enhanced antibody neutralization of human immunodeficiency virus type 1. J Virol 79(4):2042–2049. doi:10.1128/JVI.79.4.2042-2049.2005

Forthal DN, Moog C (2009) Fc receptor-mediated antiviral antibodies. Curr Opin HIV AIDS 4(5):388–393. doi:10.1097/COH.0b013e32832f0a89

French MA, Center RJ, Wilson KM, Fleyfel I, Fernandez S, Schorcht A, Stratov I, Kramski M, Kent SJ, Kelleher AD (2013) Isotype-switched immunoglobulin G antibodies to HIV Gag proteins may provide alternative or additional immune responses to 'protective' human leukocyte antigen-B alleles in HIV controllers. AIDS 27(4):519–528. doi:10.1097/QAD.0b013e32835cb720

French MA, Tanaskovic S, Law MG, Lim A, Fernandez S, Ward LD, Kelleher AD, Emery S (2010) Vaccine-induced IgG2 anti-HIV p24 is associated with control of HIV in patients with a 'high-affinity' FcgammaRIIa genotype. AIDS 24(13):1983–1990

Gavin AL, Barnes N, Dijstelbloem HM, Hogarth PM (1998a) Identification of the mouse IgG3 receptor: implications for antibody effector function at the interface between innate and adaptive immunity. J Immunol 160(1):20–23

Gavin AL, Tan PS, Hogarth PM (1998b) Gain-of-function mutations in FcgammaRI of NOD mice: implications for the evolution of the Ig superfamily. EMBO J 17(14):3850–3857

Giha HA, Nasr A, Iriemenam NC, Troye-Blomberg M, Berzins K, ElGhazali G (2012) Lack of significant influence for FcgammaRIIa-RH131 or hemoglobin AA/AS polymorphisms on immunity and susceptibility to uncomplicated malaria and existence of marked linkage between the two polymorphisms in Daraweesh. Microbes Infect 14(6):537–544. doi:10.1016/j.micinf.2012.01.003

Gomez-Roman VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, Florese R, Robert-Guroff M (2005) Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. J Immunol 174(4):2185–2189

Gupta S, Gach JS, Becerra JC, Phan TB, Pudney J, Moldoveanu Z, Joseph SB, Landucci G, Supnet MJ, Ping LH, Corti D, Moldt B, Hel Z, Lanzavecchia A, Ruprecht RM, Burton DR, Mestecky J, Anderson DJ, Forthal DN (2013) The neonatal Fc receptor (FcRn) enhances human immunodeficiency virus type 1 (HIV-1) transcytosis across epithelial cells. PLoS Pathog 9(11):e1003776. doi:10.1371/journal.ppat.1003776

Harley JB, Alarcon-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP, Moser KL, Tsao BP, Vyse TJ, Langefeld CD (2008) Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. Nat Genet 40 (2):204–210. doi:http://www.nature.com/ng/journal/v40/n2/suppinfo/ng.81_S1.html

Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH (2012) Immune-correlates analysis of an HIV-1 vaccine efficacy trial. New Engl J Med 366(14):1275–1286. doi:10.1056/NEJMoa1113425

Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, Lanigan CM, Landucci G, Forthal DN, Parren PW, Marx PA, Burton DR (2007) Fc receptor but not complement binding is important in antibody protection against HIV. Nature 449(7158):101–104

Hessell AJ, Rakasz EG, Poignard P, Hangartner L, Landucci G, Forthal DN, Koff WC, Watkins DI, Burton DR (2009) Broadly neutralizing human anti-HIV antibody 2G12 is effective in

protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog 5(5):e1000433

Hibbs ML, Bonadonna L, Scott BM, McKenzie IF, Hogarth PM (1988) Molecular cloning of a human immunoglobulin G Fc receptor. Proc Natl Acad Sci USA 85(7):2240–2244

Hogarth PM (2002) Fc receptors are major mediators of antibody based inflammation in autoimmunity. Curr Opin Immunol 14(6):798–802

Hogarth PM, Pietersz GA (2012) Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. Nat Rev Drug Discov 11(4):311–331. doi:10.1038/nrd2909

Holl V, Hemmerter S, Burrer R, Schmidt S, Bohbot A, Aubertin AM, Moog C (2004) Involvement of Fc gamma RI (CD64) in the mechanism of HIV-1 inhibition by polyclonal IgG purified from infected patients in cultured monocyte-derived macrophages. J Immunol 173(10):6274–6283

Horton HM, Chu SY, Ortiz EC, Pong E, Cemerski S, Leung IW, Jacob N, Zalevsky J, Desjarlais JR, Stohl W, Szymkowski DE (2011) Antibody-mediated coengagement of FcgammaRIIb and B cell receptor complex suppresses humoral immunity in systemic lupus erythematosus. J Immunol 186(7):4223–4233. doi:10.4049/jimmunol.1003412

Hulett MD, Brinkworth RI, McKenzie IF, Hogarth PM (1999) Fine structure analysis of interaction of FcepsilonRI with IgE. J Biol Chem 274(19):13345–13352

Hulett MD, Hogarth PM (1994) Molecular basis of Fc receptor function. Adv Immunol 57:1–127

Hulett MD, Witort E, Brinkworth RI, McKenzie IF, Hogarth PM (1995) Multiple regions of human Fc gamma RII (CD32) contribute to the binding of IgG. J Biol Chem 270(36):21188–21194

Ierino FL, Powell MS, McKenzie IF, Hogarth PM (1993) Recombinant soluble human Fc gamma RII: production, characterization, and inhibition of the Arthus reaction. J Exp Med 178(5):1617–1628

International HIVCS, Pereyra F, Jia X, McLaren PJ, Telenti A, de Bakker PI, Walker BD, Ripke S, Brumme CJ, Pulit SL, Carrington M, Kadie CM, Carlson JM, Heckerman D, Graham RR, Plenge RM, Deeks SG, Gianniny L, Crawford G, Sullivan J, Gonzalez E, Davies L, Camargo A, Moore JM, Beattie N, Gupta S, Crenshaw A, Burtt NP, Guiducci C, Gupta N, Gao X, Qi Y, Yuki Y, Piechocka-Trocha A, Cutrell E, Rosenberg R, Moss KL, Lemay P, O'Leary J, Schaefer T, Verma P, Toth I, Block B, Baker B, Rothchild A, Lian J, Proudfoot J, Alvino DM, Vine S, Addo MM, Allen TM, Altfeld M, Henn MR, Le Gall S, Streeck H, Haas DW, Kuritzkes DR, Robbins GK, Shafer RW, Gulick RM, Shikuma CM, Haubrich R, Riddler S, Sax PE, Daar ES, Ribaudo HJ, Agan B, Agarwal S, Ahern RL, Allen BL, Altidor S, Altschuler EL, Ambardar S, Anastos K, Anderson B, Anderson V, Andrady U, Antoniskis D, Bangsberg D, Barbaro D, Barrie W, Bartczak J, Barton S, Basden P, Basgoz N, Bazner S, Bellos NC, Benson AM, Berger J, Bernard NF, Bernard AM, Birch C, Bodner SJ, Bolan RK, Boudreaux ET, Bradley M, Braun JF, Brndjar JE, Brown SJ, Brown K, Brown ST, Burack J, Bush LM, Cafaro V, Campbell O, Campbell J, Carlson RH, Carmichael JK, Casey KK, Cavacuiti C, Celestin G, Chambers ST, Chez N, Chirch LM, Cimoch PJ, Cohen D, Cohn LE, Conway B, Cooper DA, Cornelson B, Cox DT, Cristofano MV, Cuchural G, Jr, Czartoski JL, Dahman JM, Daly JS, Davis BT, Davis K, Davod SM, DeJesus E, Dietz CA, Dunham E, Dunn ME, Ellerin TB, Eron JJ, Fangman JJ, Farel CE, Ferlazzo H, Fidler S, Fleenor-Ford A, Frankel R, Freedberg KA, French NK, Fuchs JD, Fuller JD, Gaberman J, Gallant JE, Gandhi RT, Garcia E, Garmon D, Gathe JC, Jr, Gaultier CR, Gebre W, Gilman FD, Gilson I, Goepfert PA, Gottlieb MS, Goulston C, Groger RK, Gurley TD, Haber S, Hardwicke R, Hardy WD, Harrigan PR, Hawkins TN, Heath S, Hecht FM, Henry WK, Hladek M, Hoffman RP, Horton JM, Hsu RK, Huhn GD, Hunt P, Hupert MJ, Illeman ML, Jaeger H, Jellinger RM, John M, Johnson JA, Johnson KL, Johnson H, Johnson K, Joly J, Jordan WC, Kauffman CA, Khanlou H, Killian RK, Kim AY, Kim DD, Kinder CA, Kirchner JT, Kogelman L, Kojic EM, Korthuis PT, Kurisu W, Kwon DS, LaMar M, Lampiris H, Lanzafame M, Lederman MM, Lee DM, Lee JM, Lee MJ, Lee ET, Lemoine J, Levy JA, Llibre JM, Liguori MA, Little SJ, Liu AY, Lopez AJ, Loutfy MR, Loy D, Mohammed DY, Man A, Mansour MK, Marconi VC, Markowitz M, Marques R, Martin JN, Martin HL, Jr, Mayer KH, McElrath MJ, McGhee TA,

McGovern BH, McGowan K, McIntyre D, McLeod GX, Menezes P, Mesa G, Metroka CE, Meyer-Olson D, Miller AO, Montgomery K, Mounzer KC, Nagami EH, Nagin I, Nahass RG, Nelson MO, Nielsen C, Norene DL, O'Connor DH, Ojikutu BO, Okulicz J, Oladehin OO, Oldfield EC, 3rd, Olender SA, Ostrowski M, Owen WF, Jr, Pae E, Parsonnet J, Pavlatos AM, Perlmutter AM, Pierce MN, Pincus JM, Pisani L, Price LJ, Proia L, Prokesch RC, Pujet HC, Ramgopal M, Rathod A, Rausch M, Ravishankar J, Rhame FS, Richards CS, Richman DD, Rodes B, Rodriguez M, Rose RC, 3rd, Rosenberg ES, Rosenthal D, Ross PE, Rubin DS, Rumbaugh E, Saenz L, Salvaggio MR, Sanchez WC, Sanjana VM, Santiago S, Schmidt W, Schuitemaker H, Sestak PM, Shalit P, Shay W, Shirvani VN, Silebi VI, Sizemore JM, Jr, Skolnik PR, Sokol-Anderson M, Sosman JM, Stabile P, Stapleton JT, Starrett S, Stein F, Stellbrink HJ, Sterman FL, Stone VE, Stone DR, Tambussi G, Taplitz RA, Tedaldi EM, Telenti A, Theisen W, Torres R, Tosiello L, Tremblay C, Tribble MA, Trinh PD, Tsao A, Ueda P, Vaccaro A, Valadas E, Vanig TJ, Vecino I, Vega VM, Veikley W, Wade BH, Walworth C, Wanidworanun C, Ward DJ, Warner DA, Weber RD, Webster D, Weis S, Wheeler DA, White DJ, Wilkins E, Winston A, Wlodaver CG, van't Wout A, Wright DP, Yang OO, Yurdin DL, Zabukovic BW, Zachary KC, Zeeman B, Zhao M (2010) The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. Science 330(6010):1551–1557. doi:10.1126/science.1195271

Isitman G, Stratov I, Kent SJ (2012) Antibody-dependent cellular cytotoxicity and NK cell-driven immune escape in HIV infection: implications for HIV vaccine development. Adv Virol 2012:637208. doi:10.1155/2012/637208

Jacobsen FW, Padaki R, Morris AE, Aldrich TL, Armitage RJ, Allen MJ, Lavallee JC, Arora T (2011) Molecular and functional characterization of cynomolgus monkey IgG subclasses. J Immunol 186:341–349

Jonsson F, Daeron M (2012) Mast cells and company. Front Immunol 3:16. doi:10.3389/fimmu.2012.00016

Karassa FB, Trikalinos TA, Ioannidis JP, Fcgamma R-SLEM-AI (2002) Role of the Fcgamma receptor IIa polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. Arthritis Rheum 46(6):1563–1571. doi:10.1002/art.10306

Kedzierska K, Azzam R, Ellery P, Mak J, Jaworowski A, Crowe SM (2003) Defective phagocytosis by human monocyte/macrophages following HIV-1 infection: underlying mechanisms and modulation by adjunctive cytokine therapy. J Clin Virol 26(2):247–263

Klein JS, Bjorkman PJ (2010) Few and far between: how HIV may be evading antibody avidity. PLoS Pathog 6(5):e1000908

Koene HR, Kleijer M, Swaak AJ, Sullivan KE, Bijl M, Petri MA, Kallenberg CG, Roos D, von dem Borne AE, de Haas M (1998) The Fc gammaRIIIA-158F allele is a risk factor for systemic lupus erythematosus. Arthritis Rheum 41(10):1813–1818. doi:10.1002/1529-0131(199810)41:10<1813:AID-ART13>3.0.CO;2-6

Kramski M, Schorcht A, Johnston AP, Lichtfuss GF, Jegaskanda S, De Rose R, Stratov I, Kelleher AD, French MA, Center RJ, Jaworowski A, Kent SJ (2012) Role of monocytes in mediating HIV-specific antibody-dependent cellular cytotoxicity. J Immunol Methods 384(1–2):51–61. doi:10.1016/j.jim.2012.07.006

Lambotte O, Pollara J, Boufassa F, Moog C, Venet A, Haynes BF, Delfraissy JF, Saez-Cirion A, Ferrari G (2013) High antibody-dependent cellular cytotoxicity responses are correlated with strong CD8 T cell viral suppressive activity but not with B57 status in HIV-1 elite controllers. PLoS ONE 8(9):e74855. doi:10.1371/journal.pone.0074855

Leeansyah E, Wines BD, Crowe SM, Jaworowski A (2007) The mechanism underlying defective Fcgamma receptor-mediated phagocytosis by HIV-1-infected human monocyte-derived macrophages. J Immunol 178(2):1096–1104

Lessard CJ, Li H, Adrianto I, Ice JA, Rasmussen A, Grundahl KM, Kelly JA, Dozmorov MG, Miceli-Richard C, Bowman S, Lester S, Eriksson P, Eloranta ML, Brun JG, Goransson LG, Harboe E, Guthridge JM, Kaufman KM, Kvarnstrom M, Jazebi H, Cunninghame Graham DS, Grandits ME, Nazmul-Hossain AN, Patel K, Adler AJ, Maier-Moore JS, Farris AD, Brennan MT, Lessard JA, Chodosh J, Gopalakrishnan R, Hefner KS, Houston GD, Huang AJ, Hughes PJ,

Lewis DM, Radfar L, Rohrer MD, Stone DU, Wren JD, Vyse TJ, Gaffney PM, James JA, Omdal R, Wahren-Herlenius M, Illei GG, Witte T, Jonsson R, Rischmueller M, Ronnblom L, Nordmark G, Ng WF, Registry UKPSsS, Mariette X, Anaya JM, Rhodus NL, Segal BM, Scofield RH, Montgomery CG, Harley JB, Sivils KL (2013) Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. Nat Genet 45(11):1284–1292. doi:10.1038/ng.2792

Li X, Wu J, Carter RH, Edberg JC, Su K, Cooper GS, Kimberly RP (2003) A novel polymorphism in the Fcγ receptor IIB (CD32B) transmembrane region alters receptor signaling. Arthritis Rheum 48(11):3242–3252. doi:10.1002/art.11313

Lichterfeld M, Yu XG (2012) The emerging role of leukocyte immunoglobulin-like receptors (LILRs) in HIV-1 infection. J Leukoc Biol 91(1):27–33. doi:10.1189/jlb.0811442

Lichtfuss GF, Cheng WJ, Farsakoglu Y, Paukovics G, Rajasuriar R, Velayudham P, Kramski M, Hearps AC, Cameron PU, Lewin SR, Crowe SM, Jaworowski A (2012) Virologically suppressed HIV patients show activation of NK cells and persistent innate immune activation. J Immunol 189(3):1491–1499. doi:10.4049/jimmunol.1200458

Lu J, Ellsworth JL, Hamacher N, Oak SW, Sun PD (2011) Crystal structure of Fc receptor I and its implication in high affinity γ-immunoglobulin binding. J Biol Chem 286(47):40608–40613

Ludlow LE, Zhou J, Tippett E, Cheng WJ, Hasang W, Rogerson SJ, Jaworowski A (2012) HIV-1 inhibits phagocytosis and inflammatory cytokine responses of human monocyte-derived macrophages to P. falciparum infected erythrocytes. PLoS ONE 7(2):e32102. doi:10.1371/journal.pone.0032102

Maenaka K, van der Merwe PA, Stuart DI, Jones EY, Sondermann P (2001) The human low affinity Fcγ receptors IIa, IIb, and III Bind IgG with fast kinetics and distinct thermodynamic properties. J Biol Chem 276(48):44898–44904. doi:10.1074/jbc.M106819200

Mahan JD, Hebert LA, McAllister C, Birmingham DJ, Shen XP, Cosio FG, Brandt J (1993) Platelet involvement in experimental immune complex-mediated glomerulonephritis in the nonhuman primate. Kidney Int 44(4):716–725

Maiga B, Dolo A, Touré O, Dara V, Tapily A, Campino S, Sepulveda N, Corran P, Rockett K, Clark TG, Troye Blomberg M, Doumbo OK (2014) Fc gamma receptor IIa-H131R polymorphism and malaria susceptibility in sympatric ethnic groups, Fulani and Dogon of Mali. Scand J Immunol 79(1):43–50. doi:10.1111/sji.12122

Manuzak JA, Dillon SM, Lee EJ, Dong ZM, Hecht DK, Wilson CC (2013) Increased Escherichia coli-induced interleukin-23 production by CD16 + monocytes correlates with systemic immune activation in untreated HIV-1-infected individuals. J Virol 87(24):13252–13262. doi:10.1128/JVI.01767-13

Maxwell KF, Powell MS, Hulett MD, Barton PA, McKenzie IF, Garrett TP, Hogarth PM (1999) Crystal structure of the human leukocyte Fc receptor, Fc gammaRIIa. Nat Struct Biol 6(5):437–442

McGovern DPB, Gardet A, Törkvist L, Goyette P, Essers J, Taylor KD, Neale BM, Ong RTH, Lagacé C, Li C, Green T, Stevens CR, Beauchamp C, Fleshner PR, Carlson M, D'Amato M, Halfvarson J, Hibberd ML, Lördal M, Padyukov L, Andriulli A, Colombo E, Latiano A, Palmieri O, Bernard E-J, Deslandres C, Hommes DW, de Jong DJ, Stokkers PC, Weersma RK, The NIDDKIBDGC, Sharma Y, Silverberg M, Cho JH, Wu J, Roeder K, Brant SR, Schumm LP, Duerr RH, Dubinsky MC, Glazer NL, Haritunians T, Ippoliti A, Melmed GY, Siscovick DS, Vasiliauskas EA, Targan SR, Annese V, Wijmenga C, Pettersson S, Rotter JI, Xavier RJ, Daly MJ, Rioux JD, Seielstad M (2010) Genome-wide association identifies multiple ulcerative colitis susceptibility loci. Nat Genet 42(4):332–337

Means TK, Latz E, Hayashi F, Murali MR, Golenbock DT, Luster AD (2005) Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. J Clin Investig 115(2):407–417. doi:10.1172/JCI23025

Metzger H (1992) Transmembrane signaling: the joy of aggregation. J Immunol 149(5):1477–1487

Meziani R, Yamada R, Takahashi M, Ohigashi K, Morinobu A, Terao C, Hiratani H, Ohmura K, Yamaguchi M, Nomura T, Vasilescu A, Kokubo M, Renault V, Hirosawa K, Ratanajaraya C,

Heath S, Mimori T, Sakaguchi S, Lathrop M, Melchers I, Kumagai S, Matsuda F (2012) A trans-ethnic genetic study of rheumatoid arthritis identified FCGR2A as a candidate common risk factor in Japanese and European populations. Mod Rheumatol 22(1):52–58. doi:10.1007/s10165-011-0467-y

Mihai S, Nimmerjahn F (2013) The role of Fc receptors and complement in autoimmunity. Autoimmun Rev 12(6):657–660. doi:10.1016/j.autrev.2012.10.008

Mizushima T, Yagi H, Takemoto E, Shibata-Koyama M, Isoda Y, Iida S, Masuda K, Satoh M, Kato K (2011) Structural basis for improved efficacy of therapeutic antibodies on defucosylation of their Fc glycans. Genes Cells 16(11):1071–1080. doi:10.1111/j.1365-2443.2011.01552.x

Moldt B, Shibata-Koyama M, Rakasz EG, Schultz N, Kanda Y, Dunlop DC, Finstad SL, Jin C, Landucci G, Alpert MD, Dugast AS, Parren PW, Nimmerjahn F, Evans DT, Alter G, Forthal DN, Schmitz JE, Iida S, Poignard P, Watkins DI, Hessell AJ, Burton DR (2012) A nonfucosylated variant of the anti-HIV-1 monoclonal antibody b12 has enhanced FcgammaRIIIa-mediated antiviral activity in vitro but does not improve protection against mucosal SHIV challenge in macaques. J Virol 86(11):6189–6196. doi:10.1128/JVI.00491-12

Moog C, Dereuddre-Bosquet N, Teillaud JL, Biedma ME, Holl V, Van Ham G, Heyndrickx L, Van Dorsselaer A, Katinger D, Vcelar B, Zolla-Pazner S, Mangeot I, Kelly C, Shattock RJ, Le Grand R (2014) Protective effect of vaginal application of neutralizing and nonneutralizing inhibitory antibodies against vaginal SHIV challenge in macaques. Mucosal Immunol 7(1):46–56. doi:10.1038/mi.2013.23

Naziruddin B, Duffy BF, Tucker J, Mohanakumar T (1992) Evidence for cross-regulation of Fc gamma RIIIB (CD16) receptor-mediated signaling by Fc gamma RII (CD32) expressed on polymorphonuclear neutrophils. J Immunol 149(11):3702–3709

Nguyen D, Sanghvi R, Scinicariello F, Pulit-Penaloza J, Hill N, Attanasio R (2014) Cynomolgus and pigtail macaque IgG subclasses: characterization of IGHG genes and computational analysis of IgG/Fc receptor binding affinity. Immunogenetics 66(6):361−377. doi:10.1007/s00251-014-0775-4

Nguyen D, Scinicariello F, Attanasio R (2011) Characterization and allelic polymorphisms of rhesus macaque (Macaca mulatta) IgG Fc receptor genes. Immunogenetics 63(6):351–362. doi:10.1007/s00251-011-0514-z

Peressin M, Proust A, Schmidt S, Su B, Lambotin M, Biedma ME, Laumond G, Decoville T, Holl V, Moog C (2014) Efficient transfer of HIV-1 in trans and in cis from Langerhans dendritic cells and macrophages to autologous T lymphocytes. Aids. doi:10.1097/QAD.0000000000000193

Perez LG, Costa MR, Todd CA, Haynes BF, Montefiori DC (2009) Utilization of immunoglobulin G Fc receptors by human immunodeficiency virus type 1: a specific role for antibodies against the membrane-proximal external region of gp41. J Virol 83(15):7397–7410. doi:10.1128/JVI.00656-09

Pietersz GA, Mottram PL, van de Velde NC, Sardjono CT, Esparon S, Ramsland PA, Moloney G, Baell JB, McCarthy TD, Matthews BR, Powell MS, Hogarth PM (2009) Inhibition of destructive autoimmune arthritis in FcgammaRIIa transgenic mice by small chemical entities. Immunol Cell Biol 87(1):3–12

Powell MS, Barnes NC, Bradford TM, Musgrave IF, Wines BD, Cambier JC, Hogarth PM (2006) Alteration of the Fc gamma RIIa dimer interface affects receptor signaling but not ligand binding. J Immunol 176(12):7489–7494

Powell MS, Barton PA, Emmanouilidis D, Wines BD, Neumann GM, Peitersz GA, Maxwell KF, Garrett TPJ, Hogarth PM (1999) Biochemical analysis and crystallisation of FcγRIIa, the low affinity receptor for IgG. Immunol Lett 68(1):17–23

Radaev S, Motyka S, Fridman W-H, Sautes-Fridman C, Sun PD (2001) The structure of a human type III Fcγ receptor in complex with Fc. J Biol Chem 276(19):16469–16477. doi:10.1074/jbc.M100350200

Ramsland PA, Farrugia W, Bradford TM, Sardjono CT, Esparon S, Trist HM, Powell MS, Tan PS, Cendron AC, Wines BD, Scott AM, Hogarth PM (2011) Structural basis for Fc

gammaRIIa recognition of human IgG and formation of inflammatory signaling complexes. J Immunol 187(6):3208–3217

Ravetch J (2010) In vivo veritas: the surprising roles of Fc receptors in immunity. Nat Immunol 11(3):183–185. doi:10.1038/ni0310-183

Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH (2009) Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. New Engl J Med 361(23):2209–2220

Rispens T, Davies AM, Ooijevaar-de Heer P, Absalah S, Bende O, Sutton BJ, Vidarsson G, Aalberse RC (2014) Dynamics of inter-heavy chain interactions in human immunoglobulin G (IgG) subclasses studied by kinetic Fab arm exchange. J Biol Chem 289(9):6098–6109. doi:10.1074/jbc.M113.541813

Rogers KA, Scinicariello F, Attanasio R (2006) IgG Fc receptor III homologues in nonhuman primate species: genetic characterization and ligand interactions. J Immunol 177(6):3848–3856

Sallusto F, Lanzavecchia A (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 179(4):1109–1118

Scharf O, Golding H, King LR, Eller N, Frazier D, Golding B, Scott DE (2001) Immunoglobulin G3 from polyclonal human immunodeficiency virus (HIV) immune globulin is more potent than other subclasses in neutralizing HIV type 1. J Virol 75(14):6558–6565. doi:10.1128/jvi.75.14.6558-6565.2001

Scinicariello F, Engleman CN, Jayashankar L, McClure HM, Attanasio R (2004) Rhesus macaque antibody molecules: sequences and heterogeneity of alpha and gamma constant regions. Immunology 111(1):66–74. doi:10.1111/j.1365-2567.2004.01767.x

Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SH, Presta LG (2002) Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 277(30):26733–26740. doi:10.1074/jbc.M202069200

Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, Xie D, Lai J, Stadlen A, Li B, Fox JA, Presta LG (2001) High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. J Biol Chem 276(9):6591–6604. doi:10.1074/jbc.M009483200

Sole-Violan J, Garcia-Laorden MI, Marcos-Ramos JA, Rodriguez de Castro F, Rajas O, Borderias L, Briones ML, Herrera-Ramos E, Blanquer J, Aspa J, Florido Y, Garcia-Bello MA, Ferrer-Aguero JM, Sologuren I, Rodriguez-Gallego C (2011) The Fc[gamma] receptor IIA-H/H131 genotype is associated with bacteremia in pneumococcal community-acquired pneumonia. Crit Care Med 39(6):1388–1393

Sondermann P, Huber R, Oosthuizen V, Jacob U (2000) The 3.2-Å crystal structure of the human IgG1 Fc fragment–Fcbig gammaRIII complex. Nature 406:267–273

Starbeck-Miller GR, Badovinac VP, Barber DL, Harty JT (2014) Cutting edge: expression of FcγRIIB tempers memory CD8 T cell function in vivo. J Immunol 192(1):35–39. doi:10.4049/jimmunol.1302232

Takai T (2005) Fc receptors and their role in immune regulation and autoimmunity. J Clin Immunol 25(1):1–18. doi:10.1007/s10875-005-0353-8

Tan Sardjono C, Mottram PL, Hogarth PM (2003) The role of FcgammaRIIa as an inflammatory mediator in rheumatoid arthritis and systemic lupus erythematosus. Immunol Cell Biol 81(5):374–381

Tan Sardjono C, Mottram PL, van de Velde NC, Powell MS, Power D, Slocombe RF, Wicks IP, Campbell IK, McKenzie SE, Brooks M, Stevenson AW, Hogarth PM (2005) Development of spontaneous multisystem autoimmune disease and hypersensitivity to antibody-induced inflammation in Fcgamma receptor IIa-transgenic mice. Arthritis Rheum 52(10):3220–3229

Tate BJ, Witort E, McKenzie IFC, Hogarth PM (1992) Expression of the high responder/non-responder human Fcbig gammaRII. Analysis by PCR and transfection into FcR COS cells. Immunol Cell Biol 70:79–87

Trist HM, Tan PS, Wines BD, Ramsland PA, Orlowski E, Stubbs J, Gardiner EE, Pietersz GA, Kent SJ, Stratov I, Burton DR, Hogarth PM (2014) Polymorphisms and interspecies differences of the activating and inhibitory FcγRII of Macaca nemestrina influence the binding of human IgG subclasses. J Immunol 192(2):792–803. doi:10.4049/jimmunol.1301554

Tse WY, Abadeh S, Jefferis R, Savage CO, Adu D (2000) Neutrophil FcgammaRIIIb allelic polymorphism in anti-neutrophil cytoplasmic antibody (ANCA)-positive systemic vasculitis. Clin Exp Immunol 119(3):574–577

van der Heijden J, Breunis WB, Geissler J, de Boer M, van den Berg TK, Kuijpers TW (2012) Phenotypic variation in IgG receptors by nonclassical FCGR2C alleles. J Immunol 188(3):1318–1324. doi:10.4049/jimmunol.1003945

van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martínez-Martínez P, Vermeulen E, den Bleker TH, Wiegman L, Vink T, Aarden LA, De Baets MH, van de Winkel JGJ, Aalberse RC, Parren PWHI (2007) Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 317(5844):1554–1557. doi:10.1126/science.1144603

van Sorge NM, van der Pol WL, Jansen MD, Geleijns KP, Kalmijn S, Hughes RA, Rees JH, Pritchard J, Vedeler CA, Myhr KM, Shaw C, van Schaik IN, Wokke JH, van Doorn PA, Jacobs BC, van de Winkel JG, van den Berg LH (2005) Severity of Guillain-Barre syndrome is associated with Fc gamma receptor III polymorphisms. J Neuroimmunol 162(1–2):157–164. doi:10.1016/j.jneuroim.2005.01.016

van Sorge NM, van Der Pol WL, van De Winkel JGJ (2003) FcγR polymorphisms: implications for function, disease susceptibility and immunotherapy. Tissue Antigens 61(3):189–202. doi:10.1034/j.1399-0039.2003.00037.x

Waltz E (2012) HIV neutralizing antibodies reignite interest in vaccine. Nat Biotechnol 30(3):204–205

Warncke M, Calzascia T, Coulot M, Balke N, Touil R, Koblinger F, Heusser C (2012) Different adaptations of IgG effector function in human and nonhuman primates and implications for therapeutic antibody treatment. J Immunol 188:4405–4411

Warnerdanm OAM, van de Winkel JGJ, Gosselin EJ, Capel PJA (1990) Molecular basis for a polymorphism of human Fc gamma receptor II (CD32). J Exp Med 172(1):19–25

Waterman PM, Cambier JC (2010) The conundrum of inhibitory signaling by ITAM-containing immunoreceptors: potential molecular mechanisms. FEBS Lett 584(24):4878–4882. doi:10.1016/j.febslet.2010.09.029

Webster RL, Johnson RP (2005) Delineation of multiple subpopulations of natural killer cells in rhesus macaques. Immunology 115(2):206–214. doi:10.1111/j.1365-2567.2005.02147.x

Weng W-K, Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to Rituximab in patients with follicular lymphoma. J Clin Oncol 21(21):3940–3947. doi:10.1200/jco.2003.05.013

Willcocks LC, Lyons PA, Clatworthy MR, Robinson JI, Yang W, Newland SA, Plagnol V, McGovern NN, Condliffe AM, Chilvers ER, Adu D, Jolly EC, Watts R, Lau YL, Morgan AW, Nash G, Smith KG (2008) Copy number of FCGR3B, which is associated with systemic lupus erythematosus, correlates with protein expression and immune complex uptake. J Exp Med 205(7):1573–1582. doi:10.1084/jem.20072413

Wren LH, Chung AW, Isitman G, Kelleher AD, Parsons MS, Amin J, Cooper DA, Investigators Asc, Stratov I, Navis M, Kent SJ (2012) Specific antibody-dependent cellular cytotoxicity responses associated with slow progression of HIV infection. Immunology 138(2):116-123. doi:10.1111/imm.12016

Part V
FcRs and Therapeutic Antibodies

FcγRIIB as a Key Determinant of Agonistic Antibody Efficacy

Ann L. White, Stephen A. Beers and Mark S. Cragg

Abstract Fc gamma Receptor (FcγR) IIB (CD32B) is an immunoreceptor tyrosine inhibitory motif (ITIM)-bearing Fc receptor that is involved in abrogating the signalling and function delivered from other receptors; archetypally those arising from other, activatory, FcγR and from the B cell receptor (BCR) for antigen. In the context of immunotherapy, it has convincingly been shown to limit a variety of clinically important therapeutic monoclonal antibodies (mAb) such as rituximab and trastuzumab in preclinical models. However, recent exploration of so-called immunomodulatory mAb, for example agonist mAb directed against various members of the TNFR super-family, has cast new light on the ability of FcγRIIB to regulate immune responses and immunotherapy. These data, accumulated by several independent groups, have shown the seemingly paradoxical ability of FcγRIIB to augment or even be absolutely required for the activity of this class of mAb. In this review we highlight the key role of FcγRIIB in regulating agonistic mAb, detail the likely mechanism of action and propose new ways in which this information may be exploited therapeutically.

