The antiinflammatory activity of IgG: the intravenous IgG paradox

Falk Nimmerjahn and Jeffrey V. Ravetch

How high doses of intravenous IgG (IVIG) suppress autoimmune diseases remains unresolved. We have recently shown that the antiinflammatory activity of IVIG can be attributed to a minor species of IgGs that is modified with terminal sialic acids on their Fc-linked glycans. Here we propose that these Fc-sialylated IgGs engage a unique receptor on macrophages that, in turn, leads to the upregulation of an inhibitory Fcγ receptor (FcγR), thereby protecting against autoantibody-mediated pathology.

IgG antibodies are the primary mediators of protective humoral immunity against pathogens, but they can also be pathogenic. Acting as cytotoxic molecules or as immune complexes, IgG autoantibodies are the principal mediators of autoimmune diseases such as immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AHA), and systemic lupus erythematosus (SLE), and may contribute to other autoimmune diseases, such as rheumatoid arthritis (RA), type I diabetes, and multiple sclerosis (1). IgG antibodies have been used therapeutically for over a century. They were first used as antitoxins for the treatment of infectious diseases in the preantibiotic era (1, 2). Today, hyperimmune sera from human donors recovering from infection with specific viruses, such as hepatitis B, cytomegalovirus, and varicella zoster, are used to provide protective immunity to susceptible populations. In addition, pooled polyclonal IgG from the serum of thousands of donors is currently used to provide replacement IVIG therapy for patients lacking immunoglobulins (3). At high doses (1 g/kg), IVIG is also widely used as an antiinflammatory agent for the treatment of autoimmune diseases. This approach is based on an observation made in 1981 that administration of IVIG attenuated platelet clearance in a child with ITP (4). Since then, high dose IVIG has been widely used to treat patients with immune system disorders and is FDA approved for the treatment of ITP and Kawasaki's Disease, an acute vasculitic syndrome, in addition to humoral immunodeficiency and bone marrow transplantation. Off label uses include the treatment of RA, SLE, multiple sclerosis, and scleroderma. Demand for IVIG has been increasing in recent years, resulting in shortages and restrictions in its use. In the United States, over 4 million grams of IVIG was used in 2004 at a cost of $500 million, more than half of which was off label use.

The mechanisms by which high doses of pooled, monomeric IgG provide antiinflammatory activity have been the subject of much speculation, stemming from the fact that IgGs can form many different binding interactions through both their antigen binding and Fc domains. In this commentary, we will address the current models of IVIG antiinflammatory activity and review recent results that argue against these models and support an alternative, novel mechanism of action. This new model accounts for the high dose requirement for IVIG in inflammatory diseases and for the dominant role of the Fc portion of the molecule, and suggests ways to improve therapeutics for autoimmune diseases.

F.N. and J.V.R. are at Laboratory of Molecular Genetics and Immunology, The Rockefeller University, New York, NY 10021.

CORRESPONDENCE
J.V.R.: ravetch@rockefeller.edu

Fc is key

In some cases, antigen binding alone might be sufficient to mediate the antiinflammatory effects attributed to IVIG, for example, by blocking interactions between a proinflammatory ligand and its receptor or by neutralizing its ability to elicit an inflammatory response. This Fab-mediated mechanism appears to underlie the therapeutic activity of IVIG in the treatment of toxic epidermal necrolysis, which has been attributed to inhibition of Fas-mediated epidermal cell death by antagonistic anti-Fas antibodies in the IVIG preparation (5). However, a generalized role for the antigen binding domain in the antiinflammatory activity of IgG is unlikely given that intact IVIG and its Fc fragments have equivalent antiinflammatory activity both in the clinical treatment of ITP (6) and in many animal models (7–9). We will therefore focus on the mechanisms by which the Fc region of IgG may function as an antiinflammatory molecule.

