Review

CellPress

Fc fusion as a platform technology: potential for modulating immunogenicity

Ditza Levin[1], Basil Golding[2], Scott E. Strome[3], and Zuben E. Sauna[1]

[1] Laboratory of Hemostasis, Division of Hematology Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA
[2] Plasma Derivatives, Division of Hematology Research and Review, Center for Biologics Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Avenue, Silver Spring, MD 20993, USA
[3] Department of Otorhinolaryngology–Head and Neck Surgery, University of Maryland School of Medicine, 16 South Eutaw Street Suite 500, Baltimore, MD 21201, USA

The platform technology of fragment crystallizable (Fc) fusion, in which the Fc region of an antibody is genetically linked to an active protein drug, is among the most successful of a new generation of bioengineering strategies. Immunogenicity is a critical safety concern in the development of any protein therapeutic. While the therapeutic goal of generating Fc-fusion proteins has been to extend half-life, there is a critical mass of literature from immunology indicating that appropriate design of the Fc component has the potential to engage the immune system for product-specific outcomes. In the context of Fc-fusion therapeutics, a review of progress in understanding Fc biology suggests the prospect of engineering products that have an extended half-life and are able to modulate the immune system.

Therapeutic Fc-fusion proteins

Biologically active proteins are playing an increasingly important role in the clinical management of some of the most challenging human diseases. Despite the vast diversity of proteins that can be exploited to modulate disease pathways, almost half of all protein therapeutics approved by the US FDA since 2009 have been monoclonal antibodies (mAbs) [1]. Given the range of protein–protein interactions, the specificity of natural ligand–receptor interactions, and the complexity of disease pathologies, it is often necessary to expand the repertoire beyond mAbs *per se*. In addition, many of the clinical benefits of protein therapeutics have come from replacement therapy for a defective protein [2], for which mAbs have limited utility.

The dominance of mAbs among protein therapeutics is largely due to their biological and pharmacological properties, many of which reside in the immunoglobulin (Ig) constant region Fc domain. The Fc domain of IgG salvages the protein from endosomal degradation by binding to the neonatal Fc receptor (FcR) (FcRn), facilitating recycling [3]. Proteins or peptides lacking a functional Fc may fail as drug products because they have a very short serum half-life due to fast renal clearance. Thus, so-called Fc-fusion technologies, in which the Ig Fc is fused genetically to a protein of interest, have emerged to confer antibody-like properties on proteins and peptides of therapeutic interest [4]. In addition to increasing serum half-life, Fc fusion has also resulted in an improvement in other properties such as stability and solubility. The use of this technology is advantageous for the manufacturing process because Fc fusion often results in increased expression and/or secretion and protein A affinity purification of Fc-fusion proteins simplifies the downstream purification of the protein drug. Consequently, Fc-fusion proteins have enjoyed considerable success in terms of the number of products approved, their application to diverse disease areas, and the value of global sales [5,6].

In the development and licensing of any therapeutic protein, immunogenicity is an important concern, as the development of neutralizing antidrug antibodies (nADAs) can affect both the safety and efficacy of the drug [7]. FDA guidance on immunogenicity (http://www.fda.gov/downloads/Drugs/./Guidances/UCM192750.pdf) states that primary sequence considerations are especially important in the evaluation of the immunogenicity of fusion proteins, because immune responses to neoantigens formed in the joining region may be elicited. Beside these considerations, which are common to all bioengineered protein therapeutics, Fc-based drugs demand additional considerations because interactions between the Fc domain and its receptors have immunological consequences. On the one hand, these interactions raise concerns about the long-term use of these products, which are often employed to treat chronic conditions. On the other hand, a suitably engineered Fc partner has the potential to improve the safety profile of biological agents or even play an active role in the treatment of disease.

Here we briefly review Fc interactions and discuss these in the context of Fc-based therapeutics. Such interactions have a potential impact on existing first-generation

Corresponding author: Sauna, Z.E. (zuben.sauna@fda.hhs.gov).
Keywords: therapeutic proteins; Fc-fusion technology; immunogenicity; neutralizing antidrug antibodies; Fc receptors; drug development.

0167-7799/
Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.tibtech.2014.11.001

Fc-fusion proteins but can also be modulated to achieve desired therapeutic outcomes [8,9]. For example, a new generation of therapies might use Fc derived from different Ig classes for specific targeting. Fc from the IgA molecule targets the IgA FcR (FcαR) predominantly found in the mucosa, while Fc from the IgE molecule targets the IgE FcR (FcεR) to inhibit allergic responses [10–12]. We describe a novel group of fully recombinant Fc multimers designed to mimic the anti-inflammatory effects of intravenous Ig (IVIG), highlighting Stradomers™ as a case study [13,14]. Finally, we discuss the translational value of these emerging principles; for example, how they may be applied to engineer the Fc region of fusion proteins to design drugs with improved safety and efficacy profiles.

Interactions between Fc and FcRs

Nine Fc-fusion drugs have been approved by the FDA. All nine drugs contain the Fc domain of human IgG1 with the primary purpose of extending the serum half-life of the active moiety [6] (Figure 1). In addition to FcRn, the canonical IgG FcRs (FcγRs) also tightly control Fc-based immune responses (Box 1). While such Fc–FcR interactions are commonly defined by the ability of the receptor to transmit either activating or inhibitory signals, the range of biological activities mediated by these receptors is far more complex and includes: (i) transmission of activating and inhibitory signals; (ii) phagocytosis of multivalent antibody–antigen complexes, which can result in antigen receptor recycling or antigen presentation, degranulation, and secretion of inflammatory mediators by innate immune cells; (iii) activation or inhibition of cytokine secretion; (iv) selection of B cells with higher-affinity B cell receptors; (v) antibody-dependent cell cytotoxicity (ADCC) [15]; and (vi) association with other surface proteins (e.g., Dectin1) [16].

