Anti-Inflammatory Actions of Intravenous Immunoglobulin

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Key Words

Fc receptor, inflammation, glycosylation, autoimmunity, autoantibody, sialic acid, IVIG

Abstract

The remarkable success story of the therapeutic application of pooled immunoglobulin G (IgG) preparations from thousands of donors, the so-called intravenous IgG (IVIG) therapy, to patients with a variety of hematological and immunological disorders began more than half a century ago. Since then, the use of this primary blood product has increased constantly, resulting in the serious danger of shortages in supply. Despite its widespread use and therapeutic success, the mechanisms of action, especially of the anti-inflammatory activity, are only beginning to be understood. In this review, we summarize the clinical use of IVIG for different diseases and discuss recent data on the molecular mechanisms that might explain how this potent drug mediates its activity in vivo.

INTRODUCTION

IgG: immunoglobulin G

IVIG: intravenous IgG therapy

ITP: idiopathic thrombocytopenic purpura

Immunoglobulins together with T cells are the key mediators of adaptive immunity, and deficiencies in either of these two arms of the adaptive immune system can result in a heightened susceptibility to bacterial, fungal, or viral infections. A variety of situations can lead to a constant or transient deficiency in immunoglobulins, including primary immunodeficiencies, such as several X-linked agammaglobulinemias and common variable immunodeficiencies (CVID) (1–3). In addition, hypogammaglobulinemic phenotypes can be caused by viral infections (for example with HIV), in the course of B cell malignancies, or after bone marrow transplantation (4–9). Thus, replacement of immunoglobulin levels, especially of the immunoglobulin G (IgG) isotype, by administration of pooled serum from healthy donors—IVIG (intravenous IgG) as a therapeutic agent—occurred more than 50 years ago. Since then, IVIG use has increased exponentially, although its therapeutic effectiveness in most of these different diseases has not been rigorously addressed, in part because of the variety of diseases it is used to treat and in part because extant studies include low patient numbers.

The popularity of IVIG in the clinic and in research is exemplified by the 360 hits that a PubMed search for IVIG yielded in 2007, when we wrote this review. The two major clinical indications for which IVIG is used are IgG replacement therapy and anti-inflammation therapy in a variety of acute and chronic autoimmune diseases (**Table 1**). This latter approach is based on an early observation that a child with idiopathic thrombocytopenic purpura (ITP) showed an attenuated platelet clearance after IVIG administration, which was confirmed in adult patients shortly thereafter (10, 11). Since then, IVIG administration has been included in the therapy of many chronic autoimmune diseases affecting a wide range of tissues and target organs, such as the skin, joints, central nervous system, and hematopoietic system (6). Aside from immunoglobulin replacement therapy, currently licensed applications for IVIG administration include Guillain-Barrè syndrome, Kawasaki disease, and chronic inflammatory demyelinating polyneuropathy

Table 1 Examples for the clinical use of IVIG (see text for details)

	Anti-inflammatory (high-dose therapy)	
Replacement (low-dose therapy)	**Licensed**	**Off-label**
Primary immunodeficiencies (CVID and others)	Idiopathic thrombocytopenia purpura	Autoimmune neutropenia
HIV infection	Guillain-Barré syndrome	Autoimmune hemolytic anemia
Bone marrow transplantation	Kawasaki disease	Anti–Factor VIII autoimmune disease
B cell lymphocytic leukemia	Chronic inflammatory demyelinating polyneuropathy (CIDP)	Multiple sclerosis
Multiple myeloma		Myesthenia gravis
		Stiff person syndrome
		Multifocal neuropathy
		Systemic vasculitis (ANCA positive)
		Polymyositis
		Dermatomyositis
		Rheumatoid arthritis
		Systemic lupus erythematosus
		Antiphospholipid syndrome
		Toxic epidermal necrolysis
		Autoimmune skin blistering diseases
		Steroid-dependent atopic dermatitis
		Graft-vs-host disease
		Sepsis syndrome

(CIDP). Licensed indications, however, only account for approximately 40%–50% of the worldwide IVIG sales, as most IVIG administrations are "off-label" (8, 12).

Although in many instances positive results have been reported, well-controlled clinical studies addressing the beneficial effects of IVIG therapy are lacking for most of these diseases. One distinguishing feature between the use of IVIG as a replacement therapy and its use as an anti-inflammatory agent is the therapeutically active dose. If used as an IgG replacement, a dose of 300–500 mg/kg body weight of IVIG is recommended to achieve serum levels of approximately 500 mg/dl, which is sufficient to prevent or substantially reduce pulmonary infections, one of the major complications in immunocompromised patients; a dose in this range is considered low-dose therapy. Consistent with the serum half-life of IgG, this treatment must be repeated every 3–4 weeks to maintain a protective serum level (6). To achieve the anti-inflammatory effect, however, doses in the range of 1–3 g/kg body weight are required repeatedly (high-dose therapy) (13). Whereas the mechanism of IVIG activity in IgG replacement therapy may be readily explained by the presence of so-called natural antibodies that have an intrinsic capacity to recognize foreign antigens or by the presence of common pathogen-specific IgG antibodies derived from previously immunized or vaccinated serum donors, the explanation of the anti-inflammatory activity is more complicated and a matter of much debate. The difficulty is to explain how a polyclonal mixture of IgG molecules can suppress the activity of the very same class of molecules, that is, other IgG antibodies recognizing autoantigens.