How IgG autoantibodies inflame: activating FcγRs, neonatal Fc receptor, and complement

To understand how IVIG reverses inflammation in autoimmune disease, it is helpful to consider how IgG autoantibodies cause inflammation. The IgG Fc region couples antigen recognition to several effector pathways, most notably the system of activating and inhibitory FcγRs, the complement family of molecules and their receptors, and the neonatal Fc receptor (FcRn) pathway, which is required for the extended in vivo half-life of IgG antibodies (10–13). Studies in animal systems and correlative studies in human populations show that the proinflammatory activities of IgG require the interaction of the Fc fragment of the antibodies with their cognate cellular FcγRs (1). Most hematopoietic cells express both activating and inhibitory FcγRs. The in vivo activity of an IgG antibody thus results

from the net effect of engaging both classes of receptors, which, in turn, is governed by the respective affinity constants of individual IgG subclasses for specific FcγRs (14).

FcγRs for IgG are the primary mediators of the proinflammatory activity of IgG in the immunopathology of autoimmune diseases and are required for the protective action of IgG therapeutics, such as the anti-CD20 monoclonal antibody (mAb) used to treat lymphoma. Thus, mice rendered genetically deficient in the activating FcγRs are protected from the pathogenic consequences of autoantibodies in many disease models, including SLE, RA, ITP, AHA, and Goodpasture's Disease (10). And tumor-specific mAbs (IgG), designed to clear tumors, also fail to protect these mice (15). Studies in humans have similarly shown that the clinical response to antitumor antibodies depends on activating FcγR genes. Indeed, patients with FcγR alleles that confer higher binding activity to the therapeutic IgG have improved clinical outcomes (16–18). FcγR alleles that confer hyperresponsiveness to IgG immune complexes are also associated with autoimmune disease susceptibility in defined populations (1).

In contrast to FcγRs, animal studies have generally failed to demonstrate a substantial contribution of the classical pathway of complement activation in models of IgG-mediated inflammation. Mice deficient in the C3 component of complement, for example, are not protected from IgG-mediated cytotoxicity for complement-fixing antibodies in models of red cell, platelet, or B cell clearance (19). In several models of immune complex-mediated inflammation, C3 has been shown to be dispensable as well (20). In some models of IgG-mediated inflammation C5a receptor (C5aR)–deficient mice are protected (21, 22). In those cases where C5aR deficiency leads to protection, the proposed mechanism is not through classical or alternative complement pathway activation of C5a, but rather through a direct activation of C5a by FcγR cross-linking on effector cells, which in turn results in C5aR-triggered up-regulation of FcγR expression on these effector cells (21, 23).

Similar to mice lacking the activating FcγRs, mice deficient in FcRn are protected from pathogenic autoantibodies. The long serum half-life of IgG results from its interaction with FcRn, a major histocompatibility complex class I homologue that associates with β2-microglobulin and is expressed on endothelial cells. FcRn remains bound to internalized IgG at the acidic pH of the lysosomal vesicle, preventing its intracellular degradation and allowing it to be recycled to the extracellular milieu (12, 13, 24). Disruption of the FcRn pathway in mice results in the rapid clearance of all IgG antibodies, including pathogenic autoantibodies, which protect these mice from autoantibody-driven inflammation (25–27).

The antiinflammatory activity of IVIG

Three possible modes of action have been proposed to account for the antiinflammatory activity of the Fc fragments of IVIG. These models take into account the mechanisms by which autoantibodies trigger inflammation and the high doses of IVIG that are required for protection (Fig. 1).

Since the in vivo data on the pathogenicity of IgG autoantibodies and immune complexes do not generally support a role for the components of the complement cascade in autoantibody-driven inflammation, we will not consider that pathway in the mechanisms of IVIG.

