Anti-inflammatory Activity of IgG-Fc

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Abstract Over 80 different autoimmune disorders have been identified. A common denominator across most of these disorders is the presence of pathogenic autoantibodies. The pathogenic and inflammatory nature of antibodies is well accepted, and over the last three decades, evidence in humans and rodent models has revealed that antibodies can induce anti-inflammatory activities. The discovery of the relationship between immunoglobulin G (IgG) glycovariants and disease activity in autoimmune patients has provided insight into the structural and functional characteristics of IgG associated with its pro- and anti-inflammatory activity. In this chapter, we discuss evidence of the anti-inflammatory nature of IgG and the mechanisms by which this activity is exerted. Current clinical evidence of this anti-inflammatory activity is also discussed.

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1 Introduction

The role of immunoglobulin G (IgG) in the pathogenesis of many autoimmune disorders such as systemic lupus erythematosus (SLE), myasthenia gravis, immune thrombocytopenia, and many other diseases is well defined. Autoantibodies can form immune complexes with their cognate antigen and trigger inflammatory processes through their interaction with cellular Fc gamma receptors (FcγRs) and complement. It is now understood that IgG can also have anti-inflammatory activities. The pro- and anti-inflammatory activity of IgG can be modulated by different properties of the Ig such as the IgG subclass, glycosylation, and valency. The composition of the Fc domain, although it is considered the invariant region of the IgG molecule, is highly heterogeneous. In addition to the four distinct subclasses of human IgG (IgG1, IgG2, IgG3, and IgG4), the Fc domain exhibits additional diversity in individual allotypes and glycosylation states. Furthermore, IgG can form complexes with target ligands, antigens, cells, or particles, leading to different degrees of Fc valency. The combination of subclass, allotype, glycosylation, and valency leads to a substantial amount of heterogeneity in Fc structure (Arnold et al. 2007; Vidarsson et al. 2014), which can influence the interaction of IgG with its receptors and ultimately influence its function. Effector functions of human IgG are carried out by complement and by the FcγRs. Humans express several activating FcγRs, including FcγRI, FcγIIa, FcγIIc, FcγIIIa, and the lone inhibitory FcγRIIb. Activating FcγRs signal via a highly conserved immunoreceptor tyrosine-based activation motif. FcγRIIa and FcγRIIc intrinsically express this motif in their γ chain, whereas FcγRI and FcγRIIIa rely on the common FcR γ chain (γ_2) to trigger activating signals. In contrast, FcγRIIb contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its α chain. Activating FcγRs are commonly found on myeloid cells, dendritic cells (DCs), natural killer (NK) cells, and neutrophils. The inhibitory FcγRIIb is typically expressed together with activating FcγRs. B cells are a notable exception as they express FcγRIIb only and do not express activating FcγRs.

FcγRs exhibit different affinities for IgG subclasses. FcγRI can bind all human IgG subclasses with relatively high affinity, with the exception of IgG2. FcγRI has the unique ability to interact with monomeric IgG. FcγRIIa, the most widely expressed receptor, can bind all IgG subclasses, and it is the only FcγR to bind to IgG2a. IgG3 binding affinity is highest for FcγRIIa, followed by IgG1, with weak but measurable binding affinity to IgG2 and IgG4. Variants of FcγRIIa have been described to modulate affinity for IgG2a, with the 131R variant having higher affinity than the 131H variant. IgG binding affinity to FcγRIIb is weak for all subclasses, and affinity for IgG2 is the lowest. FcγRIIIa, which binds all subclasses, has two well-described variants: FV158 and V158. V158 has a greatly increased affinity for all IgG subclasses and a particularly high affinity for IgG3. FcγRIIIb, which is mostly expressed on neutrophils, binds IgG1 and IgG3, with little binding to IgG2 and IgG4. FcγRIIIb also has two allotypic variants, denoted NA1 and NA2.

The NA1 allotype exhibits higher binding affinity to IgG1 and IgG3 subclasses and an increased ability to phagocytose IgG1- and IgG3-opsonized particles (Bruhns et al. 2009; Vidarsson et al. 2014).

Overall, human and mouse FcγRs, with the exception of FcγRI, have low affinity for monomeric IgG. They preferentially bind IgG in the form of immune complexes. Immune complex multivalent binding results in multimeric clustering of FcγRs and signaling cascades that involves spleen tyrosine kinase (SYK), phospholipase C (PLC), and calcium release, ultimately resulting in activation of the ERK/AKT pathways. Depending on the cell type, signaling via activating FcγRs results in cellular proliferation, generation of reactive oxygen species (ROS), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), and cytokine production. Conversely, immune complex interaction with FcγRIIb results in quelling of the proinflammatory activities of the activating FcγRs. This process is mediated by the FcγRIIb ITIM domain and the SH2-containing inositol phosphatase (SHIP). It is well accepted that FcγRIIb counterbalances signals triggered by the activating FcγRs in cells of the innate immune system and modulates signaling of the B-cell receptor (BCR) on B cells. In 2000, Clynes et al. demonstrated that FcγRIIb could modulate the in vivo ADCC activity of the anti-CD20 antibody rituximab (Clynes et al. 2000). Co-ligation of the inhibitory FcγRIIb with activating FcγRs was shown to inhibit the activation and maturation of dendritic cells and inhibit FcγRIIa-mediated cytokine release (Boruchov et al. 2005). Co-ligation of FcγRIIb and the B-cell receptor on B cells is known to inhibit BCR-mediated signaling in a SHIP-mediated manner resulting in reduced B-cell activation and proliferation (Chu et al. 2008; Heyman 2003; Kiener et al. 1997; Ono et al. 1997).

IgG glycosylation is crucial in modulating the in vivo activity of IgG. All IgG molecules contain a single conserved N-linked glycosylation site (Asn-297) in each of the constant heavy two (C_H2) domains, critical for maintaining their proinflammatory and anti-inflammatory effector functions (Arnold et al. 2007; Schwab and Nimmerjahn 2013). The sugar moiety attached to Asp-297 predominantly consists of an octasaccharide biantennary structure, composed of four *N*-acetylglucosamines (GlcNac), three mannoses, and one fucose, which may contain terminal galactose or sialic acid residues. These glycans help maintain the quaternary structure and the stability of the Fc (Mimura et al. 2000, 2001) and are vitally involved in FcγR binding through maintenance of an open conformation of the two heavy chains (Jefferis and Lund 2002; Sondermann et al. 2001). Deglycosylated IgG has been shown to lose nearly all significant FcγR and C1q binding affinity (Nose and Wigzell 1983). Evidence that deglycosylated IgG antibodies are unable to mediate in vivo inflammatory responses accounts for the requirement of the glycans for FcγR binding (Nimmerjahn and Ravetch 2006). Fc glycans are critical for maintaining both the proinflammatory and the anti-inflammatory effector functions of IgG (Arnold et al. 2007). Branching fucose residues at the core of the biantennary glycan have had a significant effect on the IgG-Fc binding affinity for FcγRIIIa, with afucosylated IgG having a much higher affinity for the receptor

(Ferrara et al. 2011; Houde et al. 2010; Nimmerjahn and Ravetch 2005; Shields et al. 2002; Shinkawa et al. 2003).