A diverse array of mechanisms, including the blockade of cellular receptors for immunoglobulins (Fc receptors, or FcRs), inhibition of the complement cascade, modulation of cytokine production, neutralization of autoantibodies, and the modulation of inhibitory FcR expression, have been suggested as being responsible for the anti-inflammatory activity of IVIG (**Table 2**) (6, 8, 14, 15). Considering the heterogeneous array of diseases treated with IVIG, one might expect a similarly complex number of mechanisms of action. In this review, we discuss these different possible mechanisms, with an emphasis on results obtained in in vivo model systems. Owing to space limitations, we do not include the immunoglobulin replacement aspect of IVIG therapy, which has been covered in several excellent recent reviews (16, 17). We start with a brief description of the drug IVIG and an introduction into IgG-mediated effector responses, and we follow with a detailed discussion of the different potential mechanisms

Table 2 Proposed mechanisms for IVIG activity

Replacement therapy	Anti-inflammatory therapy
Preventing infection with pathogenic microorganisms and improving quality of life	Autoantibody neutralization[a]
Reducing risk of graft-vs-host disease after bone marrow transplantation	Modulation of antibody production[a]
	Modulating signaling pathways[a]
	Modulation of cytokine expression and function[a,b]
	Modulating DC maturation and function[a,b]
	Complement inhibition[a,b]
	Enhancing autoantibody clearance by blocking the FcRn[b]
	Functional blockade of activating FcγRs[b]
	Upregulation of the inhibitory FcγRIIB[b]
	Restoring an anti-inflammatory milieu[b]

[a] $F(ab)_2$-mediated activity.
[b] Fc fragment–mediated activity.

that might explain the therapeutic activity of IVIG in vivo.

FcRn: neonatal Fc receptor

FcγR: Fcγ receptor

IVIG: PRODUCTION PROCESS, PRODUCT SAFETY, AND SIDE EFFECTS

IVIG is produced by many different companies and a variety of nonprofit organizations such as the American Red Cross (13). With such diverse providers, the compositions of the different IVIG preparations vary in the purity of the IgG preparation, pH, osmolarity, and sodium and sugar content (8, 13). The other most dominant immunoglobulin isotype, IgA, ranges from trace amounts up to 1–2 mg/ml in the different IVIG preparations. IVIG is a primary blood product and must be manufactured according to World Health Organization guidelines. The careful selection and testing of donors for blood transmittable viral diseases, such as HIV, HBV, and HCV, and the incorporation of virus-inactivating steps in the production process are of utmost importance (18). Similarly, the concentration step to obtain a highly enriched IgG preparation has to be selected carefully to prevent loss of biological activity and to minimize unwanted side effects such as the formation of protein aggregates.

Most of the currently used techniques were developed in the 1940s and 1950s and only slightly modified since. Thus, either a cold ethanol precipitation step or the more recently developed caprylate precipitation followed by anion exchange chromatography are used (18). With the optimization of these methods and strict quality control measures, the side effects of IVIG administration are relatively minor today, but they may include a transient headache, nausea, fever, cough, and sore throat (13, 19). In many instances, not the IgG preparation itself but rather product stabilizers such as sugar or salt content or denatured aggregated proteins are responsible for adverse effects (13). Therefore, one quality check is to prevent the presence of significant amounts of multimeric IgG aggregates that may trigger activating FcRs unspecifically. Ultimately, replacing IVIG with a recombinant product would be advantageous to circumvent these side effects and to prevent supply shortages. For this, however, we must know the molecular mechanism of IVIG activity, which is the focus of the following section.

IgG ACTIVITY IN VIVO

As indicated above, both the therapeutically active molecule in the IVIG preparation and the pathology-causing agent in autoimmune diseases are IgG molecules. Thus, we give a brief overview of how IgG antibodies are thought to mediate their activity in vivo.

IgG and other immunoglobulin isotypes are composed of an amino acid backbone that contains a sugar moiety attached to an asparagine residue in the antibody constant region (N297) (20, 21). Mice and humans have four different IgG subclasses, denominated IgG1, 2a, 2b, and 3 in mice and IgG1–4 in humans. These are present in the mg/ml range in the serum and have a relatively long half-life, in the range of one week (22). In contrast to other serum proteins, the half-life of the different IgG subclasses is not regulated by the hepatic asialo receptor, which purges the blood of nonfunctional proteins. For IgG molecules, the neonatal Fc receptor, FcRn, plays a crucial role in keeping IgG levels constant. FcRn belongs to the family of major histocompatibility class I (MHC class I) molecules, and mice deficient in this protein or its associated β2-microglobulin (β2M) chain have a dramatically reduced IgG serum concentration and half-life (23, 24).

IgG molecules' proinflammatory activity requires recruiting secondary effector functions via their Fc fragment. Cytotoxic or proinflammatory pathways that can be activated via the Fc fragment include the complement pathway and crosslinking of cellular FcRs for IgG (Fcγ receptors, FcγR) on innate immune effector cells (25, 26). The family of FcγRs consists of several activating members and one inhibitory member that are

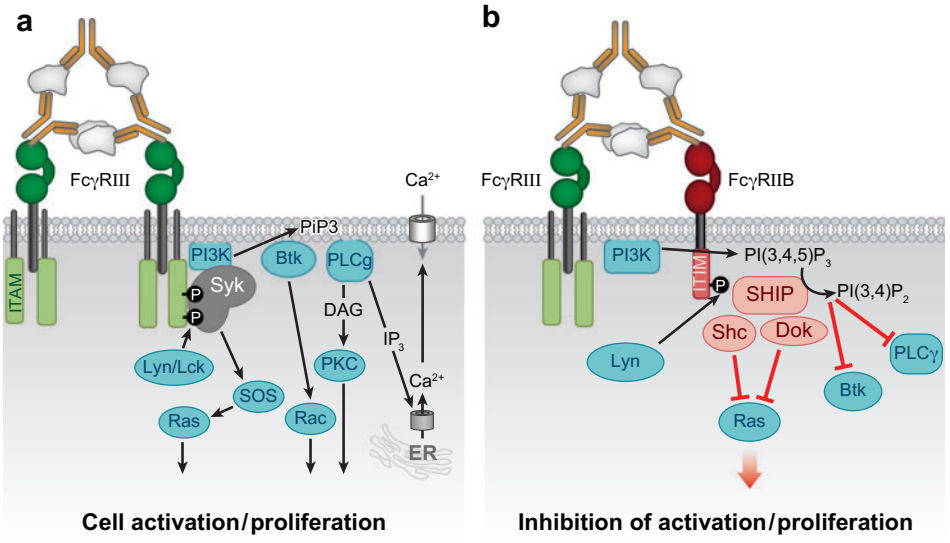

Figure 1

Activating and inhibitory Fcγ receptors set a threshold for immune effector cell activation. (*a*) Immune complex (IC) binding to activating FcγRs results in the ITAM phosphorylation of the receptor-associated γ_c-chain, creating docking sites for Syk kinases. Syk activates several downstream kinases such as the phosphatidylinositol-3 kinase (PI3K), leading to the recruitment of Bruton's tyrosine kinase (BTK) and phospholipase Cγ (PLCγ) to the plasma membrane, and ultimately leading to calcium influx into the cytosol from intracellular and extracellular sources. (*b*) The inhibitory FcγRIIB interferes with these activating signaling pathways by recruiting phosphatases such as SHIP (SH2-containing inositide phosphatase) that hydrolyze phosphatidylinositol signaling intermediates necessary for recruitment of BTK and PLCγ, thus limiting cell activation. [Reprinted from *Springer Seminars in Immunopathology* (2006), 28:305–19, Copyright 2006 with kind permission of Springer Science and Business Media.]

