CHAPTER 17

Fc Receptor Targeting in the Treatment of Allergy, Autoimmune Diseases and Cancer

Akira Nakamura,* Tomohiro Kubo and Toshiyuki Takai

Abstract

Fc receptors (FcRs) play an important role in the maintenance of an adequate activation threshold of various cells in antibody-mediated immune responses. Analyses of murine models show that the inhibitory FcR, FcγRIIB plays a pivotal role in the suppression of antibody-mediated allergy and autoimmunity. On the other hand, the activating-type FcRs are essential for the development of these diseases, suggesting that regulation of inhibitory or activating FcR is an ideal target for a therapeutic agent. Recent experimental or clinical studies also indicate that FcRs function as key receptors in the treatment with monoclonal antibodies (mAbs) therapy. This review summarizes FcR functions and highlights possible FcR-targeting therapies including mAb therapies for allergy, autoimmune diseases and cancer.

Introduction

FcRs are widely expressed on hemopoietic cells and distinguished by their structure, function, distribution and ligands, such as IgG, IgM, IgE and IgA. FcRs have homologous extracellular immunoglobulin domains, however, there are functionally two kinds of FcRs, the activation type and the inhibitory type FcRs. Recent analysis using FcRs-deficient mice have revealed that immune responses by antibodies depend upon regulation of activating and inhibitory FcR.[1-4] Deletion of activating FcRs does not elicit antibody-mediated responses, whereas deletion of inhibitory FcRs causes excessive antibody responses, resulting in the development of allergic or autoimmune disorders.

Immunotherapy using mAbs is a new strategy against allergy, autoimmune diseases and cancer. Genetic engineering enabled the development of humanized antibodies, leading to rapid progress of mAb therapy. In particular, activating FcRs play a pivotal role in the effects of mAb therapy. In addition to mAbs therapy, recent studies reveal that an inhibitory FcR, FcγRIIB, contributes to the effect of intravenous immunoglobulin (IVIg) therapy. This review summarizes the immunological functions of FcRs and focuses on FcR-targeting therapy.

*Corresponding Author: Akira Nakamura—Department of Experimental Immunology and CREST program of Japan Science and Technology Agency, Institute of Development, Aging and Cancer, Tohoku University, Seiryo 4-1, Sendai 980-8575, Japan. Email: aki@idac.tohoku.ac.jp

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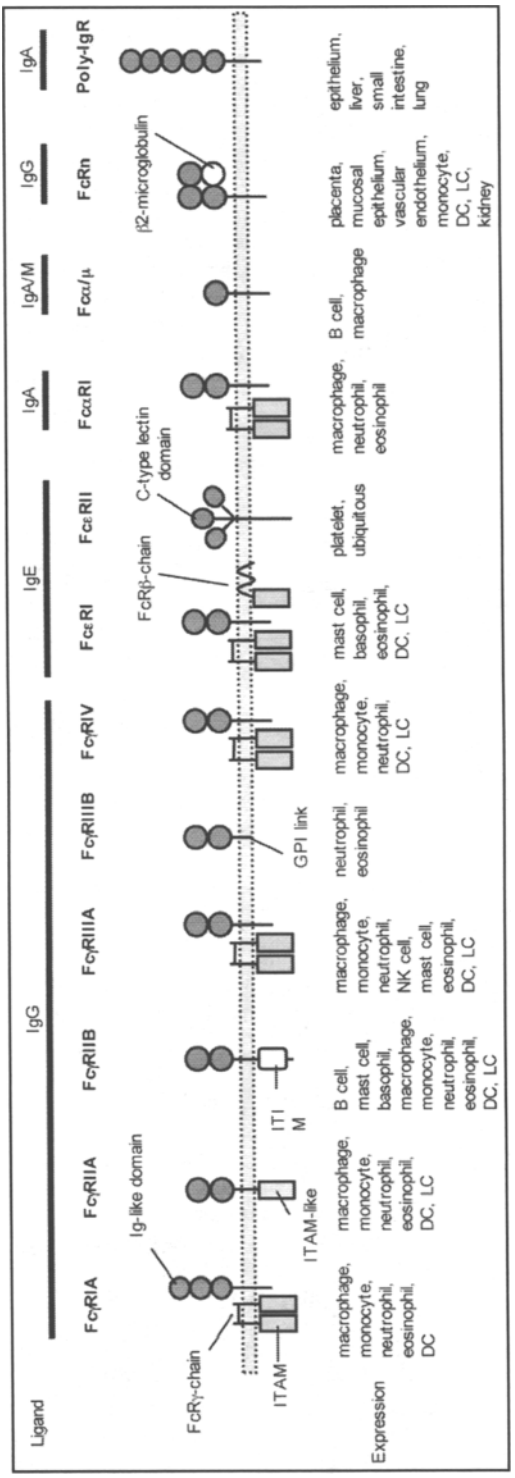

Figure 1. Schematic structure of human and murine Fc receptors. Structures of human and murine Fc receptors with their subunits, FcRγ- and FcRβ-chain, are shown. Abbreviations: DC, dendritic cell; FcRn, neonatal Fc receptor; GPI, glycosylphosphatidylinositol; Ig, immunoglobulin; ITA, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-base inhibitory motif; LC, Langerhans cell; NK, natural killer; poly-IgR, polymeric immunoglobulin receptor.

FcR Function

Structure

Most FcRs belong to immunoglobulin superfamily proteins. Since the detailed biochemistry of FcRs has been reviewed in Chapter 3 of Section I, this chapter briefly introduces the FcR structure and function. Figure 1 shows the schematic structure of representative FcγRs, FcγRIA, FcγRIIA, FcγRIIB, FcγRIIIA, FcγRIIIB and a recently discovered murine FcγRIV.[5,6] While FcγRIA binds to IgG with high affinity, FcγRIB, FcγRIC, FcγRIIs (FcγRIIA, FcγRIIB and FcγRIIC), FcγRIIIs (FcγRIIIA and FcγRIIIB) and FcγRIV have low affinity to IgG. FcγRIA, FcγRIIIA and murine FcγRIV require the FcR γ-chain (FcRγ) for their surface expression and signal delivery. Human FcRs for IgE, FcεRs, are encoded by two genes, FcεRI and FcεRII. FcεRI, which associates with the FcRγ and FcεRI β-chain (FcεγRIβ), binds to IgE with high affinity ($>10^{-10}$M). Therefore, monomeric IgE constitutively binds to FcεRI on the surface of mast cells or basophils. Low-affinity FcεRII is a C-type lectin family protein. In humans, there are three kinds of FcRs for IgA, FcαRI, polymeric Ig receptor (poly-IgR) and Fcα/μR. IgA antibodies are enriched in serum and in mucosal tissue and have three forms, monomeric, dimeric and secretory forms. The poly-IgR can transport dimeric IgA across the epithelium. Fcα/μR can bind both IgA and IgM. The neonatal FcR for IgG, FcRn, which is an MHC class I-like molecule, is responsible for perinatal IgG transport and for IgG homeostasis in adults.[7]