The terminal sugar residues in the IgG-Fc can consist of *N*-acetylglucosamine, galactose, or sialic acid. Sialylated IgG glycoforms have decreased binding affinity to several FcRs (Kaneko et al. 2006b) and can adversely impact IgG-Fc proinflammatory function (Scallon et al. 2007). In addition, changes in Fc glycosylation have been shown to modulate complement-dependent cytotoxicity where increasing amounts of terminal galactose increase the binding affinity of the complement C1q to the IgG-Fc (Raju 2008).

2 Clinical Relevance of IgG Glycosylation in Humans

A common feature of most autoimmune diseases is the formation of immune complexes between pathogenic IgG autoantibodies and cognate antigens. These immune complexes activate inflammatory effector cells expressing FcγRs and the complement cascade, contributing to tissue damage. Apart from antibody isotype and subclass, the function of autoantibodies is in part modulated by their Fc N-linked glycans. An example of the role of autoantibody glycosylation in disease pathogenicity was provided by Lood et al. The authors demonstrated that enzymatic deglycosylation of SLE immune complexes in vitro abolished their proinflammatory properties (Lood et al. 2012). Immune complex deglycosylation eliminated several important proinflammatory properties of immune complexes that are important in SLE pathogenesis: FcγR-mediated phagocytosis, interferon alfa (IFN-α) production, polymorphonuclear leukocytes chemotaxis, and classical pathway of complement activation. Furthermore, deglycosylation of immune complexes from SLE patients also had a direct effect on the molecular structure of immune complexes, causing decreased immune complex size, emphasizing the crucial role of Fc glycans for immune complex-mediated pathological signals.

In contrast to studies that link hypoglycosylation of immune complexes and the resulting loss of proinflammatory activity, results of other studies have shown that glycoforms that specifically lack terminal sialic acid or galactose residues (G0 glycoforms) might have increased proinflammatory activity. Original observations from the 1980s showed that IgG glycosylation differs in patients with rheumatoid arthritis, with decreased Fc galactosylation and sialylation compared with normal individuals (Parekh et al. 1985). Since then, a difference in the glycosylation profile of total IgG has been reported in patients with various other autoimmune diseases, including SLE, inflammatory bowel disease, myasthenia gravis, ankylosing spondylitis, primary Sjögren's syndrome, psoriatic arthritis, and multiple sclerosis (Dube et al. 1990; Selman et al. 2011; Trbojevic Akmacic et al. 2015; Vuckovic et al. 2015; Watson et al. 1999; Wuhrer et al. 2015a). In addition to their association with autoimmune diseases, agalactosylated polyclonal antibodies are associated

with increased inflammation in infectious diseases such as HIV (Ackerman et al. 2013). Besides the evidence of disparate IgG glycans in different autoimmune disease, therapeutic treatments and changes in the physiologic state can also result in differential IgG glycosylation. Namely, treatment of patients with rheumatoid arthritis with methotrexate can lead to increased levels of IgG galactosylation and hence a decrease in the abundance of agalactosylated IgG-G0 glycoforms (Pasek et al. 2006)). Furthermore, IgG-G0 glycoforms were decreased in women with rheumatoid arthritis during pregnancy, correlating with reduced incidence of flares (Rook et al. 1991; van de Geijn et al. 2009). Additional evidence suggests that serum levels of IgG-G0 glycans increase shortly before the onset of disease in patients with rheumatoid arthritis (Rombouts et al. 2015). Presumably, a window of time for initiation of preventative treatment could be estimated based on the abundance of G0 glycoforms. More recently, it was shown that treatment with estrogen in postmenopausal women with rheumatoid arthritis significantly increased IgG-Fc sialylation (Engdahl et al. 2018).

One of the best examples of specific monosaccharide residue impacting the proinflammatory activity of IgG is the core N-linked glycan fucose. Glycoforms lacking core fucose residues have been shown to have altered affinities for individual FcγRs (Shields et al. 2002). Defucosylation of IgG resulted in 10- to 50-fold enhanced affinity for mouse FcγRIV and human FcγRIII (Ferrara et al. 2011; Nimmerjahn and Ravetch 2005; Shields et al. 2002; Shinkawa et al. 2003). Consequently, defucosylation in the Fc domain of antibodies leads to increased antibody-dependent cell-mediated cytotoxic activity (Ferrara et al. 2011; Nechansky et al. 2007). Furthermore, results of a series of studies showed enhanced cytotoxic and phagocytic activity in mouse tumor and autoimmune models using these IgG glycovariants, indicating the importance of glycoengineering of antibodies for improved clinical effectiveness. In fact, therapeutic antibodies that lack fucose residues have shown enhanced tumor killing in cancer patients and have been approved as therapy for different B-cell cancers. Although there is abundant evidence of strongly increased IgG-G0 glycoforms in patients during active autoimmune disease, fucosylated IgG seems to remain stable during inflammation and vaccination in mice and humans (Kao et al. 2017; Kemna et al. 2017; Rombouts et al. 2015; Scherer et al. 2010; Selman et al. 2011; Sjowall et al. 2015). However, there are recent reports of disparate fucosylation levels of alloantibodies in hemolytic disease of the fetus or newborn (HDFN) (Kapur et al. 2014a) and fetal and neonatal alloimmune thrombocytopenia (FNAIT) (Kapur et al. 2014b; Sonneveld et al. 2016; Wuhrer et al. 2009), diseases that arise because of maternal alloimmunization against paternally inherited red blood cell antigen Rh-D or platelets, respectively. These alloantibodies are transported across the placenta and result in severe fetal anemia or thrombocytopenia. Systematical analysis of IgG-derived glycopeptides from 70 anti-D alloantibodies from pregnant women revealed a decrease in Fc-fucosylation (Kapur et al. 2014b), which correlated significantly with FcγRIIIa-mediated ADCC. Furthermore, low Fc-fucosylation

correlated with severe fetal anemia (Sonneveld et al. 2017). Thus, even though IgG fucosylation levels are generally stable during various autoimmune diseases, there is a strong correlation between antibody fucosylation and disease severity during pregnancy, and those antibodies play an active role in disease pathogenicity.