usually coexpressed on the same cell, thereby setting a threshold for cell activation by simultaneously triggering counteracting signaling pathways (**Figure 1*b***) (27). Crosslinking of activating FcγRs leads to the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) present in receptor-associated signaling adaptor proteins such as the common γ-chain (γ_c-chain) by Src family kinases. This phosphorylation induces several downstream signaling pathways, resulting in an increase in intracellular calcium levels and cell activation (**Figure 1*a***). Simultaneous triggering of FcγRIIB leads to phosphorylation of the immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytosolic domain of this receptor, which in turn recruits phosphatases such as the SH2-domain containing inositol polyphosphate 5′ phosphatase (SHIP) that interfere with these activating signaling pathways at several stages (**Figure 1**).

The individual receptors differ in their affinity and specificity for the different IgG subclasses. In mice and humans, there is one high-affinity receptor, FcγRI, which is the only FcγR that can bind to monomeric IgG. All other family members have a low to medium affinity, in the micromolar range, and exclusively bind to antibodies bound to their respective antigen, including soluble proteins, microorganisms, and malignant or virus-infected cells in the form of immune complexes (IC). Thus, the relative expression level and affinities of these different family members are important for regulating antibody activity in vivo (26). This ratio can be influenced by several cytokines and other pro- or anti-inflammatory stimuli, such

IC: immune complex

as lipopolysaccharide (LPS), several interleukins, transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), and C5a (26). Effector functions that are triggered upon IC binding to FcγRs and regulated by balanced FcγR expression include phagocytosis, degranulation, antigen presentation, release of proinflammatory cytokines, and antibody-dependent cellular cytotoxicity (ADCC) (27–29).

Although several IgG subclasses can efficiently activate the complement pathway and mediate target cell lysis in vitro, experimental evidence from in vivo model systems argues against an important role of the complement pathway for these antibody-mediated effector functions (26, 30). Because IVIG and the autoantibodies responsible for chronic inflammation and tissue destruction can bind to the same effector cells and molecules, one attractive possibility of IVIG activity is simply to compete for the same effector pathways as the pathogenic autoantibodies. This important point is addressed below.

MECHANISMS OF IVIG ACTIVITY

Several different models explain IVIG activity in various diseases and in vivo model systems. Most currently available in vivo data in mice and humans clearly point to a dominant role of the antibody Fc fragment as responsible for the anti-inflammatory activity of IVIG. There are, however, instances in which the Fab fragment may be involved in IVIG's therapeutic activity. In many of these cases, whether both Fab and Fc fragments contribute to the anti-inflammatory activity has not been addressed.

$F(ab)_2$-Dependent Effects

Two main mechanisms for the Fab fragment's anti-inflammatory function have been described in the literature. First, the $F(ab)_2$ antibody portion may bind to and neutralize the potent proinflammatory activity of the C3a- and C5a-anaphylatoxins in a mouse model of asthma and in a C5a-mediated shock model in pigs (31). However, allergic diseases are quite different from other autoimmune diseases, and IVIG treatment has not demonstrated beneficial effects in two out of three randomized controlled studies with asthma patients. Furthermore, IVIG therapy is not recommended for patients with severe asthma by the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology (32). Thus, it remains to be seen whether this is a relevant mechanism of action in other autoimmune diseases for which beneficial effects of high-dose IVIG therapy have been described.

Second, a number of studies have addressed whether antibodies with distinct specificities are present in the IVIG preparation. Indeed, several groups identified significant binding to a variety of proteins and cell surface receptors that may explain the therapeutic activity of IVIG. These include anti-idiotypic, TCRα/β, Siglec-9, CD5, anti-integrin, Fas, cytokines, and cytokine receptor specificities (6, 33–38). It is beyond our scope to discuss all these possibilities, as in vivo evidence is not yet available to demonstrate a significant contribution of many of these antigen-specific antibodies in the IVIG preparation. For special instances in which certain receptor-ligand interactions are the main cause for the disease, such as in myasthenia gravis or toxic epidermal necrolysis (TEN), these (auto-) antibody specificities may have an important therapeutic function (39, 40).

TEN, for example, is the result of an adverse drug reaction and is characterized by the detachment of large sections of the epidermis from the dermis caused by widespread death of keratinocytes (40). The CD95- or Fas-mediated pathway of apoptosis is of central importance for keratinocyte death, and CD95-specific antibodies within the IVIG preparation can block this interaction by binding to CD95 (41). Depletion of the CD95-specific antibodies within the IVIG preparation results in a loss of activity, as measured in an in vitro assay with human

keratinocytes. It is unclear, however, whether this is the sole mode of action of IVIG in TEN in vivo or if other Fc fragment–dependent activities are involved as well. Further complicating this scenario are reports that both agonistic and blocking anti-Fas antibodies are present in IVIG preparations (34, 42). Thus, apoptosis was induced upon incubation of human lymphocytes and monocytes with therapeutic preparations of IVIG, which may be responsible for eliminating or reducing effector cells involved in causing chronic inflammation (34). More studies are necessary to define the role of these antigen-specific antibodies in the IVIG preparation in suitable in vivo model systems.