Regarding FcR structural information, crystal structures of IgG-FcγRIII, IgE-FcεRI and IgA-FcαRI complexes have been reported, showing the detailed information of the binding interaction between Ig-Fc fragments and FcRs.[8-12] Atomic-level structural information on the extracellular domains of FcγRIIa, FcγRIIb, FcεRI and FcαRI was also elucidated.[13-16] These FcRs contain two Ig-like domains in their extracellular regions and bear similar structures in that the two ectodomains bend at an acute angle to each other. The structural analysis of IgG1-Fc fragment-FcγRIII complex reveals that FcγRIII binds the lower hinge region and constant region 2 (Cγ2) of the Fc fragment.[8] The IgE-Fc fragment-FcεRI complex has similar conformation to that of FcγRIII complex, in which the binding site is located in the Cε2-Cε3 linker region.[9] Furthermore, the binding site of both FcγRIII and FcεRI to the Ig-Fc fragment is situated in the D2 domain, which is the carboxyl-terminal of ectodomains.[8,9] The structure of IgA-Fc fragment-FcαRI complex is different from that of other FcRs.[10] The Ig domains of FcαRI are rotated by ~180° from the positions seen in other FcRs.[17,18] FcαRI binds to the interface of Cα2 and Cα3 domains.

Activating and Inhibitory FcRs—ITAM and ITIM Signaling

Antibody-mediated cellular activation is regulated by activating and inhibitory FcRs. Activating-type FcRs, FcγRI, FcγRIII, FcγRIV and FcεRI trigger cellular activation through FcRγ that contains an intracellular immunoreceptor tyrosine-based activation motif (ITAM). FcεRI associates with both FcRγ and FcεRIβ that also contains ITAM in the cytoplasm. On the contrary, FcγRIIB bears an immunoreceptor tyrosine-based inhibitory motif (ITIM) and inhibits ITAM-mediated cellular activation triggered through receptors upon cross-linking through immune complexes (ICs) with activating-type receptors such as FcγRI, FcγRIII, FcγRIV and FcεRI or B cell antigen receptor (BCR). Figure 2A shows the activating and inhibitory signaling in B cell responses. Upon clustering activating FcRs, intracellular Src family protein tyrosine kinases such as Lyn, Fyn, Fgr and Hck phosphorylate the tyrosine residues of the ITAM,[19,20] which then become the target for cytosolic protein kinase Syk. Simultaneously, the coengagement of activating-type receptor and FcγRIIB by ICs results in the tyrosine phosphorylation of the ITIM and the recruitment of Src-homology-2 (SH2)-domain-containing inositol 5'-phosphatase (SHIP). SHIP hydrolyzes phosphatidylinositol-3,4,5-triphosphate, PtdIns(3,4,5)P_3, to PtdIns(3,4)P_2, leading to the dissociation of Bruton's tyrosine kinase (Btk) and phospholipase-γ (PLC-γ) from the membrane and the inhibition of Ca influx into the cell.[21,22] PtdIns(3,4,5)P_3 also serves as the docking site of the anti-apoptotic kinase, Akt. Thus, Akt cannot be recruited to the membrane when SHIP hydrolyzes PtdIns(3,4,5)P_3 to PtdIns(3,4)P_2. Furthermore, phosphorylated SHIP provides binding sites for the phosphotyrosine-binding (PTB) domain of the adaptor proteins, Shc and Dok, leading to the

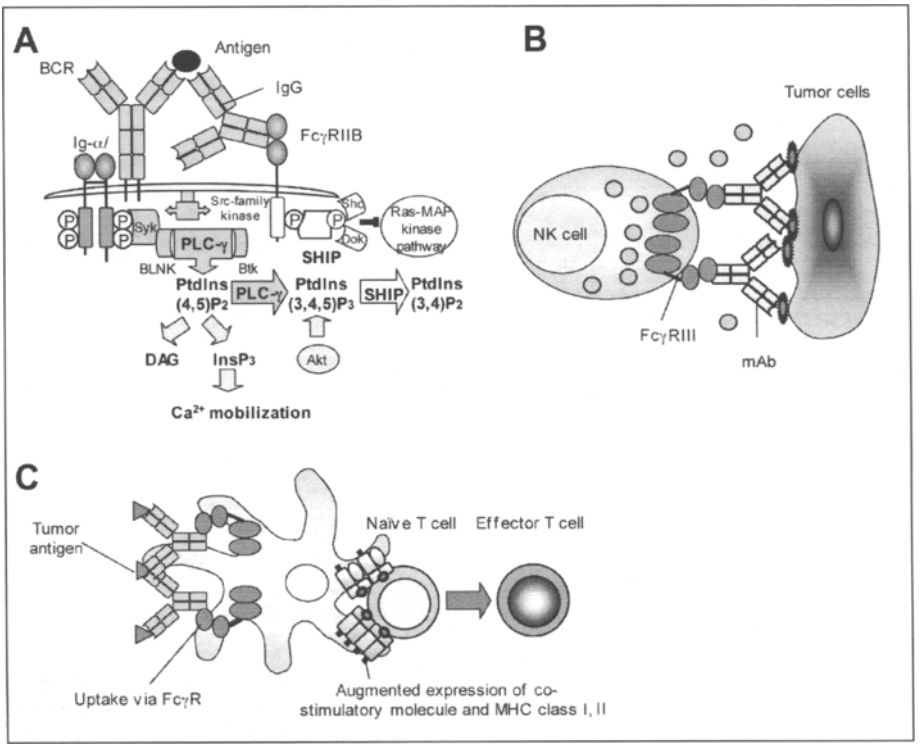

Figure 2. Three major roles of FcRs. A) Schematic signaling mechanisms mediated by activating and inhibitory receptors on B-cells. Upon cross-linking of BCR by immune complexes (ICs) on B-cells, Src family protein kinases such as Lyn, Blk and Fyn phosphorylate tyrosine residues in ITAM of Igα/β. Syk tyrosine kinase binds to the phosphorylated ITAMs and becomes activated by Src family protein kinases. The activated kinases activates BLNK, phospholipase C-γ (PLC-γ) and Tec kinases such as Btk. PLC-γ cleaves phosphatidylinositol-4,5-bisphosphate phosphatidylinositol, PtdIns(4,5)P_2, to inositol-1,4,5-triphosphate ($InsP_3$) and DAG, then initiating Ca^{2+} mobilization pathways. In contrast, SHIP hydrolyses PtdIns(3,4,5)P_3 to PtdIns(3,4)P_2. PtdIns(3,4,5)P_3 is the docking site of pleckstrin homology (PH) domain-containing proteins, including Btk and PLC-γ. Therefore, Btk and PLC-γ cannot be recruited to membrane PtdIns(3,4,5)P_3, resulting in the inhibition of Ca^{2+} mobilization pathways. PtdIns(3,4,5)P_3 also serves as the binding site of PH domain of Akt. Thus, Akt cannot be recruited to the membrane after the recruitment of SHIP to the ITIM. Phosphorylated SHIP provides binding sites for phosphotyrosine-binding (PTB) domain of adaptor proteins, Shc and Dok, leading to the blocking the downstream Ras-MAP kinase pathways. B) Antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is the killing attack of antibody-coated target cells, such as tumor cells, by NK cells or macrophages with FcγRIII. ADCC has a major role in a mAb therapy for cancer. C) FcγR-mediated effective antigen presentation on dendritic cells (DCs). FcγR efficiently uptakes antigen-antibody complexes and upregulates the expression of both MHC class I and II complexes bearing epitopes and costimulatory molecules, such as CD40, CD80 and CD86. DCs stimulated by FcγR can effectively present antigens to T-cells and initiate strong cellular and humoral responses. Abbreviations: BCR, B-cell receptor; BLNK, B-cell linker; Btk, Bruton's tyrosine kinase; DAG, diacylglycerol; Grb 2, growth-factor-receptor-bound protein 2; IC, immune complex; Ig: immunoglobulin; MHC, major histocompatibility complex; SHIP, Src-homology-2 (SH2)-domain-containing inositol 5'-phosphatase; SHP, SH2-domain-containing protein tyrosine phosphatase.