The anti-inflammatory activity of sialylated IgG is evident in patients as several studies have reported changes in IgG sialylation levels during inflammation and presence of autoimmune diseases. For instance, patients with rheumatoid arthritis, granulomatosis with polyangiitis (GPA), antiphospholipid syndrome (APS), vasculitis, and SLE have low serum levels of IgG or low autoantibody sialylation (Espy et al. 2011; Fickentscher et al. 2015; Kemna et al. 2017; Vuckovic et al. 2015; Wuhrer et al. 2015b). One particular study reported significantly higher sialylation of IgG recognizing anti-2-glycoprotein 1 autoantibodies (2GP1-IgG) isolated from the sera of healthy children and asymptomatic adults compared with that of patients with clinically apparent anti-phospholipid syndrome (APS) (Fickentscher et al. 2015). Another group demonstrated that histone IgG autoantibodies purified from SLE patients displayed significantly lower sialylation than total IgG of the same patients, suggesting that the SLE utoantibodies contain proinflammatory activities (Magorivska et al. 2016). In patients with GPA, characterized by the presence of antineutrophil cytoplasmic antibodies (ANCA) against proteinase-3 (PR3), pathogenic proteinase 3 autoantibodies are less sialylated in patients with active Wegener's vasculitis (GPA) than in those with inactive disease (Espy et al. 2011). The authors further demonstrated that purified anti-PR3 antibodies from patients with active disease were proinflammatory, and enzymatic desialylation of anti-PR3 antibodies from patients with inactive disease restored pathogenic activity (Espy et al. 2011). This study provided direct evidence of the importance of autoantibody sialylation as it pertains to the modulation of antibody activity in an autoimmune disease. Furthermore, it was shown that total IgG-Fc of patients with severe ANCA-associated vasculitis (AAV) exhibits lower levels of galactosylation, sialylation, and bisecting *N*-acetylglucosamine (GlcNAc) compared with healthy controls (Wuhrer et al. 2015b). This finding was more pronounced for PR3-ANCA antibodies than with total IgG.

Potential use of circulating levels of sialylated IgG as a disease biomarker is noteworthy. Serum levels of sialylated IgG have decreased shortly before disease relapses, similar to galactosylated IgG, showing promise as a predictive biomarker (Kemna et al. 2017; Rombouts et al. 2015). Patients with GPA exhibited low galactosylation or low sialylation of total IgG1 and were highly prone to relapse after ANCA increase. When relapsing patients were examined, total IgG1 sialylation decreased from the time of the PR3-ANCA increase to the relapse, while the glycosylation profile remained similar in nonrelapsing patients (Kemna et al. 2017). An intriguing observation is that low sialylation was observed in total IgG1 and not in antigen-specific PR3, and low sialylation of total IgG1 predicted disease reactivation in patients with GPA who experienced ANCA increase during follow-up. Along the same lines, studies have shown a reduction in sialylation of IgG in chronic inflammatory demyelinating polyneuropathy (CIDP), and this reduction

correlated with clinical severity of the disease. This observation implies potential use of sialylated IgG as a biomarker to monitor disease severity in CIDP (Wong et al. 2016). Restoring serum antibody sialylation correlated with treatment response in patients with Guillain-Barre syndrome (Fokkink et al. 2014) and Kawasaki disease (Ogata et al. 2013), where lower sialylation of endogenous IgG predicted resistance to therapy (Ogata et al. 2013). Taken together all the evidence summarized above, monitoring of sialylated IgG antibodies during different phases of an antibody-mediated disease may have diagnostic and prognostic potential and could help optimize the treatment of patients with autoimmune diseases.

3 Role of Sialylation in the Anti-inflammatory Activity of Intravenous Immunoglobulin

Despite the decade-long clinical effectiveness of intravenous immunoglobulin (IVIg) in treating inflammatory and autoimmune diseases, the mechanism of action of IVIg has remained enigmatic. IVIg is effective in a wide range of diseases in which many mechanisms of the underlying disease are not well understood, further complicating the understanding of all the details of IVIg anti-inflammatory mechanisms. Moreover, the fact that IgG can form many different binding interactions through antigen binding and Fc domains adds to the complexity. Many hypotheses have been proposed to explain the reasons for the seemingly paradoxical activity of high-dose IVIg. One theory attributes IVIg activity to the polyclonal binding specificities that are encoded in the variable region of the administered antibodies, which in turn may counteract the activity of autoantibodies and inflammatory mediators, as shown in toxic epidermal necrolysis (Viard et al. 1998). However, intact IVIg and its Fc fragments have almost identical activity in many animal models (Bruhns et al. 2003; Kaneko et al. 2006b; Samuelsson et al. 2001) and in the clinical treatment of idiopathic thrombocytopenic purpura (ITP) (Debre et al. 1993). Therefore, the emergence of evidence attributing the anti-inflammatory activity of IVIg to its Fc portion has promoted further investigation and focus on Fc-mediated mechanisms. For example, saturation of the neonatal FcRn—leading to increased catabolism of pathogenic autoantibodies—has been proposed as one of the primary mechanisms of IVIg (Akilesh 2004; Hansen and Balthasar 2002; Li et al. 2005). However, the evidence to support this proposal is limited because of the difficulty in experimentally modeling FcRn dependence since removing FcRn in mice results in rapid clearance of exogenous autoantibodies and in strongly reduced serum IgG levels (Vaccaro et al. 2005).

Another proposed mechanism is the saturation of activating FcγRs and prevention of pathogenic autoantibodies from triggering FcγRs activation, thereby limiting their pathogenic potential. For example, monoclonal antibodies that block activating FcγRs have mimicked the anti-inflammatory properties of IVIg (Siragam et al. 2006).

Furthermore, the dimeric and aggregate fraction in IVIg may facilitate multivalent avid interactions with low-affinity FcγRs and inhibit their activation by pathogenic immune complexes. Indeed, it has been experimentally shown that the protective effects of IVIg can be mimicked by the injection of opsonized red blood cells or by soluble immune complexes (Siragam et al. 2005). More recently, recombinant multivalent Fc molecules with avid binding to low-affinity FcγRs have also demonstrated similar anti-inflammatory properties to IVIg (Zuercher et al. 2016).

Another proposed mechanism of action for IVIg is the induction of anti-inflammatory activities by altering the relative surface expression of activating and inhibiting FcγRs in favor of the inhibitory FcγRIIb, thereby increasing the signal threshold necessary to activate immune cells (Anthony et al. 2011; Schwab and Nimmerjahn 2013). As it was long postulated, immune complex-induced inflammatory responses are regulated by the relative expression of activating versus inhibitory FcγRs on effector cells, dictating the threshold of immune complex-induced inflammation (Ravetch and Clynes 1998). In fact, it was demonstrated in animal models of ITP, autoimmune hemolytic anemia, rheumatoid arthritis, and nephrotoxic nephritis that the ability of IVIg to protect mice from pathogenic IgG was dependent on FcγRIIb (Akilesh 2004; Crow et al. 2003; Huang et al. 2010; Kaneko et al. 2006a, b; Samuelsson et al. 2001). Similarly, results of several studies showed that FcγRIIb is upregulated on innate immune effector cells and B cells after IVIg treatment in mice (Bruhns et al. 2003; Kaneko et al. 2006b; Samuelsson et al. 2001). Furthermore, this was confirmed in patients with CIDP, in whom FcγRIIb expression levels on B cells were impaired. Interestingly, FcγRIIb protein expression was upregulated on monocytes and B cells after clinically effective IVIg therapy (Tackenberg et al. 2009).