Keywords Antibodies · Fc receptors · FcγRIIB · Immunotherapy

Abbreviations

FcγR	Fc gamma receptor
mAb	Monoclonal antibody

Conflict statement: Prof Cragg serves as a consultant for Bioinvent International and has previously served as an ad hoc consultant for Roche. All authors contributed equally to this study.

A. L. White · S. A. Beers · M. S. Cragg (✉)
Cancer Sciences Unit, Antibody and Vaccine Group (MP88), Faculty of Medicine, Southampton University, Tremona Road, Southampton SO16 6YD, UK
e-mail: msc@soton.ac.uk

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_16,

Contents

1 Introduction 356
2 The Start of the Breakdown 358
3 Further Along the TRAIL 358
4 CD40 as a Target 360
5 What is Special About FcγRIIB? 361
6 Optimising FcγR Interations 361
7 Cross-linking Strategies for Clinical Translation 362
8 Cross-linking Versus Deletion 362
9 Anti-tumour Therapy Versus Immune Response 363
10 More than Deletors? 365
11 Other Diseases, Other Paradigms? 365
12 Direct Targeting Antibodies as Agonists 366
13 Conclusions and Further Considerations 366
References 368

1 Introduction

Fc gamma Receptor (FcγR) IIB (CD32B) is the solitary inhibitory receptor of the FcγR family in mice and humans (reviewed in (Nimmerjahn and Ravetch 2007a, b; Bruhns et al. 2009)). It is inhibitory by virtue of the immunoreceptor tyrosine inhibitory motif (ITIM) in its cytoplasmic tail (Muta et al. 1994; Ono et al. 1996; Bolland et al. 1998; Okada et al. 1998) which serves to abrogate signalling initiated from a selection of activatory receptors, principally the B cell receptor (BCR) for antigen (Sato and Ochi 1998; Enyedy et al. 2001; Li et al. 2003; Radstake et al. 2006) and the other, activatory members of the FcγR family (Daeron et al. 1995; Desai et al. 2007; Flores et al. 2009), thereby limiting their activity. Engagement of FcγRIIB typically results in activation of SHIP and SHP-1, with the former largely responsible for aborting productive signalling (Muta et al. 1994; Ono et al. 1996; Bolland et al. 1998; Okada et al. 1998). The details of the negative signalling, which is transduced by engaging FcγRIIB, is reviewed elsewhere (Daeron and Lesourne 2006).

In the context of monoclonal antibody (mAb) immunotherapy, FcγRIIB has been shown to limit the effects of a variety of clinically important mAb such as rituximab and trastuzumab. In their seminal paper Clynes and Ravetch showed that genetic deletion of FcγRIIB in mice augmented therapy, whereas loss of the activatory FcγR, through deletion of the γ chain, abrogated it (Clynes et al. 2000). This paradigm remained largely unaltered for a decade and has been confirmed in numerous studies of both normal and malignant target cell depletion (Green et al. 2002; Uchida et al. 2004; Gong et al. 2005; Hamaguchi et al. 2006; Kaneko et al. 2006; Nimmerjahn and Ravetch 2007a; Minard-Colin et al. 2008; Beers et al. 2010). Furthermore, this concept explains the observed efficacy with which

different mAb isotypes delete target cells (Ravetch and Bolland 2001; Nimmerjahn and Ravetch 2005, 2006) through their differential abilities to engage FcγR (reviewed in (Bruhns et al. 2009)) leading to the description of the activatory: inhibitory (A:I) ratio as key in determining mAb potency (described in (Nimmerjahn and Ravetch 2005), and (Nimmerjahn et al. 2005)). The relationship between FcγR and mAb isotype in mice is clear with mIgG1 binding FcγRIIB and a single activatory FcγR (FcγRIII), whereas mIgG2a engages all activatory FcγR efficiently and with enhanced affinity to both FcγRI and FcγRIV, resulting in a far higher A:I ratio and better depleting capacity. Although not directly translatable to humans, due largely to the fact that humans have a larger, more complex repertoire of FcγR and that human mAb isotypes do not possess the level of binding to the inhibitory FcγRIIB found with mIgG1, these key concepts have stimulated the development of so-called third generation mAb. These engineered mAb, as typified by the anti-CD20s (Lim et al. 2010), have been designed with altered FcγR-binding properties through various means of Fc engineering (reviewed in (Kellner et al. 2013)). The first glycomodified anti-CD20 mAb (GA101, Gazyva™ obinutuzumab) (Mossner et al. 2010) has just been approved for use in previously untreated CLL (Roche 2013). This mAb has a higher A:I ratio based upon its enhanced affinity for FcγRIIIA (Ferrara et al. 2006) and FcγRIIIB (Golay et al. 2013) whilst its binding to other FcγR remains largely unchanged. Whether further iterations will involve mAb with reduced FcγRIIB binding and whether this will further improve clinical activity on the basis of an improved A:I ratio remains to be seen.

In addition to progress with these so-called direct targeting mAb, the recent exponential explosion in therapeutic mAb development (reviewed in (Reichert and Dhimolea, 2012), has uncovered a new class of reagents; immunomodulatory mAb. Rather than binding directly to the tumour these agents engage key cells of the immune system such as T cells, dendritic cells and macrophages (reviewed in (Weiner et al. 2010). They can be sub-divided into agonists and antagonists or check-point blockers. The agonistic mAb bind to immune receptors such as CD40, CD27, 4-1BB (CD137) and OX40 (CD134) that are physiologically activated by trimeric ligands to stimulate or initiate immune responses. In contrast, antagonists or check-point blockers target receptors or their ligands that limit immune responses such as CTLA-4 (CD152), PD-1 (CD279) and PD-L1 (CD274) (reviewed in (Lee et al. 2012) and (Vonderheide and Glennie 2013)). Although both approaches are able to lead to clearance of tumour, agonistic immunostimulatory mAb offer the further possibility of activating antigen-specific responses thereby reducing the potential toxicity associated with widespread immune activation. Intriguingly, this new class of immunostimulatory mAb has begun to challenge the A:I paradigm as for some of them, such as anti-CD40, it has become clear that FcγRIIB promotes rather than inhibits their immune stimulatory and therapeutic activity.

2 The Start of the Breakdown

More than a decade ago, observations inconsistent with the A:I paradigm were first reported relating to the anti-Fas (CD95) mAb, Jo2. Xu et al. found that hepatotoxicity elicited by Jo2 was dependent on the expression of FcγRIIB in the liver (Xu et al. 2003). Following Jo2 treatment, wild-type mice die from acute liver failure, a response which is exacerbated in γ chain -/- mice, whereas FcγRIIB -/- mice survive. In vitro FcγRIIB, but not FcγRI and FcγRIII enhanced Jo2-mediated apoptosis of Fas expressing target cells and toxicity was attributed to the expression of Fas on hepatocytes and FcγRIIB on hepatic sinusoidal endothelial cells (Fig. 1a). Interestingly, in the same study, FcγRI and FcγRIII were shown as essential for the apoptosis-inducing activity of a non-hepatotoxic anti-Fas mAb HFE7A. The authors concluded that, "by interacting with the Fc region of agonistic Abs, FcγRs can modulate both the desired and undesired consequences of Ab-based therapy." This early study was perhaps the first to signal the clear dependence many mAb have on engaging the appropriate FcγR for achieving their desired therapeutic activity and also the complexity involved in understanding key modes of action.

3 Further Along the TRAIL

Further mAb targets assessed for their anti-tumour effects and FcγR-dependency were the TNF-related apoptosis-inducing ligand (TRAIL) receptors. TRAIL binds a number of receptors (reviewed in (Ashkenazi 2002)) with DR4 and DR5 in humans and DR5 in mice demonstrated as having considerable anti-tumour potential.

Early studies provided a clear indication that Fc:FcγR interactions were critical for regulating the efficacy of these reagents (Chuntharapai et al. 2001). In vitro, DR4 mAbs displayed a requirement for further cross-linking for the induction of apoptosis. In a colon cancer xenograft system a mAb of mIgG1 isotype induced apoptosis in tumour cells causing tumour regression, whereas the mIgG2a isotype switch variant of the same mAb was much less effective. Similar observations were made with other DR4 mAbs of different isotypes in vivo.

Like the human mAb, the agonistic anti-mouse DR5 mAb, MD5-1, was shown to exhibit potent anti-tumour effects through a cross-linking dependent ability to induce tumour cell apoptosis in this case provided by biotin:streptavidin conjugation (Takeda et al. 2004). As with rituximab, successful treatment of 4T1tumours was initially shown to depend on activatory FcγR expression, with therapeutic responses equivalent in FcγRIIB -/- and wild-type mice. However, in a subsequent study the same authors demonstrated that an intact B cell compartment was critical for the therapeutic activity of this same mAb, whereas CD11c+ dendritic cells were dispensable. As the authors themselves stated, "These data are intriguing and

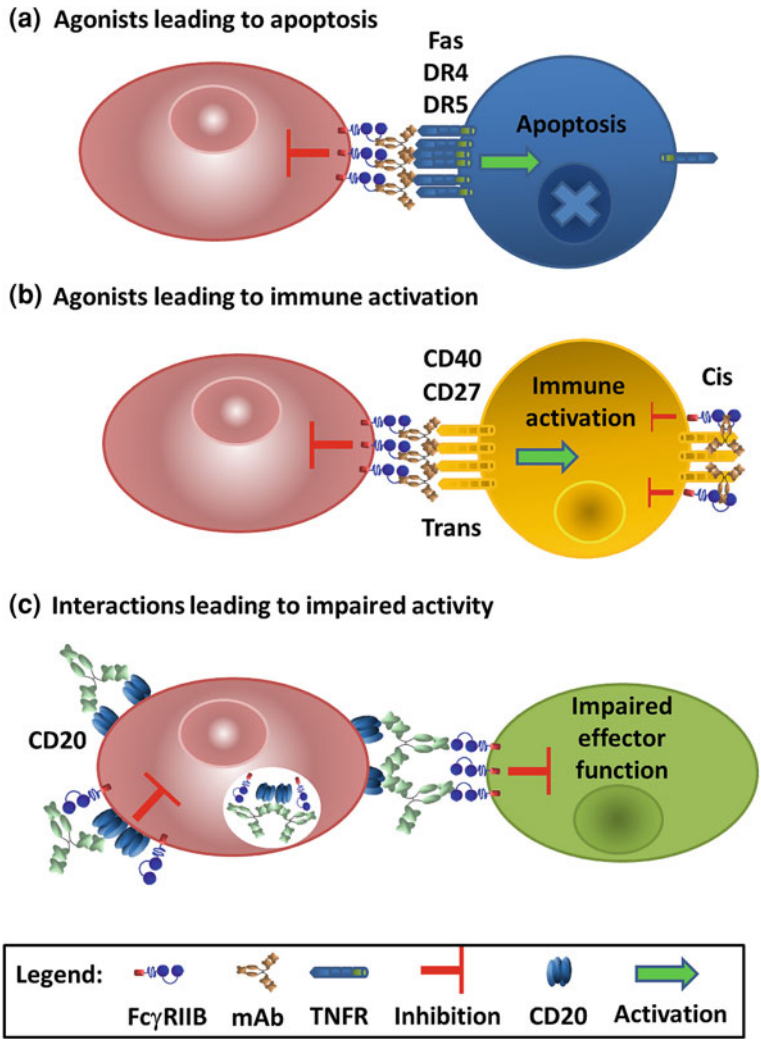


Fig. 1 Role of FcγRIIB in regulating agonistic antibodies. FcγRIIB has the potential to alter the therapeutic efficacy of agonistic mAb through various routes: **a** With mAb directed to death receptors such as DR4,5 and Fas, FcγRIIB presented on an accessory immune cell (B cell, DC, etc.) results in potent receptor cross-linking mimicking ligand-induced multimerisation, eliciting apoptosis in the target cell. **b** With mAb targeting costimulatory immune receptors, such as CD27 and CD40, FcγRIIB triggers receptor cross-linking to drive potent intracellular signalling and strong immune activation. These mAb may be engaged by FcγRIIB in either a *cis* or *trans* format. When engaged in *trans*, interaction with FcγRIIB on an accessory cell, resultant receptor cross-linking elicits productive immune responses. However, in a *cis* orientation, signalling does not result in powerful immune stimulation, potentially due to the concurrent inhibitory signalling arising from FcγRIIB in the same cell. **c** With direct targeting mAb such as rituximab, *cis* interaction with FcγRIIB on the target cell surface leads to internalisation of the receptor and abrogation of therapeutic anti-cancer activity. Further impairment of activity occurs through *trans* FcγRIIB engagement on the effector cell

establish an important platform on which to further explore the mechanism by which small numbers of B cells entering tumours might enable anti-DR5–mediated tumour apoptosis" (Haynes et al. 2010). In vitro, B cells were confirmed to trigger tumour cell apoptosis by virtue of their FcγR. As B cells only express FcγRIIB this provided further evidence that the inhibitory FcγRIIB was responsible for affording the therapeutic potential of agonistic TRAIL mAb (Fig. 1a) an observation at odds with their original findings (Takeda et al. 2004).

Wilson et al. subsequently examined this issue for the human DR5-agonistic antibody drozitumab (Wilson et al. 2011). As seen previously, FcγR promoted DR5-mediated tumour-cell apoptosis. However, drozitumab was effective when engaged by either activatory or inhibitory FcγR. In the same study similar dependency was indicated for CD40 mAb (see below), whereas rituximab as anticipated was fully reliant upon activatory FcγR expression. The question of FcγR requirement by these mAb was most recently revisited by Li et al. (Li and Ravetch 2012), who demonstrated that the anti-tumour efficacy of these mAb had an absolute requirement for FcγRIIB. Taking all of these data together it is clear that FcγRIIB can be, and often is, sufficient and necessary for the functioning of DR5 mAb with individual differences likely related to the model under study.

4 CD40 as a Target

A similar situation is observed with another TNFR family member, CD40. Anti-CD40 mAb can mimic the activities of the physiological ligand (CD154) in promoting the formation and survival of germinal centres in vivo (Liu et al. 1989; Paulie et al. 1989) as well as the proliferation of B cells in vitro (Banchereau et al. 1991). In addition to B cells (Clark and Ledbetter 1986), CD40 is expressed on other APC such as dendritic cells (Hart and McKenzie 1988; Schriever et al. 1989) and macrophages, various non-immune cells (Galy and Spits 1992) and many tumours (Paulie et al. 1985; Eliopoulos and Young 2004).

Inevitably, the presence of CD40 on B cell lymphomas and studies suggesting it was a useful target to induce cell cycle arrest and apoptosis led to its consideration as a target for clinical use. In xenograft models of human B cell lymphoma treated with anti-CD40 mAb (Law et al. 2005; Oflazoglu et al. 2009) efficacy was shown to be dependent on activatory FcγR. However, in addition to this direct targeting activity, anti-CD40 mAb were also shown to be particularly effective substitutes for T cell help, capable of powerful humoral (Dullforce et al. 1998) and cellular immunostimulatory activity (French et al. 1999). The cytotoxic T lymphocyte (CTL) responses induced in mouse models result in curative anti-tumour effects with the generation of long-term immunological memory enabling protection from tumour re-challenge. It is exactly these properties that are the holy grail of cancer immunotherapy. However, it was only recently that the in vivo requirements for anti-CD40 mAb to elicit ant-tumour and/or immunostimulation were established, with two independent groups demonstrating that FcγRIIB provides the requisite

cross-linking function (Fig. 1b) (White et al. 2011; White et al. 2013), (Li and Ravetch 2011, 2013). As such, anti-CD40 mAb of the mIgG1 isotype, which interacts well with FcγRIIB, elicits potent immunostimulatory and anti-tumour effects in various model systems, whereas mIgG2a reagents do not. Importantly, membrane redistribution of the FcγR is required for this cross-linking function, as when it is prevented by actin polymerisation inhibitors, the effects are abrogated (Wilson et al. 2011).

5 What is Special About FcγRIIB?

We believe that one of the contributing factors relating to the capacity of FcγRIIB to cross-link mAb in vivo reflects its expression pattern. In support of this, when expressed at the appropriate level and location, activatory FcγR can also provide effective cross-linking for anti-CD40 mAb both in vitro and in vivo (see below). FcγRIIB is expressed by antigen presenting cells (APC): It is the only FcγR on B cells and is expressed alongside activatory FcγR on other APC (Jonsson et al. 2013). Given that CD40 and other TNFR are highly expressed in lymphoid tissues and on APC, it is perhaps logical that FcγRIIB is ideally suited to provide cross-linking for agonistic anti-TNFR mAb. For anti-CD40 mAb cross-linking is mediated in *trans* by FcγRIIB on adjacent cells (White et al. 2011, 2013, 2014; Li and Ravetch 2013) and, although previously debated, recent results (White et al. 2011, 2014) have established that reverse signalling through the FcγRIIB ITIM (Fig. 1) does not play a role. This suggests that although *cis* interactions are likely to occur on B cells for example, they do not lead to CD40-mediated activation perhaps due to the simultaneous delivery of an inhibitory signal from the ITIM of FcγRIIB as has been demonstrated for FcγRIIB and the B cell receptor (Phillips and Parker 1983; Fong et al. 2000) (Fig. 1b).

In a therapeutic setting, B lymphoma cells themselves can provide the FcγRIIB required for effective CTL responses (Li and Ravetch 2013; White et al. 2014). This lymphoma-dependent cross-linking might give an alternative explanation for earlier observations demonstrating that effective therapy was dependent on the presence of large established tumours previously thought to reflect a required antigenic load (Tutt et al. 2002). It may relate instead to an insufficient density of cells needed for effective cross-linking.

6 Optimising FcγR Interations

When considering how these mechanistic findings can be translated to the clinic it is important to recognise that none of the human IgG isotypes bind with high affinity to FcγRIIB as monomers (Bruhns et al. 2009). Given this, what isotype might be best used for cross-linking-dependent agents in humans? Some have

suggested boosting Fc:FcγRIIB interactions through antibody engineering (Li and Ravetch 2011; Mimoto et al. 2013). Although this should lead to enhanced FcγRIIB-dependent cross-linking, these reagents would still be limited by FcγRIIB availability in vivo. An additional concern with this approach is the potential for FcγRIIB-dependent hepatotoxicity detailed above (Xu et al. 2003). A more productive avenue might be to develop reagents with FcγR-independent innate cross-linking ability. This could conceivably lead to a class of drugs with defined activity and predictable toxicity irrespective of FcγR expression and availability in vivo, leading to a broader applicability, independent of the local microenvironment.

7 Cross-linking Strategies for Clinical Translation

Agonistic mAb-based reagents which can act through FcγR-independent cross-linking have been championed by numerous groups over the years but for various reasons, such as poor half-life and linker instability, have usually faltered. However, with the availability of more stable cross-linking molecules and a better understanding of the biological factors underpinning productive in vivo activity it is perhaps time to revisit such approaches. We have recently demonstrated that multimeric forms of anti-CD40 are powerful agonists capable of inducing significant humoral and cellular immune responses in murine models and that they can produce cures in an established B cell lymphoma model under circumstances where direct targeting mAb are entirely ineffective. Similarly, potent FcγR-independent anti-tumour efficacy was demonstrated with a TRAIL-receptor binding multimeric construct that displays high inherent cross-linking capacity (Gieffers et al. 2013).

8 Cross-linking Versus Deletion

The data described above highlight the importance of the local environment in determining the effector mechanisms and FcγR dependency of therapeutic mAb. It therefore follows that changing the microenvironment will influence these factors. We recently tested this hypothesis and showed that when the TLR agonist Poly:IC is administered to mice, local activatory FcγR expression is increased and the mIgG2a isotype of anti-CD40 that under normal circumstance is not active becomes immunostimulatory in an activatory FcγR-dependent way (White et al. 2014). As well as inducing apoptosis, direct targeting of tumour cells through TNFRs expressed on their surface (for example DR5 in breast cancer (Takeda et al. 2004) or CD40 in B cell lymphoma (Law et al. 2005)) can also elicit therapeutic responses through target cell deletion. Manipulating FcγR expression profiles in this manner may therefore provide the optimal situation whereby both

tumour cell deletion as well as either stimulation of tumour immunity or target cell apoptosis can be achieved with a single agent. Interestingly, the mIgG2a isotype does not trigger deletion of the CD40+APCs in this situation, indicating that either CD40 or the APC itself is a poor target for deletion or that there are not sufficient effectors present.

Whether cross-linking-driven agonistic activity or activatory FcγR-mediated deletion is the most effective clinical approach may need to be determined empirically for each mAb target and therapeutic context. Many factors, for example epitope specificity, are likely to affect the balance between these mechanisms. Not all cell surface proteins make good targets for deleting mAb and in some cases deletion may be unwanted, such as with anti-CD40 mAb that bind APC. The optimal FcγR binding profile may also be dependent on the desired cellular target and the available FcγR repertoire when multiple cell types express the target antigen. A striking example of this is the different mechanisms by which anti-CD40 provides therapy for lymphoid and pancreatic tumours. In lymphoma models, anti-CD40 mAb induce a robust CD4-independent antigen-specific CD8 response that is able to clear tumour and generate immunological memory (French et al. 1999; Tutt et al. 2002). In contrast, in patients with metastatic pancreatic adenocarcinoma and in mouse models of this disease anti-CD40 is found to be dependent on the production of cytotoxic macrophages (Beatty et al. 2011). The important question is thus what mAb properties are required in these different settings and how they might be predicted to maximise patient responses.

9 Anti-tumour Therapy Versus Immune Response

In addition to its central role in anti-tumour responses induced by mAb directed against CD40, DR4 and DR5, we have recently found that FcγRIIB is similarly required for the immunostimulatory activity afforded by the systemic administration of mAb directed against co-stimulatory TNFRs expressed on T cells, such as 4-1BB, CD27 and OX40 (Fig. 1b) (unpublished observations). However, a recent study showed that a mAb directed against the T cell costimulatory receptor, GITR, requires activatory FcγR and not FcγRIIB engagement for therapeutic efficacy in pre-clinical cancer models (Bulliard et al. 2013). A similar situation is evident with anti-CD27 reagents (He et al. 2013) which have their T-cell stimulating activity most enhanced by mIgG1 isotypes (with cross-linking afforded by FcγRIIB) but which require activatory FcγR-engaging mIgG2a isotype for their anti-tumour activities (Vitale et al. 2012; He et al. 2013) (and unpublished observations) (Fig. 2).

These counterintuitive observations are currently explained by the ability of the anti-GITR mAb to directly deplete Treg in the tumour microenvironment. Other studies have suggested a similar mechanism of action for anti-CD27 (Roberts et al. 2010; Burris 2013) and the checkpoint blocker anti-CTLA-4 (Selby 2013; Simpson et al. 2013). These latter mAb have achieved impressive clinical success over the

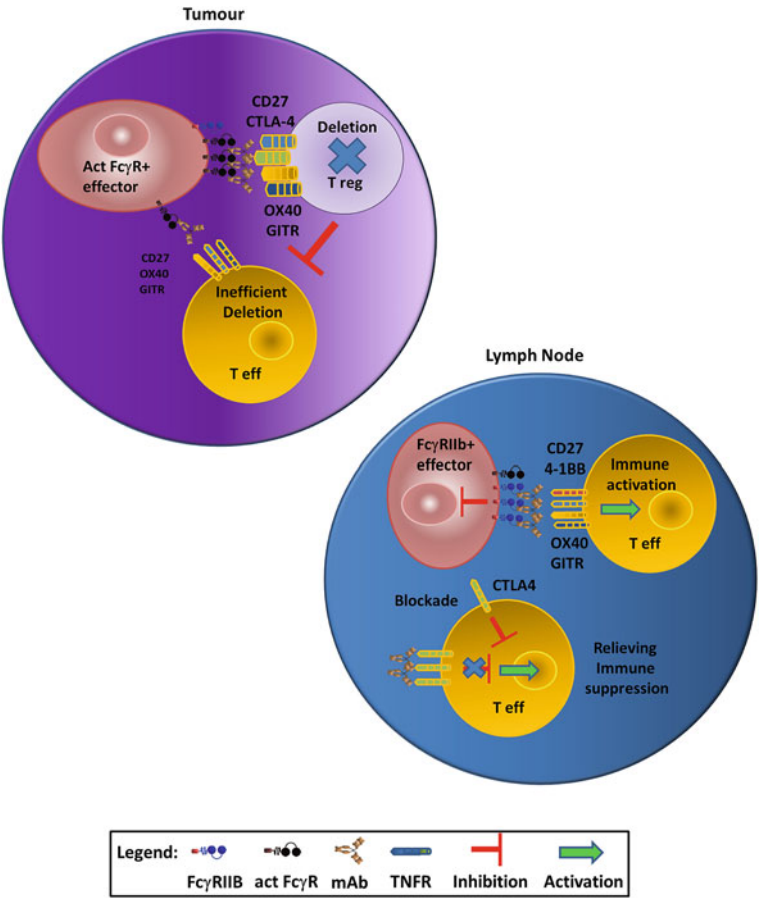


Fig. 2 Requirements for FcγR engagement in cancer immunotherapy versus immune stimulation. A number of agonistic mAb have now been assessed in terms of their FcγR requirements for stimulating immune and anti-cancer responses. These data indicate that FcγRIIB is the optimal FcγR for stimulating and augmenting immune receptor clustering and potent immune responses (both humoral and T-cell mediated). However, for optimal anti-cancer responses with some targets (e.g. GITR) it appears that activating FcγR are required, likely through their ability to delete Tregs. The dissection of these aspects is complex as many of the same molecules are expressed on both Teff and Treg. It is important therefore that the Treg express higher levels of the target receptor than the Teff and also that A:I ratio within the tumour is permissive for deletion (i.e. activatory FcγR are elevated). Potentially a cocktail of mAb, with one optimally deleting Treg and another optimally stimulating productive T cell responses may produce the most effective anti-cancer responses

last 3 years with the anti-CTLA4 mAb, ipilimumab, now approved for the treatment of metastatic melanoma (Hodi et al. 2010; Topalian et al. 2012; Brahmer et al. 2012). For these agents one would imagine that interaction with FcγRIIB would be detrimental to their therapeutic activity as cross-linking is likely to

propagate inhibitory downstream intracellular signalling. Indeed, in mouse models a mIgG2a variant of anti-mouse CTLA4 is an effective therapeutic agent, whereas the mIgG1 version is not (Selby 2013). However, as with GITR, this isotype effect appears to reflect the ability of mIgG2a to direct depletion of CTLA4-expressing Treg in the tumour microenvironment (Selby 2013; Simpson et al. 2013) (Fig. 2). It appears that two properties are crucial for these effects; (1) that the Treg differentially express higher levels of the target molecule than the Teff (seen with CTLA4 (Selby 2013; Simpson et al. 2013) as well as other receptors—unpublished data); and that (2) the local tumour environment upregulates the appropriate activatory FcγR, affording tumour-specific deletion of the Treg (Fig. 2).

10 More than Deletors?

Although these impressive data relating to Treg depletion are provocative, we should not ignore the potential of checkpoint blockers such as anti-CTLA4, to promote immune stimulation directly by overcoming deleterious inhibitory effects on effector T cells and it seems likely that a combination of immune stimulation and Treg depletion may provide optimal therapeutic efficacy (Peggs et al. 2009). In addition, although Treg depletion has been demonstrated for anti-CTLA4 and anti-GITR mAb it does not necessarily follow that mAb directed against other check point blockers, such as PD-1, will work by the same mechanism. Indeed, in early phase clinical trials objective responses to the anti-PD-1 mAb BMS-936558 were observed only in patients whose tumours expressed the ligand for this receptor, PD-L1 (Topalian et al. 2012), and similar therapeutic effects were achieved with an anti-PD-L1 mAb (Brahmer et al. 2012), suggesting that blockade of the inhibitory pathway (rather than Treg depletion) was responsible for the clinical effects. Of note, the anti-CTLA4 mAb, ipilimumab is a fully human IgG1 that would be expected to work well for depletion, whereas BMS-936558 is an IgG4 that would not. Interestingly, when ipilimumab and BMS-936558 were given in combination to patients with advanced melanoma, therapeutic effects were much more striking and rapid, with objective responses in >50 % of patients treated at the maximum tolerated dose, all with a tumour reduction of at least 80 % (Wolchok et al. 2013). This truly impressive clinical effect indicates each mAb is likely to be operating differently and supports the approach of targeting multiple, complementary, mechanisms of action.

11 Other Diseases, Other Paradigms?

The potential for FcγRIIB interaction should also be considered when these agents are used for other, non-cancer, therapeutic indications. For example, checkpoint blockers have been investigated as agents to overcome T cell exhaustion in chronic

viral infections (Ha et al. 2008; Palmer et al. 2013). In this context both activatory and inhibitory FcγR interaction would be predicted to be detrimental and isotypes, such as human IgG4, that show little FcγR interaction (Bruhns et al. 2009) may be the appropriate choice. In contrast, FcγRIIB engagement may be desirable under certain conditions, for example agonistic targeting of checkpoint blockers for the amelioration of autoimmune conditions. Thus, for each mAb and target multiple mechanisms may contribute to activity, and different mechanisms may be required for different indications.