inhibition of the Ras-MAP kinase pathway.[23] This inhibitory signaling through FcγRIIB was also observed in other immune cells including mast cells and macrophages.[3]

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

The destruction of antibody-coated target cells by NK cells or macrophages is called ADCC (Fig. 2B). Currently, ADCC seems to be the main mechanism of cytotoxicity against tumors mediated by tumor antigen-specific antibodies. In a model of B16F10 melanoma lung metastasis, the monoclonal antibody TA77 against tumor antigen gp75 is effective in the protection of metastatic expansion.[24] Since the efficiency against tumor by TA77 is significantly impaired in FcRγ-deficient mice, ADCC is mediated by an FcRγ-dependent mechanism.[25] In contrast, deletion of FcγRIIB augments cytotoxicity induced by tumor specific antibody in vivo.[26] This increased cytotoxicity in FcγRIIB deficiency is attributed to macrophage-mediated ADCC.

FcγR-Mediated Antigen Presentation on Dendritic Cells

Antigen uptake through FcγRs induces strong antigen-specific T-cell responses (Fig. 2C).[27-29] When compared to antigen alone, ICs are efficiently internalized into dendritic cells (DCs) through FcγRs. The FcγR-mediated efficient antigen-internalization initiates the DC-specific antigen transport pathway in the cytosol. In addition, FcγR-mediated antigen loading leads DCs to enhance the expression of costimulatory molecules, such as CD86 and MHC class II molecules.[27,28,30] Although targeting ICs to FcγRs on DCs significantly enhances the efficiency of antigen presentation,[27,28,30,31] it remains controversial whether inhibitory FcγRIIB contribute to antigen presentation. Our previous paper demonstrated the positive contribution of FcγRIIB to the class I and II-restricted antigen presentation because FcγRIIB as well as FcγRI and FcγRIII efficiently internalize ICs,[27,28] however, another group has shown that deletion of FcγRIIB enhances the efficiency of OVA-specific cytotoxic T-lymphocyte (CTL) activity, indicating that FcγRIIB negatively regulates the FcγR-mediated augmenting pathway for antigen presentation.[32] Moreover, a recent paper by Bergtold A, et al shows a unique role of FcγRIIB in ICs-mediated antigen presentation by DCs.[33] They showed that ICs taken up through FcγRIIB are inefficiently degraded and do not reach a vesicular compartment but are instead detected intracellularly in recycling endosomes as well as on the cell surface. The recycling effect of ICs by FcγRIIB on DCs activates B-cells well, resulting in efficient T-independent humoral responses. Since this unique effect of FcγRIIB is observed in FcRγ-deficient DCs but not in wild-type DCs, further study will be required for its physiological role.

FcR-Targeting Therapy

Antibody Therapy

Recently developed immunotherapy using mAbs is expected to provide new therapeutic agents against various disorders, especially allergy, autoimmune diseases and cancer. This is mainly due to advanced biotechnology by which genetic engineering has developed recombinant humanized antibodies. The humanize antibody is about 95% human antibody and hardly has immunogenicity, leading to the current rapid progress of mAb therapy. In addition to mAb therapy, recently, FcR-directed bispecific antibodies that target both tumor antigens and FcRs on immune effector cells are generated. In this chapter, we discuss current possible FcR-targeting mAb therapy in allergy, autoimmunity and malignant disease.

General Mechanism of Antibody Therapy

Although a number of potential mechanisms have been pointed out in mAb therapy, the main mechanism is as follows:[34,35]

1. Blocking effect. Blocking effect by mAb mainly targets the interruption of pro-inflammatory cytokines and/or cell-cell crosstalk such as receptor-ligand interaction.
2. Antigen targeting. In treatment of malignancy, the target of mAb therapy is a tumor-specific antigen. MAb bound to antigen is recognized by FcγR on immune cells, such as NK

cells and macrophages, leading to the induction of ADCC and also activates classical pathway of complement such as C1q.

3. Induction of signaling. In the mAb therapy of B-cell lymphoma, mAb crosslinks the target molecules on target cell surface and induces the target cells to signaling that impairs cell function.

Antibody Against Allergy

IgE-FcεRI interaction on mast cells or basophils triggers allergic reaction. In almost all patients who suffer allergic disease, the titers of serum IgE is elevated and thus, several passive anti-IgE therapies that aim at a neutralizing effect against IgE have been developed. One of these, a humanized anti-IgE monoclonal IgG1 antibody, Omalizumab (anti-IgE monoclonal antibody E25, E25, humanised anti-IgE MAb, IGE 025, monoclonal antibody E25, olizumab, rhuMAb-E25, Xolair), which is a nonanaphylactogenic murine anti-human IgE antibody directly against the FcεRI-binding domain of human IgE, is used for the treatment of allergic rhinitis and bronchial asthma.[36-40] Omalizumab has three separate mechanistic components based on the results of the clinical trails. Firstly, Omalizumab down-regulates IgE production. Secondly, it down-regulates FcεRI expression in receptor-expressing cells such as basophils, mast cells and DCs. Thirdly, it reduces both early allergic responses such as hypersensitivity reaction through IgE-FcεRI in mast cells and late allergic responses such as the infiltration of inflammatory cells after the release of chemical mediators from mast cells. In particular, it is important that Omalizumab binds not only to free IgE but also to the surface IgE on B-cells, which produce IgE, leading to the down-regulation of IgE production. The possible mechanism is that the Fc region of Omalizumab crosslinks the FcγRIIB on IgE-producing B-cells, resulting in the reduction of their IgE production.