Given all the evidence of anti-inflammatory activities of high-dose IVIg treatment, it has become apparent that the preparations of IVIg from normal human donors indeed contain a small fraction of active therapeutic that is not necessarily an oligomer. The first study to strongly correlate Fc glycosylation and biological activity of IVIg was reported by Kaneko 2006 and was later substantiated by results of several studies in a variety of animal models of autoimmune disease (Anthony et al. 2008a; Kaneko et al. 2006b; Schwab et al. 2012; Tackenberg et al. 2009). Biochemical characterizations of IVIg preparations that display anti-inflammatory activities in animal models pointed to the absolute requirement for the Fc fragment and its N-linked biantennary glycan attached at the Asn-297 (Kaneko et al. 2006b). The authors demonstrated that highly sialylated forms of monoclonal mouse IgG have reduced affinity for FcγR and cytotoxic function. Furthermore, the anti-inflammatory activity of IVIg was shown to be mediated mainly by a small fraction of antibodies containing terminal sialic acid on their oligosaccharide structures. Interestingly, a stronger protective effect of the IVIg fraction enriched for sialic acid was also shown. Enzymatic removal of the sialic acid residues abrogated the anti-inflammatory activity of IVIg in a mouse model of rheumatoid arthritis. These studies also showed that sialylation of the IVIg Fc fragment was sufficient for anti-inflammatory activities (Kaneko et al. 2006b).

4 Impact of Sialylation on IgG Structure and Anti-inflammatory Activity

Work by Ravetch and others has helped shed light on how different IgG glycoforms can impact the anti-inflammatory activity of IgG (Anthony et al. 2008a; Crow et al. 2003; Kaneko et al. 2006b; Samuelsson et al. 2001; Schwab et al. 2014). Structural studies revealed that the IgG-Fc may adopt "open" or "closed" confirmations depending on the glycosylation state. For example, the Fc glycans at position 297 have been shown to contribute to an "open" conformation of the IgG-Fc in which the C_H2 domains are separated by larger distances than in deglycosylated Fc structures (Krapp et al. 2003). Conversely, the deglycosylated IgG displays a conformation in which the C_H2 region adopts a "closed" state (Feige et al. 2009). More recent crystallographic work highlighted a higher conformational heterogeneity of the C_H2 domain in sialylated IgG-Fc protein fragments. The specific interactions between the N297 glycans and the amino acid backbone of the C_H2 domain are interrupted upon terminal sialylation, which can help explain these conformational changes (Ahmed et al. 2014). It was demonstrated how a specific point mutation (F241A) in the Fc domain could recapitulate the flexibility seen with sialylated glycoforms (Fiebiger et al. 2015). These structural characterizations may help explain how earlier studies showed that highly sialylated IgG significantly reduced affinity for the canonical FcγRs (Kaneko et al. 2006b). These changes in Fc structure could also help explain how highly sialylated IgG shows reduced ability to induce effector functions such as ADCC (Scallon et al. 2007). Some findings suggest otherwise, where highly sialylated species were unchanged or only slightly reduced in their ability to bind activating FcγRs (Li et al. 2017; Subedi and Barb 2016). One study suggested that Fc sialylation can also modulate an antibody Fab binding to its cognate antigen via altered flexibility of the hinge region. This reduced flexibility may restrict the antibody to monovalent Fab binding to its target, thus reducing its apparent affinity (Scallon et al. 2007).

In addition to modulating classic FcγR binding, Fc sialylation can increase IgG affinity for type II FcRs including DC-SIGN and CD23 (Anthony et al. 2008a; Sondermann et al. 2013). DC-SIGN (Dendritic Cell-Specific ICAM3-Grabbing Non-Integrin), a C-type Lectin, is found most commonly on the surface of dendritic cells and macrophages. It has been shown to recognize bacterial and viral pathogen-associated molecular patterns and is involved in HIV pathogenesis (Gringhuis et al. 2014; Mogensen et al. 2010). The murine analog of DC-SIGN, SIGN-R1, was shown to be necessary for the anti-inflammatory activity of IVIg in the KBX/N mouse model of arthritis (Anthony et al. 2008a). The activity of IVIg could be restored in SIGN-R1$^{-/-}$ mice when reconstituted with human DC-SIGN. Although SIGN-R1 is not expressed on dendritic cells in mice, it is highly expressed on a population of CSF1-dependent macrophages that reside in the marginal zone of the spleen (Anthony et al. 2008a; Schwab and Nimmerjahn 2013). In 2003, Bruhns et al. had shown that CSF1-dependent macrophages were responsible for the in vivo activity of IVIg. Studies using op/op mice deficient in

CSF1 implied that IVIg induced FcγRIIb upregulation through an indirect mechanism. It was suggested that IVIg may interact with CSF1-dependent regulatory cells, which in turn leads to the upregulated expression of FcγRIIb on separate CSF1-independent effector cells (Bruhns et al. 2003). Work by Anthony and colleagues in 2011 presented a unified hypothesis to help reconcile IVIg requirements for Fc sialylation, FcγRIIb, and SIGN-R1 for its anti-inflammatory activity in various models of autoimmunity. Using the KBX/N model, the researchers showed how IVIg, via interaction with DC-SIGN-positive splenic myeloid cells, caused the release of interleukin (IL) 33, which in turn induced the production of IL-4. IL-4 was then shown to be involved in upregulation of FcγRIIb on effector macrophages, thereby increasing their threshold of immune complex-mediated activation. Mice deficient in the IL-4 receptor were refractory to IVIg therapy, and systemic administration of IL-33 or IL-4 suppressed serum-induced arthritis. The source of IL-4 in these mice was determined to be basophils, which produce Il-4 in response to IL-33 stimulation (Anthony et al. 2011). Figure 1 summarizes the mechanism proposed by Ravetch and coworkers to explain the requirement of Fc sialylation for the anti-inflammatory activity of IVIg. This study laid the groundwork for understanding how intrinsic sialylated IgG could help maintain immune homeostasis. Both IVIg and sialylated IVIg, but not unsialylated IVIg, upregulated IL-33 mRNA in spleens of treated animals, and sialylated IVIg was more potent in a model of antiganglioside antibody-mediated inhibition of axon regeneration, supporting the previously proposed mechanisms (Zhang et al. 2016).

The question of whether this particular anti-inflammatory mechanism of IVIg plays a role in human patients is unclear given that marginal zone macrophages are absent in the human spleen (Steiniger et al. 1997). Furthermore, some ITP patients who underwent splenectomies still respond to IVIg therapy (Cines and Bussel 2005), and studies in ITP mouse models demonstrated that the inhibitory effects of sialylated IVIg were not impaired after splenectomy (Leontyev et al. 2012; Schwab et al. 2012), suggesting a potential different pathway in humans or in specific disease settings. Schwab et al. showed that, in addition to IVIg activity not being impaired in mice that underwent splenectomy, neither the activating FcγRs FcγRI and FcγRIII nor the cytokines requiring the common cytokine γ chain or the IL-33 receptor were involved in this anti-inflammatory pathway suppressing murine ITP. However, IVIg activity was still fully dependent on the terminal sialic acid and SIGN-R1, most likely on a cell type outside the spleen (Schwab et al. 2012). Perhaps IVIg mechanisms might be different depending on whether IVIg is administered before induction of autoimmune disease or inflammation (such is the case in the majority of the reported studies) versus IVIg administration during acute autoimmune disease. Recent studies addressed this question and showed that, under therapeutic conditions in mouse models of ITP, inflammatory arthritis, and the autoimmune skin blistering disease epidermolysis bullosa acquisita, IVIg activity was fully dependent on IgG sialylation and FcγRIIb. However, SIGN-R1 was only partially required, suggesting that select pathways may be involved in mediating IVIg activity when treating established autoimmune disease (Schwab et al. 2014).