12 Direct Targeting Antibodies as Agonists

Another consideration is how FcγRIIB interacts with other classes of agonistic antibody. For example, rituximab has long been recognised as capable of transmitting intracellular signals into target cells and therefore can be classed as an agonist (reviewed in (Cragg et al. 2005)). Importantly, these signals are only delivered in the context of further cross-linking which we previously proposed was due to the ability of CD20 to interact with and "hijack" the signalling properties of the BCR (Walshe et al. 2008). In vivo, such properties, if present, would be delivered by FcγR-expressing effector cells. This conjecture has been made previously and was implied largely to relate to engagement by activatory FcγR. However, given our recent findings that FcγRIIB can engage with and become activated by rituximab on the surface of malignant B cells (Lim et al. 2011b) a new aspect of this paradigm should be considered. Although FcγRIIB expression and interaction is clearly detrimental for therapeutic activity, as it drives internalisation of the tripartite CD20: rituximab: FcγRIIB complex on the target cell surface (Lim et al. 2011b), it should not be forgotten that the cross-linking of CD20 is what elicits signalling downstream from this molecule and also from FcγRIIB itself (Fig. 1c). Engagement of FcγRIIB occurs with rituximab when presented at the cell surface in both *cis* and *trans* as measured by phosphorylation of the FcγRIIB ITIM (Lim et al. 2011a; Vaughan et al. 2013). Therefore, it may be timely to re-examine the consequence of these interactions, at least in the context of target cells in which FcγRIIB is co-expressed with the target antigen such as lymphoma.

13 Conclusions and Further Considerations

Taking all of the data together the present evidence clearly indicates that for agonistic mAb, particularly those targeting members of the TNFR superfamily that require trimerisation as a prerequisite for function, engagement with FcγR leading to higher order multimerisation is critical for eliciting their desired effects. The data indicate that any FcγR will do in these circumstances, with the observed FcγR

dependence for different mAb and targets arising from the mAb isotype, the locality of mAb binding and the nature and level of FcγR expressed in this environment.

Two additional comments might be ventured. First, as with all paradigms it is the exception that makes the rule. Several examples of mAb exist targeting a variety of receptors that do not display any requirement for FcγR. Examples of these "superagonistic" reagents include mAb targeting CD40 (CP870,893 developed by Pfizer; unpublished observations), TRAIL (reviewed in (Haynes et al. 2010), and CD28 (CD28.1 (Nunes et al. 1993)). Notably, these mAb do not require additional cross-linking for function, perhaps indicating they have an inherent epitope-dependent propensity for receptor multimerisation (manuscript in preparation).

The second consideration concerns whether more than cross-linking is important, i.e. does FcγRIIB signalling have any role? Recent studies indicate this not to be the case for several TNFR but whether it is true for all remains to be seen. In order to test whether engagement and signalling downstream of the FcγRIIB is important in therapeutic situations with other mAb, an ITIM mutant akin to the NOTAM ITAM (de Haij et al. 2010) mutant mouse may be required. Alternatively, use of agonist or antagonistic mAb directed to FcγRIIB itself, such as generated by us previously (Williams et al. 2012; Williams et al. 2013) (and unpublished data) may be informative in this respect if used in combination with other therapeutic mAb.

In summary, it is clear that engagement of FcγRIIB is a critical regulator of agonistic mAb efficacy. For canonical direct targeting mAb such as rituximab, this activity is largely detrimental either in *trans* through engagement of FcγRIIB on effector cells, reducing their anti-tumour activity or through *cis* interaction leading to down-regulation of the mAb:target complex (Fig. 1c). For agonistic mAb targeting members of the TNFR super-family, such as CD40, CD27, Fas, DR4 and 5, FcγRIIB engagement appears beneficial for triggering the immediate downstream activities of these receptors; Immune stimulation, or apoptotic cell death, respectively. However, it is also clear that multiple additional factors must be taken into account before we can make clear conclusions regarding the optimal strategy for exploiting these mAb therapeutically, particularly with mAb which can potentially elicit both cross-linking-independent Treg depletion and cross-linking-dependent immune stimulation.

The concepts developed through studying direct targeting mAb and employing the principles of the A:I ratio has served us well for the last decade. No doubt, the next decade will see us update these rules to accommodate the immunomodulatory mAb as we continue to refine and improve them for therapeutic use.

Acknowledgments The authors would like to thank all of the members of the Antibody and Vaccine Group, past and present, and in particular the members of the CD20, CD40 and FcγR teams. We also apologise to those authors whose work has not been cited in this review due to space limitations.

References

Ashkenazi A (2002) Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev Cancer 2:420–430

Banchereau J, de Paoli P, Valle A, Garcia E, Rousset F (1991) Long-term human B cell lines dependent on interleukin-4 and antibody to CD40. Science 251:70–72

Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W et al (2011) CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science 331:1612–1616

Beers SA, French RR, Chan CH, Lim SH, Jarrett TC, Vidal RM et al (2010) Antigenic modulation limits the efficacy of anti-CD20 antibodies: implications for antibody selection. Blood 115:5191–5201

Bolland S, Pearse RN, Kurosaki T, Ravetch JV (1998) SHIP modulates immune receptor responses by regulating membrane association of Btk. Immunity 8:509–516

Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P et al (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 366:2455–2465

Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S et al (2009) Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113:3716–3725

Bulliard Y, Jolicoeur R, Windman M, Rue SM, Ettenberg S, Knee DA et al (2013) Activating Fc gamma receptors contribute to the antitumor activities of immunoregulatory receptor-targeting antibodies. J Exp Med 210:1685–1693

Burris H, Ansell S, Nemunaitis J, Weiss G, Sikic B, Northfelt D, Pilja L, Davis T, Yellin M, Keler T, Bullock T (2013) A phase I study of an agonist anti-CD27 human antibody (CDX-1127) in patients with advanced hematologic malignancies or solid tumors. poster; society for immunotherapy of cancer 28th annual meeting national harbor, MD, USA, 8–10 November 2013

Chuntharapai A, Dodge K, Grimmer K, Schroeder K, Marsters SA, Koeppen H et al (2001) Isotype-dependent inhibition of tumor growth in vivo by monoclonal antibodies to death receptor 4. J Immunol 166:4891–4898

Clark EA, Ledbetter JA (1986) Activation of human B cells mediated through two distinct cell surface differentiation antigens, Bp35 and Bp50. Proc Natl Acad Sci U S A 83:4494–4498

Clynes RA, Towers TL, Presta LG, Ravetch JV (2000) Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med 6:443–446

Cragg MS, Walshe CA, Ivanov AO, Glennie MJ (2005) The biology of CD20 and its potential as a target for mAb therapy. Curr Dir Autoimmun 8:140–174

Daëron M, Lesourne R (2006) Negative signaling in Fc receptor complexes. Adv Immunol 89:39–86

Daeron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S et al (1995) The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. Immunity 3:635–646

de Haij S, Jansen JH, Boross P, Beurskens FJ, Bakema JE, Bos DL et al (2010) In vivo cytotoxicity of type I CD20 antibodies critically depends on Fc receptor ITAM signaling. Cancer Res 70:3209–3217

Desai DD, Harbers SO, Flores M, Colonna L, Downie MP, Bergtold A et al (2007) Fc{gamma} receptor IIB on dendritic cells enforces peripheral tolerance by inhibiting effector T cell responses. J Immunol 178:6217–6226

Dullforce P, Sutton DC, Heath AW (1998) Enhancement of T cell-independent immune responses in vivo by CD40 antibodies. Nat Med 4:88–91

Eliopoulos AG, Young LS (2004) The role of the CD40 pathway in the pathogenesis and treatment of cancer. Curr Opin Pharmacol 4:360–367

Enyedy EJ, Mitchell JP, Nambiar MP, Tsokos GC (2001) Defective FcgammaRIIb1 signaling contributes to enhanced calcium response in B cells from patients with systemic lupus erythematosus. Clin Immunol 101:130–135

Ferrara C, Stuart F, Sondermann P, Brunker P, Umana P (2006) The carbohydrate at FcgammaRIIIa Asn-162. An element required for high affinity binding to non-fucosylated IgG glycoforms. J Biol Chem 281:5032–5036

Flores M, Desai DD, Downie M, Liang B, Reilly MP, McKenzie SE et al (2009) Dominant expression of the inhibitory FcgammaRIIB prevents antigen presentation by murine plasmacytoid dendritic cells. J Immunol 183:7129–7139

Fong DC, Brauweiler A, Minskoff SA, Bruhns P, Tamir I, Mellman I et al (2000) Mutational analysis reveals multiple distinct sites within Fc gamma receptor IIB that function in inhibitory signaling. J Immunol 165:4453–4462

French RR, Chan HT, Tutt AL, Glennie MJ (1999) CD40 antibody evokes a cytotoxic T-cell response that eradicates lymphoma and bypasses T-cell help. Nat Med 5:548–553

Galy AH, Spits H (1992) CD40 is functionally expressed on human thymic epithelial cells. J Immunol 149:775–782

Gieffers C, Kluge M, Merz C, Sykora J, Thiemann M, Schaal R et al (2013) APG350 induces superior clustering of TRAIL-Receptors and shows therapeutic anti-tumor efficacy independent of cross-linking via Fcgamma-Receptors. Mol Cancer Ther 12(12):2735–2747

Golay J, Da Roit F, Bologna L, Ferrara C, Leusen JH, Rambaldi A et al (2013) Glycoengineered CD20 antibody obinutuzumab activates neutrophils and mediates phagocytosis through CD16B more efficiently than rituximab. Blood 122:3482–3491

Gong Q, Ou Q, Ye S, Lee WP, Cornelius J, Diehl L et al (2005) Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy. J Immunol 174:817–826

Green SK, Karlsson MC, Ravetch JV, Kerbel RS (2002) Disruption of cell-cell adhesion enhances antibody-dependent cellular cytotoxicity: implications for antibody-based therapeutics of cancer. Cancer Res 62:6891–6900

Ha SJ, Mueller SN, Wherry EJ, Barber DL, Aubert RD, Sharpe AH et al (2008) Enhancing therapeutic vaccination by blocking PD-1-mediated inhibitory signals during chronic infection. J Exp Med 205:543–555

Hamaguchi Y, Xiu Y, Komura K, Nimmerjahn F, Tedder TF (2006) Antibody isotype-specific engagement of Fcgamma receptors regulates B lymphocyte depletion during CD20 immunotherapy. J Exp Med 203:743–753

Hart DN, McKenzie JL (1988) Isolation and characterization of human tonsil dendritic cells. J Exp Med 168:157–170

Haynes NM, Hawkins ED, Li M, McLaughlin NM, Hammerling GJ, Schwendener R et al (2010) CD11c + dendritic cells and B cells contribute to the tumoricidal activity of anti-DR5 antibody therapy in established tumors. J Immunol 185:532–541

He LZ, Prostak N, Thomas LJ, Vitale L, Weidlick J, Crocker A et al (2013) Agonist anti-human CD27 monoclonal antibody induces T cell activation and tumor immunity in human CD27-transgenic mice. J Immunol 191:4174–4183

Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB et al (2010) Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711–723

Jonsson F, Mancardi DA, Albanesi M, Bruhns P (2013) Neutrophils in local and systemic antibody-dependent inflammatory and anaphylactic reactions. J Leukoc Biol 94:643–656

Kaneko Y, Nimmerjahn F, Ravetch JV (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313:670–673

Kellner C, Derer S, Valerius T, Peipp M (2013) Boosting ADCC and CDC activity by Fc engineering and evaluation of antibody effector functions. Methods 65(1):105–113

Law CL, Gordon KA, Collier J, Klussman K, McEarchern JA, Cerveny CG et al (2005) Preclinical antilymphoma activity of a humanized anti-CD40 monoclonal antibody, SGN-40. Cancer Res 65:8331–8338

Lee CC, Cragg M, Glennie MJ, Johnson P (2012) Novel monoclonal antibody approaches to cancer immunotherapy. Br J Clin Pharmacol

Li F, Ravetch JV (2013) Antitumor activities of agonistic anti-TNFR antibodies require differential FcgammaRIIB coengagement in vivo. Proc Natl Acad Sci U S A 110(48):19501–19506

Li F, Ravetch JV (2012) Apoptotic and antitumor activity of death receptor antibodies require inhibitory Fcgamma receptor engagement. Proc Natl Acad Sci U S A 109:10966–10971

Li F, Ravetch JV (2011) Inhibitory Fcgamma receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. Science 333:1030–1034

Li X, Wu J, Carter RH, Edberg JC, Su K, Cooper GS et al (2003) A novel polymorphism in the Fcgamma receptor IIB (CD32B) transmembrane region alters receptor signaling. Arthritis Rheum 48:3242–3252

Lim SH, Beers SA, French RR, Johnson PW, Glennie MJ, Cragg MS (2010) Anti-CD20 monoclonal antibodies: historical and future perspectives. Haematologica 95:135–143

Lim SH, Vaughan AT, Ashton-Key M, Williams EL, Dixon SV, Chan CH et al (2011) Fc gamma receptor IIb on target B cells promotes rituximab internalization and reduces clinical efficacy. Blood 118(9):2530–2340

Liu YJ, Joshua DE, Williams GT, Smith CA, Gordon J, MacLennan IC (1989) Mechanism of antigen-driven selection in germinal centres. Nature 342:929–931

Mimoto F, Katada H, Kadono S, Igawa T, Kuramochi T, Muraoka M et al (2013) Engineered antibody Fc variant with selectively enhanced FcgammaRIIb binding over both FcgammaR-IIa(R131) and FcgammaRIIa(H131). Protein Eng Des Sel 26:589–598

Minard-Colin V, Xiu Y, Poe JC, Horikawa M, Magro CM, Hamaguchi Y et al (2008) Lymphoma depletion during CD20 immunotherapy in mice is mediated by macrophage FcgammaRI, FcgammaRIII, and FcgammaRIV. Blood 112:1205–1213

Mossner E, Brunker P, Moser S, Puntener U, Schmidt C, Herter S et al (2010) Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. Blood 115:4393–4402

Muta T, Kurosaki T, Misulovin Z, Sanchez M, Nussenzweig MC, Ravetch JV (1994) A 13-amino-acid motif in the cytoplasmic domain of Fc gamma RIIB modulates B-cell receptor signalling. Nature 369:340

Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV (2005) FcgammaRIV: a novel FcR with distinct IgG subclass specificity. Immunity 23:41–51

Nimmerjahn F, Ravetch JV (2007a) Antibodies, Fc receptors and cancer. Curr Opin Immunol 19:239–245

Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science 310:1510–1512

Nimmerjahn F, Ravetch JV (2007b) Fc-receptors as regulators of immunity. Adv Immunol 96:179–204

Nimmerjahn F, Ravetch JV (2006) Fcgamma receptors: old friends and new family members. Immunity 24:19–28

Nunes J, Klasen S, Ragueneau M, Pavon C, Couez D, Mawas C et al (1993) CD28 mAbs with distinct binding properties differ in their ability to induce T cell activation: analysis of early and late activation events. Int Immunol 5:311–315

Oflazoglu E, Stone IJ, Brown L, Gordon KA, van Rooijen N, Jonas M et al (2009) Macrophages and Fc-receptor interactions contribute to the antitumour activities of the anti-CD40 antibody SGN-40. Br J Cancer 100:113–117

Okada H, Bolland S, Hashimoto A, Kurosaki M, Kabuyama Y, Iino M et al (1998) Role of the inositol phosphatase SHIP in B cell receptor-induced Ca2 + oscillatory response. J Immunol 161:5129–5132

Ono M, Bolland S, Tempst P, Ravetch JV (1996) Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. Nature 383:263–266

Palmer BE, Neff CP, Lecureux J, Ehler A, Dsouza M, Remling-Mulder L et al (2013) In vivo blockade of the PD-1 receptor suppresses HIV-1 viral loads and improves CD4 + T cell levels in humanized mice. J Immunol 190:211–219

Paulie S, Ehlin-Henriksson B, Mellstedt H, Koho H, Ben-Aissa H, Perlmann P (1985) A p50 surface antigen restricted to human urinary bladder carcinomas and B lymphocytes. Cancer Immunol Immunother 20:23–28

Paulie S, Rosen A, Ehlin-Henriksson B, Braesch-Andersen S, Jakobson E, Koho H et al (1989) The human B lymphocyte and carcinoma antigen, CDw40, is a phosphoprotein involved in growth signal transduction. J Immunol 142:590–595

Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP (2009) Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med 206:1717–1725

Phillips NE, Parker DC (1983) Fc-dependent inhibition of mouse B cell activation by whole anti-mu antibodies. J Immunol 130:602–606

Radstake TR, Franke B, Wenink MH, Nabbe KC, Coenen MJ, Welsing P et al (2006) The functional variant of the inhibitory Fcgamma receptor IIb (CD32B) is associated with the rate of radiologic joint damage and dendritic cell function in rheumatoid arthritis. Arthritis Rheum 54:3828–3837

Ravetch JV, Bolland S (2001) IgG Fc receptors. Annu Rev Immunol 19:275–290

Reichert JM, Dhimolea E (2012) The future of antibodies as cancer drugs. Drug Discov Today 17:954–63

Roberts DJ, Franklin NA, Kingeter LM, Yagita H, Tutt AL, Glennie MJ et al (2010) Control of established melanoma by CD27 stimulation is associated with enhanced effector function and persistence, and reduced PD-1 expression of tumor infiltrating CD8(+) T cells. J Immunother 33:769–779

Roche (2013) FDA approves Roche's Gazyva (obinutuzumab) for people with previously untreated chronic lymphocytic leukemia (CLL). Press Release. http://www.roche.com/media/media_releases/med-cor-2013-11-01.htm

Sato K, Ochi A (1998) Superclustering of B cell receptor and Fc gamma RIIB1 activates Src homology 2-containing protein tyrosine phosphatase-1. J Immunol 161:2716–2722

Schriever F, Freedman AS, Freeman G, Messner E, Lee G, Daley J et al (1989) Isolated human follicular dendritic cells display a unique antigenic phenotype. J Exp Med 169:2043–2058

Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, Korman AJ (2013) Anti-CTLA-4 Antibodies of IgG2a isotype enhance antitumour activity through reduction of intratumoral regulatory T cells. Cancer Immunol Res 1(1):32–42

Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F et al (2013) Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med 210:1695–1710

Takeda K, Yamaguchi N, Akiba H, Kojima Y, Hayakawa Y, Tanner JE et al (2004) Induction of tumor-specific T cell immunity by anti-DR5 antibody therapy. J Exp Med 199:437–448

Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF et al (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366:2443–2454

Tutt AL, O'Brien L, Hussain A, Crowther GR, French RR, Glennie MJ (2002) T cell immunity to lymphoma following treatment with anti-CD40 monoclonal antibody. J Immunol 168:2720–2728

Uchida J, Hamaguchi Y, Oliver JA, Ravetch JV, Poe JC, Haas KM et al (2004) The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. J Exp Med 199:1659–1669

Vaughan AT, Iriyama C, Beers SA, Chan CHT, Lim SH, Williams EL et al (2013) Inhibitory FcγRIIb (CD32b) becomes activated by therapeutic mAb in both *cis* and *trans* and drives internalization according to antibody specificity. Blood 123(5): 669–677

Vitale LA, He LZ, Thomas LJ, Widger J, Weidlick J, Crocker A et al (2012) Development of a human monoclonal antibody for potential therapy of CD27-expressing lymphoma and leukemia. Clin Cancer Res 18:3812–3821

Vonderheide RH, Glennie MJ (2013) Agonistic CD40 antibodies and cancer therapy. Clin Cancer Res 19:1035–43

Walshe CA, Beers SA, French RR, Chan CH, Johnson PW, Packham GK et al (2008) Induction of cytosolic calcium flux by CD20 is dependent upon B Cell antigen receptor signaling. J Biol Chem 283:16971–16984

White AL, Chan HT, French RR, Beers SA, Cragg MS, Johnson PW et al (2013) FcgammaRIIB controls the potency of agonistic anti-TNFR mAbs. Cancer Immunol Immunother 62:941–948

White AL, Chan HTC, Roghanian A, French RR, Mockridge CI, Tutt AL et al (2011) Interaction with Fc gamma RIIB is critical for the agonistic activity of anti-CD40 monoclonal antibody. J Immunol 187:1754–1763

White AL, Dou L, Chan HTC, Field VL, Mockridge CI, Moss K, Williams E, Butts C, Al-Shamkhani A, Cragg MS, Verbeek SJ, Johnson P, Glennie MJ, Beers SA (2014) FcR dependence of agonistic CD40 antibody is related to anatomical location and can be overcome by antibody multimerisation. J Immunology (in press)

Williams EL, Tutt AL, Beers SA, French RR, Chan CH, Cox KL et al (2013) Immunotherapy targeting inhibitory Fcgamma receptor IIB (CD32b) in the mouse is limited by monoclonal antibody consumption and receptor internalization. J Immunol 191:4130–4140

Williams EL, Tutt AL, French RR, Chan HT, Lau B, Penfold CA et al (2012) Development and characterisation of monoclonal antibodies specific for the murine inhibitory FcgammaRIIB (CD32B). Eur J Immunol 42:2109–2120

Wilson NS, Yang B, Yang A, Loeser S, Marsters S, Lawrence D et al (2011) An Fcgamma receptor-dependent mechanism drives antibody-mediated target-receptor signaling in cancer cells. Cancer Cell 19:101–113

Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM et al (2013) Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 369:122–133

Xu Y, Szalai AJ, Zhou T, Zinn KR, Chaudhuri TR, Li X et al (2003) Fc gamma Rs modulate cytotoxicity of anti-Fas antibodies: implications for agonistic antibody-based therapeutics. J Immunol 171:562–568

Fc Receptor-Dependent Mechanisms of Monoclonal Antibody Therapy of Cancer

Jantine E. Bakema and Marjolein van Egmond

Abstract Targeted therapies like treatment with monoclonal antibodies (mAbs) have entered the arsenal of modern anticancer drugs. mAbs combine specificity with multiple effector functions that can lead to reduction of tumour burden. Direct mechanisms of action, including induction of apoptosis or growth inhibition, depend on the biology of the target antigen. Fc tails of mAbs have furthermore the potential to initiate complement-dependent lysis as well as immune effector cell-mediated tumour cell killing via binding to Fc receptors. Natural killer cells can induce apoptosis via antibody-dependent cellular cytotoxicity (ADCC), whereas macrophages are able to phagocytose mAb-opsonized tumour cells (antibody-dependent cellular phagocytosis; ADCP). Finally, neutrophils can induce non-apoptotic tumour cell death, especially in the presence of immunoglobulin A (IgA) antitumour mAbs. In spite of promising clinical successes in some malignancies, improvement of mAb immunotherapy is required to achieve overall complete remission in cancer patients. New strategies to enhance Fc receptor-mediated mechanisms of action or to overcome the immunosuppressive microenvironment of the tumour in mAb therapy of cancer are therefore currently being explored and will be addressed in this chapter.

J. E. Bakema
Tumor Biology Section, Department of Otolaryngology/Head-Neck Surgery, VU University Medical Center, De Boelelaan 1117, 1007 MB Amsterdam, The Netherlands

M. van Egmond (✉)
Department of Molecular Cell Biology and Immunology, VU University Medical Center, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands
e-mail: m.vanegmond@vumc.nl

M. van Egmond
Department of Surgery, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_17,

Contents

1 Introduction 374
2 Fc Receptor-Mediated Mechanisms of Action 375
2.1 Fc Receptor-Mediated Killing of Antibody-Opsonised Tumour Cells 375
2.2 Effector Immune Cells for Killing of mAb-Opsonized Tumour Targets 376
3 Opportunities to Improve mAb Therapy of Cancer 379
3.1 Antibody Protein and/or Glycoengineering 379
3.2 IgA as Therapeutic mAb 381
3.3 Overcoming Immunosuppressive Properties of Tumours 382
3.4 Changing Timing of mAb Therapy 383
4 Concluding Remarks 385
References 385

1 Introduction

Cancer is still one of the major causes of death in Western society in spite of significant progress to treat malignancies in the last decades. Continuous efforts to further advance the therapeutic arsenal remain therefore essential. The use of monoclonal antibodies (mAbs) as drugs to specifically target tumour cells or the tumour microenvironment is rapidly increasing, and mAbs have been included as a mainstream strategy to treat cancer patients (Scott et al. 2012; Sliwkowski and Mellman 2013). For instance, treatment with the anti-CD20 mAb rituximab has significantly improved clinical outcome of patients, and addition of rituximab to chemotherapy is now the gold standard to treat specific B cell malignancies (Amoroso et al. 2011). This unprecedented success prompted the development of a multitude of new antitumour mAbs, which are continuously modified to reduce immunogenicity and improve efficacy (Weiner et al. 2012).

Most clinically approved mAbs are unmodified chimeric or human(ised) antibodies of the immunoglobulin G (IgG) isotype. However, a multitude of novel antibody formats to potentiate effector functions are currently developed. These include for instance antibody-drug or radioisotype conjugates, bispecific biotherapeutics, glycoengineered mAbs with enhanced immune effector function or the use of different antibody isotypes. Additionally, therapeutic success may be increased by combination therapies or applying a different timing of therapy.

Several mAbs target the tumour environment or modulate immune responses. Bevacizumab (Avastin), for example targets vascular endothelial growth factor (VEGF), which interferes with angiogenesis, whereas the anti-Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) mAb Ipilimumab blocks the inhibitory mechanism of T lymphocytes. Most mAbs are, however, directed against tumour-associated antigens that are expressed by tumour cells and induce tumour cell killing via multiple mechanisms. Antitumour mAbs can have direct effects on tumour cells as they may induce programmed cell death (apoptosis) or sensitise tumour cells for chemotherapy (Glennie et al. 2007). Others, including anti-epidermal growth

factor receptor (EGFR) mAbs, interfere with ligand binding and thereby reduce proliferation (Benvenuti et al. 2007). Furthermore, IgG mAbs can initiate complement-dependent cytotoxicity (CDC) as its Fc region binds C1q, which activates the complement cascade through the classical pathway. This leads to both deposition of C3b on mAb-coated tumour cells, thereby further opsonising target cells, and to catalysation of the C5-C9 membrane attack complex (MAC) that induces tumour cell lysis (Kolev et al. 2011). Polymorphisms in the *C1QA gene* were shown to correlate with clinical responses in patients with follicular lymphoma after rituximab treatment, supporting the contribution of CDC to therapeutic efficacy (Racila et al. 2008). Finally, the Fc domain of antibodies interacts with Fc receptors. Consequently, mAbs recruit Fc receptor- expressing immune effector cells, which can result in tumour cell death and which is the focus of this chapter.

2 Fc Receptor-Mediated Mechanisms of Action

2.1 Fc Receptor-Mediated Killing of Antibody-Opsonised Tumour Cells

Two different Fcγ receptor polymorphisms that affect affinity for IgG have been described to associate with clinical success of mAb therapy in cancer. An arginine (R) to histidine (H) amino acid substitution at position 131 in FcγRIIa (FcγRIIa-131H/R) or a phenylalanine (F) to valine (V) substitution at amino acid position 158 in FcγRIIIa (FcγRIIIa-158 V/F) improved clinical response rates to rituximab (anti-CD20), cetuximab (anti-EGFR) or trastuzumab (anti-HER-2) therapy in lymphoma, colorectal or breast cancer, respectively (Weng and Levy 2003; Musolino et al. 2008; Bibeau et al. 2009). The impact of Fcγ receptor polymorphisms on clinical success of mAb monotherapy or as adjuvant to chemotherapy has recently been reviewed in detail by (Overdijk et al. 2014).

Cancer models in several Fc receptor knockout mice served to firmly establish the role of Fc receptor-mediated mechanisms in efficacy of antibody immunotherapy. Anti-gp75 mAb therapy was unable to prevent melanoma development in FcRγ chain deficient mice (lacking activating receptors FcγRI, FcγRIII and FcγRIV) (Clynes et al. 1998). Similarly, therapeutic efficacy of the clinically relevant mAbs rituximab and trastuzumab was lost in FcRγ chain$^{-/-}$ mice (Clynes et al. 2000). Murine FcγRIII was not involved in therapeutic efficacy, whereas both FcγRI and FcγRIV have been implicated in a melanoma lung metastases model (Bevaart et al. 2006; Nimmerjahn and Ravetch 2005). FcγRI and FcγRIV proved redundant in a liver metastases model as presence of either receptor was sufficient for eradication of tumour cells after mAb therapy, but absence of both abrogated therapeutic success (Otten et al. 2008; Clynes et al. 2000). Furthermore, tumour development was more effectively prevented after treatment with antitumour mAbs in mice that were deficient for the inhibitory receptor FcγRII (Clynes et al. 2000).

These studies unequivocally established the importance of Fc receptors for tumour elimination, but did not solve the main mechanisms of mAb therapy. Results with the anti-CD52 mAb Campath-1H (alemtuzumab) in a murine adult T cell leukaemia model supported the induction of antibody-dependent cellular cytotoxicity (ADCC), although the induction of apoptotic signalling in tumour cells after antigen clustering by antibody could not be excluded (Zhang et al. 2003). It was demonstrated that Fcγ receptor-mediated cross-linking of agonistic antibodies against death receptor 5 (drozitumab) promoted apoptosis in tumour cells (Wilson et al. 2011). A mouse model in which Fcγ receptors were expressed normally (allowing apoptosis induction via cross-linking of membrane-bound antibodies on tumour surfaces), but signalling via FcRγ chain was abrogated (hampering induction of antibody-dependent killing), demonstrated that antibody-dependent killing, and not apoptosis induction was a main mode of action for anti-CD20 mAbs (de Haij et al. 2010).

2.2 *Effector Immune Cells for Killing of mAb-Opsonized Tumour Targets*

Most mAbs that are currently used in the clinic are of IgG1 isotype, which are effective at engaging Fcγ receptors on immune effector cells like natural killer (NK) cells, macrophages and neutrophils (Fig. 1). Traditionally, cytotoxic activity of mAbs in clinical responses has been mostly attributed to NK cells (Zamai et al. 2007). Activation of NK cells through Fcγ receptors results in the killing of mAb-opsonized target cells via ADCC, which leads to apoptosis of tumour cells. In vitro ADCC assays showed that NK cells (the most prominent effector cell in peripheral blood mononuclear cells (PBMC) that are commonly used in cytotoxicity experiments) are effective in killing tumour cells in the presence of IgG1 mAbs (including rituximab or ofatumumab (anti-CD20), trastuzumab (anti-HER-2), alemtuzumab (anti-CD52) or cetuximab (anti-EGFR) (Overdijk et al. 2014). An anti-EGFR mAb of the IgG2 subclass (panitumumab) was, however, ineffective in recruiting NK cells as effector cells (Schneider-Merck et al. 2010). After trastuzumab treatment of patients with HER-2 positive mamma carcinoma increased numbers of tumour-associated NK cells were observed. Furthermore, enhanced expression of Granzyme B—one of the cytotoxic molecules of NK cells—was found, supporting a role for NK cell-induced ADCC in breast cancer after mAb therapy (Arnould et al. 2006). Efficacy of cetuximab, trastuzumab or rituximab therapy was furthermore dependent on the 158 V/F polymorphism in FcγRIIIa, which is the most prominent Fcγ receptor on NK cells (Weng and Levy 2003; Musolino et al. 2008; Bibeau et al. 2009). As NK cells with expression of FcγRIIIa-158 V have better ability to induce ADCC, improved clinical responses have mainly been attributed to enhanced activation of NK cells (Hatjiharissi et al. 2007).