Antibody Therapy Against Autoimmune Diseases

Although the exact mechanism of loss of self-tolerance remains unclear, the pathogenesis of these autoimmune diseases is mainly due to the excessive inflammation mediated by pro-inflammatory cytokines or autoantibodies that are initiated by autoreactive T-or B-cells.[41,42] Therefore, current mAb therapies against autoimmune diseases aim at blocking cytokines and depletion of B-cells. Particularly, passive anti-tumor necrosis factor (TNF)-α (infliximab, adalimumab) or anti-IL-6 receptor mAb (MRA) therapies show a remarkable effect in the suppression of disease progression and severity in rheumatoid arthritis (RA) patients.[43-45] However, anti-cytokine antibody treatments were considered to have no relation to FcR-mediated mechanisms. On the other hand, another strategy of mAb treatment in autoimmune diseases that aims at the depletion of autoreactive B-cells is considered as one of the FcR-targeting therapy.

Currently, in patients with RA, systemic lupus erythematosus (SLE), myasthenia gravis, idiopathic thrombocytopenic purpura (ITP) and lymphomas, a humanized anti-CD20 IgG1 mAb (Rituximab) is used for B-lymphocyte depletion therapy.[46-49] CD20 is a B-cell-specific antigen and is expressed on almost all B-cell stages from preB-cells to plasma cells but not on hematopoietic stem cell or other normal cells, indicating that CD20 is one of the ideal target molecules in B-cell depletion treatment.[50] Several studies have shown that CD20 mAb therapy is effective in improving the symptoms and disease progression. The Rituximab has three putative mechanisms that contribute to deplete B-cells as follows:[51]

1. ADCC mediated by mainly monocytes, macrophages and NK cells. The therapeutic effect is absence in FcRγ-deficient mice. In addition, a recent study shows that FcγRIIIa polymorphism, which affects affinity to IgG1, may be associated with the response rate in the treatment of SLE by affecting ADCC.[52,53]
2. Complement-dependent cytotoxicity induced by B-cell surface CD20-CD20mAb complexes and subsequent binding C1q.
3. Induction of B-cell apoptosis by CD20mAb crosslinking.

Antibody Therapy Against Malignant Disease

Recently, a targeted mAb therapy has made rapid progress in the treatment against cancers including lymphoma and solid tumor.[49,54-56] Particularly, mAb therapies for malignant lymphoma, rituximab, alemtuzumab and trastuzumab, are partially mediated by FcR-mediated immune response, ADCC.

Rituximab, which is also used in the treatment of autoimmune diseases, is the first anti-tumor mAb drug admitted by the US Food and Drug Administration (FDA) in 1997. As mentioned previously, Rituximab targets CD20 antigen, which is expressed on normal B-cells. CD20 is also expressed on 95% of B-cell lymphomas.[57] A combination of rituximab plus chemotherapy showed a remarkable effect in patients with diffuse large B-cell lymphoma and follicular lymphoma.[49] As observed in patients with SLE, the clinical response of nonHodgkin lymphoma patients is also associated with FcγRIIIa polymorphism because the patients having FcγRIIIa-V158 genotype show slightly higher response to rituximab than the patients having FcγRIIIa-F158 genotype.[58]

Alemtuzumab targets CD52, which is a glycoprotein that is expressed on malignant lymphocytes as well as peripheral lymphocytes, but not on haemopoietic stem cells. The main tumor killing mechanism with alemtuzumab involves FcγRIII on macrophages that mediate ADCC and cross-linking-induced apoptosis.[59]

Trastuzumab is a humanized anti-HER2 antibody that targets a tyrosine-kinase receptor. HER2 is overexpressed on 30% of breast cancer cells and other solid tumors, such as nonsmall cell lung cancer, ovarian and prostate cancer.[60,61] Trastuzumab binds to HER2 with high affinity and is internalized in the cytosol with its receptor through FcγR, resulting in the blockade of downstream signaling pathways. A recent report shows that trastuzumab evokes ADCC,[62] and also augments CTL against HER2-expressed cancer cells, which may be due to the FcγR-mediated effective cross-presentation by DCs.[63,64]

FcγRIIB-Targeting Therapy

FcγRIIB-Targeting Chimeric Recombinant Protein and Bispecific Antibody

As mentioned above, the mAb therapy targets activating FcRs, mainly FcγRIII on NK cells and macrophages. On the other hand, many murine studies demonstrate the inhibitory role of FcγRIIB in allergy and autoimmunity. For example, FcγRIIB-deficient mice are sensitive to IgE or IgG-mediated passive systemic anaphylaxy. FcγRIIB-deficient B6 or B6.Fas$^{lpr/lpr}$ mice also show spontaneous autoimmune glomerulonephritis,[65,66] providing supporting evidence that FcγRIIB functions as a critical suppressor gene in the development of murine autoimmunity. In human disorders, several reports indicate that FcγRIIB polymorphism may associate with SLE.[67,68] The FcγRIIB polymorphism, threonine 232-FcγRIIB, have a single amino acid substitution (from isoleucine to threonine) at position 232 within the transmembrane domain. Recently, Floto R.A. et al demonstrated that human monocytes transfected with 232Th-FcγRIIB fail to inhibit FcγRI-mediated cellular activation due to the impaired recruitment to sphingolipid rafts.[69,70] Although these findings show the involvement of FcγRIIB in the development of autoimmunity in humans and strongly suggest that FcγRIIB could be a potential therapeutic target, FcγRIIB-targeting therapy has not been generated because there is an other type of human FcγRII, activating-type FcγRIIA, that is very homologous to FcγRIIB. However, recently, FcγRIIB-targeting chimeric recombinant protein and bispecific antibody that target both FcγRI-expressing cells and FcγRIIB have been generated. The strategy of these chimeric molecules aims at the induction of inhibitory signaling by crosslinking of FcγRI with FcγRIIB on human mast cells or basophils (Fig. 3). One of these, a human IgG1 Fc fragment (γHinge-CHγ2-CHγ3) linked with IgE Fc fragment (CHε2-CHε3-CHε4), compulsorily links FcεRI to FcγRIIB (Fig. 3A).[71] This Fcγ-Fcε fusion protein decreases IgE-mediated basophil histamine release and also inhibits IgE-mediated passive cutaneous anaphylaxis in human FcεRIα transgenic mice. Another fusion protein is composed of a human IgG1 Fc fragment (Fcγ1) and a cat allergen (Fel d1) (Fig. 3B).[72] The Fcγ1-Fel d1 protein aims at the crosslink between FcγRIIB and Fel d1-specific IgE antibody bound to FcεRI. As observed in the effect of the Fcγ-Fcε protein, this Fcγ1-Fel d1 protein inhibits Fel d1-induced activation