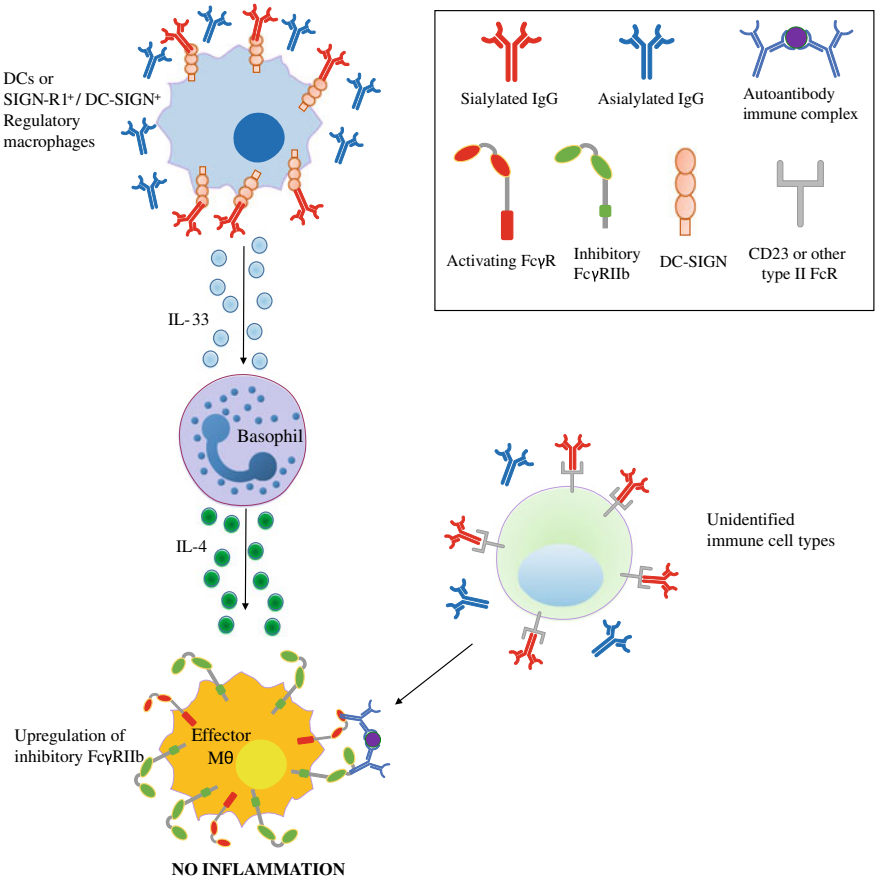


Fig. 1 Schematic representation of mechanism proposed by Ravetch and coworkers to explain the requirement of Fc sialylation for IVIg anti-inflammatory activity (Anthony et al. 2008b; Bruhns et al. 2003; Sondermann et al. 2013; Maamary et al. 2017) Ig, immunoglobulin; IL, interleukin

However, the exact pathways during patient treatment with IVIg were not addressed in those studies.

Some recent studies have helped elucidate whether this T-helper type 2 (TH2) cascade occurs in human patients treated with IVIg. IVIg studies in humans have shown that high-dose IVIg does induce IL-33 and IL-4 in patients with various inflammatory myopathies (Schwab et al. 2014; Tjon et al. 2014). Similar results with IL-33 were seen in IVIg-treated patients with Guillain-Barre syndrome, but this was not a predictor of therapeutic success (Maddur et al. 2017). The study by Tjon et al. aimed to address the variations between mice and humans and demonstrated that the IL-33 TH2 pathway by which IVIg inhibits myeloid cells in mice may also be used in humans but with slight variations (Tjon et al. 2014). Namely, instead of FcγRIIb upregulation, FcγRIIa was downregulated on circulating myeloid dendritic cells (mDCs) from IVIg-treated patients while FcγRIIb

expression remained unchanged. In vitro, IL-4 induced this change in FcR expression on purified mDCs. Although not in line with observations in mice of FcγRIIb upregulation, the change in the ratio of activating and inhibitory Fc receptors may produce the same result of higher threshold for immune complex-mediated activation of these cells. The source of IL-33 in humans remains undiscovered. One study did note an increased expression of IL-33 mRNA in human lymph node cells and monocyte-derived macrophages in the presence of IVIg and LPS, but this effect was independent of DC-SIGN (Tjon et al. 2014). More controlled studies with IVIg in the clinic are necessary to further elucidate this TH2 cascade in human patients.

Further evidence of the crucial role of Fc sialylation in IVIg efficacy was demonstrated more recently, where tetra-Fc-sialylated IVIg enhanced anti-inflammatory activity up to 10-fold higher than IVIg across different animal models (Washburn et al. 2015). Specifically, the tetra-Fc-sialylated IVIg was more potent than the parent IVIg product when dosed prophylactically in a model of collagen antibody-induced arthritis (CAIA) and a murine skin blistering model. Therapeutic anti-inflammatory benefit with the tetra-Fc-sialylated IVIg treatment at the time of active inflammation was also shown in murine K/BxN-induced arthritis and ITP models. One study also extended the importance of IVIg sialylation in bone loss in patients with rheumatoid arthritis. Desialylated immune complexes enhanced osteoclastogenesis in vivo and in vitro. Mice treated with the sialic acid precursor *N*-acetylmannosamine, which increases IgG sialylation, were less susceptible to inflammatory bone loss, suggesting a protective role of sialylated IgG in autoimmune-mediated bone loss (Harre et al. 2015).

5 Impact of IgG Sialylation on the Adaptive Immune System

The observation that T cells, purified from IVIg-treated nonimmunodeficient children, significantly suppressed pokeweed mitogen-induced B-cell and T-cell proliferation provided the first indication that IVIg could modulate the adaptive immune system (Durandy et al. 1981). In vitro experiments from the same study showed that this modulation of T-cell suppressor activity was Fc-mediated. As a follow-up, other publications noted the increase in the suppressive activities of T cells after IVIg treatment in ITP and AIDs (Delfraissy et al. 1985; Gupta et al. 1986). Since then, IVIg has been shown to increase the number of circulating T-regulatory cells (Tregs) in Guillain-Barre syndrome, SLE, eosinophilic granulomatosis with polyangiitis (Churg–Strass syndrome), Kawasaki disease, and a peripheral neuropathy known as mononeuritis multiplex, among others (Barreto et al. 2009; Burns et al. 2013; Chi et al. 2007; Tsurikisawa et al. 2012, 2014). Various mechanisms have been proposed to explain how IVIg can modulate suppressor T-cell function and frequency. One possible Fc-specific mechanism was

described in patients with Kawasaki disease where IVIg treatment induces the expansion of IL-10-producing Tregs with TCR specificity for peptides derived from IgG-Fc (Franco et al. 2014). It was later shown by the same group that these Tregs could be activated in a major histocompatibility complex (MHC)-restricted manner by autologous IgG-positive B cells in the absence of exogenous Fc. Interestingly, these Fc-specific Tregs were present in IVIg-treated patients with Kawasaki disease patients and in healthy adult donors (Burns and Franco 2015).