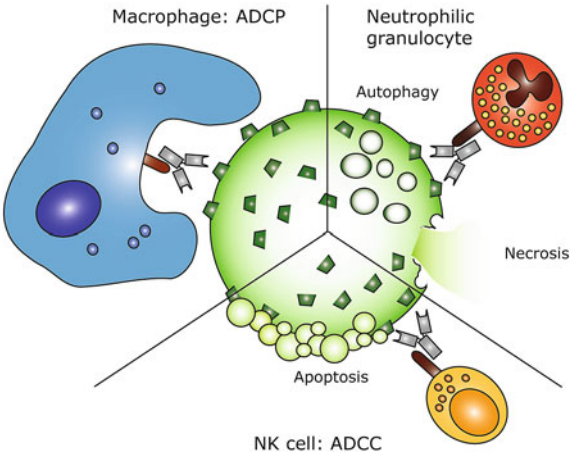


Fig. 1 *Fc receptor-mediated effector mechanisms*. Opsonization of tumour cells with mAbs results in killing by different immune effectors cells via distinct mechanisms. Macrophages execute antibody-dependent cellular phagocytosis (ADCP), whereas NK cells induce classical antibody-dependent cellular cytotoxiciy (ADCC), resulting in apoptosis. It has not yet been completely elucidated how neutrophils kill tumour cells, but it has been demonstrated that neutrophils can induce necrosis and a form of cell death that is associated with autophagic characteristics

However, macrophages proved extremely efficient in killing malignant cells in the presence of tumour-specific mAbs via antibody-dependent cellular phagocytosis (ADCP). Thus, macrophages, which also express FcγRIIIa, may play a major role in therapeutic successes of mAb therapy. Although in vitro ADCP in the presence of anticarcinoembryonic antigen (CEA), HER-2, epithelial cell adhesion molecule (EpCAM), human epithelial mucin (MUC)-1, CD20, CD30 and CD40 has been described, macrophages are likely especially efficient in removing circulating tumour cells (Akewanlop et al. 2001; Ashraf et al. 2009; Bologna et al. 2013; Huls et al. 1999; Lefebvre et al. 2006; Oflazoglu et al. 2009, 2007; Rafiq et al. 2013; Watanabe et al. 1999; Overdijk et al. 2012). Macrophage depletion abrogated therapeutic efficacy of anti-CD20 mAb in a murine lymphoma model (Minard-Colin et al. 2008; Uchida et al. 2004). Furthermore, anti-CD30 or anti-CD40 mAbs were less effective in preventing outgrowth of a Hodgkin-derived cell line in SCID mice when macrophages, but not NK cells or neutrophils, had been depleted (Oflazoglu et al. 2009, 2007). Recently, using intravital microscopy Montalvao et al. demonstrated that liver macrophages (Kupffer cells) were responsible for trapping of circulating normal and malignant B cells in the liver and subsequent ADCP after anti-CD20 mAb therapy (Montalvao et al. 2013). Clinical responses to rituximab therapy have been correlated with the 131H/R polymorphism in human FcγRIIa (Weng and Levy 2003). As macrophages, but not NK cells express FcγRIIa, a prominent role for macrophages as effector cell in anti-CD20 mAb therapy is supported.

It is less clear whether macrophages also play a role in tumour cell killing in solid malignancies. In contrast to classically activated macrophages with cytotoxic properties, it was demonstrated that macrophages in most malignancies including ovarian cancer and breast carcinoma, favour a so-called alternatively activated M2 phenotype (Lewis and Pollard 2006; Mosser and Edwards 2008). As such, they are considered pro-tumorigenic as they secrete multiple growth, angiogenic and immunosuppressive factors, and presence of macrophages has been correlated with poor prognosis and clinical outcome of patients (Leek et al. 1996). Nonetheless, tumour-associated macrophages (TAM) that were isolated from mouse breast carcinomas had ADCP capacity in the presence of anti-CD142 mAbs (Grugan et al. 2012). Moreover, in vivo depletion of macrophages abrogated successful anti-CD142 mAb therapy for prevention of breast carcinoma outgrowth and metastasis, suggesting that mAb therapy was able to re-polarise M2 macrophages into potent cytotoxic effector cells. By contrast, whereas Kupffer cells were very effective in eliminating circulating tumour cells via ADCP, they were ineffective in killing tumour cells in established (micro-) metastases, as visualised with intravital microscopy (Gül et al. 2014).

It has not yet been elucidated to which extent, if any, neutrophils contribute to current therapeutic successes. Their overall ability to recognise and kill tumour cells is limited, but is enhanced in the presence of antitumour mAbs (van Egmond and Bakema 2013). In animal models, it was shown that neutrophil depletion reduced therapeutic activity of alemtuzumab (Campath-1H) in a $CD52^{+}$ xenograft tumour model (Siders et al. 2010). Similarly, rituximab was less effective in reducing lymphoma development after depletion of neutrophils, supporting the involvement of neutrophils as cytotoxic effector cells (Hernandez-Ilizaliturri et al. 2003). It was furthermore recently demonstrated that efficacy of trastuzumab treatment in a human breast carcinoma xenograft model was abolished after neutrophil depletion (Albanesi et al. 2013).

The mechanism(s) through which neutrophils kill tumour cells is not yet fully understood. It was demonstrated that induction of tumour cell killing requires close contact between tumour cells and neutrophils, suggesting the release of toxic components (van Spriel et al. 2003). Complement receptor 3 (CR3) was essential for intimate spreading of neutrophils on tumour cells and the formation of ‘immunological synapses’ or ‘cytotoxic synapses’ (van Egmond et al. 1999; van Spriel et al. 2003). The in vivo formation of cytotoxic synapses between neutrophils and tumour cells after mAb treatment was recently shown (Hubert et al. 2011).

Neutrophils produce a plethora of cytotoxic molecules including proteases, oxidative metabolites and defensins that may be involved in tumour cell killing (Amulic et al. 2012). However, neither deficiencies in proteases (*elastase* or *myeloperoxidase*), nor in reactive oxygen species ($gp47^{phox}$ or $gp91^{phox}$-*NADPH oxidase* complex) affected in vivo therapeutic success (Albanesi et al. 2013). Similarly, absence of cytokines (tumour necrosis factor–α or interferon-γ) or inhibition of metalloproteases did not alter efficacy of mAb therapy. The ability of neutrophils to induce apoptosis in tumour cells was demonstrated, although the

mechanisms have not been elucidated (Horner et al. 2007; Stockmeyer et al. 2003). The presence of granzymes and perforin—used by NK cells to induce apoptosis—has been reported by some groups, but disputes by others (Grossman and Ley 2004; Hochegger et al. 2004; Metkar and Froelich 2004; Wagner et al. 2004). Additionally, two non-apoptotic cell death pathways have been reported after incubation of tumour cells with neutrophils in the presence of mAbs. Induction of necrosis occurred in a relatively small population of tumour cells, whereas autophagic characteristics were observed in most tumour cells (Bakema et al. 2011). Autophagy is generally regarded as a cell survival mechanism as it will generate energy for a distressed cell, but it has been proposed as an alternative cellular suicide pathway under excessive stress conditions (Mizushima et al. 2008). It is, however, still under debate whether autophagic cell death is a distinct cell death pathway or whether characteristics of autophagy are involved in a yet uncharacterized separate pathway (Shen et al. 2012). Nonetheless, targeting neutrophils may represent an attractive approach to trigger non-apoptotic cell death in tumour cells that have mutations in apoptotic pathways.

3 Opportunities to Improve mAb Therapy of Cancer

Notwithstanding the initial success of treating haematological malignancies with mAbs, therapeutic accomplishments to target solid tumours remain somewhat disappointing. For instance, trastuzumab is only effective when HER-2 is overexpressed, which is the case in ~20–25 % of the patients with breast cancer. Additionally, anti-EGFR mAbs are used to treat patients with head and neck cancer or colorectal cancer. However, mutations in the K-RAS signalling pathway seriously interfere with therapeutic success (Benvenuti et al. 2007). Furthermore, even when patients respond to mAb therapy, a substantial proportion of cancer patients fail to achieve complete remission or experience relapse. While direct mechanisms of mAbs are fully dependent on the biology of the target antigen, and as such are difficult to influence except by selecting a different target antigen, immune effector functions are mechanisms that can be improved upon. Thus, optimising Fc-mediated functions will enhance the effectiveness of therapeutic mAbs, and may significantly improve their antitumour activity in patients (Reichert and Dhimolea 2012).

3.1 Antibody Protein and/or Glycoengineering

Advances in protein and glycoengineering have enabled the production of "next generation" mAbs that may potentially be more efficacious compared to unmodified parental mAbs. Importantly, altering Fc glycosylation—in particular core-fucosylation—increases both binding of IgG to Fcγ receptors and enhances

antitumour activity of IgG (Ferrara et al. 2011; Paz-Ares et al. 2011). A non-fucosylated anti-CD20 mAb (obinutuzumab) enhanced ADCC, recruited neutrophils as effector cells, and showed clinical efficacy in patients with rituximab-refractory disease (Alduaij and Illidge 2011; Bologna et al. 2011; Golay et al. 2013). Similarly, the defucosylated anti-C–C chemokine receptor type 4 (CCR4) mAb mogamulizumab effectively induced ADCC of Adult T-cell leukaemia/lymphoma cells by NK cells, and showed clinical efficacy in patients with relapsed disease (Ishida et al. 2012). Alternatively, amino acid substitutions in the Fc domain were shown to significantly increase affinities for FcγRIIIa or FcγRIIa, and enhanced effector functions (Lazar et al. 2006; Richards et al. 2008). Structural analysis of FcγRIII binding to IgG1 revealed that FcγRIII contacts different amino acids on the two Fc polypeptide chains (Sondermann et al. 2000). Interestingly, it was recently demonstrated that Fc heterodimers with asymmetrical mutations had more stability in the CH2 domain, and were more effective in inducing ADCC (Liu et al. 2013).

In addition to modified antibodies, a variety of novel antibody formats are in development (Reichert and Dhimolea 2012). These include antibody-drug conjugates, in which a cytotoxic agent is coupled to a mAb. For example, Brentuximab vedotin is a FDA approved anti-CD30 mAb conjugated to monomethylauristatin E (MMAE), and used as treatment for relapsed Hodgkin's disease (Perini and Pro 2013). After receptor mediated internalisation, MMAE is released into the cytosol, where it induces G2/M-phase growth arrest and cell death through the induction of apoptosis (Francisco et al. 2003). However, antibody-drug conjugates do likely not involve Fcγ receptor-induced effector mechanisms, and as such are not addressed in detail (for review, see (Sievers and Senter 2013)).

Bispecific antibodies (BsAbs) are mAbs which are engineered to recognise two different targets to yield more effective therapeutics. Several BsAbs formats have been developed with varying degrees of success (reviewed by (May et al. 2012)). BsABs differ in their dual binding capacity. They can simultaneously bind cell surface receptors on the tumour and can recruit cells of the immune system. Generation of optimal functioning BsAbs in terms of stability and pharmacokinetic features in a practical and cost-effective manner has been challenging in the past as it relied on chemical linkage of two different mAbs. BsAbs have been developed to either specifically target FcγRIIIa on NK cells and macrophages or FcγRI on myeloid effector cells. Two FcγRI BsAbs, based on chemically linking of Fab' fragments (anti-HER-2 × anti-FcγRI and anti-EGFR × anti-FcγRI) have been tested in clinical trials for treatment of advanced breast cancer. Although well tolerated, neither induced significant antitumour effects, which may have been due to poor half life (for review, see (Curnow 1997; van Egmond and Bakema 2013). An anti-CD30 × FcγRIIIa BsAb did show clinical responses in patients with Hodgkin's disease in Phase I studies, but therapeutical development was halted due to low production yield and high immunogenicity (Hartmann et al. 2001).

Implementation of recombinant antibody technology has revived the BsAbs field, resulting in multiple bispecific biotherapeutic formats (e.g. tandem single chain Fv, diabodies, Triomabs and BiTes) (May et al. 2012). One advantage of BsAbs is the

possibility of recruiting cytotoxic T cells, which are effective in killing tumour cells, but normally not involved in ADCC. A HER-2 × CD3 BsAb (targeting HER-2$^+$ tumour cells and T cells) had superior in vivo activity compared with parental antibody pairs (Sen et al. 2001). The T cell-engaging CD19/CD3 BiTE (blinatumomab) was shown to transiently tether cytotoxic T cells to CD19 positive target B cells and demonstrated high response rates in patients with non-Hodgkin lymphoma (Topp et al. 2011). Furthermore, catumaxomab (CatmAb) is a bispecific tri-functional antibody directed against epithelial cell adhesion molecule (EpCAM) and CD3, and approved as intraperitoneal therapy for the treatment of malignant ascites in patients with EpCAM-positive carcinomas (Sebastian 2010). Interestingly, Catmab additionally recruits dendritic cells, macrophages and NK cells via its Fc part.

3.2 IgA as Therapeutic mAb

The most abundant effector cell population in the circulation consists of neutrophils. Moreover, whereas adoptive transfer of dendritic cells, NK cells or cytotoxic T cells requires ex vivo expansion, activation or differentiation, neutrophil numbers are easily amplified in vivo by treating patients with granulocyte-colony stimulating factor (G-CSF) or granulocyte/macrophage-colony stimulating factor (GM-CSF) (van Egmond and Bakema 2013). Thus, a formidable source of cytotoxic effector cells for mAb therapy can be recruited by targeting neutrophils. They express Fcγ receptors as well as the IgA Fc receptor FcαRI (CD89). Interestingly, it has been demonstrated that FcαRI is the most potent Fc receptor to induce tumour cell killing via a plethora of antitumour IgA mAbs (EpCAM, HER-2, EGFR, HLA class II, CD20, CD30 and CEA (Bakema et al. 2011; Deo et al. 1998; Huls et al. 1999; Valerius et al. 1997; Lohse et al. 2011). Furthermore, cross-linking of FcαRI, but not of Fcγ receptors mediates release of leukotriene B4 (LTB4)—a potent neutrophil chemoattractant (van der Steen et al. 2009). Consequently, targeting FcαRI led to neutrophil accumulation into tumour cells colonies in vitro, which were destroyed (Otten et al. 2005, 2012).

In vivo studies with IgA mAbs have, however, been hampered due to unavailability of suitable models as mice are deficient for FcαRI. Additionally, ineffective production of sufficient human IgA antitumour mAbs limited in vivo studies in the past. With the generation of human FcαRI transgenic mice (Launay et al. 2000; van Egmond et al. 1999), and improvement of IgA production processes (Beyer et al. 2009) in vivo efficacy of IgA as antitumour mAbs is currently under investigation. Injection of naked plasmid DNA encoding anti-CD20 IgA2 mAbs effectively prevented the development of B cell lymphoma (Pascal et al. 2012). In vivo antitumour activity of IgA2 EGFR mAbs against peritoneal A431 metastases was demonstrated, although FcαRI$^+$ macrophages were the likely effector cells in this model. Furthermore, IgA2 anti-EGFR mAbs prevented development of lung metastases, supporting the potential of targeting FcαRI for effective antibody therapy of cancer (Boross et al. 2013).

3.3 Overcoming Immunosuppressive Properties of Tumours

Many tumours express immune inhibitory molecules or secrete anti-inflammatory mediators that limit the efficacy of antibody immunotherapy. Thus, understanding the tumour microenvironment may lead to development of strategies that change the immunosuppressive nature of the tumour. For instance, tumour cells can circumvent CDC via expression of complement regulatory proteins (e.g. CD46, CD55 or CD59). A novel CD59 inhibitor (ILYd4) enhanced rituximab-mediated CDC in vitro, suggesting ILYd4 may be used as adjuvant in mAb therapy (You et al. 2011). It was furthermore shown that $CD47^+$ tumours are more resistant to antibody therapy in vivo as CD47 interacts with the inhibitory receptor signal regulatory protein-α (SIRPα; CD172a) that is expressed on myeloid cells. Anti-gp75 mAbs were more effective in preventing melanoma lung metastases in mice with an inhibitory signal-deficient mutant SIRPα (Zhao et al. 2011). Similarly, co-treatment of blocking anti-CD47 mAbs and rituximab enhanced ADCP of non-Hodgkin lymphoma cells by macrophages (Chao et al. 2010). Thus, interfering with the CD47-SIRPα pathway enhances antitumour mAb therapy.

Antitumour immune responses within the tumour microenvironment can be suppressed by a variety of infiltrating leukocytes, including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSC) and alternatively activated M2 macrophages. Tregs express the immune suppressor molecule CTLA4. Through inhibition of CTLA4, Tregs are no longer able to suppress antitumour immune responses, resulting in an increased number of effector immune cells and antigen presenting cells (O'Day et al. 2007). The anti-CTLA4 mAb ipilimumab was a breakthrough in the treatment of metastatic melanoma as it increased overall survival of patients with unresectable stage III and IV disease (Hodi et al. 2010). Interestingly, it was recently demonstrated that selective depletion of Tregs by Fcγ receptor-dependent mechanisms (in particular FcγRIV), contributed to the efficacy of anti-CTLA4 therapy in vivo (Simpson et al. 2013; Bulliard et al. 2013)

Suppressive mechanisms also include the secretion of cytokines such as interleukin 10 (IL-10) and transforming growth factor-β (TGF-β). An IL-10–producing B cell subset (B10 cells) was shown to limit efficacy of anti-CD20 mAb therapy in vivo through inhibition of monocyte/macrophage activation, which was counterbalanced by treatment with a toll-like receptor 3 agonist (Horikawa et al. 2011). Addition of proinflammatory cytokines to a mAb treatment regime may furthermore overcome the immunosuppressive tumour environment. It was shown that combining IL-2 and trastuzumab increased ADCC of breast cancer cells by NK cells ex vivo (Repka et al. 2003). Other immunostimulatory cytokines that activate NK cells are IL-15 and IL-18. IL-15 was demonstrated to enhance rituximab induced ADCC, even in the presence of TGF-β (Moga et al. 2011). Additionally, IL-18 augmented IFN-γ production by NK cells after activation through Fcγ receptors, and synergistically promoted regression of human lymphoma xenografts after treatment with rituximab (Srivastava et al. 2013).

In situ release of danger signals may also lead to activation of immune effector cells and initiation of antitumour immune responses. Both chemotherapy and radiotherapy have been shown to enhance efficacy of antibody therapy, putatively through induction of antitumour responses (Shuptrine et al. 2012). Alternatively, recruiting neutrophils may be an attractive approach to release danger signals and to initiate adaptive immune responses in situ. Whereas previously regarded as end-stage cells without the ability for protein synthesis, it is now clear that neutrophils can produce a multitude of cytokines, chemokines and metabolites, which influence other immune and non-immune cells (Mantovani et al. 2011). It was demonstrated that neutrophils promote NK cell proliferation, survival and cytotoxic capacity, whereas NK cells in turn release IFN-γ and GM-CSF, which activates neutrophils and prolongs survival (Costantini and Cassatella, 2011; Jaeger et al. 2012). Additionally, cross-talk between neutrophils and T helper 17 (T_h17) was reported as neutrophils secrete the T_h17 chemokines CCL2 and CCL20, and T_h17 cells release the prototypic neutrophil chemokine CXCL8 in turn (Pelletier et al. 2010). IL-17 also initiates G-CSF production by epithelial cells, which is a neutrophil growth factor. Neutrophil depletion led to reduced recruitment of $CD4^+$ T cells as well as decreased $CD8^+$ T cell activation in mouse tumour models, further supporting that neutrophils are involved in T cell function (Fridlender et al. 2009; Suttmann et al. 2006). Interestingly, it was proposed that neutrophils act as danger sensors by communicating presence of inflammation to dendritic cells. Release of TNF-α by neutrophils led to dendritic cell maturation with concomitant induction of T cell proliferation and polarisation into a Th1 phenotype (van Gisbergen et al. 2005a, b). It was also reported that neutrophils can transfer antigens into dendritic cells, resulting in specific T cell responses (Megiovanni et al. 2006). In vitro neutrophil migration into tumour colonies after targeting FcαRI (but not Fcγ receptors) resulted in release of the proinflammatory cytokines TNF-α and IL-1β. Additionally, targeting neutrophil FcαRI led to cross-talk with endothelial cells as the latter released CXCL8, resulting in enhanced neutrophil migration into tumour colonies (Otten et al. 2012). Subsequently, increased neutrophil migration into and destruction of tumour colonies was observed. Thus, a combination therapy that includes G-CSF and targeting of FcαRI on neutrophils may lead to recruitment of proinflammatory neutrophils into solid tumours, which may overcome the immunosuppressive micromilieu.

3.4 Changing Timing of mAb Therapy

It has been investigated whether mAbs can be used in an adjuvant setting, for instance after surgery of colorectal cancer. Addition of anti-EGFR mAbs to chemotherapy failed to improve outcome of patients with resected stage III disease (van Loon and Venook 2011). However, in these trials mAb therapy was usually given weeks after surgery. We recently identified a novel approach, based on the finding that mAb therapy is particularly effective in eliminating circulating tumour

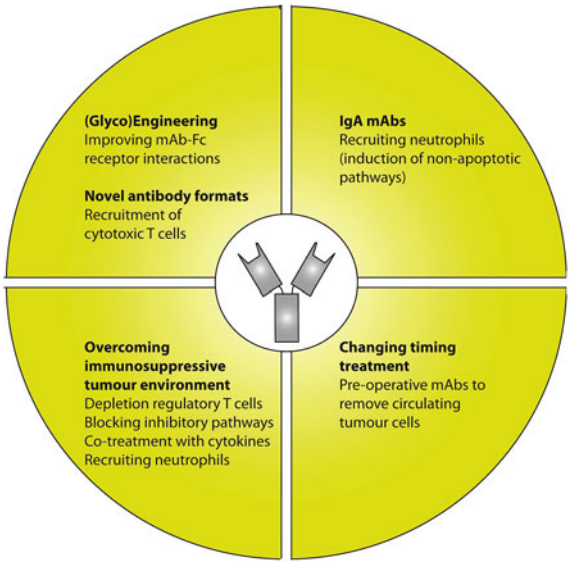


Fig. 2 Opportunities for improvements of antibody-based anticancer therapy

cells. At the time of colorectal surgery disseminated tumour cells can be detected in peripheral blood of the majority of patients, which is correlated with poor prognosis (Rahbari et al. 2010; Wind et al. 2009). Surgical removal of the primary tumour is the only therapy that can provide long-term disease free survival of patients with colorectal cancer. We, however, previously demonstrated that surgery, paradoxically, enhances the development of liver metastases, as it promotes binding of circulating tumour cells to the liver vasculature (van der Bij et al. 2008). Surgery and systemic exposure to bacterial products that are released after colectomy were shown to activate Kupffer cells, leading to release of reactive oxygen species and damage to the vascular bed of the liver (Gul et al. 2011). Subsequently, circulating tumour cells can adhere and grow out into metastases. We now show that Kupffer cells are not very effective in arrest of circulating tumour cells in the absence of mAbs, even though they are able to sample small particles of tumour cells (Gül et al. 2014). Treatment with antitumour mAbs however results in rapid phagocytosis of tumour cells by Kupffer cells, which is dependent on the presence of FcγRI and FcγRIV (Otten et al. 2008; Gül et al. 2014). ADCP of circulating tumour cells prevented the development of liver metastases, whereas depletion of Kupffer cells abrogated therapeutic efficacy (Otten et al. 2008; van der Bij et al. 2010; Gül et al. 2014). Thus, the annual 1.2 million patients that undergo resection of colorectal cancer may greatly benefit from preoperative mAb adjuvant therapy, as this may eliminate circulating tumour cells at the time of surgery.

4 Concluding Remarks

Antitumour mAbs have become an important addition for the treatment of cancer, and will continue to be included as novel treatment modalities in the years to come. After binding to the target antigen, mAbs can initiate a plethora of effector functions that can lead to killing of tumour cells. Based on in vitro and in vivo evidence as well as clinical studies in which therapeutic success depended on Fc receptor polymorphisms, it is clear that antibody- Fc receptor inactions play a prominent role to achieve eradication of the tumour. Nonetheless, there is ample room for improvements, as mAbs have not yet lived up to their full promise of being the magic bullets as was optimistically proposed in the last decades. Improvements may include optimising interactions of mAbs with Fc receptors by (glyco-) engineering, recruiting different effector cell populations like cytotoxic T lymphocytes or neutrophils, or changing the timing of treatment (Fig. 2). Additionally, combination therapies that overcome the immunosuppressive tumour environment may further advance the potential of mAbs as revolutionary drugs for the treatment of cancer.

References

Akewanlop C, Watanabe M, Singh B, Walker M, Kufe DW, Hayes DF (2001) Phagocytosis of breast cancer cells mediated by anti-MUC-1 monoclonal antibody, DF3, and its bispecific antibody. Cancer Res 61:4061–4065

Albanesi M, Mancardi DA, Jonsson F, Iannascoli B, Fiette L, di Santo JP, Lowell CA, Bruhns P (2013) Neutrophils mediate antibody-induced antitumor effects in mice. Blood 122:3160–3164

Alduaij W, Illidge TM (2011) The future of anti-CD20 monoclonal antibodies: are we making progress? Blood 117:2993–3001

Amoroso A, Hafsi S, Militello L, Russo AE, Soua Z, Mazzarino MC, Stivala F, Libra M (2011) Understanding rituximab function and resistance: implications for tailored therapy. Front Biosci (Landmark Ed) 16:770–782

Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A (2012) Neutrophil function: from mechanisms to disease. Annu Rev Immunol 30:459–489

Arnould L, Gelly M, Penault-Llorca F, Benoit L, Bonnetain F, Migeon C, Cabaret V, Fermeaux V, Bertheau P, Garnier J, Jeannin JF, Coudert B (2006) Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? Br J Cancer 94:259–267

Ashraf SQ, Umana P, Mossner E, Ntouroupi T, Brunker P, Schmidt C, Wilding JL, Mortensen NJ, Bodmer WF (2009) Humanised IgG1 antibody variants targeting membrane-bound carcinoembryonic antigen by antibody-dependent cellular cytotoxicity and phagocytosis. Br J Cancer 101:1758–1768

Bakema JE, Ganzevles SH, Fluitsma DM, Schilham MW, Beelen RH, Valerius T, Lohse S, Glennie MJ, Medema JP, van Egmond M (2011) Targeting FcalphaRI on polymorphonuclear cells induces tumor cell killing through autophagy. J Immunol 187:726–732

Benvenuti S, Sartore-Bianchi A, di Nicolantonio F, Zanon C, Moroni M, Veronese S, Siena S, Bardelli A (2007) Oncogenic activation of the RAS/RAF signalling pathway impairs the

response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 67:2643–2648

Bevaart L, Jansen MJ, van Vugt MJ, Verbeek JS, van de Winkel JG, Leusen JH (2006) The high-affinity IgG receptor, FcgammaRI, plays a central role in antibody therapy of experimental melanoma. Cancer Res 66:1261–1264

Beyer T, Lohse S, Berger S, Peipp M, Valerius T, Dechant M (2009) Serum-free production and purification of chimeric IgA antibodies. J Immunol Methods 346:26–37

Bibeau F, Lopez-Crapez E, di Fiore F, Thezenas S, Ychou M, Blanchard F, Lamy A, Penault-Llorca F, Frebourg T, Michel P, Sabourin JC, Boissiere-Michot F (2009) Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. J Clin Oncol 27:1122–1129

Bologna L, Gotti E, da Roit F, Intermesoli T, Rambaldi A, Introna M, Golay J (2013) Ofatumumab is more efficient than rituximab in lysing B-chronic lymphocytic leukemia cells in whole blood and in combination with chemotherapy. J Immunol 190:231–239

Bologna L, Gotti E, Manganini M, Rambaldi A, Intermesoli T, Introna M, Golay J (2011) Mechanism of action of type II, glycoengineered, anti-CD20 monoclonal antibody GA101 in B-chronic lymphocytic leukemia whole blood assays in comparison with rituximab and alemtuzumab. J Immunol 186:3762–3769

Boross P, Lohse S, Nederend M, Jansen JH, van Tetering G, Dechant M, Peipp M, Royle L, Liew LP, Boon L, van Rooijen N, Bleeker WK, Parren PW, van de Winkel JG, Valerius T, Leusen JH (2013) IgA EGFR antibodies mediate tumour killing in vivo. EMBO Mol Med 5:1213–1226

Bulliard Y, Jolicoeur R, Windman M, Rue SM, Ettenberg S, Knee DA, Wilson NS, Dranoff G, Brogdon JL (2013) Activating Fc gamma receptors contribute to the antitumor activities of immunoregulatory receptor-targeting antibodies. J Exp Med 210:1685–1693

Chao MP, Alizadeh AA, Tang C, Myklebust JH, Varghese B, Gill S, Jan M, Cha AC, Chan CK, Tan BT, Park CY, Zhao F, Kohrt HE, Malumbres R, Briones J, Gascoyne RD, Lossos IS, Levy R, Weissman IL, Majeti R (2010) Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. Cell 142:699–713

Clynes R, Takechi Y, Moroi Y, Houghton A, Ravetch JV (1998) Fc receptors are required in passive and active immunity to melanoma. Proc Natl Acad Sci USA 95:652–656

Clynes RA, Towers TL, Presta LG, Ravetch JV (2000) Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 6:443–446

Costantini C, Cassatella MA (2011) The defensive alliance between neutrophils and NK cells as a novel arm of innate immunity. J Leukoc Biol 89:221–233

Curnow RT (1997) Clinical experience with CD64-directed immunotherapy. An overview. Cancer Immunol Immunother 45:210–215

de Haij S, Jansen JH, Boross P, Beurskens FJ, Bakema JE, Bos DL, Martens A, Verbeek JS, Parren PW, van de Winkel JG, Leusen JH (2010) In vivo cytotoxicity of type I CD20 antibodies critically depends on Fc receptor ITAM signalling. Cancer Res 70:3209–3217

Deo YM, Sundarapandiyan K, Keler T, Wallace PK, Graziano RF (1998) Bispecific molecules directed to the Fc receptor for IgA (Fc alpha RI, CD89) and tumor antigens efficiently promote cell-mediated cytotoxicity of tumor targets in whole blood. J Immunol 160:1677–1686

Ferrara C, Grau S, Jager C, Sondermann P, Brunker P, Waldhauer I, Hennig M, Ruf A, Rufer AC, Stihle M, Umana P, Benz J (2011) Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcgammaRIII and antibodies lacking core fucose. Proc Natl Acad Sci USA 108:12669–12674

Francisco JA, Cerveny CG, Meyer DL, Mixan BJ, Klussman K, Chace DF, Rejniak SX, Gordon KA, Deblanc R, Toki BE, Law CL, Doronina SO, Siegall CB, Senter PD, Wahl AF (2003) cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. Blood 102:1458–1465

Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM (2009) Polarisation of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell 16:183–194

Glennie MJ, French RR, Cragg MS, Taylor RP (2007) Mechanisms of killing by anti-CD20 monoclonal antibodies. Mol Immunol 44:3823–3837

Golay J, da Roit F, Bologna L, Ferrara C, Leusen JH, Rambaldi A, Klein C, Introna M (2013) Glycoengineered CD20 antibody obinutuzumab activates neutrophils and mediates phagocytosis through CD16B more efficiently than rituximab. Blood 122:3482–3491