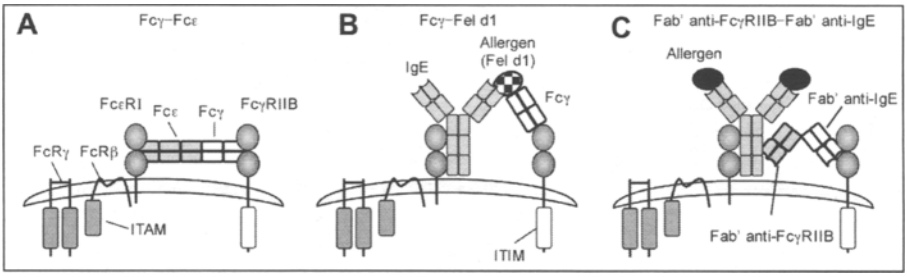

Figure 3. FcγRIIB-targeting chimeric recombinant protein and bispecific antibody. A) The Fcγ-Fcε fusion protein binds to both FcεRI and FcγRIIB, resulting in competing with IgE-FcεRI binding and the possible induction of inhibitory signaling through FcγRIIB. B) The Fcγ1-Fel d1 protein aims at the crosslink between FcγRIIB and the major cat allergen, Fel d1-specific IgE antibody bound to FcεRI. C) The bispecific antibody, Fab' anti-FcγRIIB-Fab' anti-IgE protein, that crosslinks IgE and FcγRIIB and inhibits IgE-induced histamine release of human mast cells and basophils.

of human mast cells and basophils and also Fel d1-induced systemic anaphylaxis. Although these two fusion proteins aim at the same strategy that is the induction of FcγRIIB-mediated inhibitory signaling, currently, there is no direct evidence whether Fel d1 can induce inhibitory signaling into the cells or not. In addition to these fusion proteins, a bispecific antibody against human IgE and FcγRII has been generated (Fig. 3C).[73] This bispecific antibody is composed of Fab' fragment of anti-human IgE and Fab' fragment of anti-human FcγRII. The Fab' anti-FcγRIIB-Fab' anti-IgE protein, which aims at the coengagement of IgE with FcγRIIB, inhibits IgE-induced histamine release of human mast cells and basophils.

Anti-Human FcγRIIB Antibody Therapy

As mentioned above, there has been no specific anti-human FcγRIIB antibody due to the high homology to FcγRIIA (96% homology). However, very recently, by immunizing human FcγRIIA-transgenic mice, Rankin CT et al have succeeded in generating high affinity mAb against human FcγRIIB that does not cross-react with FcγRIIA.[74]

Administration of the humanized anti-human FcγRIIB antibody, 2B6, can eliminate FcγRIIB-expressing B-cell lymphoma cell line in vitro or in vivo by ADCC. However, currently, it remains unclear whether 2B6 can induce inhibitory signaling. Moreover, unlike rituximab-targeting antigen CD20, FcγRIIB is expressed on various normal immune cells as well as on B-cell lymphomas, thus, further investigation will be required for the adaptation of clinical study.

IVIg—Possible FcγRIIB Targeting Therapy

Intravenous immunoglobulin (IVIg) has been used as the standard treatment for primary and secondary immunodeficient disorders such as hypo- and agammaglobulinemia for prevention of infectious disease. Currently, IVIg therapy has been established as an effective treatment for some autoimmune diseases including ITP,[75] Guillain-Barré syndrome,[76] multiple sclerosis,[77] myasthenia gravis,[78] and vasculitis.[79,80] Putative mechanisms of IVIg treatment are considered as follows:[81-83]

1. Interaction mediated by the antibody variable regions in the $F(ab')_2$ portion. An example is antagonistic effect against superantigens which could be related to the development of Kawasaki disease.
2. Effects mediated by the Fc portion.
3. Inhibitory effect against deposition of activated complement such as the interception of the conformation of complement membrane attack complex.
4. Effects mediated by immunoregulatory substances including soluble cytokine inhibitors other than immunoglobulin.

In particular, the Fc fragment plays an important role in these effects because intact IgG antibody has more suppressive effects than the $F(ab')_2$ antibody in the treatment of the disease model such as ITP.[33] It remains unclear whether the suppressive effect of the Fc fragment of IVIg interacts with FcγRIIB, however, in a murine model of ITP, FcγRIIB has been shown to play an essential role in the effects of IVIg.[84] In ITP, autoantibody-coated platelets bind to FcγRIII on macrophages, resulting in the FcγRIII cross-linking that triggers the phagocytosis of platelets. In a murine ITP, administration of the Fc fragment as well as IVIg to wild-type mice prevents platelet deletion induced by pathogenic anti-platelet antibody. However, IVIg treatment does not have a therapeutic effect in FcγRIIB-deficient mice or in mice treated with a blocking monoclonal antibody against FcγRIIB and FcγRIII, showing the requirement of FcγRIIB for the protection of ITP by IVIg.[84] In the nephrotoxic nephritis model induced by heterologus anti-glomerular basement membrane antiserum, IVIg down-regulates FcγRIV expression, while up-regulates the surface expression of FcγRIIB on macrophage infiltrating in the kidney and protects mice from fatal disease, suggesting that FcγRIV as well as FcγRIIB also contribute to the effect of IVIg.[85] Moreover, in the arthritis model induced by serum transfer from K/BxN mice, IVIg treatment is effective in protecting arthritis in wild-type mice but not in FcγRIIB-deficient mice.[86] The protective mechanism is partially associated with the increased population of FcγRIIB-expressing splenic macrophages, resulting in the induction of inhibitory signaling through FcγRIIB triggered by the cross-linking of FcγRIIB and FcγRIII or RIV by platelet-antibody ICs (Fig. 4A). These findings support the hypothesis that IVIg is a possible FcγRIIB-targeting therapy, however, it remains unclear whether the exact inhibitory signaling mediated by FcγRIIB involves the effect of IVIg treatment because mice deficient in SHIP, SHP-1 and Btk respond to the ameliorating effects of IVIg with the same kinetics as control mice.[87]

On the other hand, a recent report shows that the effect of IVIg in mouse ITP model seems to involve the acute interaction of activating FcγRs on dendritic cells, while FcγRIIB has a role in the late phase of IVIg action.[88] The adoptive transfer of IVIg-primed wild-type DCs ameliorated ITP, but donor cells from FcRγ chain-deficient mice did not inhibit ITP. Expression of FcγRIIB on donor DCs was not required for the amelioration of ITP, however, the effect of IVIg-treated DCs was observed in FcγRIIB-sufficient recipients but not FcγRIIB-deficient mice. These findings suggest that the interaction of IVIg with FcRγ-associating receptors, including activating FcγRI or paired immunoglobulin-like receptor (PIR)-A on DC, down-regulates the function of phagocytic macrophages (Fig. 4B).

In addition to the contribution of activating FcγR, it is reported that FcRn plays an essential role in the effects of IVIg in the arthritis model induced by serum transfer from K/BxN mice[89] and autoimmune skin blistering disease model induced by pathogenic IgG transfer.[90] FcRn-deficient mice show less susceptibility to both arthritis and skin blistering disease models and these mice are also resistant to the IVIg treatment. These findings show that the recycling of antibody by FcRn is critical for both the disease development and the therapeutic effect of IVIg in the pathogenic autoantibody-induced diseases. It is possible that IVIg blocks the recycling of autoantibodies by interacting with FcRn (Fig. 4C). These findings above suggest that both activating and inhibitory FcRs contribute to the effect of IVIg treatment at the various phases.