Model 1: competition for activating FcγRs. The first model posits that high doses of intravenous IgG compete with pathogenic IgGs for activating FcγRs, thereby limiting their pathogenic potential. Studies showing that mAbs that block activating FcγRs mimic the antiinflammatory activity of IVIG appear to support this model (28). Although this model recognizes the primary role of FcγRs in the pathogenesis of autoimmune-mediated inflammation, it fails to take into account the low affinity of these receptors. IgGs interact with activating FcγRs (FcγRIII and IV) with affinity constants in the range of 10^{-6} to 10^{-7}. This ensures that these receptors are activated by IgG immune complexes, which can form high avidity interactions, and not by monomeric, circulating IgG. Competition for activating FcγR might occur if IVIG preparations were contaminated with immune complexes. This is unlikely, however, as whole IgG and isolated IgG Fc regions have comparable antiinflammatory activities. Even at the high doses of IVIG required to elicit antiinflammatory activity, the serum IgG concentrations that can be achieved by injecting exogenous IgG into individuals with normal levels of circulating IgG are insufficient to compensate for the low affinity constants of FcγRs for monomeric IgGs. In fact, at high doses of IgG, the resulting saturation of FcRn increases IgG catabolism, thus restoring the circulating concentration of IgG to a relatively narrow range. The fact that mAbs that block activating FcγRs mimic the antiinflammatory activity of IVIG simply reflects the fact that pathogenic immune complexes trigger inflammation through these activation receptors; it does not indicate that IVIG and anti-FcγRs mAbs have a common mechanism of action (9, 28). Indeed, as will be described below, the antiinflammatory activity of IgGs in IVIG preparations increases as their affinity for activating FcγRs decreases.

Model 2: high dose IgG saturation of FcRn. An alternative scenario to model 1 is that saturation of FcRn by high levels of exogenous IgG leads to the catabolism of pathogenic autoantibodies. It is difficult to evaluate this theory experimentally, however, as removing or blocking FcRn in mice results in a rapid clearance of exogenous autoantibodies and drastically reduced serum IgG levels (27). Investigations of the effect of IVIG in FcRn-deficient mice have relied either on repeated infusion of IVIG, a situation that does not accurately model the use of IVIG, or evaluation of the purported protective effect of IVIG at time points far beyond the peak of the disease. For example, a common animal model used to evaluate IVIG action is platelet depletion in mice mediated by