In addition to such constitutive functions, Fc–FcR interactions can be 'fine-tuned' by the degree of Fc aggregation and the disease state of the host. Because most of these receptors are of low affinity, the size and strength of their interactions with antibodies or IgG-based fusion proteins is correlated with the degree of Fc multimerization [17]. Treatment with Fc-fusion drugs can induce aggregates

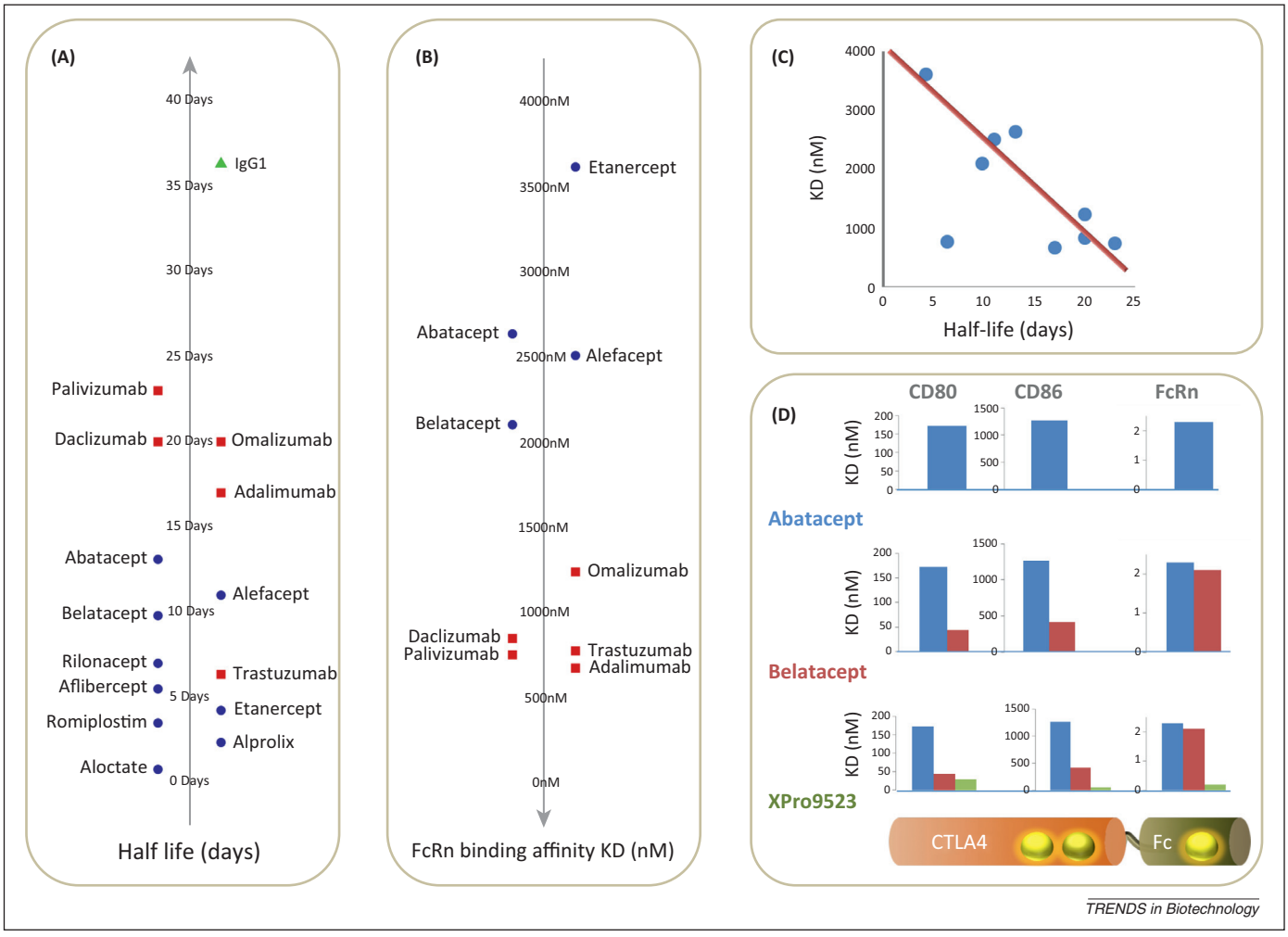

Figure 1. Properties of the fragment crystallizable (Fc) partner of fusion-protein drugs compared with therapeutic monoclonal antibodies (mAbs) and human IgG1. **(A)** Comparison of the half-life of Fc-fusion drugs (blue circles), therapeutic mAbs (red squares), and IgG1 (green triangle). **(B)** Neonatal Fc receptor (FcRn)-binding affinities (nM) of Fc-fusion drugs (blue circles) and therapeutic mAbs (red squares). **(C)** Correlation between half-life and FcRn-binding affinities of Fc-fusion drugs and therapeutic mAbs [36]. **(D)** Binding affinities for therapeutic targets (CD80, CD86) and FcRn are depicted for three generations of cytotoxic T lymphocyte-associated protein 4 (CTLA-4)–Fc-fusion drugs [72,73]. The active moiety CTLA-4 (orange cylinder) and Fc (green cylinder) were both engineered. Abatacept is the parent molecule and affinities are depicted as blue bars. The CTLA-4 domain was engineered in the second-generation belatacept, resulting in increased binding affinity for CD80 and CD86 but with no effect on affinity for FcRn (red bars). In the third-generation XPro9523, both the CTLA-4 and Fc domains were engineered, resulting in increased affinity for CD80, CD86, and FcRn (green bars).

Box 1. The human canonical FcγRs

The Fc of IgG molecules has critical biological effector functions like activating complement, mediating phagocytosis, and ADCC. The various subclasses of IgG antibodies and both canonical (Figure I) and noncanonical FcRs (see Figure I in Box 2) are key molecules involved in defining biological phenomena such as the half-lives of antibodies and in determining whether an immune response will be stimulatory or suppressive.

The FcγRs are glycoproteins that belong to the Ig superfamily and are widely expressed throughout the hematopoietic system. FcγRs bind the Fc domain of IgG isotypes (1–4) in humans, near the hinge-proximal region, in a 1:1 ratio (see Table 1 in main text). These molecules provide an important link between innate, humoral, and cellular immunity. The human FcγRs are classified as FcγRI, FcγRII (A, B, C), and FcγRIII (A, B). The FcγRs are functionally divided into activating and inhibitory receptors [18]. The activating receptors contain or are associated with ITAM in their intracytoplasmic structure. The only inhibitory receptor in both humans and mice, namely FcγRIIB, has an immunoreceptor tyrosine-based inhibition motif (ITIM). The receptors also differ in their affinity for the IgG isotypes and their expression patterns by different cells of the immune system (see Table 1 in main text).

FcRn, which is a homolog of MHC class I molecules, is ubiquitously expressed by almost all blood cells as well as endothelial cells, epithelial cells, and syncytiotrophoblasts of the placenta. The receptor is located within vacuoles and tubules inside the cell. In humans, FcRn expression is lifelong. FcRn interacts with critical amino acid residues within the CH2 and CH3 regions of the Fc domain of IgG in a ratio of 2:1 [39]. Binding of endocytosed IgG or Fc-fusion proteins to FcRn occurs in acidic pH and results in recycling by endosomes rather than entering lysosomes and undergoing degradation. This mechanism is responsible for the long half-life of IgG molecules and Fc-fusion proteins. In addition, FcRn molecules mediate the transfer of IgG and Fc-fusion proteins across cellular barriers, tissues, and mucosa and through the placenta from mother to fetus [3,75].