Successful induction of Treg responses requires signals in the form of soluble cytokines and costimulatory molecules, typically provided by professional antigen-presenting cells (APCs) such as dendritic cells, macrophages, and B cells. IVIg has been shown to modulate dendritic cell function in various ways. Decreased expression of costimulatory markers such as CD80/86 and MHC class II on IVIg-exposed dendritic cells results in decreased capacity for these cells to drive lymphocyte proliferation (Bayry et al. 2003; Kaufman et al. 2011; Qian et al. 2011). In addition, IVIg has been shown to induce dendritic cell production of inhibitory and anti-inflammatory cytokines such as IL-10 (Bayry et al. 2003; Massoud et al. 2012; Ohkuma et al. 2007; Ramakrishna et al. 2011). Work from De Groot et al. and others has suggested a role for dendritic cells in the presentation of IgG-derived T-cell epitopes known as "Tregitopes," which contain peptides from both the Fab and the Fc fragments of IgG (De Groot et al. 2008; Ephrem et al. 2008). In the DeGroot model, dendritic cells uptake IVIg and process it for loading of peptides onto MHC class II, allowing for activation and expansion of Tregs.

Specifically, it is debatable which receptors on APC IVIg might interact with. The obvious candidates are the type I and type II FcRs. FcγRIIa and FcγRIIb are found on subsets of dendritic cells and macrophages. Activating FcγRs on dendritic cells are necessary for successful treatment of ITP (Siragam et al. 2006). In this model, the researchers hypothesized that IVIg formed immune complexes in vivo, which acted on dendritic cells, thus priming their regulatory activity. The investigators developed an adoptive transfer model in which isolated dendritic cells were primed with IVIg in vitro and subsequently transferred to mice with active ITP. The IVIg-primed dendritic cells were able to ameliorate ITP in these mice even when the dendritic cells lacked FcγRIIb. Interestingly, the IVIg-primed dendritic cells were unable to ameliorate ITP when the host mouse lacked FcγRIIb expression. This suggests that the IVIg-dendritic cell interaction is independent of FcγRIIb, but the receptor was still necessary for later phases of IVIg action (Siragam et al. 2006). Substantiating this finding, IVIg and immune complexes containing sialylated IgGs were able to inhibit the LPS-induced maturation of bone marrow-derived dendritic cells independently of FcγRIIb (Oefner et al. 2012). One study, using a similar model of adoptive transfer, showed that IVIg-primed bone marrow-derived dendritic cells (BMDCs) could induce Treg activity and be used to successfully treat mice with ovalbumin-induced airway hyperresponsiveness (AHR), but IVIg effectiveness was not disturbed when the BMDCs lacked activating FcRs (Massoud et al. 2014). In this same study, the researchers found that IVIg ability to ameliorate AHR was almost entirely dependent on the dendritic cell immunoreceptor (DCIR). DCIR is an ITIM-bearing C-type lectin receptor found mostly on monocyte and

dendritic cell populations (Kanazawa 2007). Massoud et al. showed that Treg induction in AHR by IVIg was dependent on Cd11c-positive dendritic cells, but they were unable to detect expression of the type II Fc receptor SIGN-R1 on these cells. Instead, they observed DCIR-dependent binding of sialylated IVIg glycoforms and DCIR-dependent induction of Tregs.

The type II FcγR SIGN-R1 is expressed mainly on macrophages in mice, whereas DC-SIGN is expressed on myeloid and plasmacytoid dendritic cells and on some macrophages in humans. Fieberger et al. reported that IVIg required DC-SIGN/SIGN-R1 in amelioration of both T-cell-mediated and autoantibody-mediated diseases (Fiebiger et al. 2015). They demonstrated that a single-point mutation at F241A in recombinant Fc, even when lacking terminal sialic acid, conferred the same protections as sialylated Fc in the KBX/N model of arthritis. IVIg and the F241A Fc but not unsialylated IVIg were shown to expand FoxP3+ Tregs in KBX/N-naive and KBX/N-challenged mice. Both IVIg and F241A Fc were also able to expand Treg and suppress T-cell-mediated autoimmunity in the experimental autoimmune encephalitis (EAE) model in a SIGN-R1/DC-SIGN-dependent manner. Finally, inhibition of IL-33 signaling abrogated IVIg and F241A Fc ability to induce Treg and suppress inflammation, lending more support to this group's original proposal of TH2 cytokine cascade as the underlying cause of sialylated IVIg anti-inflammatory activity (Fiebiger et al. 2015). Do DCIR and DC-SIGN/SIGN-R1 operate independently of one another to induce Treg expansion and activity in response to sialylated IVIg treatment? More studies are necessary to address how these receptors and the cells upon which they are expressed mediate IVIg ability to induce Treg.

Modulation of T-cell responses in models of delayed-type hypersensitivity (DTH) by sialylated IgGs was reported by Oefner et al. in 2012. DTH reactions require antigen uptake, processing, and presentation to T-helper cells, which in turn produce proinflammatory cytokines, resulting in tissue inflammation. In the Oefner et al. model of ovalbumin-induced DTH injection of sialic-rich ovalbumin-specific IgG, but not antibodies of different antigen specificity, they were able to suppress DTH responses. Sialylated immune complexes were shown to inhibit dendritic cell maturation in an FcγRIIb-independent manner, which could explain this inhibition of DTH. Although both the antigen specificity and the FcγRIIb independence of the sialylated IgG ability to inhibit DTH in this model are in contrast to reports of IVIg-mediated suppression of antibody responses, modulation of T-cell responses is still a common denominator (Oefner et al. 2012).

6 Modulation of Autoantibody Pathogenicity by Sialylation

Although most understanding of the anti-inflammatory activity of IgG comes from experience with IVIg, more recent studies have helped translate the IVIg findings to antigen-specific IgG autoantibodies. One particular study elegantly demonstrated

how antigen-specific antibodies, when sialylated, could be used not only to reverse autoantibody-mediated inflammation, but also to prevent development of disease. Ohmi et al. showed that genetic knockdown of mSt6gal1, a glycotransferase responsible for transferring sialic acid to galactose, specifically in activated B cells resulted in higher incidence and earlier development of collagen-induced arthritis (CIA), along with more severe joint swelling in the affected mice (Ohmi et al. 2016). Enforcing sialylation of the ACC4 monoclonal antibody used to stimulate collagen antibody-induced arthritis in DBA-1 mice abolished the development of disease in all mice. Using the CIA model, the researchers demonstrated that, after immunization with bovine type II collagen, treatment of mice with sialylated ACPAs resulted in delayed incidence of disease and less severe joint swelling. In addition, treatment of CIA with the sialylated ACPAs resulted in in vivo production of sialylated anti-type II collagen antibodies (Ohmi et al. 2016).