Fc-Dependent Effects

In contrast to the role of the Fab fragment, considerable evidence suggests that many autoimmune diseases for which IVIG has beneficial effects involve the Fc fragment as playing the central role in IVIG's anti-inflammatory activity. In a clinical trial with ITP patients and in many mouse autoimmune models, such as arthritis, nephrotoxic nephritis (NTN), and ITP, the Fc fragment was as effective as the whole IVIG preparation (43–46). The potential mechanisms for this activity are mirrored by the different effector pathways or receptors/ligands that can interact with the Fc fragment (**Figure 2**), including the complement pathway, FcRn, and classical activating and inhibitory FcγRs (23, 25, 26). As is now well established, the complement pathway is not significantly involved in the activity of different cytotoxic and autoantibodies in most mouse in vivo model systems; therefore, we do not cover this topic in depth, although IVIG does bind to activated C3b and C4b and prevents the tissue deposition of these activated complement proteins (47–49). However, tissue deposition of activated complement proteins is not necessarily predictive of complement-mediated tissue destruction. Indeed, in many mouse models of antibody-mediated inflammation, autoantibody activity and tissue destruction are abrogated in the absence of cellular FcγRs, although activated complement components are still present in high amounts in noninflamed tissues (50, 51). Similar results were reported for many other in vivo models of antibody-mediated inflammation in vivo (30). Direct evidence against a role for complement in the mechanism of IVIG activity comes from studies in which complement inactivation by injection of cobra venom factor had no impact on IVIG activity (52, 53). In addition to these well-described Fc fragment–dependent effector pathways, recent evidence points to a novel anti-inflammatory function of the Fc fragment–associated sugar moiety, which is discussed at the end of this review.

IVIG-mediated saturation of FcRn. The FcRn is the crucial regulator of IgG half-life (**Figure 2*a***). Therefore, one rationale to block autoantibody activity is to interfere with their interaction with FcRn, thus shortening their half-life and clearing them from the circulation before they can cause major damage. Indeed, a generation of antibody mutants with enhanced affinity for FcRn or for blocking the IgG-FcRn interaction by antibodies specific for FcRn or its β2M subunit competes with serum antibodies for binding to FcRn and inducing their accelerated clearance (54–56). One attractive possibility is that IVIG, by means of its high dose, may also mediate its anti-inflammatory activity by competing with autoantibodies for FcRn binding (**Figure 2*b***) (57–61). Antibody half-life studies in ITP models in mice and rats demonstrated that IVIG at a dose of 1 g/kg can shorten the half-life of a platelet-specific autoantibody from 79 to 54 h (58). Importantly, however, the maximal levels of platelet depletion and of IVIG-mediated protection essentially occur immediately after autoantibody injection (within the first 1–4 h) in mice and rats, which makes it rather unlikely that this change in antibody serum half-life will have an effect (46, 58, 62). Indeed, in this early phase hardly any difference in the serum level of the