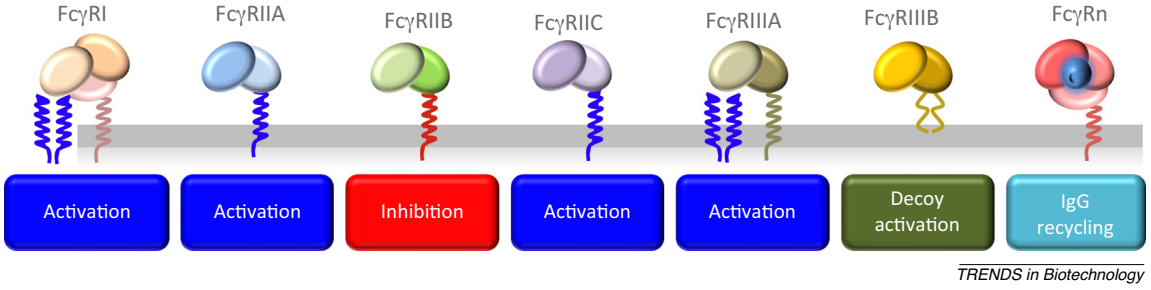

Figure I. Schematic showing canonical IgG fragment crystallizable (Fc)-binding receptors (FcγRs) and their major functions.

with higher avidity for the FcγR, which enhances binding and signaling capacity [17–19]. For example, tumor necrosis factor alpha (TNFα) naturally exists as a homotrimer [20]. Recent structural data suggest that TNFα is bound in a 3:3 ratio by the antigen-binding fragment (Fab) of the anti-TNFα mAb infliximab [21]. It is likely that the Fc-fused TNFα receptor (TNFαR) also generates multimer complexes. Similarly, Fc–drug complexes can be generated when patients develop ADAs or when Fc-fusion drugs like Fc–TNFαR and Fc–cytotoxic T lymphocyte-associated protein 4 (CTLA-4) target multiple cell-surface molecules [22,23].

The expression patterns of the receptors can be modified under pathological conditions or following therapeutic interventions. The cytokine milieu plays an important role in the cell expression and function of FcγR. For example, interferon gamma (IFNγ) upregulates while interleukin-4 (IL-4) downregulates the expression of the inhibitory FcγRIIB on B cells. By contrast, IFNγ downregulates while IL-4 upregulates FcγRIIB on monocytes and peritoneal macrophages [24,25]. Beside the regulation of FcRs by cytokines, cytokine effects are sometimes dependent on Fc–FcR engagement. For example, a gain of antimicrobial function by peritoneal macrophages via IFNγ-mediated Jak–STAT signaling is dependent on immunoreceptor tyrosine-based activating motif (ITAM) signaling by FcγRI-bound IgG monomers [26]. IL-3 induces increased expression of both activating and inhibitory FcγRII (A and B) by basophils [27] and increases the ability of the high-affinity FcγRI on monocytes to bind IgG complexes and deliver intracellular signals [28].

Fc biology is also partially dependent on varying patterns of N-glycosylation and FcγR polymorphism [17,29–31]. Thus, specific alleles of human FcγR are associated with autoimmune diseases, the success of antibody-based therapy [32], and the development of nADAs to Factor VIII replacement therapy in hemophilia A patients [33]. Taken in concert, the immunological outcomes of Fc–FcR interactions are multivariable and need to be critically evaluated for each new Fc-fusion protein and, possibly, individual patient populations.

Mechanisms by which Fc–FcR interactions can modulate the immunogenicity of Fc-fusion proteins

Activating mechanisms

The IgG1 Fc can interact with the canonical FcγRs (Box 1) or with noncanonical FcRs (Box 2) expressed by many effector immune cells (Table 1). IgG monomers, with or without antigen, constitutively engage the high-affinity activating FcγRI on macrophages *in vivo* [26,34,35]. Studies that measured the binding of Fc domains derived from Fc-fusion proteins to the soluble FcγRI demonstrated that the binding affinities were comparable with those observed for mAbs [36]. However, the nature of the active molecule fused markedly affects the affinity of Fc for FcγRs [14]. This is an important observation as it suggests that Fc-fusion proteins should be considered on a case-by-case basis and it is difficult to generalize based on the properties of the Fc-fusion partner alone.

Immunogenicity to therapeutic mAbs and Fc-fusion drugs is mostly triggered by a humoral immune response dependent on CD4 T helper cells and mediated by B cells with crucial involvement of the professional antigen-presenting cells [e.g., dendritic cells (DCs) and macrophages]. These cells express the activating FcγRI and FcγRIIA and

Box 2. Noncanonical IgG FcRs

In addition to the canonical FcγRs, an increasing number of 'nontraditional' receptors that regulate the function of IgG homodimers and aggregates continues to be identified (Figure I). Examples of such molecules include, but are not limited to: (i) TRIM21, a cytosolic FcR that binds all Ig classes, targets opsonized intracellular pathogens for proteasome-mediated degradation, and activates the type 1 IFN pathway [76]; (ii) CD22, expressed by B cells, is postulated to inhibit their function [77]; (iii) FcRL5, expressed by B cells, is recognized to modulate B cell antigen receptor signaling [78]; (iv) CD23, expressed by B cells, DCs, macrophages, monocytes, and activated T cells, is involved in positive selection of B cells and directing allergens/antigens to B cells by IgG and IgE immune complexes [79]; and (v) the C-type lectin receptor SIGN-R1 in mice/DC-SIGN in humans (CD209), expressed by DCs, macrophages, and monocytes, is reported to bind α-2,6-sialylated Fc and to mediate many of the anti-inflammatory properties of IVIG in a mouse model [80,81]. The α-2,6-sialylated Fc-mediated mechanism for the anti-inflammatory properties of IVIG is highly controversial and the reader is referred to these references for more information [19,82,83]. CD209 and CD23 were reported to bind the Fc domain of IgG only in a closed conformation defined as the type II receptor, which is associated with the anti-inflammatory activity of IgG [15].