Grossman WJ, Ley TJ (2004) Granzymes A and B are not expressed in human neutrophils. Blood 104:906-907 (author reply 907–8)

Grugan KD, McCabe FL, Kinder M, Greenplate AR, Harman BC, Ekert JE, van Rooijen N, Anderson GM, Nemeth JA, Strohl WR, Jordan RE, Brezski RJ (2012) Tumor-associated macrophages promote invasion while retaining Fc-dependent antitumor function. J Immunol 189:5457–5466

Gül N, Babes LKS, KorthouwerR, Bögels M, Braster R, Vidarsson G, ten Hagen TLM, Kubes P, van Egmond M (2014) Antibody-dependent phagocytosis by macrophages is a main effector mechanism to prevent development of liver metastases. J Clin Invest 124(2):812–813

Gul N, Bogels M, Grewal S, van der Meer AJ, Rojas LB, Fluitsma DM, van den Tol MP, Hoeben KA, van Marle J, de Vries HE, Beelen RH, van Egmond M (2011) Surgery-induced reactive oxygen species enhance colon carcinoma cell binding by disrupting the liver endothelial cell lining. Gut 60:1076–1086

Hartmann F, Renner C, Jung W, da Costa L, Tembrink S, Held G, Sek A, Konig J, Bauer S, Kloft M, Pfreundschuh M (2001) Anti-CD16/CD30 bispecific antibody treatment for Hodgkin's disease: role of infusion schedule and costimulation with cytokines. Clin Cancer Res 7:1873–1881

Hatjiharissi E, Xu L, Santos DD, Hunter ZR, Ciccarelli BT, Verselis S, Modica M, Cao Y, Manning RJ, Leleu X, Dimmock EA, Kortsaris A, Mitsiades C, Anderson KC, Fox EA, Treon SP (2007) Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the Fc{gamma}RIIIa-158 V/V and V/F polymorphism. Blood 110:2561–2564

Hernandez-Ilizaliturri FJ, Jupudy V, Ostberg J, Oflazoglu E, Huberman A, Repasky E, Czuczman MS (2003) Neutrophils contribute to the biological antitumor activity of rituximab in a non-Hodgkin's lymphoma severe combined immunodeficiency mouse model. Clin Cancer Res 9:5866–5873

Hochegger K, Eller P, Rosenkranz AR (2004) Granzyme A: an additional weapon of human polymorphonuclear neutrophils (PMNs) in innate immunity? Blood 103:1176

Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ (2010) Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711–723

Horikawa M, Minard-Colin V, Matsushita T, Tedder TF (2011) Regulatory B cell production of IL-10 inhibits lymphoma depletion during CD20 immunotherapy in mice. J Clin Invest 121:4268–4280

Horner H, Frank C, Dechant C, Repp R, Glennie M, Herrmann M, Stockmeyer B (2007) Intimate cell conjugate formation and exchange of membrane lipids precede apoptosis induction in target cells during antibody-dependent, granulocyte-mediated cytotoxicity. J Immunol 179:337–345

Hubert P, Heitzmann A, Viel S, Nicolas A, Sastre-Garau X, Oppezzo P, Pritsch O, Osinaga E, Amigorena S (2011) Antibody-dependent cell cytotoxicity synapses form in mice during tumor-specific antibody immunotherapy. Cancer Res 71:5134–5143

Huls G, Heijnen IA, Cuomo E, van der Linden J, Boel E, van de Winkel JG, Logtenberg T (1999) Antitumor immune effector mechanisms recruited by phage display-derived fully human IgG1 and IgA1 monoclonal antibodies. Cancer Res 59:5778–5784

Ishida T, Joh T, Uike N, Yamamoto K, Utsunomiya A, Yoshida S, Saburi Y, Miyamoto T, Takemoto S, Suzushima H, Tsukasaki K, Nosaka K, Fujiwara H, Ishitsuka K, Inagaki H, Ogura M, Akinaga S, Tomonaga M, Tobinai K, Ueda R (2012) Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T cell leukemia-lymphoma: a multicenter phase II study. J Clin Oncol 30:837–842

Jaeger BN, Donadieu J, Cognet C, Bernat C, Ordonez-Rueda D, Barlogis V, Mahlaoui N, Fenis A, Narni-Mancinelli E, Beaupain B, Bellanne-Chantelot C, Bajenoff M, Malissen B, Malissen M, Vivier E, Ugolini S (2012) Neutrophil depletion impairs natural killer cell maturation, function, and homeostasis. J Exp Med 209:565–580

Kolev M, Towner L, Donev R (2011) Complement in cancer and cancer immunotherapy. Arch Immunol Ther Exp (Warsz) 59:407–419

Launay P, Grossetete B, Arcos-Fajardo M, Gaudin E, Torres SP, Beaudoin L, Patey-Mariaud DE, Serre N, Lehuen A, Monteiro RC (2000) Fcalpha receptor (CD89) mediates the development of immunoglobulin A (IgA) nephropathy (Berger's disease). Evidence for pathogenic soluble receptor-Iga complexes in patients and CD89 transgenic mice. J Exp Med 191:1999–2009

Lazar GA, Dang W, Karki S, Vafa O, Peng JS, Hyun L, Chan C, Chung HS, Eivazi A, Yoder SC, Vielmetter J, Carmichael DF, Hayes RJ, Dahiyat BI (2006) Engineered antibody Fc variants with enhanced effector function. Proc Natl Acad Sci USA 103:4005–4010

Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL (1996) Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res 56:4625–4629

Lefebvre ML, Krause SW, Salcedo M, Nardin A (2006) Ex vivo-activated human macrophages kill chronic lymphocytic leukemia cells in the presence of rituximab: mechanism of antibody-dependent cellular cytotoxicity and impact of human serum. J Immunother 29:388–397

Lewis CE, Pollard JW (2006) Distinct role of macrophages in different tumor microenvironments. Cancer Res 66:605–612

Liu Z, Gunasekaran K, Wang W, Razinkov V, Sekirov L, Leng E, Sweet H, Foltz I, Howard M, Rousseau AM, Kozlosky C, Fanslow W, Yan W (2013) Asymmetrical Fc engineering greatly enhances ADCC effector function and stability of the modified antibodies. J Biol Chem

Lohse S, Derer S, Beyer T, Klausz K, Peipp M, Leusen JH, van de Winkel JG, Dechant M, Valerius T (2011) Recombinant dimeric IgA antibodies against the epidermal growth factor receptor mediate effective tumor cell killing. J Immunol 186:3770–3778

Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 11:519–531

May C, Sapra P, Gerber HP (2012) Advances in bispecific biotherapeutics for the treatment of cancer. Biochem Pharmacol 84:1105–1112

Megiovanni AM, Sanchez F, Robledo-Sarmiento M, Morel C, Gluckman JC, Boudaly S (2006) Polymorphonuclear neutrophils deliver activation signals and antigenic molecules to dendritic cells: a new link between leukocytes upstream of T lymphocytes. J Leukoc Biol 79:977–988

Metkar SS, Froelich CJ (2004) Human neutrophils lack granzyme A, granzyme B, and perforin. Blood 104:905–906 (author reply 907-8)

Minard-Colin V, Xiu Y, Poe JC, Horikawa M, Magro CM, Hamaguchi Y, Haas KM, Tedder TF (2008) Lymphoma depletion during CD20 immunotherapy in mice is mediated by macrophage FcgammaRI, FcgammaRIII, and FcgammaRIV. Blood 112:1205–1213

Mizushima N, Levine B, Cuervo AM, Klionsky DJ (2008) Autophagy fights disease through cellular self-digestion. Nature 451:1069–1075

Moga E, Canto E, Vidal S, Juarez C, Sierra J, Briones J (2011) Interleukin-15 enhances rituximab-dependent cytotoxicity against chronic lymphocytic leukemia cells and overcomes transforming growth factor beta-mediated immunosuppression. Exp Hematol 39:1064–1071

Montalvao F, Garcia Z, Celli S, Breart B, Deguine J, van Rooijen N, Bousso P (2013) The mechanism of anti-CD20-mediated B cell depletion revealed by intravital imaging. J Clin Invest 123:5098–5103

Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8:958–969

Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, Laccabue D, Zerbini A, Camisa R, Bisagni G, Neri TM, Ardizzoni A (2008) Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol 26:1789–1796

Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science 310:1510–1512

O'Day SJ, Hamid O, Urba WJ (2007) Targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4): a novel strategy for the treatment of melanoma and other malignancies. Cancer 110:2614–2627

Oflazoglu E, Stone IJ, Brown L, Gordon KA, van Rooijen N, Jonas M, Law CL, Grewal IS, Gerber HP (2009) Macrophages and Fc receptor interactions contribute to the antitumor activities of the anti-CD40 antibody SGN-40. Br J Cancer 100:113–117

Oflazoglu E, Stone IJ, Gordon KA, Grewal IS, van Rooijen N, Law CL, Gerber HP (2007) Macrophages contribute to the antitumor activity of the anti-CD30 antibody SGN-30. Blood 110:4370–4372

Otten MA, Bakema JE, Tuk CW, Glennie MJ, Tutt AL, Beelen RH, van de Winkel JG, van Egmond M (2012) Enhanced FcalphaRI-mediated neutrophil migration towards tumor colonies in the presence of endothelial cells. Eur J Immunol 42:1815–1821

Otten MA, Rudolph E, Dechant M, Tuk CW, Reijmers RM, Beelen RH, van de Winkel JG, van Egmond M (2005) Immature neutrophils mediate tumor cell killing via IgA but not IgG Fc receptors. J Immunol 174:5472–5480

Otten MA, van der Bij GJ, Verbeek SJ, Nimmerjahn F, Ravetch JV, Beelen RH, van de Winkel JG, van Egmond M (2008) Experimental antibody therapy of liver metastases reveals functional redundancy between Fc gammaRI and Fc gammaRIV. J Immunol 181:6829–6836

Overdijk MB, Verploegen S, Bleeker WK, Parren PW (2014) Role of IgG Fc Receptors in monoclonal antibody therapy of cancer. Chapter 13, Antibody Fc: Linking Adaptive and Innate Immunity. Elsevier, New York

Overdijk MB, Verploegen S, Ortiz Buijsse A, Vink T, Leusen JH, Bleeker WK, Parren PW (2012) Crosstalk between human IgG isotypes and murine effector cells. J Immunol 189:3430–3438

Pascal V, Laffleur B, Debin A, Cuvillier A, van Egmond M, Drocourt D, Imbertie L, Pangault C, Tarte K, Tiraby G, Cogne M (2012) Anti-CD20 IgA can protect mice against lymphoma development: evaluation of the direct impact of IgA and cytotoxic effector recruitment on CD20 target cells. Haematologica 97:1686–1694

Paz-Ares LG, Gomez-Roca C, Delord JP, Cervantes A, Markman B, Corral J, Soria JC, Berge Y, Roda D, Russell-Yarde F, Hollingsworth S, Baselga J, Umana P, Manenti L, Tabernero J (2011) Phase I pharmacokinetic and pharmacodynamic dose-escalation study of RG7160 (GA201), the first glycoengineered monoclonal antibody against the epidermal growth factor receptor, in patients with advanced solid tumors. J Clin Oncol 29:3783–3790

Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C, Cosmi L, Lunardi C, Annunziato F, Romagnani S, Cassatella MA (2010) Evidence for a cross-talk between human neutrophils and Th17 cells. Blood 115:335–343

Perini GF, Pro B (2013) Brentuximab vedotin in CD30+ lymphomas. Biol Ther 3:15–23

Racila E, Link BK, Weng WK, Witzig TE, Ansell S, Maurer MJ, Huang J, Dahle C, Halwani A, Levy R, Weiner GJ (2008) A polymorphism in the complement component C1qA correlates with prolonged response following rituximab therapy of follicular lymphoma. Clin Cancer Res 14:6697–6703

Rafiq S, Butchar JP, Cheney C, Mo X, Trotta R, Caligiuri M, Jarjoura D, Tridandapani S, Muthusamy N, Byrd JC (2013) Comparative assessment of clinically utilized CD20-directed antibodies in chronic lymphocytic leukemia cells reveals divergent NK cell, monocyte, and macrophage properties. J Immunol 190:2702–2711

Rahbari NN, Aigner M, Thorlund K, Mollberg N, Motschall E, Jensen K, Diener MK, Buchler MW, Koch M, Weitz J (2010) Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. Gastroenterology 138:1714–1726

Reichert JM, Dhimolea E (2012) The future of antibodies as cancer drugs. Drug Discov Today 17:954–963

Repka T, Chiorean EG, Gay J, Herwig KE, Kohl VK, Yee D, Miller JS (2003) Trastuzumab and interleukin-2 in HER2-positive metastatic breast cancer: a pilot study. Clin Cancer Res 9:2440–2446

Richards JO, Karki S, Lazar GA, Chen H, Dang W, Desjarlais JR (2008) Optimization of antibody binding to FcgammaRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther 7:2517–2527

Schneider-Merck T, Lammerts van Bueren JJ, Berger S, Rossen K, van Berkel PH, Derer S, Beyer T, Lohse S, Bleeker WK, Peipp M, Parren PW, van de Winkel JG, Valerius T, Dechant M (2010) Human IgG2 antibodies against epidermal growth factor receptor effectively trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG1, only by cells of myeloid lineage. J Immunol 184:512–520

Scott AM, Wolchok JD, Old LJ (2012) Antibody therapy of cancer. Nat Rev Cancer 12:278–287

Sebastian M (2010) Review of catumaxomab in the treatment of malignant ascites. Cancer Manag Res 2:283–286

Sen M, Wankowski DM, Garlie NK, Siebenlist RE, van Epps D, Lefever AV, Lum LG (2001) Use of anti-CD3 x anti-HER2/neu bispecific antibody for redirecting cytotoxicity of activated T cells toward HER2/neu+ tumors. J Hematother Stem Cell Res 10:247–260

Shen S, Kepp O, Kroemer G (2012) The end of autophagic cell death? Autophagy 8:1–3

Shuptrine CW, Surana R, Weiner LM (2012) Monoclonal antibodies for the treatment of cancer. Semin Cancer Biol 22:3–13

Siders WM, Shields J, Garron C, Hu Y, Boutin P, Shankara S, Weber W, Roberts B, Kaplan JM (2010) Involvement of neutrophils and natural killer cells in the anti-tumor activity of alemtuzumab in xenograft tumor models. Leuk Lymphoma 51:1293–1304

Sievers EL, Senter PD (2013) Antibody-drug conjugates in cancer therapy. Annu Rev Med 64:15–29

Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, Roddie C, Henry JY, Yagita H, Wolchok JD, Peggs KS, Ravetch JV, Allison JP, Quezada SA (2013) Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med 210:1695–1710

Sliwkowski MX, Mellman I (2013) Antibody therapeutics in cancer. Science 341:1192–1198

Sondermann P, Huber R, Oosthuizen V, Jacob U (2000) The 3.2-A crystal structure of the human IgG1 Fc fragment-Fc gammaRIII complex. Nature 406:267–273

Srivastava S, Pelloso D, Feng H, Voiles L, Lewis D, Haskova Z, Whitacre M, Trulli S, Chen YJ, Toso J, Jonak ZL, Chang HC, Robertson MJ (2013) Effects of interleukin-18 on natural killer cells: costimulation of activation through Fc receptors for immunoglobulin. Cancer Immunol Immunother 62:1073–1082

Stockmeyer B, Beyer T, Neuhuber W, Repp R, Kalden JR, Valerius T, Herrmann M (2003) Polymorphonuclear granulocytes induce antibody-dependent apoptosis in human breast cancer cells. J Immunol 171:5124–5129

Suttmann H, Riemensberger J, Bentien G, Schmaltz D, Stockle M, Jocham D, Bohle A, Brandau S (2006) Neutrophil granulocytes are required for effective Bacillus Calmette-Guerin immunotherapy of bladder cancer and orchestrate local immune responses. Cancer Res 66:8250–8257

Topp MS, Kufer P, Gokbuget N, Goebeler M, Klinger M, Neumann S, Horst HA, Raff T, Viardot A, Schmid M, Stelljes M, Schaich M, Degenhard E, Kohne-Volland R, Bruggemann M, Ottmann O, Pfeifer H, Burmeister T, Nagorsen D, Schmidt M, Lutterbuese R, Reinhardt C, Baeuerle PA, Kneba M, Einsele H, Riethmuller G, Hoelzer D, Zugmaier G, Bargou RC (2011) Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. J Clin Oncol 29:2493–2498

Uchida J, Hamaguchi Y, Oliver JA, Ravetch JV, Poe JC, Haas KM, Tedder TF (2004) The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. J Exp Med 199:1659–1669

Valerius T, Stockmeyer B, van Spriel AB, Graziano RF, Van den Herik-Oudijk IE, Repp R, Deo YM, Lund J, Kalden JR, Gramatzki M, van de Winkel JG (1997) FcalphaRI (CD89) as a novel trigger molecule for bispecific antibody therapy. Blood 90:4485–4492

van der Bij GJ, Bogels M, Otten MA, Oosterling SJ, Kuppen PJ, Meijer S, Beelen RH, van Egmond M (2010) Experimentally induced liver metastases from colorectal cancer can be prevented by mononuclear phagocyte-mediated monoclonal antibody therapy. J Hepatol 53:677–685

van der Bij GJ, Oosterling SJ, Bogels M, Bhoelan F, Fluitsma DM, Beelen RH, Meijer S, van Egmond M (2008) Blocking alpha2 integrins on rat CC531s colon carcinoma cells prevents operation-induced augmentation of liver metastases outgrowth. Hepatology 47:532–543

van der Steen L, Tuk CW, Bakema JE, Kooij G, Reijerkerk A, Vidarsson G, Bouma G, Kraal G, de Vries HE, Beelen RH, van Egmond M (2009) Immunoglobulin A: Fc(alpha)RI interactions induce neutrophil migration through release of leukotriene B4. Gastroenterology 137(2018–29):e1–e3

van Egmond M, Bakema JE (2013) Neutrophils as effector cells for antibody-based immunotherapy of cancer. Semin Cancer Biol 23:190–199

van Egmond M, van Vuuren AJ, Morton HC, van Spriel AB, Shen L, Hofhuis FM, Saito T, Mayadas TN, Verbeek JS, van de Winkel JG (1999) Human immunoglobulin A receptor (FcalphaRI, CD89) function in transgenic mice requires both FcR gamma chain and CR3 (CD11b/CD18). Blood 93:4387–4394

van Gisbergen KP, Geijtenbeek TB, van Kooyk Y (2005a) Close encounters of neutrophils and DCs. Trends Immunol 26:626–631

van Gisbergen KP, Sanchez-Hernandez M, Geijtenbeek TB, van Kooyk Y (2005b) Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. J Exp Med 201:1281–1292

van Loon K, Venook AP (2011) Adjuvant treatment of colon cancer: what is next? Curr Opin Oncol 23:403–409

van Spriel AB, van Ojik HH, Bakker A, Jansen MJ, van de Winkel JG (2003) Mac-1 (CD11b/CD18) is crucial for effective Fc receptor-mediated immunity to melanoma. Blood 101:253–258

Wagner C, Iking-Konert C, Denefleh B, Stegmaier S, Hug F, Hansch GM (2004) Granzyme B and perforin: constitutive expression in human polymorphonuclear neutrophils. Blood 103:1099–1104

Watanabe M, Wallace PK, Keler T, Deo YM, Akewanlop C, Hayes DF (1999) Antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC) of breast cancer cells mediated by bispecific antibody, MDX-210. Breast Cancer Res Treat 53:199–207

Weiner LM, Murray JC, Shuptrine CW (2012) Antibody-based immunotherapy of cancer. Cell 148:1081–1084

Weng WK, Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 21:3940–3947

Wilson NS, Yang B, Yang A, Loeser S, Marsters S, Lawrence D, Li Y, Pitti R, Totpal K, Yee S, Ross S, Vernes JM, Lu Y, Adams C, Offringa R, Kelley B, Hymowitz S, Daniel D, Meng G, Ashkenazi A (2011) An Fcgamma receptor-dependent mechanism drives antibody-mediated target-receptor signalling in cancer cells. Cancer Cell 19:101–113

Wind J, Tuynman JB, Tibbe AG, Swennenhuis JF, Richel DJ, van Berge Henegouwen MI, Bemelman WA (2009) Circulating tumor cells during laparoscopic and open surgery for primary colonic cancer in portal and peripheral blood. Eur J Surg Oncol 35:942–950

You T, Hu W, Ge X, Shen J, Qin X (2011) Application of a novel inhibitor of human CD59 for the enhancement of complement-dependent cytolysis on cancer cells. Cell Mol Immunol 8:157–163

Zamai L, Ponti C, Mirandola P, Gobbi G, Papa S, Galeotti L, Cocco L, Vitale M (2007) NK cells and cancer. J Immunol 178:4011–4016

Zhang Z, Zhang M, Goldman CK, Ravetch JV, Waldmann TA (2003) Effective therapy for a murine model of adult T-cell leukemia with the humanised anti-CD52 monoclonal antibody, Campath-1H. Cancer Res 63:6453–6457

Zhao XW, van Beek EM, Schornagel K, van der Maaden H, van Houdt M, Otten MA, Finetti P, van Egmond M, Matozaki T, Kraal G, Birnbaum D, van Elsas A, Kuijpers TW, Bertucci F, van den Berg TK (2011) CD47-signal regulatory protein-alpha (SIRPalpha) interactions form a barrier for antibody-mediated tumor cell destruction. Proc Natl Acad Sci U S A 108:18342–18347

Sweet and Sour: The Role of Glycosylation for the Anti-inflammatory Activity of Immunoglobulin G

Sybille Böhm, Daniela Kao and Falk Nimmerjahn

Abstract The importance of immunoglobulin G (IgG) molecules for providing long-term sterile immunity as well as their major contribution to tissue inflammation during autoimmune diseases is generally accepted. In a similar manner, studies over the last years have elucidated many details of the molecular and cellular pathways underlying this protective activity in vivo, emphasizing the role of cellular Fc receptors recognizing the constant antibody fragment. In contrast, the active anti-inflammatory activity of IgG, despite being known and actually identified in human autoimmune patients more than 30 years ago, is much less defined. Recent evidence from several independent model systems suggests that IgG glycosylation is critical for the immunomodulatory activity of IgG and that both monomeric IgG as well as IgG immune complexes can diminish Fc receptor and complement dependent inflammatory processes. Moreover, there is increasing evidence that IgG molecules also modulate B and T cell responses, which may suggest that IgG is centrally involved in the establishment and maintenance of immune homeostasis.

Contents

1 Introduction 394
2 The Impact of IgG Glycosylation on Antibody Activity 395
3 The Role of Sialic Acid for the Anti-inflammatory Activity of Intravenous Immunoglobulins 399
4 Impact of Sialic Acid-Rich IgG on the Adaptive Immune System 404
4.1 Impact of IVIg Therapy on B Cells 404
4.2 Inhibition of the T Cell Response by Sialylated IgG 406

S. Böhm · D. Kao · F. Nimmerjahn (✉)
Department of Biology, Institute of Genetics, University of Erlangen-Nürnberg, Erwin-Rommelstr. 3, 91058 Erlangen, Germany
e-mail: falk.nimmerjahn@fau.de

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0_18,

5 The Role of Galactose in the Anti-inflammatory Activity of Immune Complexes 408
6 Conclusion 410
References 410

1 Introduction

The notion that immunoglobulin G (IgG) molecules can trigger two completely opposing effects, that is tissue inflammation and resolution of inflammation at the same time, has sparked the interest to decipher the molecular and cellular pathways underlying this dual activity (Durandy et al. 2009; Negi et al. 2007; Schwab and Nimmerjahn 2013; Seite et al. 2008). Although it is well known that the pro-inflammatory phase of normal immune as well as of many autoimmune responses are followed by a resolution phase in which pro-inflammatory processes are stopped and replaced by a phase of tissue repair and ultimately the return to the steady state, we are far from understanding the molecular and cellular pathways underlying this final phase of an immune response. With respect to the pro-inflammatory activity of IgG, it is generally accepted that the IgG Fc-fragment is critical for initiating effector functions including the release of reactive oxygen species, chemokines, cytokines such as TNFα, IL1, and IFNγ, phagocytosis, complement activation, and antibody-dependent cellular cytotoxicity (ADCC), which are all involved in establishing and maintaining tissue inflammation (Hogarth and Pietersz 2012; Nimmerjahn 2013; Takai 2002). On a cellular basis, this is achieved by binding of the IgG Fc fragment to Fcγ receptors (FcγR), which are broadly expressed on cells of the innate immune system including basophils, eosinophils, mast cells, monocytes, macrophages, and neutrophils. In addition, certain IgG subclasses including mouse IgG2a, IgG2b, and IgG3 and human IgG1 and IgG3 can activate the classical complement pathway via C1q resulting in the generation of the pro-inflammatory anaphylatoxins C3a and C5a, which can trigger innate immune effector cell recruitment (Carroll 1998). Furthermore, deposition of C3b on target cells enables their recognition through C3b receptors expressed on phagocytic cells, which also will result in removal of the opsonized antigen via phagocytosis. Despite this capacity to activate the complement system via the classical pathway, in the majority of mouse model systems of autoimmune diseases or therapeutic antibody activity, IgG binding to cellular FcγRs was demonstrated to be responsible for autoantibody pathology (Beers et al. 2009; Nimmerjahn et al. 2007; Nimmerjahn and Ravetch 2005; Sylvestre et al. 1996; Sylvestre and Ravetch 1994; Clynes and Ravetch 1995; Kaneko et al. 2006a). Of note, however, in several model systems, such as inflammatory arthritis, nephrotoxic nephritis, and skin blistering diseases, the alternative complement pathway was also involved in tissue inflammation in addition to the FcγR pathway (Mihai and Nimmerjahn 2012; Schmidt and Gessner 2005). In brief, FcγRs are a protein

family which can be distinguished by two major factors (Fig. 1). The first is their differential affinity for the individual IgG subclasses, with FcγRI being a high affinity receptor with the capacity to bind to select IgG subclasses (IgG2a in mice and IgG1, IgG3, and IgG4 in humans) as IgG monomers (Hogarth and Pietersz 2012; Nimmerjahn and Ravetch 2006; Takai 2002). All the other receptors can only bind IgG in the form of immune complexes, corresponding to their lower affinity for the different IgG subclasses. Second, there is one inhibitory FcγR, FcγRIIB, in mice and humans which is very broadly expressed together with activating FcγRs and thereby may set a threshold for cell activation by immune complexes as demonstrated by several studies (Bolland and Ravetch 1999; Daeron and Lesourne 2006; Smith and Clatworthy 2010). Depending on the experimental model system and IgG subclass responsible for the respective effector function, different activating FcγRs have been shown to be responsible for IgG activity (Nimmerjahn and Ravetch 2006). The genetic studies in mouse model systems are supported by reports from human patient cohorts showing enhanced therapeutic antibody activity if patients carry allelic variants of activating FcγRs with a higher affinity for the therapeutic antibody (Cartron et al. 2002; Musolino et al. 2008; Weng et al. 2004; Weng and Levy 2003). As the details of the involvement of the activating FcγRs for the pro-inflammatory activity of IgG will be the focus of several other reviews in this volume, we will not describe this in further depth and direct the reader to the reviews of DiLillo et al. and Bakema and van Egmond, for example.

2 The Impact of IgG Glycosylation on Antibody Activity

Several studies over the last years have emphasized the importance of IgG glycosylation for modulating its activity in vivo (Arnold et al. 2007; Dalziel et al. 2014). Thus, the genetic or enzymatic removal of this moiety results in a loss of both the pro- and the anti-inflammatory activities of IgG (Arnold et al. 2007; Ghirlando et al. 1999; Krapp et al. 2003; Rudd et al. 2001; Walker et al. 1989; Schwab and Nimmerjahn 2013; Shields et al. 2001). Whereas most immunoglobulin isotypes, such as IgA, IgM, and IgE contain multiple N-linked sugar moieties, IgG has only one single sugar domain attached to the IgG Fc fragment at the asparagine 297 (N297) residue in the CH2 domain. This sugar moiety is confined within the hydrophobic space between the two IgG heavy chains and consists of a heptameric biantennary structure (Fig. 2). The core of this domain is made up of a conserved array of N-acetylglucosamine and mannose residues. In contrast to this core structure, there is a great variability with respect to the presence of branching fucose and N-acetylglucosamine residues and terminal galactose and sialic acid residues. Thus, in humans and mice more than 30 different IgG glycoforms were identified in the serum, which may result in several hundreds of different individual IgG glycovariants if one considers that the two individual IgG Fc fragments may contain different sugar domains (Arnold et al.