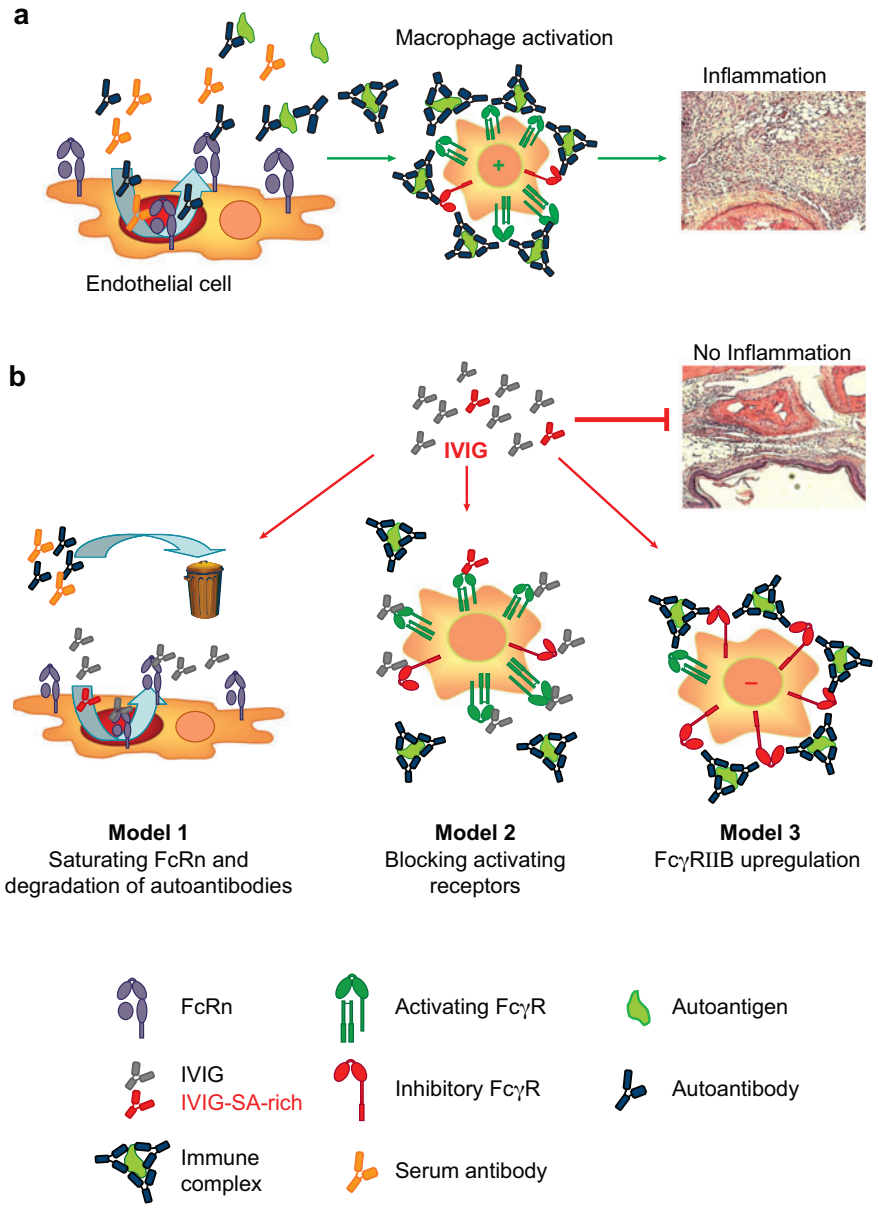

Figure 2

Proposed Fc fragment–dependent mechanisms of IVIG activity. (*a*) The neonatal Fc receptor (FcRn) binds to serum IgG at low pH after it has been endocytosed and transported into acidic vesicles. FcRn-bound IgG molecules are then recycled to the cell surface and released into the circulation at physiologic pH. (*b*) Three different models have been proposed to mediate the anti-inflammatory activity of the IVIG Fc fragment in vivo. First, the high dose of IgG molecules present in the IVIG preparation may compete with autoantibodies for FcRn binding and thus result in their enhanced clearance. Second, immune complexes present in the IVIG preparation may bind to activating FcγRs and thereby prevent binding of autoantibody immune complexes. In the third model, IVIG activity is crucially dependent on the presence of the inhibitory FcγRIIB. (Reproduced from the *Journal of Experimental Medicine*, 2007, 204:11–15. Copyright 2007 Rockefeller University Press.)

autoantibody in the presence or absence of IVIG was observed. Moreover, the therapeutic dose of IVIG can be reduced from roughly 25 mg per mouse to 600–700 μg if a specific glycovariant of the IVIG Fc fragment is used (45). This dramatically lower dose, while maintaining its anti-inflammatory activity, has no effect on the half-life of serum or autoantibodies and thus argues against a role for FcRn in these fast models of autoantibody-mediated platelet depletion (12, 45).

In addition to ITP, two other mouse model systems of autoantibody-mediated arthritis and autoantibody-induced skin blistering diseases demonstrated a role for IVIG in mediating its activity via FcRn (57, 60). In the serum transfer arthritis model, serum from arthritogenic mice is injected into wild-type animals, which develop a transient joint swelling over the next 20 days (63). Treatment of mice with IVIG is very efficient at blocking autoantibody-induced inflammation, which makes this model an attractive system to study the mechanism of IVIG activity. IVIG treatment resulted in a reduced serum half-life of the arthritogenic antibodies, and IVIG activity was reduced in mice deficient in FcRn and the inhibitory FcγRIIB (57). The central role of FcγRIIB for IVIG activity in vivo has been shown in many model systems and is discussed later in this review (43, 45, 46, 50). The major problem with using FcRn-deficient animals for these experiments is that the arthritogenic autoantibodies as well as IVIG have a dramatically reduced serum half-life. This necessitates very high doses of arthritogenic serum to obtain a level of inflammation that is still far below the response normally achieved in wild-type mice (57). In addition, the reduction in serum half-life of the autoantibodies was only investigated after administration of four consecutive 1 g/kg doses of IVIG, whereas one dose is normally sufficient to block arthritis in this model, making it difficult to evaluate whether the enhanced clearance rate induced by this high dose of IVIG is required for anti-inflammatory activity.

Direct evidence against a role for FcRn in mediating IVIG activity in the serum transfer arthritis model comes from studies that analyzed the interaction of aglycosylated IgG with FcRn and the effect of the absence of this Fc fragment–attached sugar moiety on IVIG anti-inflammatory activity in vivo (45, 64). A mutation at position 297 that changes an arginine to an alanine (N297A) residue abrogated binding to classical FcγRs but kept its affinity for FcRn (64). Thus, aglycosylated IVIG should still be functional without this sugar moiety if FcRn saturation and enhanced autoantibody degradation are the mechanisms of action. This is not the case, however, as aglycosylated IVIG loses its anti-inflammatory activity (45).

In a similar serum transfer approach, the capacity of IVIG to suppress antibody-mediated skin blistering diseases was investigated (60). Diseases such as bullous pemphigoid, pemphigus foliaceus, and pemphigus vulgaris are characterized by subepidermal or intraepidermal blisters and by autoantibodies of the IgG isotype specific for hemidesmosomal or epidermal proteins, which are able to transfer the disease if injected into newborn mice (65). In this model, injection of IVIG leads to a reduction in the serum half-life of the autoantibodies and efficiently prevents the development of blisters. In contrast to other model systems, in this model IVIG activity is independent of the inhibitory FcγRIIB. Moreover, deletion of this negative regulator of antibody activity does not further exacerbate the autoimmune symptoms, which again is not consistent with most of the results obtained in adult mouse model systems to date (26, 27). Thus, FcγRIIB deletion results in a lower threshold for B cell and innate immune effector cell activation, leading to the generation of class switched autoantibodies, spontaneous dendritic cell maturation, higher levels of autoantibody-mediated tissue destruction, and the development of autoimmune diseases on otherwise resistant genetic backgrounds (66–74). Indeed, the expression

and functionality of activating and inhibitory FcRs have not been studied in depth in newborn mice, making it hard to predict whether these findings can be generalized and are comparable with results obtained in adult mice and the adult immune system.

Taken together, technical difficulties in designing an unequivocal experimental setting make it hard to predict whether IVIG-mediated FcRn saturation is responsible for its activity in vivo. Nonetheless, reducing autoantibody half-life by modulating the antibody-FcRn interaction may be a promising therapeutic strategy.

Activating Fc receptor blockade and cytokine modulation. The second major theory for how IVIG mediates its anti-inflammatory activity is via blockade of activating FcγRs (**Figure 2*b***). Indeed, because FcγRs are centrally important in antibody-mediated effector functions, blocking of individual or all activating FcRs results in the abrogation of antibody activity in a variety of autoimmune and tumor models, such as ITP, NTN, arthritis, and several models of antibody-mediated tumor cell destruction (26, 50, 51, 62, 63, 75–81). Similarly, blocking human FcγRIIIA in a transgenic mouse model and in human ITP patients interferes with platelet depletion (46, 82–84). In contrast, blocking or deleting the high-affinity FcγRI in vivo in human ITP patients or in mice was therapeutically ineffective, highlighting the importance of the low-affinity FcγRs for antibody-mediated effector functions (62, 85). Thus, we focus on the low-affinity FcγRs in discussing the possible impact of IVIG-mediated interaction with activating FcγRs. Low- or medium-affinity receptors, such as mouse FcγRIIB, FcγRIII, and FcγRIV and even more so their human counterparts FcγRIIA/B/C and FcγRIIIA, cannot interact with monomeric IgG but can only bind to IgG in the form of an IC (27). Therefore, monomeric IgG, which constitutes more than 97% of the IVIG preparation, cannot be responsible for directly blocking activating FcγRs.