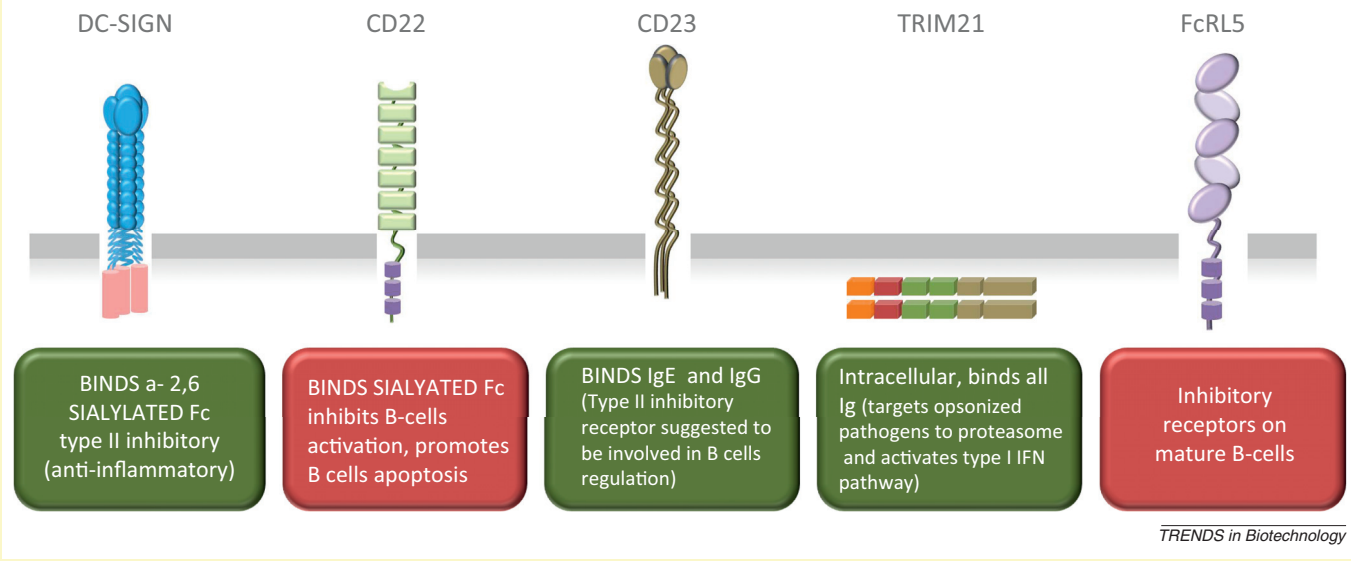

Figure I. Schematic showing the noncanonical fragment crystallizable (Fc) receptors and their functions.

the inhibitory FcγRIIB (Table 1). Crosslinking of activating FcγRs on APCs enhances antigen uptake and maturation, expression of MHC class II and costimulatory molecules, and secretion of proinflammatory cytokines, all of which substantially increase antigen presentation to T cells [18,35,37,38].

Several studies suggest that, in addition to playing a role in recycling, Fc–FcRn interactions may be more nuanced and, under alternative conditions, the receptor can be involved in antigen presentation. For example, in DCs, FcRn bound to IgG–antigen complexes traffics cargo into endosomes and lysosomes, where antigen processing

Table 1. Human canonical FcγR, functions, binding affinities to various IgG subclasses, and expression in cells

Canonical FcγR	FcγRI	FcγRIIA		FcγRIIB	FcγRIIC	FcγRIIIA		FcγRIIIB	FcRn
Function	Activation	Activation		Inhibition	Activation	Activation		Decoy	Recycling
Subclass	*Binding affinity* (KD, μM)								
		His131	Arg131			Val158	Phe158		
IgG1	0.6	5	3	10	10	20	10	20	0.8
IgG2	No binding	40	10	200	200	700	300	No binding	0.5
IgG3	0.6	90	90	20	20	0.1	8	1	0.3
IgG4	0.3	20	20	20	20	20	20	No binding	0.2
Expression									
	MQs/Monos[a] DCs PMNs[b] (induced) Mast cells (induced)	MQs/Monos DCs Neutrophils Basophils Eosinophils Mast cells Platelets		B cells MQs/Monos DCs Basophils	MQs/Monos DCs Neutrophils NK[c] cells	MQs/Monos Neutrophils NK cells		Neutrophils Basophils	MQs/Monos DCs Neutrophils Endothelium Epithelium Syncytiotrophoblasts

Affinities of human IgG subclasses for FcγR alleles and FcRn are expressed as KD (μM). Non-immune cells expressing FcRn are colored in gray [8,15,32,37,84].

[a]Macrophages and/or monocytes.

[b]Polymorphonuclear leukocytes.

[c]Natural killer.

can occur for cross-presentation via the MHC class I and II pathways [39].

Inhibitory mechanisms

The importance of Fc–FcR interactions that inhibit or downregulate immune responses is also now well recognized [24]. Mice that lack the gene encoding the inhibitory receptor FcγRIIB spontaneously develop autoimmune diseases [40]. Engagement of the FcγRIIB by immune complexes results in downregulation of the maturation of DCs and macrophages [41–43]. Binding to FcγRIIB by monomeric IgG on human DCs induces higher expression of this inhibitory receptor [41]. Thus, it is plausible that long-term treatment with monomeric Fc-fusion drugs could have a similar effect and thereby modulate immunogenicity.

B cells and plasma cells express the FcγRIIB inhibitory receptor. Multivalent engagement of the FcγRIIB on B cells by Fc can induce apoptosis of B cells. However this effect can be attenuated by coligation of the B cell receptor [44]. This mechanism favors the selection and survival of B cells with higher affinity for the antigen. By contrast, plasma cells, which have little or no expression of the B cell receptor and express elevated levels of FcγRIIB, are subject to apoptosis by immune complexes and have no survival mechanism [45].

Exceptions to the role of inhibitory/activating receptors

The concept of distinct activating and inhibitory roles for receptors is also undergoing reevaluation [46]. Under some conditions, the inhibitory FcγRIIB can mediate activation of the immune response. For instance, DCs present native, unprocessed antigen to B cells following immune complex internalization via the inhibitory FcγRIIB. These DCs directly activate B cells specific for epitopes on the native Ag [47]. The CD40 agonist mAb has immune-adjuvant properties dependent on the inhibitory FcγRIIB when administered by the intravenous (IV) route. However, the non-FcγRIIB-binding isotype of this mAb stimulates the immune response, but only when injected subcutaneously [48]. These data suggest that the site of administration and the local repertoire of cells and their FcγR profiles can determine the immune consequences of therapy.

There are also reports that activating FcγRs can induce an inhibitory signal (ITAMi) when engaged with low-valence immune complexes [49]. Uncomplexed IgG1 was shown to deliver an inhibitory signal, mediated by the activating FcγRIII. This ITAMi signal reduced phagocytosis, endocytosis, calcium response, and oxygen species production by mouse and human monocytes and macrophages *in vitro* and blocked the development of inflammatory disease *in vivo*. Neutrophils, which also express this activation receptor, are not affected [50,51]. In some cases, such as administration of pooled IVIG therapy, ligation of the activating receptors on splenic macrophages induces tolerance. Thus the terms 'activating' and 'inhibitory', as strictly applied to downstream outcomes of FcR ligation, may be misnomers [52]. Alternatively, it is possible, and perhaps even likely, that powerful activating signals have the potential to induce immunological tolerance.