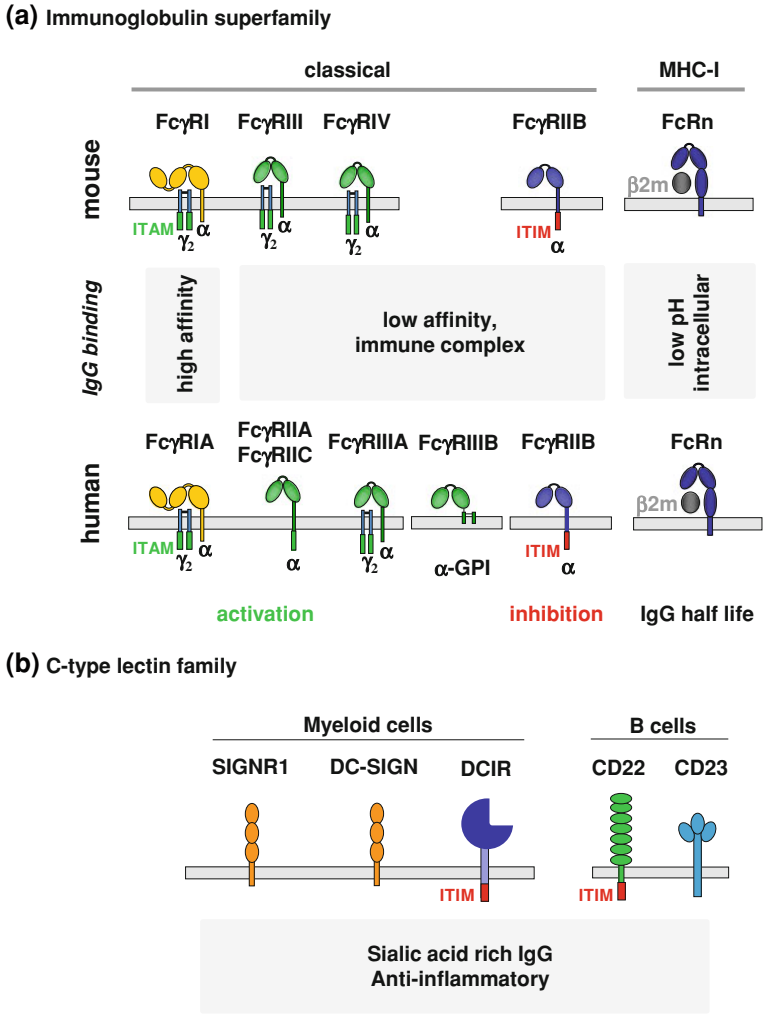


Fig. 1 The expanding family of IgG receptors. **a** Shown is the family of classical Fcγ-receptors belonging to the immunoglobulin superfamily in mice and humans. Apart from their differential affinity for IgG, they can be separated into activating and inhibitory receptors. Some of these receptors such as mouse FcγRIV and human FcγRIIIA have the capacity to differentially recognize IgG glycosylation variants. See text for further details. **b** Shown are members of the C-type lectin family, which have been shown to have the capacity to recognize sialic acid-rich IgG glycosylation variants. While all of these receptors have been suggested to negatively regulate myeloid cell or B cell activation, only some of these receptors contain ITIM motifs. See text for further details

2007). It is known for a long time that this IgG glycosylation status is not stable but can change during inflammation. Glycoforms lacking terminal sialic acid and galactose residues (the so-called G0 glycoforms), for example, increase during

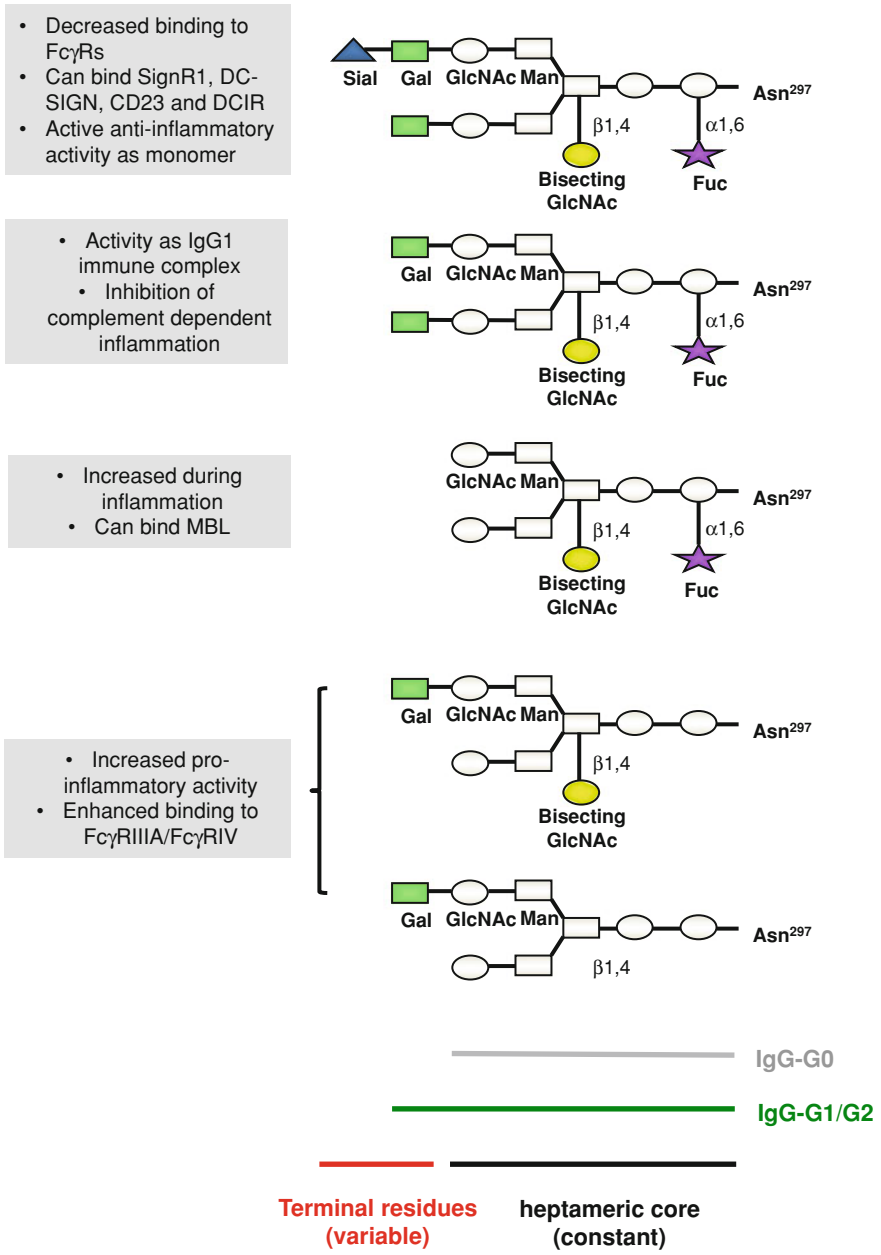


Fig. 2 IgG glycoforms with altered activity. Depicted are the sugar moieties of IgG glycosylation variants with altered activity or function. Whereas IgG glycoforms rich in terminal sialic acid residues have the ability to suppress FcγR-dependent effector functions and DC activation, IgG rich in terminal galactose residues interferes with the pro-inflammatory activity of activated complement components. In contrast, other variants lacking terminal galactose or branching fucose residues may have a heightened pro-inflammatory activity

acute inflammation, which may be caused by autoimmune diseases, viral and bacterial infections, or during the course of vaccinations with certain adjuvants (Fig. 2) (Kaneko et al. 2006b, Mehta et al. 2008; Parekh et al. 1988; Tomana et al. 1988; Dube et al. 1990; Holland et al. 2002; Scherer et al. 2010). Apart from inflammation, IgG-G0 glycoforms increase during aging, which correlates with the increasing likelihood to develop an autoimmune disease (Arnold et al. 2007; Bohm et al. 2012). Conversely, IgG-G0 glycoforms decrease during pregnancy which correlates with the reduced incidence of flares in pregnant women with arthritis (van de Geijn et al. 2009). Although there is evidence that IgG-G0 glycovariants may have an enhanced pro-inflammatory activity via gaining the capacity to bind to MBL (Malhotra et al. 1995; Rademacher et al. 1994), which is the first component of the lectin pathway of complement activation, studies performed in MBL knockout mice showed that the lectin pathway may not be a dominant factor responsible for the activity of these glycoforms at least in these animal models (Nimmerjahn et al. 2007). In a similar manner, the ability of an IgG3 rheumatoid factor antibody derived from lupus prone MRL-lpr mice to induce kidney damage upon deposition in glomeruli was not different if the antibody was in the G0 or G1/G2 glycoform (Otani et al. 2012). Moreover, the hemolytic ability of red blood cell specific antibodies inversely correlated with the amount of G0 glycoforms (Hadley et al. 1995; Kumpel et al. 1995). In humans, high levels of MBL are rather associated with milder pathology in rheumatoid arthritis, also arguing against a major involvement of this pathway for enhancing autoimmune inflammation (van de Geijn et al. 2008, 2011; Garred et al. 2000). Taken together, no clear picture with respect to an enhanced activity of agalactosyl antibodies is emerging. As we will discuss later, the galactosylation as well as the sialylation state may, however, confer the antibody with an immunomodulatory and anti-inflammatory activity.

The most convincing evidence that the specific IgG glycosylation status can have a dramatic impact on the pro-inflammatory activity of IgG was demonstrated for IgG glycoforms lacking branching fucose residues. In mice and humans, this results in a 10- to 50-fold enhanced affinity of IgG for mouse FcγRIV and human FcγRIIIA, respectively (Figs. 1, 2) (Ferrara et al. 2011; Nimmerjahn and Ravetch 2005; Shields et al. 2002; Shinkawa et al. 2003). Consistent with this increased affinity these IgG glycovariants showed an enhanced cytotoxic or phagocytic activity in mouse tumor and autoimmune model systems and in a variety of immunodeficient mouse strains xenotransplanted with human tumor cells (Nimmerjahn and Ravetch 2012). The first glycoengineered tumor-specific antibodies, lacking branching fucose residues have now completed the clinical testing phase and have been approved for general use in human therapy of cancer.

3 The Role of Sialic Acid for the Anti-inflammatory Activity of Intravenous Immunoglobulins

It is well known since more than three decades that IgG itself can have an immunomodulatory activity and is able to suppress IgG-driven autoimmune diseases such as ITP (Imbach 2012; Imbach et al. 1981). This type of therapeutic intervention is known as IVIg therapy, which is the intravenous infusion of IgG pooled from several thousand donors at 1–3 g/kg (Bayry et al. 2011b; Imbach 2012; Schwab and Nimmerjahn 2013). Whereas the most obvious explanation for this capacity to block the effector phase of autoantibodies would have been an inhibition of autoantibody binding to activating FcγRs, reports published over the last decade argue against this simple scenario as the major pathway (Nimmerjahn and Ravetch 2007). In brief, monomeric IgG even if infused at high doses may not be able to interfere with the binding of immune complexes to activating FcγRs. Moreover, IVIg activity was demonstrated to be independent of the small amount of IgG dimers present in most IVIg preparations and can be recapitulated with the IgG Fc fragment in mice and humans (Crow et al. 2001; Tremblay et al. 2012; Anthony et al. 2008a; Kaneko et al. 2006b; Samuelsson et al. 2001; Debre et al. 1993). Finally, IgG glycovariants with enhanced therapeutic activity have a reduced affinity for most activating FcγRs (Anthony et al. 2008a, b; Anthony and Ravetch 2010; Kaneko et al. 2006b; Schwab et al. 2012a; Scallon et al. 2007). Nonetheless, some studies imply a role of activating FcγRIII most likely on dendritic cells or macrophages as being a part of IVIg-mediated immunomodulation, although other studies demonstrated that IVIg-dependent amelioration of ITP and nephrotoxic nephritis were not impaired in FcγRIII-deficient mice (Kaneko et al. 2006a; Park-Min et al. 2007; Schwab et al. 2012a; Siragam et al. 2006; Huang et al. 2010). Thus, model system-specific effects may underlie these different experimental results.

In contrast to activating FcγRs, the inhibitory FcγRIIB was shown to be required for IVIg activity by several groups in different model systems (Fig. 3). Mice deficient for the inhibitory FcγRIIB were no longer protected by IVIg therapy from the induction of ITP, nephrotoxic nephritis, epidermolysis bullosa acquisita, and inflammatory arthritis (Huang et al. 2010; Bruhns et al. 2003; Kaneko et al. 2006a; Samuelsson et al. 2001). As sialic acid-rich IgG also shows a reduced binding to FcγRIIB, not a direct induction of signaling via IVIg binding to FcγRIIB, but a more indirect pathway involving this receptor was likely to be involved. Several studies demonstrated that FcγRIIB becomes upregulated on innate immune effector cells and B cells following IVIg infusion in mice (Bruhns et al. 2003; Kaneko et al. 2006a, b; Samuelsson et al. 2001). This finding was confirmed in human patients with chronic inflammatory demyelinating polyneuropathy (CIDP), a disease in which autoantibodies against neuronal tissue cause a progressive nerve inflammation and paralysis (Tackenberg et al. 2009). Similar to the Guillain-Barré syndrome, which is an acute form of this disease, IVIg infusion is a licensed first-line treatment for CIDP allowing to directly study IVIg-mediated

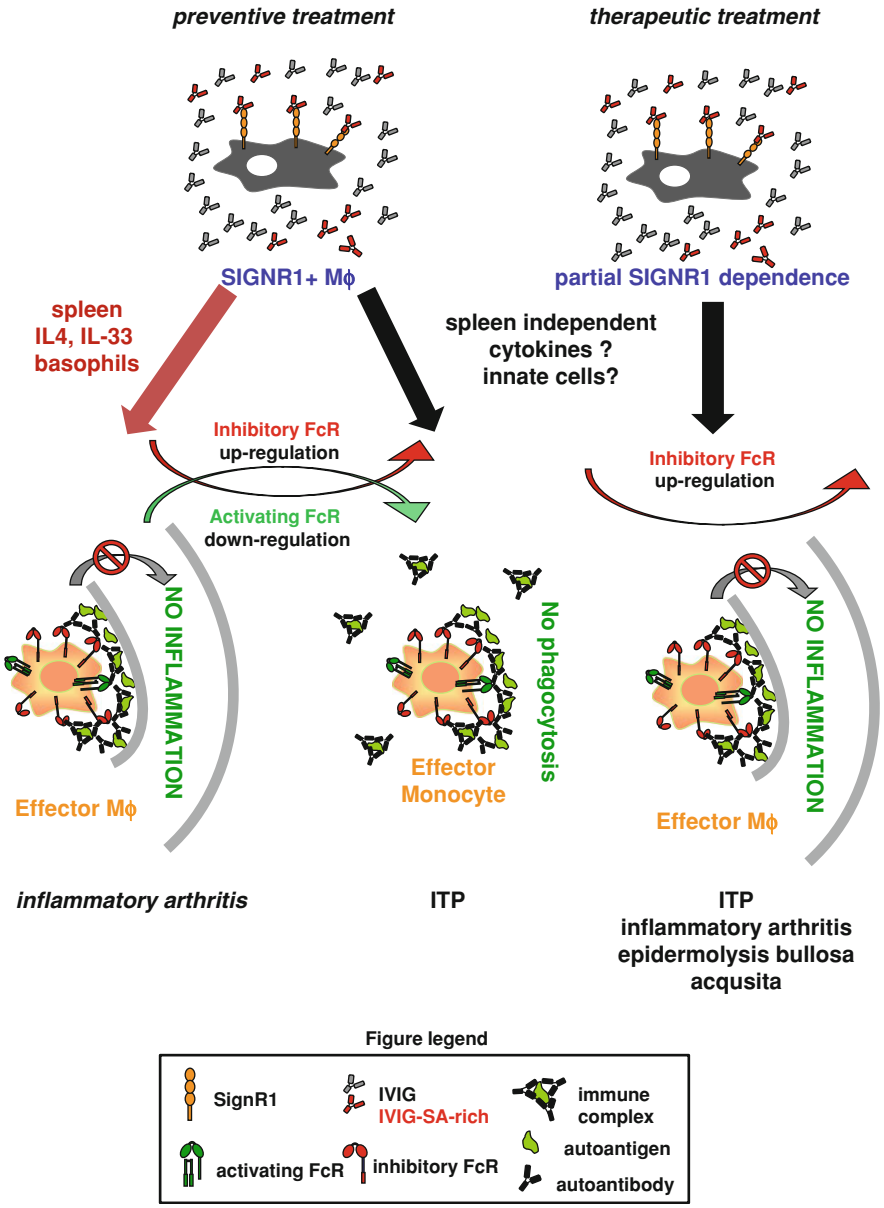


Fig. 3 Pathways involved in the anti-inflammatory activity of IVIg. Shown are the details of the molecular and cellular components involved in the anti-inflammatory activity of IVIg under preventive and therapeutic treatment conditions in mice. Whereas some components such as terminal sialic acid residues and the inhibitory FcγRIIB are critical under both preventive and therapeutic treatment conditions, SIGNR1 may only be partially involved in the anti-inflammatory pathway under therapeutic conditions. See text for further details

immunomodulatory effects in human patient populations. In parallel to the upregulation of FcγRIIB, a downregulation of the activating FcγRIV was observed in a mouse model of nephrotoxic nephritis upon IVIg treatment, supporting the notion that IVIg induces a higher threshold for activation of innate immune effector cells via immune complexes (Fig. 3) (Kaneko et al. 2006a). More recently, a novel TH2 cytokine pathway involving IL33, which in turn leads to the release of IL4 was shown to be involved in the upregulation of FcγRIIB in a model of inflammatory arthritis (Anthony et al. 2011). Thus, mice deficient in the IL4 receptor were no longer protected by IVIg therapy and it was further demonstrated that IL33 was responsible for the IL4 release by basophils. Indeed, IL4 is well known to have the capacity to upregulate FcγRIIB on myeloid effector cells. As will be discussed later in this chapter, this pathway may not be involved in upregulation of FcγRIIB on B cells, as IL4 induces a reduction of FcγRIIB on this cell type (Rudge et al. 2002). In contrast to inflammatory arthritis, IVIg-mediated protection from ITP was demonstrated to be independent of this TH2 cytokine pathway and of basophils (Crow et al. 2007; Schwab et al. 2012a). Despite these differences in the cellular players involved in the anti-inflammatory pathway some common denominators of IVIg activity have become apparent, one of which is the need for IgG glycosylation.

The first evidence that IgG glycosylation may play a role in the anti-inflammatory and immunomodulatory activity of IVIg was provided in 2006 and confirmed by several studies thereafter in a variety of model systems of autoimmune disease (Kaneko et al. 2006b; Anthony et al. 2008a, b, 2011; Schwab et al. 2012a, b). By using either *PNGaseF* treated and hence aglycosylated or *neuraminidase* treated and hence asialylated IVIg, it was demonstrated that the capacity of IVIg to suppress inflammatory arthritis was lost in the absence of IgG glycosylation or sialylation, respectively (Kaneko et al. 2006b; Schwab et al. 2012a). A more detailed analysis demonstrated that especially 2,6 linked terminal sialic acid residues were responsible for this amelioration of joint inflammation, allowing to generate a recombinant IVIg replacement by using a highly sialylated monoclonal human IgG1 Fc fragment (Anthony et al. 2008a). This IVIg replacement was able to suppress inflammatory arthritis at a 30-fold lower dose than untreated IVIg, further demonstrating that at least for the studied model systems the polyclonal nature of the IgG preparation is dispensable. As about 15–20 % of serum IgG can contain an additional sugar moiety in the IgG variable region, which is exposed on the surface of the IgG molecules and has a high level of terminal sialic acid residues, it was critical to determine whether this F(ab)2 attached sugar moiety plays a role in the immunomodulatory activity as well (Arnold et al. 2007). Of note, however, enriching IVIg for sialic acid residues in the IgG variable region had no impact on activity, whereas enriching the IgG Fc fragment for high levels of sialic acid increased the immunosuppressive activity (Guhr et al. 2011; Kaneko et al. 2006b). This is in line with results showing that it is not the sialic acid residues themselves, which are sufficient for the anti-inflammatory activity, but rather the IgG amino acid backbone itself (Kaneko et al. 2006b). Indeed, other serum proteins such as fetuin or transferrin, which also carry a bi-antennary sugar structure with high levels of sialic acid residues did not show

therapeutic activity. Although the crystal structure of highly sialylated IgG did not show major structural alterations, a recent study using different biophysical methods suggests that high levels of sialic acid may impact IgG structure (Crispin et al. 2013; Sondermann et al. 2013). According to this, the IgG Fc fragment may acquire a closed structure if high levels of terminal sialic acid residues are present in the sugar moiety. This may explain earlier data showing that sialic acid-rich IgG binds with lower affinity to the family of canonical FcγRs in mice and humans (Kaneko et al. 2006b; Scallon et al. 2007). While having a reduced capacity to bind to classical FcγRs, these IgG glycovariants gain the capacity to bind to SIGNR1, which belongs to the family of C-type lectins and can recognize pathogens such as HIV (Anthony et al. 2008b). Consistent with these in vitro binding data, IVIg was no longer able to protect SIGNR1-deficient mice or mice injected with a blocking SIGNR1-specific antibody from the development of inflammatory arthritis or ITP (Fig. 3) (Anthony et al. 2008b, 2011; Schwab et al. 2012a). In mice, a major cell population expressing SIGNR1 are marginal zone macrophages in the spleen. Supporting an important role of this macrophage subpopulation, mice deficient in MCSF1 or Rag1, which either have a reduced number of these macrophages or a disorganized splenic structure, did not respond to IVIg therapy in a model of inflammatory arthritis (Anthony et al. 2008b; Bruhns et al. 2003). With respect to the human system, the most closely related human protein to SIGNR1 is DC-SIGN, which has a different expression pattern. Most forward, DC-SIGN is abundantly expressed on dendritic cells, which largely lack SIGNR1 expression in mice. Reconstituting SIGNR1 deficient mice with human DC-SIGN expression was able to restore IVIg activity, indicating that not necessarily the cell population but especially the features of the receptor molecule being able to recognize sialic acid-rich IgG is a key element of the anti-inflammatory pathway. This concept is corroborated by the fact that in contrast to the critical requirement of the spleen for IVIg activity in the inflammatory arthritis model, IVIg-mediated amelioration of ITP was independent of the spleen (Fig. 3) (Schwab et al. 2012a). A similar lack of requirement of splenic resident cells can be observed in human ITP patients, who respond well to IVIg therapy even after splenectomy.

As the two sugar moieties attached to the N297 residue in the IgG Fc fragment may differ in composition, or potentially even contain two terminal sialic acid residues each, it was critical to determine which of these combinations are actually present in IVIg preparations enriched for sialic acid-rich IgG glycoforms via the lectin SNA. This analysis revealed that the dominant IgG glycoforms enriched via SNA contained maximally one sialic acid residue in each of the two sugar moieties and suggest that this sialic acid residue was mostly present in the 1,3 branch in the majority of sugar domains (Stadlmann et al. 2009; Wormald et al. 1997). Fully processed IgG glycovariants containing two sialic acid residues in each sugar moiety were virtually absent from this preparation suggesting that especially the IgG glycovariant containing a single sialic acid residue on the 1,3 sugar arm was critical for the anti-inflammatory activity at least within an IVIg preparation (Stadlmann et al. 2009; Bohm et al. 2012). This is consistent with results demonstrating that enriching IgG molecules for glycovariants containing F(ab)2-linked

sugar moieties, which can be generated through de novo generation of N-linked glycosylation sites during somatic hypermutation and which carry sialic acid residues mostly attached to the 1,6 sugar arm, do not have any enhanced therapeutic activity (Guhr et al. 2011; Kasermann et al. 2012).

The general importance of IgG Fc-sialylation for IVIg activity in vivo was demonstrated more recently in two model systems of ITP, inflammatory arthritis and a model of epidermolysis bullosa acquisita (EBA), in which collagen type VII specific autoantibodies induce a widespread skin blister formation (Schwab et al. 2012a, 2014; Kasperkiewicz et al. 2012a, b). Importantly, this also holds true for therapeutic treatment conditions where IVIg is administered after autoimmune disease is established, which closely reflects the clinical situation (Schwab et al. 2014). Thus, sialic acid-deficient IVIg was no longer able to block ITP, inflammatory arthritis, and EBA if administered during ongoing autoimmune disease. As demonstrated before for IVIg treatment of ITP under therapeutic conditions, FcγRIIB was essential for IVIg activity in inflammatory arthritis and EBA (Fig. 3). An interesting difference with respect to preventive treatment conditions became apparent with respect to the requirement for SIGNR1, however. Although an initial delay of IVIg activity was observed in models of EBA and inflammatory arthritis, ultimately IVIg was able to ameliorate autoantibody tissue inflammation independently of SIGNR1 (Schwab et al. 2014). One possible explanation for this differential requirement for SIGNR1 may be that once inflammation is established other molecules with the capacity to bind sialic acid-rich IgG may take over this function. A candidate receptor with such an activity may be DCIR (dendritic cell immunoreceptor), another C-type lectin receptor expressed on dendritic cells, which was identified most recently as another potential receptor involved in the anti-inflammatory activity of IVIg (Dalziel et al. 2014). Of note, there are data suggesting that by increasing the autoantibody dose IVIg may also have the capacity to suppress ITP independent of the sialic acid-rich IgG fraction within the IVIg preparation (Leontyev et al. 2012). Whether an according increase in the dose of sialic acid-rich IVIg may be sufficient to regain therapeutic activity was not studied, making it hard to fully appreciate the results obtained in this experimental model system. The use of a rat-derived platelet-specific antibody as a surrogate for a naturally arising autoantibody cannot explain these results as a recent study using the same ITP model system demonstrated an absolute requirement for sialic acid-rich IgG glycoforms for IVIg activity (Schwab et al. 2014). Ultimately, human clinical trials or humanized mouse model systems at a pre-clinical stage will provide conclusive evidence if these findings are of relevance for the human immune system. The essential role of sialic acid-rich glycoforms for preventive and therapeutic treatment regimens in model systems of ITP, inflammatory arthritis, and epidermolysis bullosa acquisita, however, strongly argue for a generalized importance of this IgG glycoform as an immunomodulatory molecule. As we have discussed before, this model is also supported by epidemiologic data in humans, showing that the level of sialic acid-rich IgG glycoforms seems to be closely regulated during different stages of an immune response, with strongly reduced levels being present during acute inflammation (Schwab and Nimmerjahn

2013). Further along these lines, there is convincing data showing that the pathogenicity of mouse rheumatoid factor antibodies, that is antibodies specific for other antibody isotypes, which results in the deposition of large amounts of immune complexes in the lungs and kidneys of affected animals, is critically determined by the level of IgG sialylation (Otani et al. 2012). Interestingly, sialylation did not affect the antigen specificity, that is the binding of the IgG3 autoantibody to other serum IgG isotypes, but rather modulated its capacity to precipitate at temperatures below 37 °C. Thus, despite the generation of immune complexes in the blood, they no longer became deposited in the glomeruli of the kidneys (Otani et al. 2012).

4 Impact of Sialic Acid-Rich IgG on the Adaptive Immune System

Besides the well-established inhibitory effect of IVIg on cells of the innate immune system, there is accumulating evidence that IVIg may also modulate the adaptive immune response. As indicated before, several groups observed an upregulation of FcγRIIB on mouse and human B cells upon IVIg infusion (Nikolova et al. 2009; Tackenberg et al. 2009). This finding is of great interest, as the inhibitory Fc receptor may be an important checkpoint for maintaining humoral tolerance and prevent the production of autoantibodies by B cells. Moreover, there is very recent data that sialic acid containing IgG glycovariants in the form of immune complexes may be able to interfere with T cell-mediated delayed type hypersensitivity (DTH) reactions arguing for a broad impact of IVIg on the adaptive immune response.

4.1 Impact of IVIg Therapy on B Cells

The first evidence that IVIg therapy may regulate the threshold for B cell activation was noted by Tackenberg and colleagues in human patients with chronic inflammatory demyelinating polyneuropathy (CIDP), who receive IVIg as a first-line treatment to suppress the autoantibody mediated inflammation of the peripheral nervous system (Tackenberg et al. 2009). As had been demonstrated for human SLE patients before, this study confirmed that also CIDP patients showed a lower level of FcγRIIB expression on B cells and failed to upregulate the receptor on memory B cells (Mackay et al. 2006; Tackenberg et al. 2009). Together with studies demonstrating that a nonfunctional allele of the inhibitory FcγRIIB, which can no longer associate with lipid rafts due to the exchange of the isoleucine residue 232 for a threonine residue (I232T), is associated with SLE development in several human SLE cohorts, this suggests that FcγRIIB is involved in maintaining

humoral tolerance (Willcocks et al. 2010; Schwab et al. 2014; Chu et al. 2004; Kyogoku et al. 2002; Kono et al. 2005; Floto et al. 2005). Moreover, the treatment of patients with common variable immunodeficiencies with IVIg resulted in B cell proliferation and immunoglobulin synthesis (Bayry et al. 2011a). Reversely, a block of B cell proliferation and induction of B cell apoptosis was noted with human B cells in in vitro assays (Seite et al. 2010). Regardless of these different outcomes on B cell survival, these results suggest that IVIg can have a direct effect on the B cell response.

Whereas FcγRIIB counterbalances signals triggered by activating FcγRs on cells of the innate immune system, it regulates signaling pathways initiated via the B cell receptor (BCR) on B cells. A possible situation where such a co-cross-linking of FcγRIIB with the BCR could occur is during the germinal center reaction in which B cells are selected for their antigen specificity and BCR affinity in the presence of immune complexes which are presented on the surface of follicular dendritic cells (Espeli et al. 2012). Thus, the threshold set by FcγRIIB binding to IgG antibodies present in these immune complexes may ensure that B cells only become activated if the BCR has a very high affinity for the antigen (Lehmann et al. 2012; Smith and Clatworthy 2010). Consistent with this model, mice deficient for FcγRIIB develop an SLE-like autoimmune disease characterized by the loss of humoral tolerance and a glomerulonephritis depending on the mouse genetic background (Bolland and Ravetch 2000; Bolland et al. 2002; Boross et al. 2011; McGaha et al. 2008; Fukuyama et al. 2005). Furthermore, CIDP and SLE patients, most autoimmune prone mouse strains, and the majority of wild mouse strains have a lower expression level of this receptor, which is due to promotor polymorphisms resulting in reduced transcription of the gene (Pritchard et al. 2000; Espeli et al. 2012; Su et al. 2004a, b, 2007). Restoring the level of FcγRIIB expression either ubiquitously or selectively on B cells has been shown to block autoantibody production and reduce pathology in autoimmune prone mouse strains or in models of induced autoimmune disease, strongly supporting the concept that FcγRIIB is a gatekeeper of humoral tolerance (Brownlie et al. 2008; McGaha et al. 2005). Providing direct evidence that FcγRIIB may have a related function in the human immune system, a study using immunodeficient mice reconstituted with human hematopoietic stem cells expressing either the fully functional or the functionally impaired FcγRIIB-232T variant, demonstrated a loss of humoral tolerance and the production of autoantibodies typical for human rheumatoid arthritis and SLE in the humanized animals (Baerenwaldt et al. 2011). Thus, restoring FcγRIIB expression levels on B cells in autoimmune patients via IVIg therapy may reinstate the threshold for B cell activation and reduce the production of autoantibodies in the long term.

Apart from a function on mature B cells, restoring FcγRIIB expression may also have an effect on the antibody producing plasma cell itself. The situation on plasma cells is unique in the way that this B cell subset no longer expresses the BCR but high levels of FcγRIIB (Baerenwaldt et al. 2011). In the absence of simultaneous activating signals, the isolated cross-linking of the inhibitory FcγRIIB may induce a pro-apoptotic signal, which has been shown to be

independent of the classical ITIM-dependent signaling pathway (Pearse et al. 1999; Tzeng et al. 2005). There is evidence that cross-linking of FcγRIIB on plasma cells in the bone marrow, may result in the death of a proportion of plasma cells occupying a limited number of niches, which allow plasma cell survival. By this mechanism, niches may become available for supporting the survival of new plasma cells, which have been generated during a subsequent immune response (Xiang et al. 2007; Radbruch et al. 2006). Quite interestingly, the lower level of FcγRIIB expression on B cells in autoimmune prone mice also correlates with a higher amount of plasma cells, suggesting that these cells may be more resistant to being eliminated via this pathway. Thus, normalizing FcγRIIB expression by IVIg treatment may allow restoring the FcγRIIB-dependent pro-apoptotic pathway on autoantibody producing plasma cells, which may result in the elimination of these cells via circulating immune complexes.

With respect to the mechanism of FcγRIIB upregulation on B cells, several open questions remain. While recent studies have demonstrated that IVIg can directly interact with B cells, it is still unclear which receptor is responsible for this binding. As we have discussed before, a direct binding to FcγRIIB seems unlikely, as sialic acid-rich IgG has a strongly reduced affinity for this Fcγ receptor (Kaneko et al. 2006b). In vitro experiments with human B cells demonstrated that sialic acid-rich IgG may bind to B cells via CD22, a cell surface molecule broadly expressed on mouse and human B cells with a known specificity for sialic acid residues (Fig. 1) (Seite et al. 2010; Jellusova and Nitschke 2011). In CD22 knockout mice, however, IVIg was still able to bind to B cells suggesting that other or additional proteins may be involved in this binding (Schwab et al. 2012b). This may also be due to the fact that CD22 is usually occupied with ligands present on proteins on the same cell, such as other CD22 molecules or the B cell receptor. More recently, CD23, which is the low affinity receptor for IgE expressed on B cells, was suggested to be another candidate receptor with the capacity to bind sialic acid-rich IgG (Fig. 1) (Sondermann et al. 2013). CD23 is a prime example demonstrating that proteins, which have been identified as members of the C-type lectin family member due to their capacity to recognize sugar structures directly, can also bind ligands (IgE) via direct binding to the protein backbone and hence independent of carbohydrates. Future studies using CD23 knockout mice will be necessary to determine if CD23 is indeed the critical link explaining how IVIg modulates B cell functions and upregulates FcγRIIB.