As discussed above, however, different IVIG preparations may contain varying levels of dimeric or multimeric IgG molecules. Aged IVIG preparations with a higher content of IgG dimers have an enhanced anti-inflammatory activity in a mouse model of ITP, although whether these dimeric molecules are able to block autoantibody binding to activating FcγRs has not been investigated (53).

A more recent study reported that antibodies specific for soluble proteins in the presence of their antigen or antibodies specific for cell surface proteins on red blood cells, for example, can exhibit IVIG-like activity in ITP (52, 86). This finding is consistent with earlier data from human ITP patients treated with the so-called anti-D IgG, which is a pooled polyclonal IgG fraction that comes selectively from donors immunized to the rhesus D antigen [anti-Rh0(D)-positive].

Anti-D, which, compared with IVIG, is used at a 40,000-fold lower dose, also can prevent platelet consumption in human ITP, and this activity is lost in patients who do not express the rhesus D antigen (87–90). However, the use of a monoclonal anti-D antibody of the IgG1 subclass that can efficiently interact with human FcRs was not able to prevent platelet depletion in seven anti-Rh0(D)-positive patients, arguing against a simple IC-formation mechanism (91). Clearly, the use of anti-D or other hyperimmune sera in the presence of the respective antigen will create a high level of IC that may compete with the autoantibody-antigen complexes and inhibit their phagocytosis by a mechanism similar to directly blocking activating FcγRs with an FcγR-specific antibody. With respect to the many specificities present in the IVIG preparation, these complexes may form after administration into patients. Whether the level of IC formation is high enough to achieve a significant level of binding to activating FcγRs is unclear. More importantly, the use of ICs poses quite some danger to the patient as they will systemically

trigger activating FcγRs and may even enhance inflammation by inducing release of proinflammatory cytokines from innate immune effector cells.

Recently, two other mechanisms of IVIG activity involving FcγRIII were proposed. First, triggering activating FcγRs on dendritic cells in vitro and then adoptively transferring them into mice can have IVIG-like effects and can ameliorate murine ITP; thus, investigators concluded that IVIG can be replaced by antibodies specific for activating FcγRs (92). Although an intriguing finding, it remains to be seen whether this is the actual mechanism of IVIG activity, as IVIG preparations with enhanced activity have a reduced affinity for activating FcγRs in mice and humans (45, 93). Second, investigators suggested that IVIG mediates its anti-inflammatory activity by inhibiting the release of the proinflammatory cytokine IFN-γ from myeloid cells in an FcγRIII-dependent manner (94). Although many studies described cytokine-modulating effects by IVIG, the relevance of these findings for the therapeutic activity of IVIG in vivo remains unclear (95). Indeed, IVIG is fully functional in preventing platelet depletion in mouse strains deficient in a variety of proinflammatory cytokines and cytokine receptors, such as the IFN-γR, CCL3, IL-12β, and TNF-α (96). More direct evidence against a central role for FcγRIII in IVIG activity comes from a recent study using a model of accelerated NTN. In this model, autoantibody-mediated kidney inflammation is mainly mediated by the activating FcγRIV, and IVIG is very potent in suppressing kidney destruction (49). Thus, we could investigate the role of activating FcγRIII in IVIG activity without simultaneously interfering with the interaction between the autoantibody and its activating FcγR. Importantly, deletion of FcγRIII had no influence on IVIG activity in vivo, whereas the absence of the inhibitory FcγRIIB abrogated the anti-inflammatory effect of IVIG.

Taken together, the data suggest that IVIG-like effects can be achieved by blocking the responsible activating FcγRs either with competing ICs or with FcγR-specific monoclonal antibodies that have the capacity to block binding of ICs to activating FcγRs. This highlights the importance of FcγRs in murine and human autoimmune diseases. At present, however, it seems unlikely that this is the actual mechanism of IVIG activity.

Upregulation of the inhibitory FcγRIIB. As indicated briefly in the previous paragraphs, the other classical FcγR that has been associated repeatedly with the anti-inflammatory activity of IVIG in vivo is the low-affinity inhibitory FcγRIIB (**Figure 2*b***). Animals deficient in this protein are no longer protected by administration of IVIG in mouse models of ITP, rheumatoid arthritis, and NTN (43, 45, 46, 50, 57, 97). Moreover, IVIG therapy resulted in the upregulation of the inhibitory FcγRIIB on effector macrophages (43, 46, 50). In addition, a dramatic decrease in the expression of the triggering activating FcγRIV was observed in a model of NTN (50). This altered expression level of activating and inhibitory FcγRs will heighten the threshold for innate immune effector cell activation, which ultimately leads to a lower level of inflammation and platelet consumption. Consistent with the low affinity of FcγRIIB, a direct interaction with IVIG seems rather unlikely. In addition, the IVIG-mediated upregulation of FcγRIIB on effector macrophages seems to be indirect as determined by the loss of IVIG activity and increased expression of FcγRIIB on effector macrophages in op/op mice (43). These mice lack the hematopoietic growth factor colony-stimulating factor (CSF)-1, which results in the loss of select monocyte and macrophage subpopulations (98). Because IVIG activity is lost but antibody-mediated inflammation is maintained, investigators suggested that a CSF-1-dependent macrophage population has a regulatory function and is responsible for the upregulation of the inhibitory FcγR on effector macrophages (**Figure 3**) (12). Although this provides some information about