Involvement of innate immune cells

Studies in mice and human FcγR transgenic mice identified the involvement of various activating FcγRs and the innate immune cells (macrophages, monocytes, neutrophils, basophils, and mast cells) in inflammatory diseases and systemic hyperacute anaphylaxis [29,32]. Administration of therapeutic mAbs carries the risk of immune reactions such as acute anaphylaxis [53]. Fc-fusion drugs can interact with innate cells expressing FcγRs, but there are almost no reports of acute anaphylaxis.

Fully recombinant therapeutic Fc multimers for the induction of tolerance

There is growing interest in exploring the use of multimerized Fc alone for the treatment of disease. IVIG therapy is now increasingly used in the treatment of inflammatory and autoimmune diseases. Several studies supported the idea that the anti-inflammatory effects of IVIG are primarily localized to the Fc portion of immune aggregates. The clinical utility of this concept was first demonstrated when an enzymatically generated Fc was successfully used in pediatric patients with autoimmune disease [54]. The anti-inflammatory activities of IVIG can be recapitulated with preformed antibody–antigen complexes [55], antibodies directed against endogenous antigen [56], or genetically engineered Fc fusion polymers [14]. For the sake of completeness and accuracy, it is important to reinforce the notion that several non-Fc aggregate-related mechanisms are also proposed to mediate the tolerogenic effects of IVIG. These include: (i) binding to the activating FcγRIIIA on monocytes and macrophages, generating ITAMi signaling to suppress their proinflammatory functions [50,51]; (ii) regulatory epitopes contained within Fc (Tregitopes), which bind with high-affinity to human and mouse MHC class II, activating T regulatory cells (Tregs) [57–59]; (iii) Fab-mediated effects; and (iv) binding of α-2,6-sialylated IgG Fc to mouse SIGN-R1 or human DC-SIGN on myeloid cells inducing IL-33 and IL-4 secretion, resulting in elevation of the inhibitory FcγRIIB on macrophages [60]. It must be emphasized that the last mechanism is highly controversial. For example, several studies have demonstrated that IL-4, IL-33, and sialylation are not required for the immunomodulatory effects of IVIG [19]. Moreover, in humans IVIG treatment suppresses DC function via stimulation of IL-33, IL-4, and IL-13 production, which is not mediated by the DC-specific DC-SIGN receptor [61]. It is relevant to note that the initial effects of IVIG *in vivo* are proinflammatory – which may be necessary for the secondary induction of tolerance [62]. Our understanding of the exact role of these individual mechanisms, alone and in combination, in regulating the anti-inflammatory functions of IVIG continues to develop.

Armed with the knowledge that artificial Fc-bearing immune aggregates can induce tolerance, investigators sought to develop fully recombinant IgG multimers for both clinical translation and mechanistic experiments. Translation of murine IgG2a Fc cDNA that incorporated specific 'multimerization domain' sequences (e.g., the hinge region of human IgG2 [63,64], the isoleucine zipper) yielded Fc multimers. These fully recombinant Fc multimers were called Stradomers™ to distinguish them from

naturally occurring Fc homodimers. The Stradomers™ bound low-affinity FcγRs with significantly lower K_d values than Fc monomers and the affinity correlated directly with the degree of multimerization [13]. Functional evaluation of these Stradomers™ in murine models of autoimmunity revealed their ability to ameliorate idiopathic thrombocytopenic purpura and collagen-induced arthritis, but not graft-versus-host disease [13]. Stradomers™ can also effectively treat experimental autoimmune neuritis [65] and myasthenia gravis (MG) [66]. Importantly, the studies in MG provided significant mechanistic insights, demonstrating that drug administration was associated with a reduction in pathogenic antibodies, decreased T and B cell responsiveness to the acetylcholine receptor, and increases in both Tregs and immunosuppressive cytokines. Collectively, these data support the idea that Fc-bearing immune complexes may serve as a protective mechanism against inflammation and, as a corollary, that fully recombinant Fc Stradomers™ might have therapeutic value for the treatment of autoimmunity.

Technological and regulatory considerations

A new generation of engineered protein therapeutics that incorporate the Fc moiety has entered the drug development pipeline and many of these have gained approval. Any of the scenarios described above can plausibly occur with Fc-based drugs. An understanding of the nuanced biology of Fc–FcR interactions is helpful in assessing the risks associated with this class of therapies. More importantly, this knowledge can be used to improve the safety profile of the drugs. The complexity and diversity of the physiological responses, which are dependent on factors such as the isotype of the Fc, the site of injection, the populations of APCs, and the cytokine milieu, can be exploited based on the clinical needs of individual drugs. However, a critical lacuna remains: despite the emergence of a critical mass of literature on Fc biology, very few research studies have used therapeutic fusion proteins *per se*.

TNFα-blocking drugs can provide interesting insights into the role of the Fc moiety. These drugs include: anti-TNFα mAbs, which contain a Fc as part of the mAb, a Fc-fusion TNFα decoy receptor, and an anti-TNFα Fab domain linked to polyethylene glycol (PEG) that has no Fc component. The affinity of the Fc–TNFαR to FcRn is tenfold lower than that of either of the mAb-based drugs. Moreover, once the decoy receptor binds TNFα there is a further decrease in the affinity to FcRn. This suggests reduced protection from degradation of the Fc–TNFαR–TNFα complexes, availability to other FcRs, or immune reactions [36].

About 50% of patients who initially respond to the mAbs or the Fc decoy receptor show subsequent unresponsiveness to therapy due largely to the development of ADAs [67]. A clinical trial with the newer drug, Fab–PEG, suggests that it can be used successfully and safely in patients showing secondary unresponsiveness [68]. This might indicate an advantage of this Fab-based drug over drugs that can interact with FcRs. The TNFα-blocking drugs can induce hypersensitivity, which although rare, is a serious and potentially life-threatening, adverse event. Post-marketing experience has shown that the Fc-fusion decoy receptor has an advantage over the mAb infliximab in this regard [69,70]. This is consistent with results showing that mice immunized with *Schistosoma mansoni* cercarial elastase antigen induced notable levels of IgE, while no IgE was detectable in animals immunized with this antigen fused to mouse IgG2a Fc [14].

Exploiting Fc biology may also offer strategies to engineer the Fc partner to achieve desirable properties in the drug [6,71]. Examples of such rational protein design include engineering both the active moiety and the Fc partner of the CTLA4–Fc drug to improve functional outcomes (Figure 1) [72,73] and using an anti-IgE fused to a Fc domain that was mutated to enhance targeting to the inhibitory FcγRIIB, to block basophil activation and release of allergy mediators [10].

