# 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

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# 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

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[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
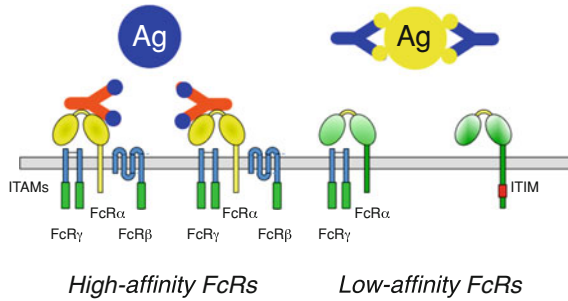
**Fig. 1** *Differential binding of antibodies and antigen to high-affinity and to low-affinity FcRs.* Antibodies bind first to high-affinity FcRs, then antigen binds to receptor-bound antibodies (*left*). Antigen binds first to antibodies and form immune complexes that can then bind to low-affinity 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):

$$[\text{Ab}][\text{FcR}] \underset{k_d}{\overset{k_a}{\rightleftharpoons}} [\text{Ab} - \text{FcR}]$$
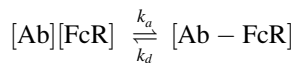
The affinity of FcRs is characterized by an affinity constant ($K_a$) which is the quotient of an association constant ($k_a$) divided by a dissociation constant ($k_d$). The affinity constant is a characteristic of the FcR.

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
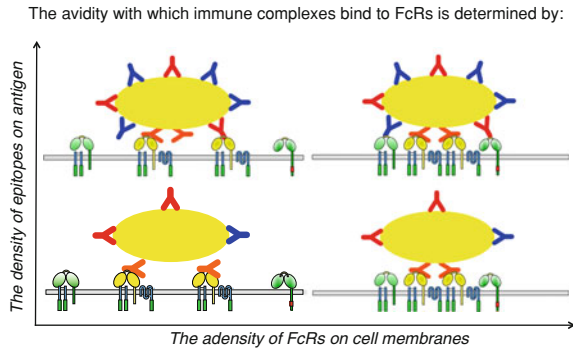


## 4.2 Modulation of the Binding Avidity/Affinity with Which Antibodies Bind to FcRs

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)



Two modes of interactions with FcRs of
antibodies against cell surface antigens

*Trans-binding*

*Cis-binding*
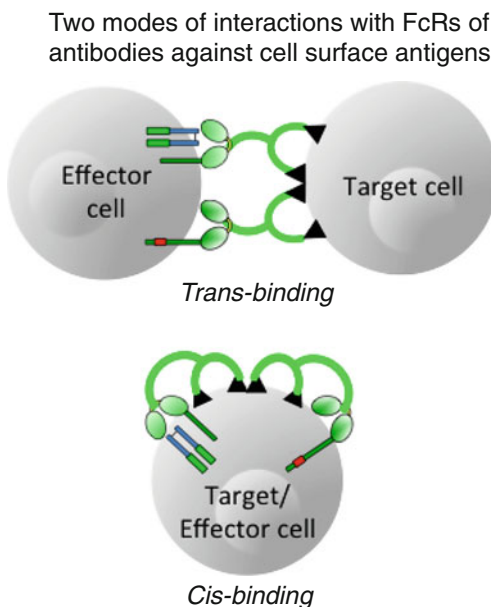
conserved $N_{297}$ residue in its $CH_2$ domain. Point mutations of this glycosylation
site abrogate the ability of IgG antibodies to bind to FcγRs. If engineered with
such a mutation (e.g., $N_{297}Q$), aglycosylated antibodies therefore no longer engage
FcγRs and they can be used as blocking-only 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 immuno-
globulins, 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 immuno-
globulins. 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 $3 \times 10^5$ and $3 \times 10^6$ M$^{-1}$, their affinity for mouse IgE is in the order of $2 \times 10^4$ 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.
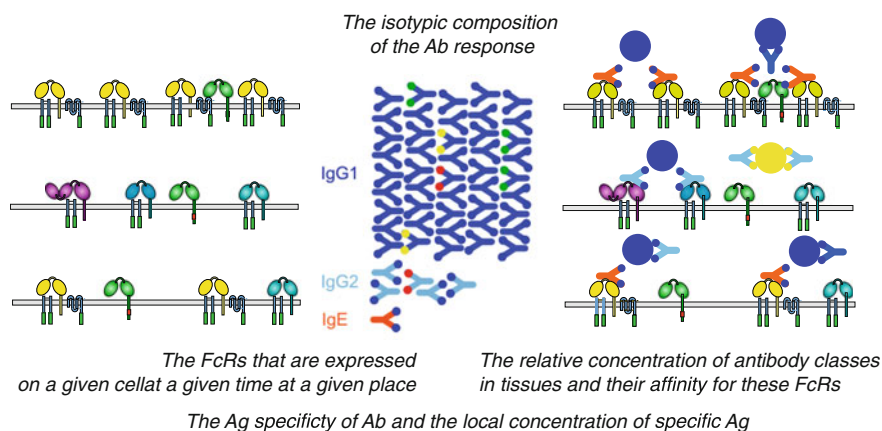
The composition of FcR superstructures isdetermined by:



*The isotypic composition*
*of the Ab response*

IgG1

IgG2

IgE

*The FcRs that are expressed*
*on a given cellat a given time at a given place*

*The relative concentration of antibody classes*
*in tissues and their affinity for these FcRs*

*The Ag specificty of Ab and the local concentration of specific Ag*

**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 $\gamma$/$\delta$T 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 Fc$\gamma$RIIIA were however found on a subset of murine CD8 T cells and they efficiently triggered antibody-dependent cell-mediated cytotoxicity (Dhanji et al. 2005).

Fc$\gamma$RIIB are expressed by most myeloid cells and by B lymphocytes. NK cells and T cells, which do not express Fc$\gamma$RIIB, express several other inhibitory receptors involved in cell-cell interactions (Long 1999). Fc$\gamma$RIIB 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 Fc$\gamma$RIIB only in both mice and humans, and NK cells, which typically express Fc$\gamma$RIIIA only in both mice and humans. A SNP in the *FCGR2C* gene, however, determines the expression Fc$\gamma$RIIC 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
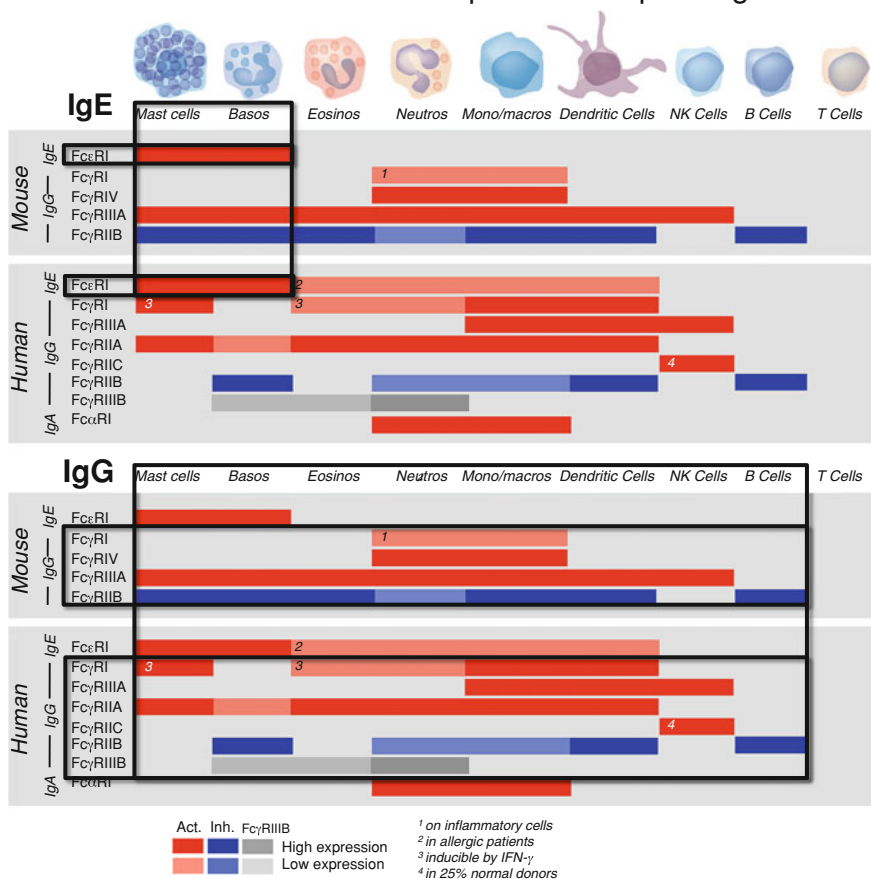
**Fig. 5** *Tissue distribution of FcRs and their engagement by antibodies of different classes.* FcRs expressed by the various cell types in mice and humans are differentially engaged by antibodies of different classes. As a consequence, different cell types are involved

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 transfer 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, antihuman 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.

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