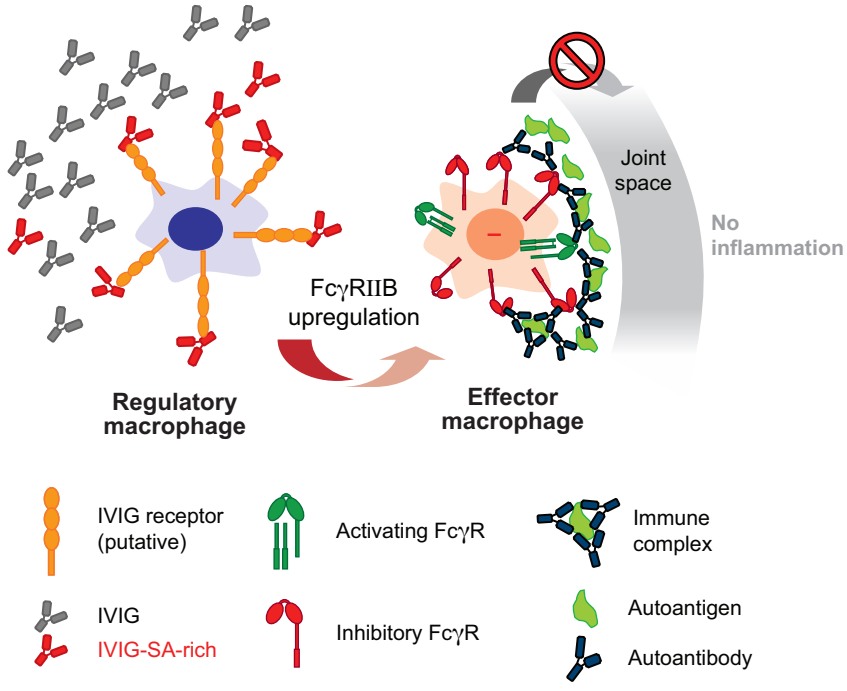

Figure 3

The role of the inhibitory FcγRIIB for IVIG activity. Binding of SA-rich immunoglobulins in the IVIG preparation to an as yet unknown receptor on CSF-1-dependent regulatory macrophages results in the upregulation of the inhibitory FcγRIIB on effector macrophages, which leads to an increased threshold for cell activation and ultimately reduces or blocks chronic inflammatory reactions. (Reproduced from the *Journal of Experimental Medicine*, 2007, 204:11–15. Copyright 2007 Rockefeller University Press.)

the cell types that are involved in IVIG activity, the fact of this regulatory function does not explain why such high doses are required to achieve it. Rather, it suggests that only a minor component in the IVIG preparation is the active component. This, at first sight, seems to be at odds with the limited heterogeneity within the IVIG preparation (which consists of only four different IgG subclasses) if one focuses on IgG Fc fragment–mediated effects.

Differential antibody glycosylation. IgG antibodies are glycoproteins that contain a sugar moiety attached to each of the asparagine 297 (N297) residues in the two chains of the antibody Fc fragment (99). This glycan moiety is an integral structural component of the IgG molecule, forming part of the scaffold for FcγR binding (**Figure 4**). In addition, depending on the variable region sequences, roughly 20% of serum IgG antibodies have a Fab fragment–attached N-linked sugar side chain. These sugar moieties consist of a biantennary heptameric core sugar structure high in mannose and N-acetylglucosamine with variable amounts of branching and terminal sugar residues such as galactose, sialic acid (SA), N-acetylglucosamine, and fucose (**Figure 4*b***) (100–102). Whereas Fab fragment–attached sugar moieties and the sugar domains of other serum proteins such as tranferrin or fetuin are usually fully processed (meaning that they contain all possible sugar residues), the Fc fragment–associated moiety is much more heterogeneous. In fact, more than 30 different antibody glycovariants have been detected in human serum, with about 25%–30% of