The anti-inflammatory activity of specific sialylated antibodies was also shown in models of IgG- and IgE-mediated anaphylaxis. Although allergen-induced anaphylaxis is mostly induced by IgE, it can also be mediated by IgG produced in response to high levels of allergen. In the presence of excess allergen, these IgGs have the potential to activate complement and FcγRs on various effector cells, leading to anaphylaxis. However, allergen-specific IgG antibodies, which are often induced by allergen-specific immunotherapies (AITs), have also been shown to inhibit IgE-mediated anaphylaxis (Burton et al. 2014; Strait et al. 2006). This is accomplished through a combination of allergen masking and FcγRIIb engagement. Just as FcγRIIb can mediate inhibition of the activating FcγRs, cross-linking of FcγRIIb with the IgE receptor FcεRI on basophils and mast cells can prevent activation of these cells and, thus, limit release of various proinflammatory mediators implicated in allergen-induced anaphylaxis (Strait et al. 2006). In line with these observations, TNP-specific IgG1 antibodies were unable to inhibit anti-TNP IgE-mediated anaphylaxis in response to challenge with TNP-Ova in FcγRIIb$^{-/-}$ mice (Epp et al. 2018). In a similar model, it was shown that desialylated and degalactosylated IgG1 and IgG2 anti-TNP antibodies induce more severe anaphylaxis in response to challenge with TNP-Ova. In contrast, sialylated anti-TNP IgG1 effectively inhibited TNP-Ova-induced anaphylaxis. This effect was dependent on FcγRIIb and SIGN-R1. In humans, AITs typically elicit allergen-specific IgG4 antibodies. These studies used mouse IgG1 antibodies, which resemble human IgG4 in their complement and classical FcγR binding (Epp et al. 2018). This study highlights the possibility that AIT protocols that induce sialylated IgGs might limit IgG-mediated anaphylaxis in patients exposed to high doses of allergen. To this end, the researchers showed that immunization of mice with Ova in the adjuvant monophosphoryl lipid A, which was recently approved for AIT, resulted in production of sialylated and galactosylated IgG1 and IgG2, which were limited in their ability to induce IgG-mediated anaphylaxis (Epp et al. 2018).

Immune complexes formed by autoantibodies and their cognate antigens have also been shown, in certain scenarios, to mediate anti-inflammatory processes. As discussed in previous sections, it is clear that IgG glycosylation changes during

disease progression and sialylation is often correlated with low disease activity (Espy et al. 2011; Fickentscher et al. 2015; Kemna et al. 2017; Parekh et al. 1985; Pasek et al. 2006). In vitro analysis of these sialylated autoantibodies and immune complexes has helped shed light on some of the mechanisms by which inflammation is controlled by antibody glycosylation. In the case of SLE, antihistone antibodies isolated from patients were shown to be mostly asialylated and were able to mediate efficient phagocytosis when used to opsonize necrotic cells, a hallmark of SLE pathology (Magorivska et al. 2016). Sialylated autoantibodies isolated from these patients were less effective at mediated neutrophil phagocytosis and switched inflammatory cytokine production form IL-6/IL-8 to IL-1b/TNF-α (Magorivska et al. 2016). Earlier studies had shown similar results in anti-PR3 antibodies isolated from patients with active GPA (Espy et al. 2011). The authors demonstrated that autoantibodies isolated from patients with active disease, exhibiting low sialic acid content, induced a greater oxidative burst in neutrophils than those from patients with quiescent disease. Enzymatic desialylation of anti-PR3 antibodies from those patients with inactive GPA resulted in restoration of pathogenic activity (Espy et al. 2011). In rheumatoid arthritis, anticitrullinated protein antibodies (ACPAs) are known to induce innate cell-mediated inflammation in and around joints, resulting in bone destruction (Kurowska et al. 2017). These ACPAs can contain less Fc sialylation than other circulating IgGs (Ohmi et al. 2016; Rombouts et al. 2015; Scherer et al. 2010). Studies in mice have shown that these ACPAs promote osteoclastogenesis, resulting in bone resorption. In following up on these findings, it was found that desialylated immune complexes increased osteoclastogenesis in an FcγRII- and FcγRIII-mediated manner. Furthermore, treatment of CIA in mice with ManNAc, a precursor to sialic acid, resulted in increased sialylation of circulating IgG1, delayed induction of arthritis, and reduced severity of the disease (Harre et al. 2015).

Taken together, these studies show that, when sialylated, antigen-specific autoantibodies possess anti-inflammatory properties. The results of these studies have large implications for the development of antigen-specific immunotherapies for autoimmune disorders whose autoantigens are well defined. In line with this notion, a recent study took a different approach and attempted to sialylate antibodies in vivo as a means of making them anti-inflammatory. The researchers investigated how administration of B4GALT1 and ST6GAL1, glycotransferases that transfer galactose and sialic acid, respectively, modulated autoantibody function in murine models of arthritis. Coadministration of these enzymes before the transfer of K/BxN sera resulted in marked reduction in inflammation similar to that seen with IVIg treatment (Pagan et al. 2018). This treatment was also effective in a model of Goodpasture syndrome, in which coadministration of the enzymes markedly inhibited kidney disease. Keeping with the aforementioned TH2 anti-inflammatory cascade initiated by sialylated IgG, in vivo sialylation and the resulting anti-inflammatory activity achieved with these enzymes had a strict requirement of FcγRIIb and SIGN-R1 (Pagan et al. 2018).

7 Anti-inflammatory Activity of Multivalent IgG-Fc

Immune complexes formed by the interaction between autoantibodies and self-antigens are drivers of pathogenicity in many inflammatory and autoimmune diseases. The inflammatory mechanisms are driven by activation of complement pathways or through FcγR-mediated cellular activation. Briefly, all FcγRs except FcγRI bind Fc domains with low affinity. For effective cell activation, FcγRs require immune complexes for their aggregation, resulting in ultimate activation of downstream signaling. Inhibition of these pathways through competition with IgG-Fc molecules with high valency has been explored as a potential therapeutic alternative. In fact, commercial IVIg preparations may contain a small fraction of higher-order IgG structures in the form of dimers and aggregates, which presumably are important for the anti-inflammatory activities of IVIg (Teeling et al. 2001).