Conclusion

The deregulation of balancing by FcR-mediated activation or inhibition signaling affects the maintaining of peripheral tolerance, the prevention of allergic responses and the therapeutic effects by mAbs or IVIg, supporting the idea that FcR-mediated signaling pathways as well as FcR itself could be effective therapeutic targets. Recent accumulating structural analyses of FcR complexes provide detailed information of several peptides blocking the interaction between FcRs and immunoglobulin.[91-94] On the other hand, a molecule that targets FcR-mediated activation or inhibitory signaling has been hindered. If FcγRIIB-targeting mAbs or chimeric recombinant proteins can induce inhibitory signaling into immune or malignant cells, it will be launched as an effective therapeutic agent to treat allergy, autoimmune diseases and cancer.

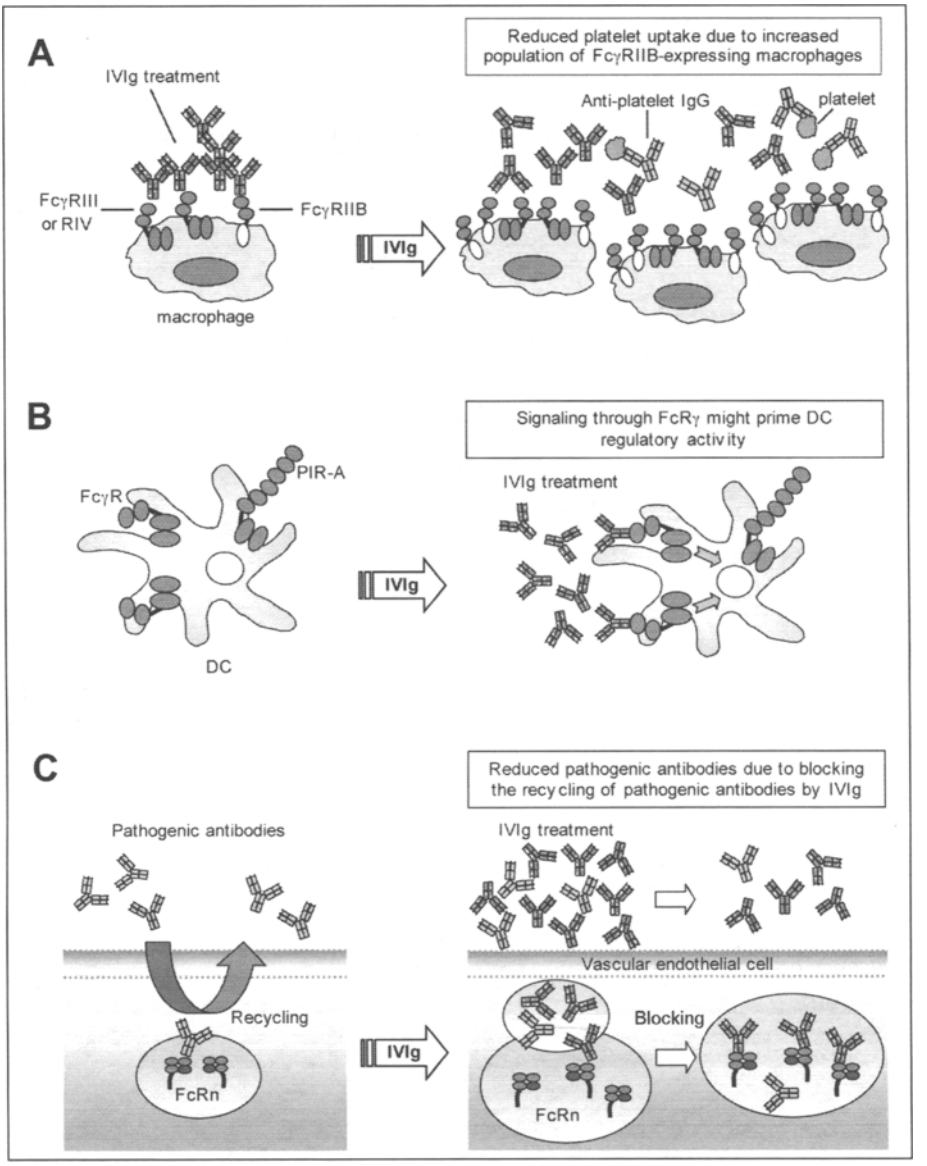

Figure 4. Possible mechanisms in IVIg treatment. A) In the murine model of ITP, anti-platelet antibody-coated platelets bind to FcγRIII or IV on macrophages, resulting in the FcγRIII or RIV cross-linking that triggers the phagocytosis of platelets. Administration of IVIg increases the population of FcγRIIB expressing splenic macrophages, resulting in the induction of inhibitory signaling through FcγRIIB triggered by the cross-linking of FcγRIIB and FcγRIII or RIV by platelet-antibody ICs. B) Interaction of IVIg with FcRγ-associating activating receptors including FcγR or PIR-A on DC, might down-regulate the function of phagocytic macrophage. C) The therapeutic saturation of FcRn by IVIg might contribute to blocking the recycling of pathogenic autoantibodies.