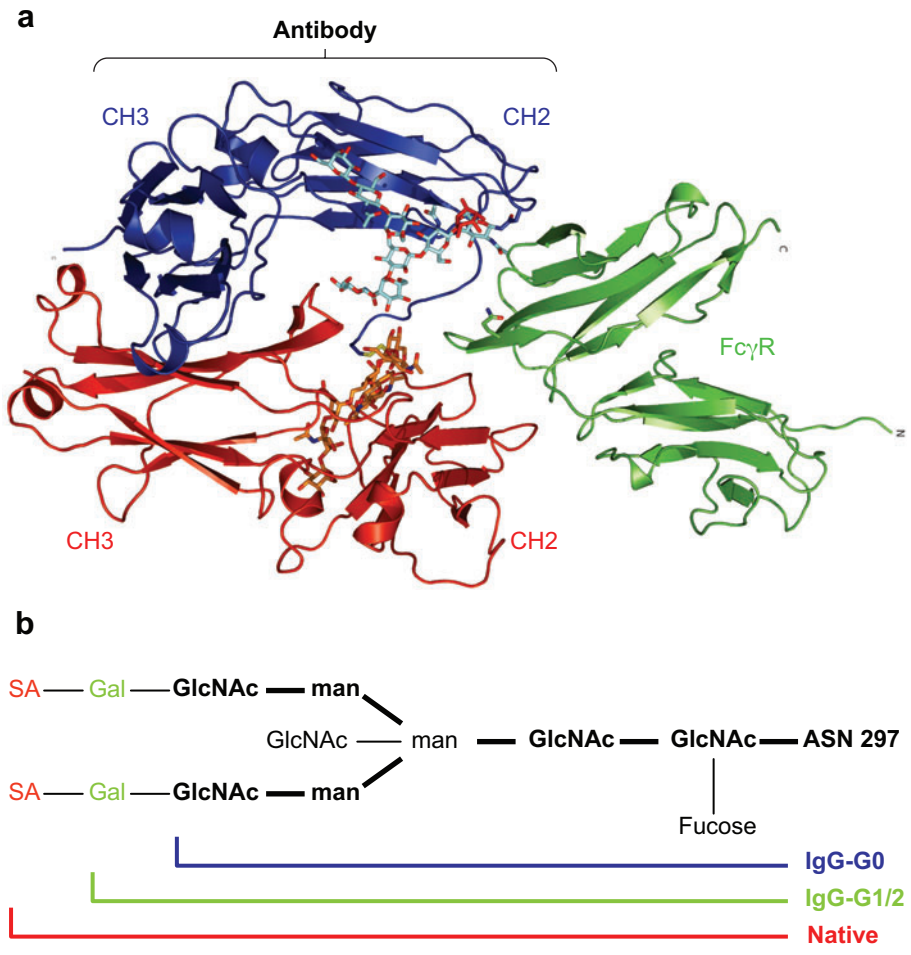

Figure 4

The sugar domain of the antibody Fc fragment. (*a*) Shown is the crystal structure of the extracellular domain of human FcγRIIIA in complex with an immunoglobulin Fc fragment (CH2-CH3 domains). The Fc fragment–attached sugar moieties that protrude into the central cavity between the two antibody heavy chains are shown as a stick and ball model. (*b*) Cartoon of the antibody-attached sugar moiety. The heptameric core sugar structure consisting of mannose (man) and N-acetylglucosamine (GlcNAc) residues is shown in bold, and terminal or branching sugar residues are indicated. Most IgG glycoforms can be distinguished by the absence of terminal sialic acid (SA) or galactose (Gal) residues (IgG-G0 glycoform), the presence of one or two galactose residues (IgG-G1/2 glycoform), and the presence of one or two terminal SA residues (fully processed or native form).

them in the IgG-G0 glycoform (**Figure 4*b***) without terminal SA or galactose residues (103). Thus, these 30 variants, multiplied by the four different IgG subclasses, result in more than 120 different glycoproteins in the IVIG preparation that could contain the active anti-inflammatory component. The importance of this sugar moiety is highlighted by the loss of therapeutic activity of deglycosylated IVIG preparations (45). As discussed above, this excludes a simple FcRn competition model because the FcRn, unlike classical FcγRs, retains its affinity for deglycosylated Fc fragments. The loss of classical

FcγR binding could indicate that a direct interaction with these molecules is important. However, there is strong evidence against this scenario, as IVIG preparations as well as isolated Fc fragments enriched for terminal SA residues have a more than 10-fold higher anti-inflammatory activity. These SA-rich IgG glycovariants have a decreased affinity for classical FcγRs in mice and humans, consistent with their strongly impaired activity in vivo (45, 93). This excludes the possibility that SA-rich IVIG preparations block access of autoantibody ICs to activating FcγRs and rather argues for a novel receptor on regulatory macrophages that can specifically recognize SA-rich IgG and promote an anti-inflammatory milieu. Data in favor of an important role of differential antibody glycosylation and especially sialylation in vivo come from human arthritis patients and mice with a variety of autoimmune diseases, such as systemic lupus erythematosus, arthritis, and NTN (45, 104–110). Especially during acute disease phases, a significant reduction in terminal SA residues in serum and antigen-specific antibodies can be observed, and the IgG-G0 glycovariant constitutes more than 50% of serum IgG (106). Thus, IVIG infusion would restore the level of SA-rich IgG that is necessary to regain an anti-inflammatory environment that suppresses the activity of autoantibodies by increasing inhibitory FcγRIIB expression.

SUMMARY AND OUTLOOK

Despite the widespread use of IVIG, we have only recently begun to shed some light on the mechanism of IVIG activity in vivo. Elucidating the molecular details of this activity is crucially important for gaining some general insights into the regulation of pro- and anti-inflammatory immune responses as well as for optimizing IVIG activity or even replacing it by a recombinant product in the future. Although probably none represent the actual mechanism of IVIG activity, several possibilities have already been described, such as enhancing autoantibody clearance by blocking the neonatal FcR or interfering with the interaction of autoimmune complexes with activating FcγRs. Although attractive strategies, both have drawbacks: Continuous blocking of FcRn will result in a more rapid clearance not only of autoantibodies but also of all other serum IgG molecules that have protective functions against microbial infections. Blocking activating FcγRs is, of course, the most direct strategy to interfere with innate effector cell activation, but it is far downstream in the inflammatory cascade and may also pose some significant dangers as the blocking antibodies or competing soluble IC might result in unspecific cell activation and the massive systemic release of proinflammatory cytokines, a so-called cytokine storm. Thus, potential FcγR-blocking antibodies or soluble ICs have to be carefully evaluated to prevent these severe side effects. In contrast, restoring a balanced immune response might be a very promising strategy in the long-term. Although we are just at the very beginning, the recent identification of antibody glycovariants with immunoregulatory activity might enable us to pursue more efficient and long-lasting therapeutic responses, especially in combination with therapies that aim at a specific depletion of autoantibody-producing B cells.

Important topics to be addressed include determining whether genetic polymorphisms exist in the population that result in a differential antibody glycosylation pattern and which signals regulate antibody glycosylation in B cells during the steady state and during immune or autoimmune responses. A better understanding of the molecular details of differential antibody glycosylation might enable us to specifically modify these pathways during autoimmune responses and interfere with chronic inflammatory processes.

SUMMARY POINTS

1. The two major clinical uses of IVIG are Ig replacement and anti-inflammatory therapy.
2. IVIG is produced from the pooled serum of thousands of donors enriched for the IgG fraction.
3. The anti-inflammatory activity of IVIG requires a high-dose treatment regimen.
4. IVIG contains autoreactive antibody species of varying specificity.
5. A $F(ab)_2$-mediated mechanism may account for IVIG activity for select diseases.
6. Most in vivo evidence suggests that the Fc fragment contains the anti-inflammatory activity.
7. Blocking FcRn or activating FcγRs results in an IVIG-like activity by interfering with autoantibody half-life or binding to triggering activating FcγRs.
8. The inhibitory FcγRIIB is required for IVIG activity in vivo.
9. More than 30 different IgG glycovariants can be detected in human serum.
10. IgG antibodies from human arthritis patients and autoimmune mouse strains have a reduced level of terminal SA residues.
11. The SA-rich IgG fraction of IVIG has an enhanced anti-inflammatory activity.

FUTURE ISSUES

1. Why do autoimmune patients have aberrantly glycosylated antibodies?
2. Are there genetic factors that impact antibody glycosylation?
3. How is antibody glycosylation regulated?
4. Can recombinantly produced SA-rich IgG replace IVIG?
5. What is the cellular receptor that can recognize SA-rich IgG?

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Contents