All these observations have stimulated the development of recombinant oligomeric Fc proteins as potential replacement for IVIg (Zuercher et al. 2016). Figure 2 provides an overview of multimeric Fc drug candidates currently in preclinical or clinical studies. Stradomer (GL-2045) generated by Gliknik/Pfizer (Gliknik. Gliknik pipeline products. http://www.gliknik.com/pipeline/) is a drug candidate consisting of a mixture of heterogenous IgG-Fc oligomeric structures. In particular, stradomers are fully recombinant fusion proteins composed of murine IgG2a-Fc hinge and either the human IgG2H or isoleucine zipper multimerization domains, existing as homodimers and as a high percentage of covalently linked highly

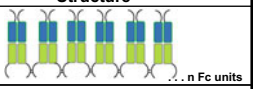
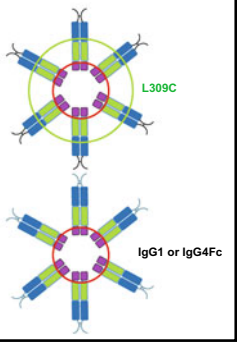
Drug Candidate	Description	Institution	Structure
Stradomer GL2045	Heterogeneous IgG-Fc multimer with IgG2 hinge	**Gliknik/Pfizer**	
Fc3Y or M230/CSL730	**3 IgG1-Fc domains**	**Momenta Pharmaceuticals/CSL**	
Hexamers	HexaGard: Hexameric IgG-Fc with IgM tailpiece with L309/H310L CSL777: Hexameric IgG-Fc with IgM tailpiece with L309C γ1 or γ4: IgG1 or IgG4 Hexameric Fc	Liverpool School of Tropical Medicine **CSL** **UCB**	

Fig. 2 Summary of multimeric Fc drug candidates being explored as anti-inflammatory agents. Ig, immunoglobulin

ordered multimers (Jain et al. 2012). Stradomers prevented the onset of ITP and ameliorated symptoms in other autoimmune disease models, including collagen-induced arthritis (Jain et al. 2012), experimental autoimmune neuritis (Niknami et al. 2013), and autoimmune myasthenia gravis (Thiruppathi et al. 2014). This drug candidate is in preclinical development, and, in 2015, it received orphan drug status in the USA for the treatment of chronic inflammatory demyelinating polyradiculoneuropathy (Adis Insight. Research program: autoimmune disorder therapeutics—Gliknik GL-2045. http://adisinsight.springer.com/drugs/800035077).

The knowledge of how IgM polymerizes via Fc domains was leveraged to develop a novel approach to generating recombinant polymeric Fc-fusion proteins. Specifically, more controlled oligomers—hexameric Fc proteins—were generated by Richard Pleass as potential alternatives to IVIg (Czajkowsky et al. 2015; Mekhaiel et al. 2011). These hexameric proteins were shown to bind low-affinity inhibitory receptors FcRL5, FcγRIIb, and DC-SIGN with high avidity and specificity, although they can also activate complement pathways (Czajkowsky et al. 2015). These proteins have yet to advance to the clinic, but they have great potential as more effective and less expensive to produce than IVIg. Another hexameric Fc-fusion protein was created by UCB, which was shown to alter receptor density and trigger internalization and degradation of FcγRs in vitro (Qureshi et al. 2017). As a result, the molecule caused Fc binding disruption and phagocytosis in vitro and the inhibition of platelet phagocytosis in a mouse ITP model. However, the molecule also exhibited a short half-life in mice and cynomolgus monkeys. This work pointed to yet another mechanism through which oligomeric Fc compounds can exert anti-inflammatory activity. This molecule has not been advanced to clinical use. Most recently, CSL generated an rFc hexamer, termed Fc-mTP-L309C, by fusing IgM μ-tailpiece to the C terminus human IgG1-Fc (Spirig et al. 2018). The hexamer bound FcγRs with high avidity and could inhibit a range of in vitro FcγR-mediated effector functions such as ADCC, phagocytosis, and respiratory burst. In addition, the molecule potently inhibited the activation of the classic complement pathway. In vivo, Fc-mTP-L309C suppressed inflammatory arthritis in mice at a 10-fold lower dose than IVIg, and, in a mouse model of immune thrombocytopenia, it successfully restored platelet counts. The authors hypothesize that the mechanism of action exerted by this hexamer is through blockade of FcγRs and through its unique inhibition of complement activation.

The concept of selectively antagonizing the activating FcγR system without activating proinflammatory pathways was introduced by Momenta Pharmaceuticals (Ortiz et al. 2016). Through novel insight into the modulation of the FcγR pathways, a trivalent-IgG-Fc recombinant product candidate—Fc3Y, or M230—was designed. M230 was designed to preferentially assemble into a homogeneous trimeric IgG-Fc using knobs into hole and electrostatic steering molecular heterodimerization technology. Trivalent M230 exerts avid binding to FcγRs, without inadvertently activating immune cell effector functions, resulting in a potent molecule that inhibits immune complex-driven processes in many cells that express FcγRs. A main driver for the design of M230 was the identification of a threshold for activation of the FcγR signaling pathways with Fc-multimers containing five or

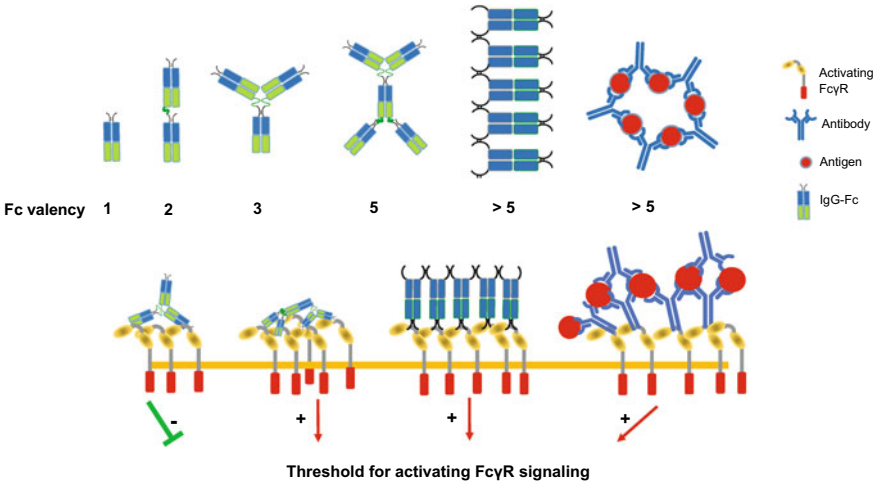


Fig. 3 Schematic representation of the IgG1-Fc valency threshold required for FcγR signaling identified by Bosques and coworkers

more Fc units but not with multivalent Fc containing three or less Fc units (Fig. 3). Furthermore, M230 has been shown to have 40-fold greater potency than IVIg in animal models of autoimmune diseases such as arthritis, immune thrombocytopenia, and epidermolysis bullosa acquisita skin blistering. This drug candidate is in clinical development in collaboration with CSL, and it may represent an effective therapeutic candidate for FcγR-mediated autoimmune diseases.

8 Conclusion

Studies from the past 15 years have shed new light on the mechanisms by which IgG can perform pro- and anti-inflammatory activities. Through detailed biophysical characterization, use of in vitro and in vivo models, and biomarker studies in clinical investigation, the role of glycosylation in the anti-inflammatory and immunoregulatory properties of IgG has been well established. At the molecular level, studies have shown the far-reaching effects of terminal Fc sialylation and have demonstrated the ability of this sugar moiety to modulate Fc structure and interactions with FcRs. In addition to the use of IVIg in the clinic, the relationship between Fc glycosylation states and disease activity in various autoimmune disorders has provided valuable human evidence of the immunoregulatory effects of IgG. Detailed in vitro and in vivo studies have elucidated the key receptors, cells, and signaling cascades involved in these anti-inflammatory processes. This knowledge has enabled the evaluation of new therapeutics, such as multivalent Fc drug candidates and hypersialylated IVIg, which are being developed to harness the anti-inflammatory effects of IgG.

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