Concluding remarks and future perspectives

The Fc-fusion platform technology has had an enviable track record in terms of generating successful drug products. Nevertheless, there continues to be considerable potential for extending the serum half-life. Attempts to engineer the Fc partner for enhanced binding to FcRn are already underway both in experimental systems and in drug-development programs. In addition, progress in understanding Fc biology extends far beyond Fc–FcRn interactions *per se*. There is a sufficient knowledge base to

Box 3. Fc fusion and drug delivery

Unlike during drug development for small molecules, placental transport of biological agents is not studied extensively, most likely because of the common belief that placental transport is minimal for these drugs. However, the situation has changed with the wave of Fc-fusion proteins, mAbs, and other Fc-based products that have entered the drug-development pipeline.

Maternal antibodies are transferred through the placenta to the fetus and by breastfeeding through the neonatal gut [3]. It therefore follows that drug products that include the Fc domain can be delivered to the fetus. This was demonstrated in mice, when a Fc-tagged β-glucuronidase was shown to pass from mother to fetus with mucopolysaccharidosis VII [85]. This pathway is not only a means of *in utero* replacement therapy for the fetus, but may induce tolerance to replacement therapy later in life and prevent the formation of inhibitory antibodies. In contrast to the above, maternal transfer of antibodies to the fetus may have negative consequences. If the mother develops an autoimmune disease with autoantibodies, these antibodies can cross the placenta and cause harm. Examples are complications in the fetuses and neonates of mothers with MG, immune thrombocytopenic purpura, lupus, or other autoimmune based pathologies [86]. While autoantibodies have been detected in patients with neonatal autoimmune disease, the pathogenic role of autoantibodies has not been well defined. FcRn can facilitate bidirectional passage of Fc-bearing molecules across mucosal surfaces and can reach the circulation when given orally or by inhalation [3]. Successful delivery of Fc-fusion proteins via the respiratory tract by inhalation has been described for erythropoietin. FcRn-mediated delivery via the oral route of Fc–follicle-stimulating hormone (FSH) in rats led to an increase in ovarian and testicular weight in females and males respectively and prolonged the half-life of FSH compared with unfused FSH [87]. Fc-bearing molecules given systemically (IV) can reach mucosal surfaces and provide effective therapy. HIV-neutralizing mAb administered by the IV route provided greater protection in primates challenged intrarectally with the lentivirus simian/human HIV (SHIV) when the mAb carried a Fc mutation that increased binding to FcRn [88].

warrant the rational engineering of the Fc partner in fusion proteins to modulate immune responses for desirable outcomes. In addition to immune responses, Fc engineering also offers new strategies for drug delivery and targeting, including fetal targeting (Box 3). Finally, Fc biology is fostering the development of therapies using Fc proteins alone. Translating these concepts and results into Fc-fusion drugs rationally designed for specific functional outcomes require validated assays, reagents, and animal models for proof-of-concept and preclinical studies. Fc–FcR interactions are species specific and experimental systems should use either chimeric fusion proteins with the species-specific Fc or 'humanized' mice with the appropriate receptors [29,74]. Recent progress in basic science bodes well for the development of a new generation of Fc-fusion protein therapies that has immense potential.

Disclaimer statement

The authors' contribution is an informal communication and represents their own best judgment. These comments do not bind or obligate the FDA. S.E.S. is a cofounder of and major stockholder in Gliknik, Inc., a biotechnology company. He also receives royalties for intellectual property, related to B7-H1 (PD-L1), licensed by the Mayo Clinic College of Medicine to third parties.

Acknowledgments

The authors thank Jonathan Simon for assistance with Figure 1. This work was supported by funds from the Laboratory of Hemostasis, Center for Biologics Evaluation and Research (B.G. and Z.E.S.), the FDA's Modernization of Science Program (Z.E.S.), The Critical Path initiative (Z.E.S.), and the FDA Chief Scientist's Challenge Grant (Z.E.S.).