4.2 Inhibition of the T Cell Response by Sialylated IgG

More recently, a feedback of sialylated antibodies on the T cell response was noted as well. Injection of IgG1 antibodies specific for the model antigen ovalbumin was able to reduce the severity of ovalbumin-dependent T cell-mediated delayed type hypersensitivity (DTH) reactions including allergic asthma, if they contained high levels of terminal sialic acid residues (Oefner et al. 2012). Compared to the

immediate hypersensitivity reactions, which develop within minutes after antigen encounter and depend on antibody mediated cross-linking of Fc receptors on mast cells for example, DTH reactions take days to develop and are dependent on antigen uptake and processing by dendritic cells followed by recognition of T-helper cells, which in turn release pro-inflammatory cytokines, resulting in tissue inflammation. In contrast to the IVIg-mediated suppression of antibody responses, however, the antigen specificity of the injected antibodies was critical in this model system. Thus, injection of antibodies of the same IgG subclass and carrying a similar degree of sialylation but a different antigen specificity were not able to suppress an ovalbumin-dependent DTH response, suggesting that the formation of immune complexes with sialic acid-rich ovalbumin-specific antibodies with ovalbumin was somehow involved in this immunoregulatory process. Moreover, a much lower amount of sialic acid-rich IgG was necessary to reduce the DTH response, again arguing for the formation of immune complexes (Oefner et al. 2012). Similar results were obtained in a model of ovalbumin-dependent allergic airway inflammation, where the pre-injection of OVA-specific IgG1 antibodies reduced the recruitment of eosinophils to the lung following an intranasal challenge with the antigen. A potential pathway how the sialic acid-rich antibodies might ameliorate DTH reactions was provided by experiments showing that sialic acid-rich IgG immunocomplexes were able to inhibit dendritic cell maturation which might limit their capacity to activate T-helper cells and thereby limit the release of pro-inflammatory cytokines (Oefner et al. 2012). Again in contrast to the IVIg-induced immunomodulatory pathway, the inhibitory FcγRIIB was not required for this inhibition of DC maturation, consistent with the reduced affinity of sialic acid-rich IgG1 for the inhibitory FcγRIIB (Kaneko et al. 2006b; Oefner et al. 2012). An involvement of dendritic cells and regulatory T cells (Treg) in the anti-inflammatory pathway of IVIg activity was shown by several other studies (Bayry et al. 2003). Thus, IVIg (or IgG in general) was demonstrated to contain epitopes in its Fc fragment with the capacity to expand regulatory T cells (so-called Tregitopes) (De Groot et al. 2008; Ephrem et al. 2008). Upon uptake of IVIg via dendritic cells and processing of IgG in endosomal compartments, this would lead to the loading of these peptide sequences onto MHC class II molecules, which then enables the expansion of Tregs. More recently, a receptor called dendritic cell inhibitory receptor (DCIR) was shown to be essential for the IVIg dependent induction of Tregs. This receptor specifically recognized highly sialylated IgG species within the IVIg preparation, providing further evidence that enriching IVIg for its sialic acid-rich glycoforms may not only enhance its capacity to modulate the effector phase of IgG activity but also enhance the generation of Tregs, resulting in a more potent suppression of pro-inflammatory T cell responses (Dalziel et al. 2014).

Quite interestingly, a similar inhibition of a DTH reaction was observed if mice were pre-immunized with the model antigen in the absence of adjuvants or if T cell-independent antigens such as TNP-LPS or TNP-Ficoll were used (Hess et al. 2013; Oefner et al. 2012). It is well established that a successful T cell-dependent vaccination with protein-based antigens usually requires co-stimulatory signals

which may be provided via Toll-like receptor ligands as present in complete Freunds' adjuvant or more directly by co-injection of CD40-specific antibodies, for example. In the absence of such co-stimulatory signals, only a limited proliferation of T cells occurs, and most of these antigen-specific T cells are no longer detectable after one week, consistent with a concept of tolerance induction by deletion of potentially self-reactive T cells in the absence of infection or inflammation and/or by the induction of regulatory T cells (Yamazaki et al. 2008). With respect to effects on antibody glycosylation, immunization of mice with T cell-dependent antigens in complete Freunds' adjuvant was shown to result in the production of IgG antibodies with low levels of terminal sialic acid residues, which therefore lose their ability to trigger anti-inflammatory pathways (Kaneko et al. 2006b; Hess et al. 2013; Oefner et al. 2012). This reduction in IgG sialylation was absent in interferon-γ receptor and interleukin 17 receptor double knockout mice, suggesting that these two cytokines are involved in changing IgG sialylation patterns (Hess et al. 2013). Of note, an involvement of these cytokines in altering IgG glycosylation was not observed with human B cells in an in vitro culture system (Wang et al. 2011). Here only the treatment with all-trans retinoic acid (ATRA) resulted in a production of antibodies with a reduced level of sialic acid, whereas treatment with IFNγ and IL21 rather increased galactosylation and/or sialylation and IL17 had no measurable effect on IgG glycosylation (Wang et al. 2011).

With respect to the antibody response induced in the absence of co-stimulatory signals, largely IgG antibodies with no reduction in terminal sialic acid residues compared to serum IgG during steady-state conditions were produced. As discussed for the monoclonal antibodies before, transfer of these serum antibodies protected mice from a subsequent DTH response in a sialic acid-dependent manner. Apart from the effect noted with respect to the activation of dendritic cells, also a suppression of subsequent B cell responses was noted, although again independent of FcγRIIB. Further studies will be critical to understand this immunomodulatory effect in greater detail and it will be important to understand whether previous studies describing antigen-dependent negative feedback loops on antibody production by B cells are also dependent on specific IgG glycoforms (Heyman 2000).

5 The Role of Galactose in the Anti-inflammatory Activity of Immune Complexes

More recently, another residue of the IgG heavy chain sugar moiety was identified to be important for a novel immunomodulatory activity of IgG immune complexes. As we have discussed before, immune complexes are well known to have a potent pro-inflammatory activity as polymeric IgG acquires the capacity to ligate low affinity FcγRs on cells of the innate immune system and to activate the classical complement pathway (Fig. 1). The activated complement components

C3a and C5a recruit a variety of innate immune effector cells including monocytes and neutrophils, which will further enhance inflammation. With respect to complement activation, individual IgG subclass members differ in their capacity to trigger the classical complement pathway. Whereas in mice IgG2a and IgG2b are most potent in triggering complement activation, in humans IgG1 and IgG3 have this capacity. Mouse IgG1, in contrast, does only have a very low affinity toward C1q and hence has a very limited ability to initiate the complement cascade. Indeed, mouse IgG1 activity is usually abrogated in mice deficient in the activating FcγRIII (Nimmerjahn and Ravetch 2006). Although not being able to initiate the complement pathway, IgG1 glycovariants in the form of immune complexes were able to actively block the effector pathways triggered by complement activation (Karsten et al. 2012). Thus, IgG1 carrying high levels of terminal galactose residues (IgG–G2; Fig. 2) interfered with the recruitment of neutrophils in a C5a-dependent model of peritonitis. Moreover, injection of these immune complexes abrogated the formation of skin blisters induced by the injection of collagen-type VII specific antibodies in a passive model of epidermolysis bullosa acquisita, consistent with the co-dominant role of C5 and activating FcγRs in autoimmune diseases such as nephritis, inflammatory arthritis, peritonitis, skin blistering diseases, and immune complex-dependent alveolitis (Mihai and Nimmerjahn 2012; Karsten et al. 2012).

Of note, IgG2a immune complexes did not suppress neutrophil recruitment irrespective of their level of galactosylation, suggesting that not only the glycosylation state but also the IgG subclass is critical for this activity. Interestingly, this effect was dependent on the inhibitory FcγRIIB, as mice deficient for this receptor did not respond to high galactose IgG1 immune complexes. This may be consistent with earlier studies demonstrating that mouse IgG1 has an about 10-fold higher affinity for the inhibitory FcγRIIB compared to IgG2a (Ravetch and Nimmerjahn 2008). Whether high levels of galactose further enhance this affinity for FcγRIIB, remains to be established. In vitro studies revealed that the immune complexes blocked neutrophil migration through an FcγRIIB mediated reduction of MAP kinase and calcium-dependent signaling pathways initiated via the C5aR. Apart from the requirement for FcγRIIB, which has the capacity to directly interact with IgG1 immune complexes, this immunomodulatory pathway was also shown to be dependent on the Dectin-1 (clec7a), yet another C-type lectin family member. Dectin-1 has an intracellular ITAM motif and is co-expressed with FcγRIIB on neutrophils, but cannot interact with IgG immune complexes directly. Upon high galactose IgG1 immune complex binding, however, an interaction of Dectin-1 and FcγRIIB was noted, providing a possible explanation for the co-dominant role of both receptors in the modulation of complement pathway-dependent effector functions (Karsten et al. 2012).

Considering previous studies, showing that C5a may be critical to allow the upregulation of activating FcγRs on innate immune effector cells, such as tissue resident macrophages, one may expect a more generalized immunomodulatory activity of these high galactose immune complexes in diseases such as inflammatory arthritis for example (Schmidt and Gessner 2005; Syed et al. 2009). Taken

together, high galactose IgG1 immune complexes may block the local C5a-dependent positive feedback loop, which seems critical to allow a full-blown activation of the innate immune system.

6 Conclusion

Taken together, experiments over the last eight years have firmly established the capacity of IgG to mediate immunoregulatory and most notably potent anti-inflammatory activities. New studies investigating the anti-inflammatory activity of monomeric IgG have provided convincing evidence that sialic acid-rich IgG glycoforms within the IVIg preparation are broadly required for its ability to suppress a wide variety of autoimmune diseases under preventive and therapeutic treatment conditions. These studies have also demonstrated that the molecular and cellular players in the downstream pathway may vary, but finally converge on the important function of FcγRIIB to inhibit the activation of innate immune effector cells. Future studies will need to show the direct relevance of this pathway for the human immune system either directly in human clinical trials or in small animal models reflecting the complexity of the human immune system. More recently, an inhibitory effect of sialic acid-rich immune complexes on T cell-dependent DTH reactions was demonstrated. This effect was independent of FcγRIIB, dependent on the antigen specificity of the antibody and may require the modulation of dendritic cell activity. Finally, IgG1 immune complexes containing high levels of galactose had a potent activity to inhibit complement mediated recruitment of innate immune effector cells, which required the inhibitory FcγRIIB and Dectin-1. Despite these intriguing findings, we are only at the beginning of understanding how these novel immune complex-dependent immunomodulatory pathways and the increasing numbers of C-type lectin family members involved in these activities work precisely.

Acknowledgments We apologize to all our colleagues whose important work could not be cited directly due to limited amount of space. These references can be found in the different review articles cited in the manuscript. This work was supported by grants from the German Research Foundation the Bavarian Genome Research Network, and by the CAVD network within the Bill and Melinda Gates Foundation.

References

Anthony RM, Ravetch JV (2010) A novel role for the IgG Fc glycan: the anti-inflammatory activity of sialylated IgG Fcs. J Clin Immunol 30(Suppl 1):S9–S14

Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV (2008a) Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science 320:373–376

Anthony RM, Wermeling F, Karlsson MC, Ravetch JV (2008b) Identification of a receptor required for the anti-inflammatory activity of IVIG. Proc Natl Acad Sci USA 105:19571–19578

Anthony RM, Kobayashi T, Wermeling F, Ravetch JV (2011) Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. Nature 475:110–113

Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA (2007) The impact of glycosylation on the biological function and structure of human immunoglobulins. Annu Rev Immunol 25:21–50

Baerenwaldt A, Lux A, Danzer H, Spriewald BM, Ullrich E, Heidkamp G, Dudziak D, Nimmerjahn F (2011) Fcgamma receptor IIB (FcgammaRIIB) maintains humoral tolerance in the human immune system in vivo. Proc Natl Acad Sci USA 108:18772–18777

Bayry J, Lacroix-Desmazes S, Carbonneil C, Misra N, Donkova V, Pashov A, Chevailler A, Mouthon L, Weill B, Bruneval P, Kazatchkine MD, Kaveri SV (2003) Inhibition of maturation and function of dendritic cells by intravenous immunoglobulin. Blood 101:758–765

Bayry J, Fournier EM, Maddur MS, Vani J, Wootla B, Siberil S, Dimitrov JD, Lacroix-Desmazes S, Berdah M, Crabol Y, Oksenhendler E, Levy Y, Mouthon L, Sautes-Fridman C, Hermine O, Kaveri SV (2011a) Intravenous immunoglobulin induces proliferation and immunoglobulin synthesis from B cells of patients with common variable immunodeficiency: a mechanism underlying the beneficial effect of IVIg in primary immunodeficiencies. J Autoimmun 36:9–15

Bayry J, Negi VS, Kaveri SV (2011b) Intravenous immunoglobulin therapy in rheumatic diseases. Nat Rev Rheumatol 7:349–359

Beers SA, Cragg MS, Glennie MJ (2009) Complement: help or hindrance? Blood 114:5567–5568 (author reply 5568)

Bohm S, Schwab I, Lux A, Nimmerjahn F (2012) The role of sialic acid as a modulator of the anti-inflammatory activity of IgG. Semin Immunopathol 34:443–453

Bolland S, Ravetch JV (1999) Inhibitory pathways triggered by ITIM-containing receptors. Adv Immunol 72:149–177

Bolland S, Ravetch JV (2000) Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. Immunity 13:277–285

Bolland S, Yim YS, Tus K, Wakeland EK, Ravetch JV (2002) Genetic modifiers of systemic lupus erythematosus in FcgammaRIIB(-/-) mice. J Exp Med 195:1167–1174

Boross P, Arandhara VL, Martin-Ramirez J, Santiago-Raber ML, Carlucci F, Flierman R, van der Kaa J, Breukel C, Claassens JW, Camps M, Lubberts E, Salvatori D, Rastaldi MP, Ossendorp F, Daha MR, Cook HT, Izui S, Botto M, Verbeek JS (2011) The inhibiting Fc receptor for IgG, FcgammaRIIB, is a modifier of autoimmune susceptibility. J Immunol 187:1304–1313

Brownlie RJ, Lawlor KE, Niederer HA, Cutler AJ, Xiang Z, Clatworthy MR, Floto RA, Greaves DR, Lyons PA, Smith KG (2008) Distinct cell-specific control of autoimmunity and infection by FcgammaRIIb. J Exp Med 205:883–895

Bruhns P, Samuelsson A, Pollard JW, Ravetch JV (2003) Colony-stimulating factor-1-dependent macrophages are responsible for IVIG protection in antibody-induced autoimmune disease. Immunity 18:573–581

Carroll MC (1998) The role of complement and complement receptors in induction and regulation of immunity. Annu Rev Immunol 16:545–568

Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, Watier H (2002) Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. Blood 99:754–758

Chu ZT, Tsuchiya N, Kyogoku C, Ohashi J, Qian YP, Xu SB, Mao CZ, Chu JY, Tokunaga K (2004) Association of Fcgamma receptor IIb polymorphism with susceptibility to systemic lupus erythematosus in Chinese: a common susceptibility gene in the Asian populations. Tissue Antigens 63:21–27

Clynes R, Ravetch JV (1995) Cytotoxic antibodies trigger inflammation through Fc receptors. Immunity 3:21–26

Crispin M, Yu X, Bowden TA (2013) Crystal structure of sialylated IgG Fc: Implications for the mechanism of intravenous immunoglobulin therapy. Proc Natl Acad Sci USA 110:E3544–E3546

Crow AR, Song S, Semple JW, Freedman J, Lazarus AH (2001) IVIg inhibits reticuloendothelial system function and ameliorates murine passive-immune thrombocytopenia independent of anti-idiotype reactivity. Br J Haematol 115:679–686

Crow AR, Song S, Semple JW, Freedman J, Lazarus AH (2007) A role for IL-1 receptor antagonist or other cytokines in the acute therapeutic effects of IVIg? Blood 109:155–158

Daeron M, Lesourne R (2006) Negative signaling in Fc receptor complexes. Adv Immunol 89:39–86

Dalziel M, Crispin M, Scanlan CN, Zitzmann N, Dwek RA (2014) Emerging principles for the therapeutic exploitation of glycosylation. Science 343:1235681

de Groot AS, Moise L, McMurry JA, Wambre E, van Overtvelt L, Moingeon P, Scott DW, Martin W (2008) Activation of natural regulatory T cells by IgG Fc-derived peptide "Tregitopes". Blood 112:3303–3311

Debre M, Bonnet MC, Fridman WH, Carosella E, Philippe N, Reinert P, Vilmer E, Kaplan C, Teillaud JL, Griscelli C (1993) Infusion of Fc gamma fragments for treatment of children with acute immune thrombocytopenic purpura. Lancet 342:945–949

Dube R, Rook GA, Steele J, Brealey R, Dwek R, Rademacher T, Lennard-Jones J (1990) Agalactosyl IgG in inflammatory bowel disease: correlation with C-reactive protein. Gut 31:431–434

Durandy A, Kaveri SV, Kuijpers TW, Basta M, Miescher S, Ravetch JV, Rieben R (2009) Intravenous immunoglobulins–understanding properties and mechanisms. Clin Exp Immunol 158(Suppl 1):2–13

Stadlmann J, Weber A, Pabst M, Anderle H, Kunert R, Ehrlich, HJ, Peter Schwarz H, Altmann F (2009) A close look at human IgG sialylation and subclass distribution after lectin fractionation. Proteomics 9:4143–4153

Ephrem A, Chamat S, Miquel C, Fisson S, Mouthon L, Caligiuri G, Delignat S, Elluru S, Bayry J, Lacroix-Desmazes S, Cohen JL, Salomon BL, Kazatchkine MD, Kaveri SV, Misra N (2008) Expansion of CD4+CD25+ regulatory T cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis. Blood 111:715–722

Espeli M, Clatworthy MR, Bokers S, Lawlor KE, Cutler AJ, Kontgen F, Lyons PA, Smith KG (2012) Analysis of a wild mouse promoter variant reveals a novel role for FcgammaRIIb in the control of the germinal center and autoimmunity. J Exp Med 209(12):2307–2319

Ferrara C, Grau S, Jager C, Sondermann P, Brunker P, Waldhauer I, Hennig M, Ruf A, Rufer AC, Stihle M, Umana P, Benz J (2011) Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcgammaRIII and antibodies lacking core fucose. Proc Natl Acad Sci USA 108:12669–12674

Floto RA, Clatworthy MR, Heilbronn KR, Rosner DR, Macary PA, Rankin A, Lehner PJ, Ouwehand WH, Allen JM, Watkins NA, Smith KG (2005) Loss of function of a lupus-associated FcgammaRIIb polymorphism through exclusion from lipid rafts. Nat Med 11:1056–1058

Fukuyama H, Nimmerjahn F, Ravetch JV (2005) The inhibitory Fcgamma receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G+ anti-DNA plasma cells. Nat Immunol 6:99–106

Garred P, Madsen HO, Marquart H, Hansen TM, Sorensen SF, Petersen J, Volck B, Svejgaard A, Graudal NA, Rudd PM, Dwek RA, Sim RB, Andersen V (2000) Two edged role of mannose binding lectin in rheumatoid arthritis: a cross sectional study. J Rheumatol 27:26–34

Ghirlando R, Lund J, Goodall M, Jefferis R (1999) Glycosylation of human IgG-Fc: influences on structure revealed by differential scanning micro-calorimetry. Immunol Lett 68:47–52

Guhr T, Bloem J, Derksen NI, Wuhrer M, Koenderman AH, Aalberse RC, Rispens T (2011) Enrichment of sialylated IgG by lectin fractionation does not enhance the efficacy of immunoglobulin G in a murine model of immune thrombocytopenia. PLoS ONE 6:e21246

Hadley AG, Zupanska B, Kumpel BM, Pilkington C, Griffiths HL, Leader KA, Jones J, Booker DJ, Stamps R, Sokol RJ (1995) The glycosylation of red cell autoantibodies affects their functional activity in vitro. Br J Haematol 91:587–594

Hess C, Winkler A, Lorenz AK, Holecska V, Blanchard V, Eiglmeier S, Schoen AL, Bitterling J, Stoehr AD, Petzold D, Schommartz T, Mertes MM, Schoen CT, Tiburzy B, Herrmann A, Kohl J, Manz RA, Madaio MP, Berger M, Wardemann H, Ehlers M (2013) T cell-independent B cell activation induces immunosuppressive sialylated IgG antibodies. J Clin Invest 123:3788–3796

Heyman B (2000) Regulation of antibody responses via antibodies, complement, and Fc receptors. Annu Rev Immunol 18:709–737

Hogarth PM, Pietersz GA (2012) Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. Nat Rev Drug Discov 11:311–331

Holland M, Takada K, Okumoto T, Takahashi N, Kato K, Adu D, Ben-Smith A, Harper L, Savage CO, Jefferis R (2002) Hypogalactosylation of serum IgG in patients with ANCA-associated systemic vasculitis. Clin Exp Immunol 129:183–190

Huang HS, Sun DS, Lien TS, Chang HH (2010) Dendritic cells modulate platelet activity in IVIg-mediated amelioration of ITP in mice. Blood 116:5002–5009

Imbach P (2012) Treatment of immune thrombocytopenia with intravenous immunoglobulin and insights for other diseases. A historical review. Swiss Med Wkly 142:w13593

Imbach P, Barandun S, D'Apuzzo V, Baumgartner C, Hirt A, Morell A, Rossi E, Schoni M, Vest M, Wagner HP (1981) High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. Lancet 1:1228–1231

Jellusova J, Nitschke L (2011) Regulation of B cell functions by the sialic acid-binding receptors siglec-G and CD22. Front Immunol 2:96

Kaneko Y, Nimmerjahn F, Madaio MP, Ravetch JV (2006a) Pathology and protection in nephrotoxic nephritis is determined by selective engagement of specific Fc receptors. J Exp Med 203:789–797 (Epub 2006 Mar 6)

Kaneko Y, Nimmerjahn F, Ravetch JV (2006b) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313:670–673

Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, McDonald JU, Orr SJ, Berger M, Petzold D, Blanchard V, Winkler A, Hess C, Reid DM, Majoul IV, Strait RT, Harris NL, Kohl G, Wex E, Ludwig R, Zillikens D, Nimmerjahn F, Finkelman FD, Brown GD, Ehlers M, Kohl J (2012) Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcgammaRIIB and dectin-1. Nat Med 18:1401–1406

Kasermann F, Boerema DJ, Ruegsegger M, Hofmann A, Wymann S, Zuercher AW, Miescher S (2012) Analysis and functional consequences of increased Fab-sialylation of intravenous immunoglobulin (IVIG) after lectin fractionation. PLoS ONE 7:e37243

Kasperkiewicz M, Nimmerjahn F, Wende S, Hirose M, Iwata H, Jonkman MF, Samavedam U, Gupta Y, Moller S, Rentz E, Hellberg L, Kalies K, Yu X, Schmidt E, Hasler R, Laskay T, Westermann J, Kohl J, Zillikens D, Ludwig RJ (2012a) Genetic identification and functional validation of FcgammaRIV as key molecule in autoantibody-induced tissue injury. J Pathol 228:8–19

Kasperkiewicz M, Schmidt E, Zillikens D (2012b) Current therapy of the pemphigus group. Clin Dermatol 30:84–94

Kono H, Kyogoku C, Suzuki T, Tsuchiya N, Honda H, Yamamoto K, Tokunaga K, Honda Z (2005) FcgammaRIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. Hum Mol Genet 14:2881–2892 (Epub 2005 Aug 22)

Krapp S, Mimura Y, Jefferis R, Huber R, Sondermann P (2003) Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. J Mol Biol 325:979–989

Kumpel BM, Wang Y, Griffiths HL, Hadley AG, Rook GA (1995) The biological activity of human monoclonal IgG anti-D is reduced by beta-galactosidase treatment. Hum Antibodies Hybridomas 6:82–88

Kyogoku C, Dijstelbloem HM, Tsuchiya N, Hatta Y, Kato H, Yamaguchi A, Fukazawa T, Jansen MD, Hashimoto H, van de Winkel JG, Kallenberg CG, Tokunaga K (2002) Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. Arthritis Rheum 46:1242–1254

Lehmann B, Schwab I, Bohm S, Lux A, Biburger M, Nimmerjahn F (2012) FcgammaRIIB: a modulator of cell activation and humoral tolerance. Expert Rev Clin Immunol 8:243–254

Leontyev D, Katsman Y, Ma XZ, Miescher S, Kasermann F, Branch DR (2012) Sialylation-independent mechanism involved in the amelioration of murine immune thrombocytopenia using intravenous gammaglobulin. Transfusion 52:1799–1805

Mackay M, Stanevsky A, Wang T, Aranow C, Li M, Koenig S, Ravetch JV, Diamond B (2006) Selective dysregulation of the FcgammaIIB receptor on memory B cells in SLE. J Exp Med 203:2157–2164

Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB (1995) Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nat Med 1:237–243

McGaha TL, Sorrentino B, Ravetch JV (2005) Restoration of tolerance in lupus by targeted inhibitory receptor expression. Science 307:590–593

McGaha TL, Karlsson MC, Ravetch JV (2008) FcgammaRIIB deficiency leads to autoimmunity and a defective response to apoptosis in Mrl-MpJ mice. J Immunol 180:5670–5679

Mehta AS, Long RE, Comunale MA, Wang M, Rodemich L, Krakover J, Philip R, Marrero JA, Dwek RA, Block TM (2008) Increased levels of galactose-deficient anti-Gal immunoglobulin G in the sera of hepatitis C virus-infected individuals with fibrosis and cirrhosis. J Virol 82:1259–1270

Mihai S, Nimmerjahn F (2012) The role of Fc receptors and complement in autoimmunity. Autoimmun Rev 12(6):657–660

Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, Laccabue D, Zerbini A, Camisa R, Bisagni G, Neri TM, Ardizzoni A (2008) Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol 26:1789–1796

Negi VS, Elluru S, Siberil S, Graff-Dubois S, Mouthon L, Kazatchkine MD, Lacroix-Desmazes S, Bayry J, Kaveri SV (2007) Intravenous immunoglobulin: an update on the clinical use and mechanisms of action. J Clin Immunol 27:233–245

Nikolova KA, Tchorbanov AI, Djoumerska-Alexieva IK, Nikolova M, Vassilev TL (2009) Intravenous immunoglobulin up-regulates the expression of the inhibitory FcgammaIIB receptor on B cells. Immunol Cell Biol 87:529–533

Nimmerjahn F (ed) (2013) Molecular and cellular mechanisms of antibody activity. Springer, New York

Nimmerjahn F, Ravetch JV (2005) Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science 310:1510–1512

Nimmerjahn F, Ravetch JV (2006) Fcgamma receptors: old friends and new family members. Immunity 24:19–28

Nimmerjahn F, Ravetch JV (2007) The antiinflammatory activity of IgG: the intravenous IgG paradox. J Exp Med 204:11–15

Nimmerjahn F, Ravetch JV (2012) Translating basic mechanisms of IgG effector activity into next generation cancer therapies. Cancer Immun 12:13

Nimmerjahn F, Anthony RM, Ravetch JV (2007) Agalactosylated IgG antibodies depend on cellular Fc receptors for in vivo activity. Proc Natl Acad Sci USA 104:8433–8437

Oefner CM, Winkler A, Hess C, Lorenz AK, Holecska V, Huxdorf M, Schommartz T, Petzold D, Bitterling J, Schoen AL, Stoehr AD, Van Vu D, Darcan-Nikolaisen Y, Blanchard V, Schmudde I, Laumonnier Y, Strover HA, Hegazy AN, Eiglmeier S, Schoen CT, Mertes MM, Loddenkemper C, Lohning M, Konig P, Petersen A, Luger EO, Collin M, Kohl J, Hutloff A, Hamelmann E, Berger M, Wardemann H, Ehlers M (2012) Tolerance induction with T cell-dependent protein antigens induces regulatory sialylated IgGs. J Allergy Clin Immunol 129:1647–1655 e13

Otani M, Kuroki A, Kikuchi S, Kihara M, Nakata J, Ito K, Furukawa J, Shinohara Y, Izui S (2012) Sialylation determines the nephritogenicity of IgG3 cryoglobulins. J Am Soc Nephrol 23:1869–1878

Parekh RB, Roitt IM, Isenberg DA, Dwek RA, Ansell BM, Rademacher TW (1988) Galactosylation of IgG associated oligosaccharides: reduction in patients with adult and juvenile onset rheumatoid arthritis and relation to disease activity. Lancet 1:966–969

Park-Min KH, Serbina NV, Yang W, Ma X, Krystal G, Neel BG, Nutt SL, Hu X, Ivashkiv LB (2007) FcgammaRIII-dependent inhibition of interferon-gamma responses mediates suppressive effects of intravenous immune globulin. Immunity 26:67–78

Pearse RN, Kawabe T, Bolland S, Guinamard R, Kurosaki T, Ravetch JV (1999) SHIP recruitment attenuates Fc gamma RIIB-induced B cell apoptosis. Immunity 10:753–760

Pritchard NR, Cutler AJ, Uribe S, Chadban SJ, Morley BJ, Smith KG (2000) Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor FcgammaRII. Curr Biol 10:227–230

Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, Hiepe F (2006) Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol 6:741–750

Rademacher TW, Williams P, Dwek RA (1994) Agalactosyl glycoforms of IgG autoantibodies are pathogenic. Proc Natl Acad Sci USA 91:6123–6127

Ravetch JV, Nimmerjahn F (2008) Fc receptors. In: Paul WE (ed) Fundamental immunology, 5th edn. Lippincott-Raven, Philadelphia

Rudd PM, Elliott T, Cresswell P, Wilson IA, Dwek RA (2001) Glycosylation and the immune system. Science 291:2370–2376

Rudge EU, Cutler AJ, Pritchard NR, Smith KG (2002) Interleukin 4 reduces expression of inhibitory receptors on B cells and abolishes CD22 and Fc gamma RII-mediated B cell suppression. J Exp Med 195:1079–1085

Samuelsson A, Towers TL, Ravetch JV (2001) Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. Science 291:484–486

Scallon BJ, Tam SH, McCarthy SG, Cai AN, Raju TS (2007) Higher levels of sialylated Fc glycans in immunoglobulin G molecules can adversely impact functionality. Mol Immunol 44:1524–1534

Scherer HU, van der Woude D, Ioan-Facsinay A, el Bannoudi H, Trouw LA, Wang J, Haupl T, Burmester GR, Deelder AM, Huizinga TW, Wuhrer M, Toes RE (2010) Glycan profiling of anti-citrullinated protein antibodies isolated from human serum and synovial fluid. Arthritis Rheum 62:1620–1629

Schmidt RE, Gessner JE (2005) Fc receptors and their interaction with complement in autoimmunity. Immunol Lett 100:56–67

Schwab I, Nimmerjahn F (2013) Intravenous immunoglobulin therapy: how does IgG modulate the immune system? Nat Rev Immunol 13:176–189

Schwab I, Biburger M, Kronke G, Schett G, Nimmerjahn F (2012a) IVIg-mediated amelioration of ITP in mice is dependent on sialic acid and SIGNR1. Eur J Immunol 42:826–830