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References

1. Heyman B. Regulation of antibody responses via antibodies, complement and Fc receptors. Annu Rev Immunol 2000; 18:709-737.
2. Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol 2001; 19:275-290.
3. Takai T. Roles of Fc receptors in autoimmunity. Nat Rev Immunol 2002; 2:580-592.
4. Hogarth PM. Fc receptors are major mediators of antibody based inflammation in autoimmunity. Curr Opin Immunol 2002; 14:798-802.
5. Nimmerjahn F, Ravetch JV. Fcγ receptors: Old friends and new family members. Immunity 2006; 24:19-28.
6. Nimmerjahn F, Bruhns P, Horiuchi K et al. FcγRIV: A novel FcR with distinct IgG subclass specificity. Immunity 2005; 23:41-51.
7. Roopenian DC, Christianson GJ, Sproule TJ et al. The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG-Fc-coupled drugs. J Immunol 2003; 170:3528-3233.
8. Sondermann P, Huber R, Oosthuizen V et al. The 3.2-Å crystal structure of the human IgG1 Fc fragment-FcγRIII complex. Nature 2000; 406:267-273.
9. Garman SC, Wurzburg BA, Tarchevskaya SS et al. Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcεRIα. Nature 2000; 406:259-266.
10. Herr AB, Ballister ER, Bjorkman PJ. Insights into IgA-mediated immune responses from the crystal structures of human FcαRI and its complex with IgA1-Fc. Nature 2003; 423:614-620.
11. Radaev S, Motyka S, Fridman WH et al. The structure of a human type III Fcγ receptor in complex with Fc. J Biol Chem 2001; 276:16469-16477.
12. Woof JM, Burton DR. Human antibody-Fc receptor interactions illuminated by crystal structures. Nat Rev Immunol 2004; 4:89-99.
13. Maxwell KF, Powell MS, Hulett MD et al. Crystal structure of the human leukocyte Fc receptor, FcγRIIa. Nat Struct Biol 1999; 6:437-442.
14. Sondermann P, Huber R, Jacob U. Crystal structure of the soluble form of the human Fcγ-receptor IIb: A new member of the immunoglobulin superfamily at 1.7 A resolution. EMBO J 1999; 18:1095-1103.
15. Garman SC, Kinet JP, Jardetzky TS. Crystal structure of the human high-affinity IgE receptor. Cell 1998; 95:951-961.
16. Ding Y, Xu G, Yang M et al. Crystal structure of the ectodomain of human FcαRI. J Biol Chem 2003; 278:27966-27970.
17. Willcox BE, Thomas LM, Bjorkman PJ. Crystal structure of HLA-A2 bound to LIR-1, a host and viral major histocompatibility complex receptor Nat Immunol 2003; 4:913-919.
18. Chapman TL, Heikema AP, West AP Jr et al. Crystal structure and ligand binding properties of the D1D2 region of the inhibitory receptor LIR-1 (ILT2) Immunity 2000; 13:727-736.
19. Ghazizadeh S, Bolen JB, Fleit HB. Physical and functional association of Src-related protein tyrosine kinases with FcγRII in monocytic THP-1 cells. J Biol Chem 1994; 269:8878-8884.
20. Wang AV, Scholl PR, Geha RS. Physical and functional association of the high affinity immunoglobulin G receptor (FcγRI) with the kinases Hck and Lyn. J Exp Med 1994; 180:1165-1170.
21. Bolland S, Pearse RN, Kurosaki T et al. SHIP modulates immune receptor responses by regulating membrane association of Btk. Immunity 1998; 8:509-516.
22. Fluckiger AC, Li Z, Kato RM et al. Btk/Tec kinases regulate sustained increases in intracellular Ca^{2+} following B-cell receptor activation. EMBO J 1998; 17:1973-1985.
23. Tamir I, Stolpa JC, Helgason CD et al. The RasGAP-binding protein p62dok is a mediator of inhibitory FcγRIIB signals in B-cells. Immunity 2000; 12:347-358.
24. Hara I, Takechi Y, Houghton AN. Implicating a role for immune recognition of self in tumor rejection: passive immunization against the brown locus protein. J Exp Med 1995; 182:1609-1614.
25. Clynes R, Takechi Y, Moroi Y et al. Fc receptors are required in passive and active immunity to melanoma. Proc Natl Acad Sci USA 1998; 95:652-656.
26. Clynes RA, Towers TL, Presta LG et al. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. Nat Med 2000; 6:443-446.

27. Akiyama K, Ebihara S, Yada A et al. Targeting apoptotic tumor cells to FcγR provides efficient and versatile vaccination against tumors by dendritic cells. J Immunol 2003; 170:1641-1648.
28. Yada A, Ebihara S, Matsumura K et al. Accelerated antigen presentation and elicitation of humoral response in vivo by FcγRIIB- and FcγRI/III-mediated immune complex uptake. Cell Immunol 2003; 225:21-32.
29. Regnault A, Lankar D, Lacabanne V et al. Fcγ receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune comple internalization. J Exp Med 1999; 189:371-380.
30. Rodriguez A, Regnault A, Kleijmeer M et al. Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. Nat Cell Biol 1999; 1:362-368.
31. Dhodapkar KM, Krasovsky J, Williamson B et al. Antitumor monoclonal antibodies enhance cross-presentation of cCellular antigens and the generation of myeloma-specific killer T-cells by dendritic cells. J Exp Med 2002; 195:125-133.
32. Kalergis AM, Ravetch JV. Inducing tumor immunity through the selective engagement of activating Fcγ receptors on dendritic cells. J Exp Med 2002; 195:1653-1659.
33. Bergtold A, Desai DD, Gavhane A et al. Cell surface recycling of internalized antigen permits dendritic cell priming of B-cells. Immunity 2005; 23:503-514.
34. Sinclair NR. Fc-signalling in the modulation of immune responses by passive antibody. Scand J Immunol 2001; 53:322-330.
35. Nakamura A, Akiyama K, Takai T. Fc receptor targeting in the treatment of allergy, autoimmune disease and cancer. Expert Opin Ther Targets 2005; 9:169-190.
36. Kinet JP. Atopic allergy and other hypersensitivities. Curr Opin Immunol 1999; 11: 603-605.
37. Weinberger M. Innovative therapies for asthma: anti-IgE—The future? Paediatr Respir Rev 2004; 5 Suppl A:S115-118.
38. Busse W, Neaville W. Anti-immunoglobulin E for the treatment of allergic disease. Curr Opin Allergy Clin Immunol 2001; 1:105-108.
39. Bruhns P, Fremont S, Daeron M. Regulation of allergy by Fc receptors. Curr Opin Immunol 2005; 17:662-669.
40. Strunk RC, Bloomberg GR. Omalizumab for asthma. N Engl J Med 2006; 354:2689-2695.
41. Goodnow CC. Pathways for self-tolerance and the treatment of autoimmune diseases. Lancet 2001; 357:2115-2121.
42. Scofield RH. Autoantibodies as predictors of disease. Lancet 2004; 363:1544-1546.
43. Olseni NJ, Stein CM. New drugs for rheumatoid arthritis. N Engl J Med 2004; 350:2167-2179.
44. Choy EH, Isenberg DA, Garrood T et al. Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: A randomized, double-blind, placebo-controlled, dose-escalation trial. Arthritis Rheum 2002; 46:3143-3150.
45. Nishimoto N, Yoshizaki K, Miyasaka N et al. Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: A multicenter, double-blind, placebo-controlled trial. Arthritis Rheum 2004; 50:1761-1769.
46. Gorman C, Leandro M, Isenberg D. B-cell depletion in autoimmune disease. Arthritis Res Ther 2003; 5:S17-21.
47. Edwards JC, Szczepanski L, Szechinski J et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004; 350:2572-2581.
48. Pescovitz MD. Rituximab, an anti-CD20 monoclonal antibody: History and mechanism of action. Am J Transplant 2006; 6:859-866.
49. Coiffier B. Monoclonal antibody as therapy for malignant lymphomas. CR Biol 2006; 329:241-254.
50. Gopal AK, Press OW. Clinical applications of anti-CD20 antibodies. J Lab Clin Med 1999; 134:445-450.
51. Uchida J, Hamaguchi Y, Oliver JA et al. The innate mononuclear phagocyte network depletes B-lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy. J Exp Med 2004; 199:1659-6169.
52. Anolik JH, Campbell D, Felgar RE et al. The relationship of FcγRIIIa genotype to degree of B-cell depletion by rituximab in the treatment of systemic lupus erythematosus. Arthritis Rheum 2003; 48:455-459.
53. Weng WK, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 2003; 21:3940-3947.
54. Glennie MJ, Johnson PW. Clinical trials of antibody therapy. Immunol. Today 2000; 21:403-410.
55. Gelderman KA, Tomlinson S, Ross GD et al. Complement function in mAb-mediated cancer immunotherapy. Trends Immunol 2004; 25:158-164.
56. Harris M. Monoclonal antibodies as therapeutic agents for cancer. Lancet Oncol 2004; 5:292-302.