References

1 Kariolis, M.S. *et al.* (2013) Beyond antibodies: using biological principles to guide the development of next-generation protein therapeutics. *Curr. Opin. Biotechnol.* 24, 1072–1077
2 Sauna, Z.E. *et al.* (2012) Plasma derivatives: new products and new approaches. *Biologicals* 40, 191–195
3 Roopenian, D.C. and Akilesh, S. (2007) FcRn: the neonatal Fc receptor comes of age. *Nat. Rev. Immunol.* 7, 715–725
4 Huang, C. (2009) Receptor–Fc fusion therapeutics, traps, and MIMETIBODY technology. *Curr. Opin. Biotechnol.* 20, 692–699
5 Beck, A. and Reichert, J.M. (2011) Therapeutic Fc-fusion proteins and peptides as successful alternatives to antibodies. *MAbs* 3, 415–416
6 Rath, T. *et al.* (2013) Fc-fusion proteins and FcRn: structural insights for longer-lasting and more effective therapeutics. *Crit. Rev. Biotechnol.* (http://dx.doi.org/10.3109/07388551.2013.834293)
7 Shankar, G. *et al.* (2006) Scientific and regulatory considerations on the immunogenicity of biologics. *Trends Biotechnol.* 24, 274–280
8 Hogarth, P.M. and Pietersz, G.A. (2012) Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. *Nat. Rev. Drug Discov.* 11, 311–331
9 Liang, Y. *et al.* (2011) Immunity against a therapeutic xenoprotein/Fc construct delivered by gene transfer is reduced through binding to the inhibitory receptor FcγRIIb. *J. Gene Med.* 13, 470–477
10 Buschor, P. *et al.* (2014) Improved FcγRIIb targeting functionally translates into enhanced inhibition of basophil activation. *Int. Arch. Allergy Immunol.* 163, 206–214
11 Saxon, A. *et al.* (2008) Accentuate the negative, eliminate the positive": engineering allergy therapeutics to block allergic reactivity through negative signaling. *J. Allergy Clin. Immunol.* 121, 320–325
12 Van Scott, M.R. *et al.* (2008) Systemic administration of an Fcγ–Fcε-fusion protein in house dust mite sensitive nonhuman primates. *Clin. Immunol.* 128, 340–348
13 Jain, A. *et al.* (2012) Fully recombinant IgG2a Fc multimers (Stradomers) effectively treat collagen-induced arthritis and prevent idiopathic thrombocytopenic purpura in mice. *Arthritis Res. Ther.* 14, R192
14 Mekhaiel, D.N. *et al.* (2011) Polymeric human Fc-fusion proteins with modified effector functions. *Sci. Rep.* 1, 124
15 Pincetic, A. *et al.* (2014) Type I and type II Fc receptors regulate innate and adaptive immunity. *Nat. Immunol.* 15, 707–716
16 Karsten, C.M. *et al.* (2012) Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIB and Dectin-1. *Nat. Med.* 18, 1401–1406
17 Lux, A. *et al.* (2013) Impact of immune complex size and glycosylation on IgG binding to human FcγRs. *J. Immunol.* 190, 4315–4323
18 Nimmerjahn, F. and Ravetch, J.V. (2008) Fcγ receptors as regulators of immune responses. *Nat. Rev. Immunol.* 8, 34–47
19 Schwab, I. and Nimmerjahn, F. (2013) Intravenous immunoglobulin therapy: how does IgG modulate the immune system? *Nat. Rev. Immunol.* 13, 176–189
20 Eck, M.J. and Sprang, S.R. (1989) The structure of tumor necrosis factor-α at 2.6 Å resolution. Implications for receptor binding. *J. Biol. Chem.* 264, 17595–17605
21 Liang, S. *et al.* (2013) Structural basis for treating tumor necrosis factor alpha (TNFα)-associated diseases with the therapeutic antibody infliximab. *J. Biol. Chem.* 288, 13799–13807
22 Kaymakcalan, Z. *et al.* (2009) Comparisons of affinities, avidities, and complement activation of adalimumab, infliximab, and etanercept in binding to soluble and membrane tumor necrosis factor. *Clin. Immunol.* 131, 308–316
23 Moreland, L. *et al.* (2006) Abatacept. *Nat. Rev. Drug Discov.* 5, 185–186
24 Smith, K.G. and Clatworthy, M.R. (2010) FcγRIIB in autoimmunity and infection: evolutionary and therapeutic implications. *Nat. Rev. Immunol.* 10, 328–343
25 Pricop, L. *et al.* (2001) Differential modulation of stimulatory and inhibitory Fc gamma receptors on human monocytes by Th1 and Th2 cytokines. *J. Immunol.* 166, 531–537
26 Bezbradica, J.S. *et al.* (2014) A role for the ITAM signaling module in specifying cytokine-receptor functions. *Nat. Immunol.* 15, 333–342
27 Cassard, L. *et al.* (2012) Fcγ receptors inhibit mouse and human basophil activation. *J. Immunol.* 189, 2995–3006
28 van der Poel, C.E. *et al.* (2010) Cytokine-induced immune complex binding to the high-affinity IgG receptor, FcγRI, in the presence of monomeric IgG. *Blood* 116, 5327–5333
29 Bruhns, P. (2012) Properties of mouse and human IgG receptors and their contribution to disease models. *Blood* 119, 5640–5649
30 Beck, A. *et al.* (2008) Trends in glycosylation, glycoanalysis and glycoengineering of therapeutic antibodies and Fc-fusion proteins. *Curr. Pharm. Biotechnol.* 9, 482–501
31 Strome, S.E. *et al.* (2007) A mechanistic perspective of monoclonal antibodies in cancer therapy beyond target-related effects. *Oncologist* 12, 1084–1095
32 Gillis, C. *et al.* (2014) Contribution of human FcγRs to disease with evidence from human polymorphisms and transgenic animal studies. *Front. Immunol.* 5, 254
33 Eckhardt, C.L. *et al.* (2014) The Fc gamma receptor IIa R131H polymorphism is associated with inhibitor development in severe hemophilia A. *J. Thromb. Haemost.* 12, 1294–1301
34 Unkeless, J.C. and Eisen, H.N. (1975) Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages. *J. Exp. Med.* 142, 1520–1533
35 Ioan-Facsinay, A. *et al.* (2002) FcγRI (CD64) contributes substantially to severity of arthritis, hypersensitivity responses, and protection from bacterial infection. *Immunity* 16, 391–402
36 Suzuki, T. *et al.* (2010) Importance of neonatal FcR in regulating the serum half-life of therapeutic proteins containing the Fc domain of human IgG1: a comparative study of the affinity of monoclonal antibodies and Fc-fusion proteins to human neonatal FcR. *J. Immunol.* 184, 1968–1976
37 Guilliams, M. *et al.* (2014) The function of Fcγ receptors in dendritic cells and macrophages. *Nat. Rev. Immunol.* 14, 94–108
38 Swanson, J.A. and Hoppe, A.D. (2004) The coordination of signaling during Fc receptor-mediated phagocytosis. *J. Leukoc. Biol.* 76, 1093–1103
39 Rath, T. *et al.* (2013) The immunologic functions of the neonatal Fc receptor for IgG. *J. Clin. Immunol.* 33 (Suppl. 1), S9–S17
40 Bolland, S. and Ravetch, J.V. (2000) Spontaneous autoimmune disease in FcγRIIB-deficient mice results from strain-specific epistasis. *Immunity* 13, 277–285