Schwab I, Seeling M, Biburger M, Aschermann S, Nitschke L, Nimmerjahn F (2012b) B cells and CD22 are dispensable for the immediate antiinflammatory activity of intravenous immunoglobulins in vivo. Eur J Immunol 42(12):3302–3309

Schwab I, Mihai S, Seeling M, Kasperkiewicz M, Ludwig R, Nimmerjahn F (2014) Broad requirement for terminal sialic acid residues and FcgRIIB for the preventive and therapeutic activity of intravenous immunoglobulins in vivo. Eur J Immunol 44(5):1444–1453

Seite JF, Shoenfeld Y, Youinou P, Hillion S (2008) What is the contents of the magic draft IVIg? Autoimmun Rev 7:435–439

Seite JF, Cornec D, Renaudineau Y, Youinou P, Mageed RA, Hillion S (2010) IVIg modulates BCR signaling through CD22 and promotes apoptosis in mature human B lymphocytes. Blood 116:1698–1704

Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, Xie D, Lai J, Stadlen A, Li B, Fox JA, Presta LG (2001) High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem 276:6591–6604

Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SH, Presta LG (2002) Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 277:26733–26740 (Epub 2002 May 1)

Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, Uchida K, Anazawa H, Satoh M, Yamasaki M, Hanai N, Shitara K (2003) The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466–3473 (Epub 2002 Nov 8)

Siragam V, Crow AR, Brinc D, Song S, Freedman J, Lazarus AH (2006) Intravenous immunoglobulin ameliorates ITP via activating Fc gamma receptors on dendritic cells. Nat Med 12:688–692 (Epub 2006 May 21)

Smith KG, Clatworthy MR (2010) FcgammaRIIB in autoimmunity and infection: evolutionary and therapeutic implications. Nat Rev Immunol 10:328–343

Sondermann P, Pincetic A, Maamary J, Lammens K, Ravetch JV (2013) General mechanism for modulating immunoglobulin effector function. Proc Natl Acad Sci USA 110:9868–9872

Su K, Li X, Edberg JC, Wu J, Ferguson P, Kimberly RP (2004a) A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. II. Differential binding of GATA4 and Yin-Yang1 transcription factors and correlated receptor expression and function. J Immunol 172:7192–7199

Su K, Wu J, Edberg JC, Li X, Ferguson P, Cooper GS, Langefeld CD, Kimberly RP (2004b) A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. I. Regulatory FCGR2B polymorphisms and their association with systemic lupus erythematosus. J Immunol 172:7186–7191

Su K, Yang H, Li X, Gibson AW, Cafardi JM, Zhou T, Edberg JC, Kimberly RP (2007) Expression profile of FcgammaRIIb on leukocytes and its dysregulation in systemic lupus erythematosus. J Immunol 178:3272–3280

Syed SN, Konrad S, Wiege K, Nieswandt B, Nimmerjahn F, Schmidt RE, Gessner JE (2009) Both FcgammaRIV and FcgammaRIII are essential receptors mediating type II and type III autoimmune responses via FcRgamma-LAT-dependent generation of C5a. Eur J Immunol 39:3343–3356

Sylvestre DL, Ravetch JV (1994) Fc receptors initiate the Arthus reaction: redefining the inflammatory cascade. Science 265:1095–1098

Sylvestre D, Clynes R, Ma M, Warren H, Carroll MC, Ravetch JV (1996) Immunoglobulin G-mediated inflammatory responses develop normally in complement-deficient mice. J Exp Med 184:2385–2392

Tackenberg B, Jelcic I, Baerenwaldt A, Oertel WH, Sommer N, Nimmerjahn F, Lunemann JD (2009) Impaired inhibitory Fcgamma receptor IIB expression on B cells in chronic inflammatory demyelinating polyneuropathy. Proc Natl Acad Sci USA 106:4788–4792

Takai T (2002) Roles of Fc receptors in autoimmunity. Nat Rev Immunol 2:580–592

Tomana M, Schrohenloher RE, Koopman WJ, Alarcon GS, Paul WA (1988) Abnormal glycosylation of serum IgG from patients with chronic inflammatory diseases. Arthritis Rheum 31:333–338

Tremblay T, Pare I, Bazin R (2012) Immunoglobulin G dimers and immune complexes are dispensable for the therapeutic efficacy of intravenous immune globulin in murine immune thrombocytopenia. Transfusion 53(2):261–269

Tzeng SJ, Bolland S, Inabe K, Kurosaki T, Pierce SK (2005) The B cell inhibitory Fc receptor triggers apoptosis by a novel c-Abl-family kinase dependent pathway. J Biol Chem 22:22

van de Geijn FE, Hazes JM, Geleijns K, Emonts M, Jacobs BC, Dufour-van den Goorbergh BC, Dolhain RJ (2008) Mannose-binding lectin polymorphisms are not associated with rheumatoid arthritis: confirmation in two large cohorts. Rheumatology (Oxford) 47:1168–1171

van de Geijn FE, Wuhrer M, Selman MH, Willemsen SP, de Man YA, Deelder AM, Hazes JM, Dolhain RJ (2009) Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study. Arthritis Res Ther 11:R193

van de Geijn FE, de Man YA, Wuhrer M, Willemsen SP, Deelder AM, Hazes JM, Dolhain RJ (2011) Mannose-binding lectin does not explain the course and outcome of pregnancy in rheumatoid arthritis. Arthritis Res Ther 13:R10

Walker MR, Lund J, Thompson KM, Jefferis R (1989) Aglycosylation of human IgG1 and IgG3 monoclonal antibodies can eliminate recognition by human cells expressing Fc gamma RI and/or Fc gamma RII receptors. Biochem J 259:347–353

Wang J, Balog CI, Stavenhagen K, Koeleman CA, Scherer HU, Selman MH, Deelder AM, Huizinga TW, Toes RE, Wuhrer M (2011) Fc-glycosylation of IgG1 is modulated by B-cell stimuli. Mol Cell Proteomics 10(M110):004655

Weng WK, Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 21:3940–3947 (Epub 2003 Sep 15)

Weng WK, Czerwinski D, Timmerman J, Hsu FJ, Levy R (2004) Clinical outcome of lymphoma patients after idiotype vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. J Clin Oncol 22:4717–4724 (Epub 2004 Oct 13)

Willcocks LC, Carr EJ, Niederer HA, Rayner TF, Williams TN, Yang W, Scott JA, Urban BC, Peshu N, Vyse TJ, Lau YL, Lyons PA, Smith KG (2010) A defunctioning polymorphism in FCGR2B is associated with protection against malaria but susceptibility to systemic lupus erythematosus. Proc Natl Acad Sci USA 107:7881–7885

Wormald MR, Rudd PM, Harvey DJ, Chang SC, Scragg IG, Dwek RA (1997) Variations in oligosaccharide-protein interactions in immunoglobulin G determine the site-specific glycosylation profiles and modulate the dynamic motion of the Fc oligosaccharides. Biochemistry 36:1370–1380

Xiang Z, Cutler AJ, Brownlie RJ, Fairfax K, Lawlor KE, Severinson E, Walker EU, Manz RA, Tarlinton DM, Smith KG (2007) FcgammaRIIb controls bone marrow plasma cell persistence and apoptosis. Nat Immunol 8:419–429

Yamazaki S, Dudziak D, Heidkamp GF, Fiorese C, Bonito AJ, Inaba K, Nussenzweig MC, Steinman RM (2008) CD8+ CD205+ splenic dendritic cells are specialized to induce Foxp3 + regulatory T cells. J Immunol 181:6923–6933

Thematic Index

Organs

Thymus, 41, 42
Bone marrow, 20, 41–43, 77, 89, 101, 114, 117, 213, 229, 255, 406
Germinal center, 20, 32, 33, 204, 205, 207, 210, 283, 405
Marginal zone, 32, 33, 205, 211, 402
MALT, 33, 43, 257–258
Blood-brain barrier, 261
Nephron, 258
  slit diaphragm, 260
  glomerular basement membrane, 229, 260
  glomerular convoluted tubule, 260
  glomerular capsule, 258, 259

Cells

Myeloid cells, 11, 12, 20, 23, 103, 134, 145, 153, 287, 288, 326, 382, 396
  macrophages, 4, 32, 77, 97, 103, 117, 145–151, 153, 167, 168, 173, 177, 183, 184, 188, 189, 190, 223, 225, 231, 232, 255, 257, 262, 304, 308, 309, 310, 311, 325, 326, 334, 335, 342, 357, 360, 363, 373, 376, 377, 378, 381, 382, 394, 399, 402, 409
  monocytes, 97, 103, 142, 145–148, 150, 151, 153, 168, 173, 183, 184, 187, 223, 225, 226, 231, 232, 239, 240, 242, 255, 256, 284, 308–311, 324–326, 335, 336, 337, 342, 382, 394, 400, 409
  kupffer cells, 223, 225, 377, 378, 384
  mast cells, 22, 69, **70–90**, 97, 98, 101, 103, **111**, **112**, **118**, **123**, 136, 137, 140, 142–144, 147, 148, 151, 154, 174, 176, 177, 185, 283, 292, 308–310, 323, 325, 326, 394
  basophils, 22, 97, 112, 113, 115–117, 122, 136, 140, 142, 144, 146, 148, 151, 154, 174, 176, 177, 292, 308, 325–327, 394, 400, 401
  neutrophils, 97, 103, 142, 145, 147, 148, 151, 168, 173, 174, 183–185, 188, 223, 225, 231, 239, 240, 242, 278, 281–284, 286–288, 304, 308–311, 313, 323–327, 342, 373, 376–379, 381, 383, 385, 394, 409
  eosinophils, 97, 142, 151, 173, 176, 185, 223, 225, 325, 394, 407
  platelets, 11, 12, 151, 152, 185, 226, 242, 243, 308, 323, 325, 326, 342, 403
  dendritic cells, 7, 25, 101, 145, 146, 149, 167, 172, 173, 189, 201, 205, 210, 222, 223, 225, 239, 242, 244, 255, 286, 310, 324, 335, 336, 357, 358, 360, 381, 383, 399, 402, 403, 405, 407, 410
  follicular Dendritic Cells, 201, 205
Lymphoid cells, 20, 22, 96, 145, 240
  B cells, 5, 7, 9, 12–16, 18, 20–25, 29, 31–34, 36–45, 84, 96, 97, 104–106, 113, 117, 134, 135, 137, 142, 144–146, 149, 150, 152, 154, 168, 170, 177, 178, 181, 183, 186, 190, 204–206, 210, 211, 213, 240, 242–244, 255, 257, 262, 282–285, 287, 292, 293, 304, 305, 307, 310–312, 323, 325, 326, 342, 355, 356, 358–362, 366, 374, 377, 381, 382, 399, 401, 404–406, 408
    B1 B cells, 33
    marginal zone B cells, 205
  T cells, 5, 7, 9, 10, 12–16, 18, 20, 22–24, 33, 36, 40–42, 44, 45, 96–101, 104, 131, 134, 135, 140, 145, 146, 149,

M. Daëron and F. Nimmerjahn (eds.), *Fc Receptors*, Current Topics in Microbiology and Immunology 382, DOI: 10.1007/978-3-319-07911-0,

154, 187, 201, 207, 208, 210–213, 222, 224, 242–244, 255, 257, 258, 262, 263, 304, 307, 308, 312, 323–326, 337, 357, 360, 363–365, 380–382, 393, 404, 406–408, 410
natural Killer cells (NK cells), 4, 11–13, 30, 32, 36, 103, 144–146, 153, 168, 183, 186, 187, 190, 240, 242, 282, 285, 308, 325, 326, 334–337, 342, 376, 377, 379–383
innate lymphoid cells (ILCs), 20, 145
plasma cells, 4, 13, 21, 23, 25, 32, 33, 38, 135, 155, 172, 211, 405, 406

Receptors

Fc Receptors
FcR tissue distribution/cellular expression, 11, 12–16, 20–22, 31–34, 36, 38, 41–45, 62, 63, 131, 134, 140, 145–147, 178, 183, 184–189, 213, 223, 225, 231, 239–242, 244, 245, 251, 253, 255–258, 278–287, 290–292, 306–312, 321, 325, 335, 341, 342, 361, 362, 366, 385, 404–406
FcR binding specificity, 8–10, 34, 138, 139, 144, 331, 333, 337–339
FcR-Ig interaction sites
FcγR, 327–329
IgG, 328–332
tryptophane sandwich, 327–329
FcαRI, 4, 12, 15, 97, 106, 139, 142, 166, 168, **170–174**, **221–229**, 231, 232, 246, 279, 283, 288, 381, 383
FcεRI, 4, 7, 12, **69–77**, 83, 86–90, 101–103, 106, **111–123**, 136, 140, 142–144, 147–149, 151, 154, 174–178, 208, 212, 213, 225, 227, 228, 246, 279, 283, 292, 323, 326
FcεRII, 140, 147, 149, 167, 175, 177, 178, 212, 279, 283, 292
FcγRI, 4, 11, 31, 36, 142, 146, 147, 151, 152, 169, 182–184, 206, 208, 210, 239–242, 306, 308–311, 322, 324, 325, 327–330, 333, 335, 338–340, 357, 358, 375, 380, 384, 395, 396
FγRIIA, 11, 26, 102, 138, 140, 142, 144, 146, 151, 152, 179, 182, 183, 185, 186, 188, 238–242, 244, 245, 276–278, 281, 286–288, 292, 293, 306, 310–313, 322, **314–331**, 333, 335, 336, 338–340, 342, 375, 377, 380, 396
FγRIIB, 14, 36, 39, 42, 76, 83, 86–88, 104–107, 138–140, 143–146, 148–155, 182–189, 206, 208–213, 239, 240, 242–245, 251, 277, 278, 281, 282, 284, 286–289, 292, 293, 306, 310–313, 322–331, 333, 335, 338–340, 342, **355–367**, 395, 396, 399–401, 403–410
FγRIIC, 140, 142, 145, 146, 185, 186, 239, 240, 277, 282, 285, 306, 322, 324, 325, 396
FcγRIII, 103, 106, 138–140, 142, 144–148, 151, 152, 154, 179, 182–189, 206, 208, 212, 238–242, 244, 245, 251, 278, 282, **284–289**, 293, 306, 309–313, 322, 324–331, 333, 335–340, 342, 357, 358, 375–377, 380, 396–399, 409
FcγRIIIA, 103, 138, 139, 142, 144–148, 151–152, 154, 179, 182, 183, 186–188, 238, 240–242, 244, 245, 278, 282, 285–287, 289, 293, 306, 312, 313, 322, 324–326, 329, 331, 333, 335–337, 339, 342, 357, 375–377, 380, 396–398
FcγRIIIB, 140, 146, 182, 183, 187–189, 239, 240, 242, 251, 278, 282, 284, 286–289, 306, 313, 322, 324–328, 330, 331, 333, 342, 357, 396
FcγRIV, 4, 136, 139, 142, 146–148, 152, 206, 208, 212, 239, 240, 306, 309, 310, 357, 375, 382, 384, 396, 397, 401
FcμR, **3–25**, 97, 168, 204, 205
FcRn, 54, 55, 58, 138–140, 145, 146, 154, 166, 167, 169, 189, 206, 246, **249–265**, 279, 280, 284, 334, 396
domains, 251
expression, 255
function in BBB, 261
function in podocytes, 260
function in proximal tubule, 260
function in genital tract, 258
function in intestine, 257
interactions, 252
pH dependent binding to, 252
role in Ag presentation, 262
targeted therapies, 263
trafficking, 252
blockers, 264
pIgR, 4, 5, 7, 9, 10, 14, 15, 19, 24, 138, 140, 173, 222–224
FCRL, **29–45**, 221, 223, 275, 279, 280, 284, 290, 291

FCRH, 31
TRIM21, 14, 15, 27, **51–64**, 150, 151, 160
Toso/FAIM3, 3, 8, 9, 20, 22, 25, 204
BCR, 14, 23, 24, 37–41, 44, 45, 85, 87, 88, 96, 97, 101, 102, 104–106, 113, 131, 134, 135, 139, 140, 144, 145, 149, 152, 154, 168, 282, 292, 355, 356, 366, 405
TCR, 21, 96, 97, 101–103, 131, 134, 135, 139, 140, 142, 145, 307
DCIR (DC immunoreceptor), 396, 397, 403, 407
DC-SIGN, 168, 181, 221, 223, 226, 229, 230, 232, 246, 396, 402, 407
SIGNR1, 221, 222, 223, 226, 229, 230, 232, 396, 397, 400, 402, 403
Dectin-1, 168, 409, 410
TLR, 23, 28, 33, 38, 40, 41, 45, 63, 167, 229, 255, 312, 336, 362
TNFR, 154, 355, 360–363, 366, 367
Complement receptors, 3, 168, 188, 204, 205, 211, 378
FAIM3/Toso, 3, 8, 9, 25, 204
FAS (CD95), 8, 9, 10, 25, 358, 359, 367

Clusters of Differentiation (CD)
CD16, 186, 322, 330, 336, 337, 406
CD22, 406
CD22/siglec, 14
CD23, 20, 23, 40, 177, 178, 201, 209, 212–214, 246, 283, 292, 396, 397, 406
CD32, 36, 39, 185, 293, 322, 355, 356
CD32a, 322
CD32b, 355, 356
CD89, 15, 173, 223, 225, 227, 230, 232, 246, 283, 381
CD95, 8, 358

Major Histocompatibility Complex
MHC, 35, 36, 63, 137, 140, 149, 189, 213, 249–251, 262, 263, 307, 396, 407
Class I, 63, 137, 140, 189, 249, 251, 262, 263
Class II, 35, 36, 149, 262

Antibodies/immunoglobulins
IgA, 15, 31, 34–36, 41, 44, 106, 138–140, 145, 146, 166–168, 170–174, 190, 203, 212, **221–232**, 246, 257, 258, 279, 283, 288, 304, 373, 374, 381, 395
IgE, 8, 11, 22, 30, 31, 35, 70, 75, 87, 88, 101–103, 106, **111–120**, 136, 139, 140, 143, 144, 146–149, 151, 154, 155, 166, 168, 170, 174–178, 190, 201–203, 209, 211–214, 223, 246, 275, 279, 283, 292, 304, 323, 395, 406
IgG, 8–11, 14–17, 20, 23, 24, 26, 27, 30, 34–36, 39, 41, 42, 51, 53–55, 57–62, 75, 76, 103, 104, 106, 136–141, 144–149, 151, 154, 155, 165–170, 174–176, 178–185, 187–190, 201–203, 205–212, 214, 223, 224, 227, 231, 237–246, 249–265, 275–278, 281, 282, 284–286, 288, 289, 292, 303, 304, 306, 311, 312, 321–325, 327–343, 357–363, 365, 366, 374–376, 380, 393–399, 401–410
half life, 34, 51, 53–55, 57–59, 237–240, 242, 243, 245, 255
half life enhancement, 263
homeostasis, 255
maternofetal transfer, 256
suppression by, 205
enhancement by, 207, 210
IgM, 3–5, **7–25**, 31, 35, 36, 41, 51, 58, 138, 140, 155, 168, 170, 173, 201–205, 211, 212, 214, 223, 304, 395
Immune complexes, 4, 7, 9, 11, 14, 18, 24, 51, 53–55, 59–63, 101–103, 105, 106, **133–145**, 147, 150, 151, 154, 201, 202, 207–214, 227–230, 242, 249–251, 257, 258, 260–262, 281, 284, 285, 287, 289, 292, 303, 306–308, 311, 322–324, 326, 327, 329–331, 333, 338, 341, 393–397, 399–401, 404–410
Autoantibodies, 14, 15, 23, 24, 52, 53, 152, 154, 184, 204, 264, 287, 289–291, **303–313**, 394, 399, 400, 403–406
Therapeutic antibodies, 133, 153–155, 166, 170, 181, 190, 231, 232, 237–241, 244, 245, 249, 251, 256, 263, 265, 293, 321, 323, 324, 336, 338, 342, 355, **357–367**, **374–381**, 384, 385, 394, 395, 399, 400, 402, 403, 410
Monoclonal antibody (therapy), 381–383
abdegs, 264
IVIg, 35, 181, 264, 265, 288, 393, **399–410**
Immunotherapy, 43, 133, 137, 153, 155, 237, 263–265, **355–367**, **373–385**, **393–410**

Vaccines, 133, 155, 321, 324, 334, 336–338, 342, 343

Complement

Complement (involvement of), 3, 13, 61, 133, 168, 178, 181, 188, **201–208**, 210–212, 214, 285, 286, 304, 305, 309, 373, 375, 382, 393, 394, 397, 398, 408–410
Complement components
C1q, 204, 206, 409
C3, 204, 205, 207, 210, 375, 394, 409
C5, 375, 394, 409, 410
Complement receptors, 2, 3, 168, 188, 204, 205, 211, 378

Cytokines

Cytokines, 4, 12, 44, 63, 77, 86–90, 96, 111, 112, 115–122, 146, 174, 184, 225, 227–230, 240, 241, 255, 262, 283, 285, 304, 308, 310, 311, 323, 325, 336, 378, 382, 383, 394, 400, 401, 407, 408
IL-4, 400, 401
IL-10, 230, 382
IL-33, 401
TNF-α, 24, 112, 154, 225, 232, 255, 283, 304, 310, 311, 337, 355, 358, 383, 394
IFN-γ, 62, 103, 146, 183, 184, 241, 337, 382, 383, 394, 408
TGF-β, 44, 225, 382

Glycobiology of Ig-FcR interactions

Glycosylation, 5, 15, 35, 136, 137, 138, 154, **165–190**, **393–410**
FcR glycosylation, 5, 15, 166, 169, 170, 171, 173, 176, 178, 179, **182–190**, 226, 227, 282, 340
Ig glycosylation, 35, 36, 136, 137, 138, 154, 166, 170, 171, 172, 175, 176, 177, 179, 180, 190, 224, 379, **393–410**
Galctosylation, 179–181, 230, 398, 408, 409
G0 glycoforms, 180, 396–398
N-glycans, 137, 165, 172, 175, 177, 178, 180, 184, 185, 187, 189, 190, 226, 252, 328
N-glycosylation, 175, 177, 178, 184, 186, 226, 340
O-glycans, 172, 230
O-glycosylation, 170, 172, 226
Sialic acid, 6, 7, 14, 35, 172, 178, 180, 181, 188, 395–397, **399–404**, 406–408, 410
Sialylation/sialylated, 172, 173, 175, 179, 181, 184–186, 188, 246, 398, **401–404**, 406–408, 410
Sugar domains, 395

Signaling

Modeling & logical models, **69–90**, 256, 276
Molecular motifs
ITAM, 11, 31, 32, 36, 37, 39, 75, 76, 89, 97, 102, 106, 113, 116, 120, 134, 135, 139, 140, 142–145, 174, 182, 185, 208, 209, 221, 224, 226–229, 239, 251, 282, 306, 324–326, 337, 340, 367, 378, 396, 409
ITIM, 11, 31, 32, 37–39, 76, 97, 103, 104, 106, 134, 135, 140, 143, 144, 182, 185, 186, 229, 239, 251, 306, 310, 324–326, 355, 356, 361, 366, 367, 396, 406
ITSM, 31
Ig Tail tyrosine, 11
Activation/inhibition
kinases
src kinases, 31, 39, 75, 102, 104, 113, 114, 120, 142, 143, 228, 283, 326
Lyn, 39, 75–77, 86–90, 113, 115, 116, 119–121, 228, 283
Syk, 38, 40, 75–77, 86–89, 102, 103, 113, 121, 222, 227–229
MAPK, 38–40, 71, 76, 79, 89, 119, 121
phosphatases
protein phosphatases, 31, 37, 53, 96, 221, 227, 229, 253, 337
SHP-1, 37–40, 106, 221, 227–229, 337
SHP-2, 37, 40
lipid phosphatases, 76, **111–122**, 326
SHIP1, 41, 76, 87, 88, 89, **111–122**, 143, 326
SHIP2, 122
adaptors, 11, 37, 75, 76, 102, 113, 116, 119, 121, 142, 227, 228, 253
NFκB, 86–89
BLIMP1, 40
SPAP, 31
calcium (Ca^{2+}), 24, 37–40, 76, 77, 86–88, 89, **95–106**, 115, 119, 121, 122, 142, 174, 214, 228, 229, 252, 282, 409
Channels, 96–101

ORAI, 98–100, 102, 103, 105, 106, 115
STIM1, 98, 99, 102–106, 115, 121
TRPM4, 101, 102, 106

Effector/regulatory functions

Antibody response, 11, 23, 25, 59, 61, 135, 142, 149, 168, 190, **201–214**, 222, 311, 404–408
Feedback regulation, 202, 203
Phagocytosis, 4, 61, 96, 103, 146, 150, 153, 168, 172, 174, 178, 206, 227, 228, 281, 282, 284, 286, 308, 322, 323, 326, 335, 373, 377, 384, 394, 400
Endocytosis, 4, 22, 60, 146, 210, 252, 256, 264, 335
Internalization, 11, 22, 103, 150, 154, 172, 254, 256, 262, 263, 336, 359, 366, 380
Antigen presentation, 4, 147, 149, 154, 189, 201, 210, 213, 228, 249–251, 262, 263, 265, 308, 324, 336
ADCC, 154, 168, 174, 178, 181, 186, 187, 282, 293, 322, 323, 326, 328, 334–338, 373, 376, 377, 380–382, 394
ADCP, 377, 378, 382, 384
Secretion, 3, 4, 22, 24, 41, 44, 63, 95, 96, 119, 121, 122, 170, 173, 223, 225, 227, 230, 257, 258, 262, 336, 337, 382
Complement activation, 398
Apoptosis, 8–10, 15, 23, 25, 38, 96, 100, 105, 106, 282, 358–360, 362, 363, 374, 376–380, 405

Immunity & hypersensitivity

Innate immunity, 63, 71, 134
Adaptive immunity, 30, 37, 71, 134, 170
Immediate hypersensitivity, 407
Type III hypersensitivity, 326
Delayed-type hypersensitivity, 404, 406

Diseases

Allergy, 70, 71, 111–113, 116, 117, 122, 123, 132, 151, 154, 155, 174–177, 190, 211, 229–231, 275, 279, 292, 293, 323, 406, 407
  anaphylaxis, 76, 77, 101, 116, 122, 132, 147, 148, 151, 242, 243
  asthma, 174, 229, 232, 279, 292, 406
Autoimmune diseases/autoimmunity, 14, 24, 40, 52, 53, 105, 133, 152, 153, 190, 222, 230, 231, 237, 239, 251, 263–265, 275, 277, 280, 284, 285, 287, 289–291, 293, 303, 304, 310, 312, 323, 331, 333, 342, 366, 393, 394, 398, 399, 401, 403, 405, 406, 409, 410
  thrombocytopenic Purpura (ITP), 152, 230, 231, 242, 243, 277, 278, 399–403
  rheumatoid arthritis, 44, 52, 152, 154, 180, 278, 290, **303–305**, 323, 331, 398, 405
  juvenile idiopathic arthritis, 290
  spondyloarthropathies, 290
  systemic lupus erythematosus (SLE), 44, 52, 105, 152, 155, 186, 260, 264, 277–279, 281, 289–291, 323, 331, 398, 404, 405
  multiple sclerosis, 291
  inflammatory bowel disease, 291, 323
  diabetes mellitus, 291
  vasculitides, 278, 287, 288, 323, 331
  wegener granulomatosis/Granulomatosis with polyangitis (GPA), 278, 288, 331
  chronic inflammatory demyelinating polyneuropathy (CIDP), 399
  epidermolysis bullosa acquisita (EBA), 403
  Guillain-Barré, 331, 399
Inflammation, 79, 101, 111, 152, 230, 231, 237, 241, 242, 246, 255, 264, 277, 287, 288, 292, 304, 307, 309, 321–324, 326, 331, 333, 336, 342, 383, 394, 396–401, 403, 404, 407, 409
Inflammatory, 4, 53, 63, 70, 106, 112, 115, 117, 118, 121, 146, 147, 148, 153, 155, 172, 174, 176, 177, 181, 185, 221–233, 237, 238, 240, 246, 255, 256, 263, 275, 283, 285, 287–289, 291, 303, 304, 308–312, 321–324, 331, 333, 335, 341, 342, 382, 383, 393–410
Nephrotoxic nephritis, 399
Infection, 44, 52, 61–64, 133, 150, 151, 153, 155, 168, 169, 189, 190, 213, 229–231, 237–241, 243, 249, 257, 258, 277, 286, 287, 321–324, 331, **334–338**, 343, 366, 398, 408
  infectious disease, 43, 133, 237, 238, 241, 246, 285
  viruses, 5, 8, 9, 16, 18, 36, 40, 43, 44, 51, 52, 60–66, 102, 112, 133, 150, 151,

155, 168, 185, 213, 224, 243, 245, 258, 275, 287, 321, 323, 334–338, 342, 356, 398
HIV, 43, 44, 53, 150, 240, 258, 287, 321, 324, 331, 333, **334–338**, 341, 342, 402
human, 324, 327, 329–333
macaque, 329, 330, 340
bacteria, 24, 52, 60, 63, 64, 101, 103, 133, 150, 151, 167, 170–172, 174, 222, 224, 265, 285, 286, 322, 336, 384, 398
parasites, 112, 174, 190, 203, 204, 211
malaria, 44, 203, 204, 331, 333
Cancer/tumor, 117, 122, 145, 152–155, 178, 181, 190, 211, 225, 243–245, 255, 263, 264, 323, 336, 358–367, **373–385**, 398
myeloma, 16, 31, 175, 211, 227
leukemia, 5, 41–43, 116
melanoma, 34, 243, 244, 364, 365, 375, 382

Genetics

Polymorphism, 8, 44, 152, 153, 186, 239, **275–293**, 312, 323, 327, 331, 333, 335–337, 340, 343, 375–377, 385, 405
SNPs, 44, 45, 145, 186, 276, **281–292**, 312, 313
FγRIIA H131R, 276, 277, 281, 286, 287, 289, 292, 327, 329, 331, 333, 336, 375
FγRIIB T232, 281, 405
FγRIIIA V158F, 278, 286, 287, 313, 330, 331, 333, 338, 339, 375
FγRIIIB NA1/NA2, 138, 276, 278, 282, 284, 286, 288, 289, 313, 331
FcRn polymorphism, 284
copy number variations (CNV), 284
Disease susceptibility, 44, 45, 239, 275, **288–291**, 293, 304, **309–313**, 331, 333, 337
MicroRNA, 117
Chromosomal translocation, 31

Animal models

Mouse models
murine models of human diseases, 41, 43, 45, 122, 152, 201, 203, 206, 231, 232, 257, 258, 263–265, 285, 303, 306–311, 313, 355, 362, 363, 365, 375–378, 383, 395, 398, 399, 401–403, 405–410
knock-out mice, 34, 36, 42, 64, 87–90, 181, 201, 206, 231, 238, 255, 308, 311, 375, 398, 406, 408
knock-in mice, 204, 214, 246
knock-down mice, 44, 77, 98, 122
transgenic mice, 16, 41, 42, 151, 152, 204, 212, 227, 229, 231, 232, 237, 241, 245, 246, 257, 265, 307, 310, 326, 381
humanized mice, **237–246**, 405
Non-human primate (macaque) models, **321–343**