57. Anderson KC, Bates MP, Slaughenhoupt BL et al. Expression of human B-cell-associated antigens on leukemias and lymphomas: A model of human B-cell differentiation. Blood 1984; 63:1424-1433.
58. Cartron G, Dacheux L, Salles G et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. Blood 2002; 99:754-758.
59. Zhang Z, Zhang M, Goldman CK et al. Effective therapy for a murine model of adult T-cell leukemia with the humanized anti-CD52 monoclonal antibody, Campath-1H. Cancer Res 2003; 63:6453-6457.
60. Slamon DJ, Godolphin W, Jones LA et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244:707-712.
61. Mosesson Y, Yarden Y. Oncogenic growth factor receptors: implications for signal transduction therapy. Semin Cancer Biol 2004; 14:262-270.
62. Sliwkowski MX, Lofgren JA, Lewis GD et al. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). Semin Oncol 1999; 4 Suppl 12:60-70.
63. zum Buschenfelde CM, Hermann C, Schmidt B et al. Antihuman epidermal growth factor receptor 2 (HER2) monoclonal antibody trastuzumab enhances cytolytic activity of class I-restricted HER2-specific T-lymphocytes against HER2-overexpressing tumor cells. Cancer Res 2002; 62: 2244-2247.
64. Kono K, Sato E, Naganuma H et al. Trastuzumab (Herceptin) enhances class I-restricted antigen presentation recognized by HER-2/neu-specific T cytotoxic lymphocytes. Clin Cancer Res 2004; 10:2538-2544.
65. Bolland S, Ravetch JV. Spontaneous autoimmune disease in FcγRIIB-deficient mice results from strain-specific epistasis. Immunity 2000; 13:277-285.
66. Yajima K, Nakamura A, Sugahara A et al. FcγRIIB deficiency with Fas mutation is sufficient for the development of systemic autoimmune disease. Eur J Immunol 2003; 33:1020-1029.
67. Kyogoku C, Dijstelbloem HM, Tsuchiya N et al. Fcγ receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus. Arthritis Rheum 2002; 46:1242-1254.
68. Siriboonrit U, Tsuchiya N, Sirikong M et al. Association of Fcγ receptor IIb and IIIb polymorphisms with susceptibility to systemic lupus erythematosus in Thais. Tissue Antigens 2003; 61:374-383.
69. Floto RA, Clatworthy MR, Heilbronn KR et al. Loss of function of a lupus-associated FcγRIIb polymorphism through exclusion from lipid rafts. Nat Med 2005; 11:1056-1058.
70. Kono H, Kyogoku C, Suzuki T et al. FcγRIIB 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 2005; 14:2881-2892.
71. Zhu D, Kepley CL, Zhang M et al. A novel human immunoglobulin Fcγ-Fcε bifunctional fusion protein inhibits FcεRI-mediated degranulation. Nat Med 2002; 8:518-521.
72. Zhu D, Kepley CL, Zhang K et al. A chimeric human-cat fusion protein blocks cat-induced allergy. Nat Med 2005; 11:446-449.
73. Tam SW, Demissie S, Thomas D et al. A bispecific antibody against human IgE and human Fcgamma-RII that inhibits antigen-induced histamine release by human mast cells and basophils. Allergy 2004; 59:772-780.
74. Rankin CT, Veri MC, Gorlatov S et al. CD32B, the human inhibitory Fcγ receptor IIB, as a target for monoclonal antibody therapy of B-cell lymphoma. Blood 2006; 108:2384-2391.
75. Cines DB, Blanchettei VS. Immune thrombocytopenic purpura. N Engl J Med 2002; 346:995-1008.
76. Yuki N. Infectious origins of and molecular mimicry in, Guillain-Barre and Fisher syndromes. Lancet Infect Dis 2001; 1:29-37.
77. Durelli L, Isoardo G. High-dose intravenous immunoglobulin treatment of multiple sclerosis. Neurol Sci 2002; 23:S39-48.
78. Latov N, Chaudhry V, Koski CL et al. Use of intravenous γ globulins in neuroimmunologic diseases. J Allergy Clin Immunol 2001; 108:S126-132.
79. Wiles CM, Brown P, Chapel H et al. Intravenous immunoglobulin in neurological disease: A specialist review. J Neurol Neurosurg Psychiatry 2002; 72:440-448.
80. Burns JC. Kawasaki disease. Adv Pediatr 2001; 48:157-177.
81. Sewell WA, Jolles S. Immunomodulatory action of intravenous immunoglobulin. Immunology 2002; 107:387-393.
82. Simon HU, Spath PJ. IVIG—Mechanisms of action. Allergy 2003; 58:543-552.
83. Bayry J, Thirion M, Misra N et al. Mechanisms of action of intravenous immunoglobulin in autoimmune and inflammatory diseases. Transfus Clin Biol 2003; 10:165-169.
84. Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. Science 2001; 291:484-486.
85. Kaneko Y, Nimmerjahn F, Madaio MP et al. Pathology and protection in nephrotoxic nephritis is determined by selective engagement of specific Fc receptors. J Exp Med 2006; 203:789-797.
86. Bruhns P, Samuelsson A, Pollard JW et al. Colony-stimulating factor-1-dependent macrophages are responsible for IVIG protection in antibody-induced autoimmune disease. Immunity 2003; 18:573-581.

87. Crow AR, Song S, Freedman J et al. IVIg-mediated amelioration of murine ITP via FcγRIIB is independent of SHIP1, SHP-1 and Btk activity. Blood 2003; 102:558-560.
88. Siragam V, Crow AR, Brinc D et al. Intravenous immunoglobulin ameliorates ITP via activating Fcγ receptors on dendritic cells. Nat Med 2006; 12:688-692.
89. Akilesh S, Petkova S, Sproule TJ et al. The MHC class I-like Fc receptor promotes humorally mediated autoimmune disease. J Clin Invest 2004; 113:1328-1333.
90. Li N, Zhao M, Hilario-Vargas J et al. Complete FcRn dependence for intravenous Ig therapy in autoimmune skin blistering diseases. J Clin Invest 2005; 115:3440-3450.
91. McDonnell JM, Beavil AJ, Mackay GA et al. Structure based design and characterization of peptides that inhibit IgE binding to its high-affinity receptor. Nat Struct Biol 1996; 5:419-426.
92. Marino M, Ruvo M, De Falco S et al. Prevention of systemic lupus erythematosus in MRL/lpr mice by administration of an immunoglobulin-binding peptide. Nat Biotechnol 2000; 18:735-739.
93. Uray K, Medgyesi D, Hilbert A et al. Synthesis and receptor binding of IgG1 peptides derived from the IgG Fc region. J Mol Recognit 2004; 17:95-105.
94. Medgyesi D, Uray K, Sallai K et al. Functional mapping of the FcγRII binding site on human IgG1 by synthetic peptides. Eur J Immunol 2004; 34:1127-1135.