41 Boruchov, A.M. *et al.* (2005) Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J. Clin. Invest.* 115, 2914–2923
42 Dhodapkar, K.M. *et al.* (2005) Selective blockade of inhibitory Fcγ receptor enables human dendritic cell maturation with IL-12p70 production and immunity to antibody-coated tumor cells. *Proc. Natl. Acad. Sci. U.S.A.* 102, 2910–2915
43 van Montfoort, N. *et al.* (2012) Fcγ receptor IIb strongly regulates Fcγ receptor-facilitated T cell activation by dendritic cells. *J. Immunol.* 189, 92–101
44 Pearse, R.N. *et al.* (1999) SHIP recruitment attenuates Fc gamma RIIB-induced B cell apoptosis. *Immunity* 10, 753–760
45 Xiang, Z. *et al.* (2007) FcγRIIb controls bone marrow plasma cell persistence and apoptosis. *Nat. Immunol.* 8, 419–429
46 Pfirsch-Maisonnas, S. *et al.* (2011) Inhibitory ITAM signaling traps activating receptors with the phosphatase SHP-1 to form polarized "inhibisome" clusters. *Sci. Signal.* 4, ra24
47 Bergtold, A. *et al.* (2005) Cell surface recycling of internalized antigen permits dendritic cell priming of B cells. *Immunity* 23, 503–514
48 White, A.L. *et al.* (2013) FcγRIIB controls the potency of agonistic anti-TNFR mAbs. *Cancer Immunol. Immunother.* 62, 941–948
49 Blank, U. *et al.* (2009) Inhibitory ITAMs as novel regulators of immunity. *Immunol. Rev.* 232, 59–71
50 Aloulou, M. *et al.* (2012) IgG1 and IVIg induce inhibitory ITAM signaling through FcγRIII controlling inflammatory responses. *Blood* 119, 3084–3096
51 Ben, M.S. *et al.* (2014) Role of FcγRIIIA (CD16) in IVIg-mediated anti-inflammatory function. *J. Clin. Immunol.* 34 (Suppl. 1), S46–S50
52 Siragam, V. *et al.* (2006) Intravenous immunoglobulin ameliorates ITP via activating Fc gamma receptors on dendritic cells. *Nat. Med.* 12, 688–692
53 Hansel, T.T. *et al.* (2010) The safety and side effects of monoclonal antibodies. *Nat. Rev. Drug Discov.* 9, 325–338
54 Imbach, P. *et al.* (1981) High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet* 1, 1228–1231
55 Bazin, R. *et al.* (2006) Reversal of immune thrombocytopenia in mice by cross-linking human immunoglobulin G with a high-affinity monoclonal antibody. *Br. J. Haematol.* 135, 97–100
56 Bussel, J.B. *et al.* (1991) Intravenous anti-D treatment of immune thrombocytopenic purpura: analysis of efficacy, toxicity, and mechanism of effect. *Blood* 77, 1884–1893
57 De Groot, A.S. *et al.* (2008) Activation of natural regulatory T cells by IgG Fc-derived peptide "Tregitopes". *Blood* 112, 3303–3311
58 De Groot, A.S. *et al.* (2013) Tregitope peptides: the active pharmaceutical ingredient of IVIG? *Clin. Dev. Immunol.* 2013, 493138
59 Su, Y. *et al.* (2013) Regulatory T cell epitopes (Tregitopes) in IgG induce tolerance *in vivo* and lack immunogenicity *per se*. *J. Leukoc. Biol.* 94, 377–383
60 Anthony, R.M. *et al.* (2011) Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature* 475, 110–113
61 Tjon, A.S. *et al.* (2014) Intravenous immunoglobulin treatment in humans suppresses dendritic cell function via stimulation of IL-4 and IL-13 production. *J. Immunol.* 192, 5625–5634
62 Aukrust, P. *et al.* (1994) Release of cytokines, soluble cytokine receptors, and interleukin-1 receptor antagonist after intravenous immunoglobulin administration *in vivo*. *Blood* 84, 2136–2143
63 Wypych, J. *et al.* (2008) Human IgG2 antibodies display disulfide-mediated structural isoforms. *J. Biol. Chem.* 283, 16194–16205
64 Yoo, E.M. *et al.* (2003) Human IgG2 can form covalent dimers. *J. Immunol.* 170, 3134–3138
65 Niknami, M. *et al.* (2013) Beneficial effect of a multimerized immunoglobulin Fc in an animal model of inflammatory neuropathy (experimental autoimmune neuritis). *J. Peripher. Nerv. Syst.* 18, 141–152
66 Thiruppathi, M. *et al.* (2014) Recombinant IgG2a Fc (M045) multimers effectively suppress experimental autoimmune myasthenia gravis. *J. Autoimmun.* 52, 64–73
67 Bendtzen, K. (2012) Anti-TNF-α biotherapies: perspectives for evidence-based personalized medicine. *Immunotherapy* 4, 1167–1179
68 Schiff, M.H. *et al.* (2014) Rheumatoid arthritis secondary non-responders to TNF can attain an efficacious and safe response by switching to certolizumab pegol: a Phase IV, randomised, multicentre, double-blind, 12-week study, followed by a 12-week open-label phase. *Ann. Rheum. Dis.* 73, 2174–2177
69 Puxeddu, I. *et al.* (2012) Hypersensitivity reactions during treatment with infliximab, etanercept, and adalimumab. *Ann. Allergy Asthma Immunol.* 108, 123–124
70 Krieckaert, C.L. *et al.* (2012) Comparison of long-term clinical outcome with etanercept treatment and adalimumab treatment of rheumatoid arthritis with respect to immunogenicity. *Arthritis Rheum.* 64, 3850–3855
71 Czajkowsky, D.M. *et al.* (2012) Fc-fusion proteins: new developments and future perspectives. *EMBO Mol. Med.* 4, 1015–1028
72 Larsen, C.P. *et al.* (2005) Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am. J. Transplant.* 5, 443–453
73 Bernett, M.J. *et al.* (2013) Immune suppression in cynomolgus monkeys by XPro9523: an improved CTLA4–Ig fusion with enhanced binding to CD80, CD86 and neonatal Fc receptor FcRn. *MAbs* 5, 384–396
74 Smith, P. *et al.* (2012) Mouse model recapitulating human Fcγ receptor structural and functional diversity. *Proc. Natl. Acad. Sci. U.S.A.* 109, 6181–6186
75 Roopenian, D.C. *et al.* (2003) The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG–Fc-coupled drugs. *J. Immunol.* 170, 3528–3533
76 McEwan, W.A. *et al.* (2013) Intracellular antibody-bound pathogens stimulate immune signaling via the Fc receptor TRIM21. *Nat. Immunol.* 14, 327–336
77 Muller, J. and Nitschke, L. (2014) The role of CD22 and Siglec-G in B-cell tolerance and autoimmune disease. *Nat. Rev. Rheumatol.* 10, 422–428
78 Franco, A. *et al.* (2013) Human Fc receptor-like 5 binds intact IgG via mechanisms distinct from those of Fc receptors. *J. Immunol.* 190, 5739–5746
79 Meulenbroek, L.A. *et al.* (2013) IgG antibodies in food allergy influence allergen–antibody complex formation and binding to B cells: a role for complement receptors. *J. Immunol.* 191, 3526–3533
80 Schwab, I. *et al.* (2014) Broad requirement for terminal sialic acid residues and FcγRIIB for the preventive and therapeutic activity of intravenous immunoglobulins *in vivo*. *Eur. J. Immunol.* 44, 1444–1453
81 Sondermann, P. *et al.* (2013) General mechanism for modulating immunoglobulin effector function. *Proc. Natl. Acad. Sci. U.S.A.* 110, 9868–9872
82 Campbell, I.K. *et al.* (2014) Therapeutic effect of IVIG on inflammatory arthritis in mice is dependent on the Fc portion and independent of sialylation or basophils. *J. Immunol.* 192, 5031–5038
83 Crow, A.R. *et al.* (2007) A role for IL-1 receptor antagonist or other cytokines in the acute therapeutic effects of IVIg? *Blood* 109, 155–158
84 Bruhns, P. *et al.* (2009) Specificity and affinity of human Fcγ receptors and their polymorphic variants for human IgG subclasses. *Blood* 113, 3716–3725
85 Grubb, J.H. *et al.* (2008) Infused Fc-tagged β-glucuronidase crosses the placenta and produces clearance of storage *in utero* in mucopolysaccharidosis VII mice. *Proc. Natl. Acad. Sci. U.S.A.* 105, 8375–8380
86 Carvalheiras, G. *et al.* (2012) Fetal outcome in autoimmune diseases. *Autoimmun. Rev.* 11, A520–A530
87 Low, S.C. *et al.* (2005) Oral and pulmonary delivery of FSH–Fc fusion proteins via neonatal Fc receptor-mediated transcytosis. *Hum. Reprod.* 20, 1805–1813
88 Ko, S.Y. *et al.* (2014) Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature* 514, 642–645