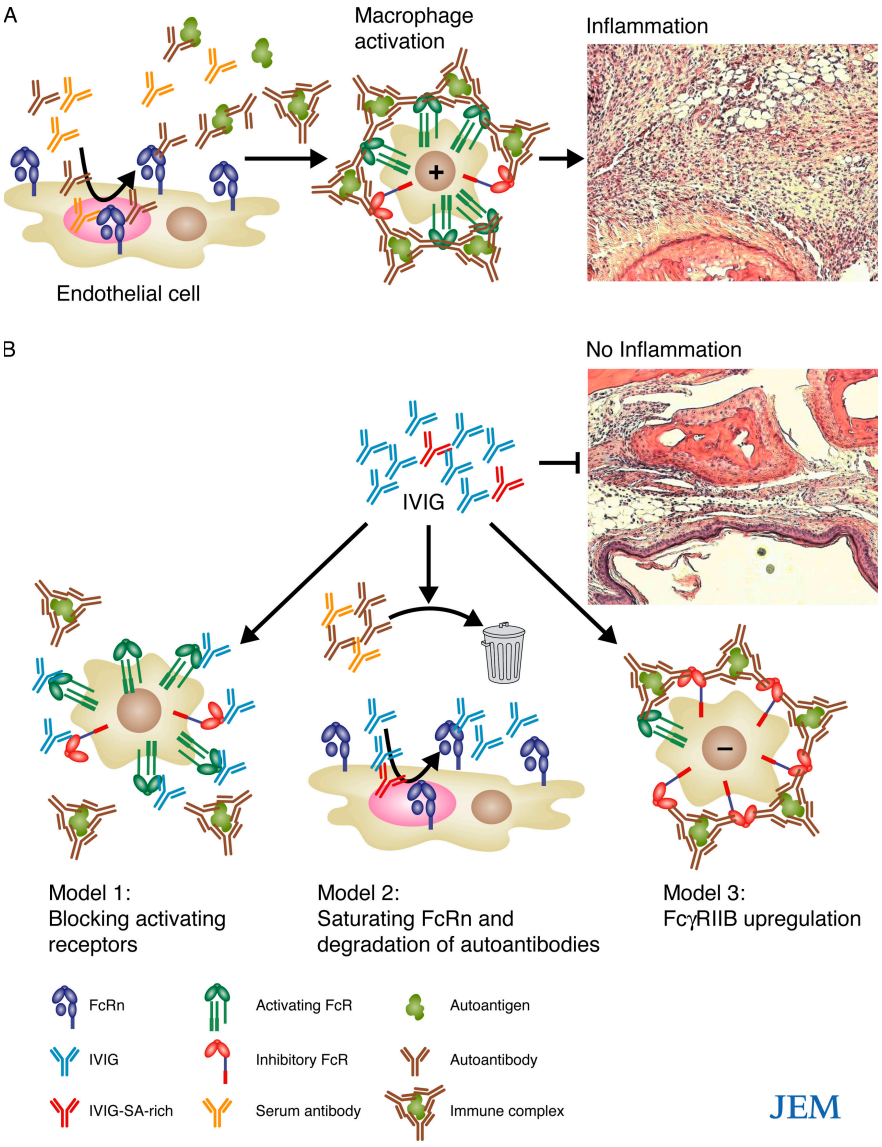

Figure 1. Models for the mechanism of IVIG-mediated antiinflammatory activity. (A) During an inflammatory response immune complexes consisting of autoantibodies (brown) and self-antigens (green) activate innate immune effector cells (e.g., macrophages) by cross-linking cell surface FcγRs, which can lead to the destruction of self-tissues. Serum antibody half-life of normal (light brown) and autoreactive antibodies is regulated by FcRn (purple) expressed on endothelial cells. (B) Three models have been proposed to explain the antiinflammatory activity of IVIG. In the first model, IVIG (consisting of a mixture of sialic acid–rich [red] and sialic acid–low [blue] antibodies) binds to activating FcγRs on immune effector cells, thereby blocking access of immune complexes to these receptors and inhibiting cell activation. The second model proposes that IVIG competes with serum IgG (including autoreactive antibodies) for recycling mediated by FcRn. Thus, serum and autoreactive antibodies would be cleared more rapidly and not reach the threshold level for initiating tissue destruction. In the third model, IVIG leads to up-regulation of the inhibitory FcγRIIB on immune effector cells, thus increasing the threshold level for cell activation by immune complexes.

an antiplatelet antibody. In this model, platelet consumption reaches a nadir 4 hours after introduction of the cytotoxic antibody; IVIG prevents platelet depletion through this time point. In FcRn-deficient mice, however, IVIG-induced protection against platelet depletion is not observed until 48–72 h after infusion of the cytotoxic antibody (25, 29). In another model, human polyclonal antibodies found in human bullous diseases (bullous pemphigoid, pemphigus foliaceus, and pemphigus vulgaris) were passively transferred to neonatal mice to induce disease. IVIG protected wild-type mice but did not protect neonatal FcRn-deficient mice. Little is known about the mechanisms by which these human antibodies trigger disease in this model or about the expression of FcγRs or FcRn in neonatal mice, making it difficult to draw any general conclusions about the mechanism of IVIG protection (26).

Ruling out a role for FcRn saturation in the antiinflammatory activity of IVIG would require an IVIG preparation that binds FcRn and has a normal serum half-life but has no antiinflammatory activity. There are several experimental systems that fulfill these requirements. Deglycosylation of the conserved, Asn297 N-linked glycan on IgG or its Fc fragment with PNGase, for example, yields an IVIG preparation that retains FcRn binding and normal serum half-life but lacks its antiinflammatory activity (30, 31). Desialylation of the terminal sialic acid residues on the Asn297-linked glycan of IVIG has the same effect (31). Thus, if saturation of FcRn by high dose IVIG is involved in its antiinflammatory mechanism of action, these glycan-modified preparations of IVIG should not have lost activity. Conversely, enriching IVIG for sialylated Fc glycoforms enhances its antiinflammatory activity, thus reducing the dose of IVIG required for protection without affecting the half-life of either the IVIG antibodies (31) or the pathogenic mouse serum antibodies (unpublished data). Again, this provides evidence against a role for FcRn in the antiinflammatory activity of IVIG.

Model 3: induction of inhibitory FcγR expression. The final model posits that the antiinflammatory effect of IVIG Fc fragments involves the inhibitory FcγRIIB. Triggering of effector cells, such as macrophages, by IgG bound to target cells or by immune complexes occurs when FcγR signaling reaches a critical threshold. Changing the affinity of IgG for either activating or

inhibitory receptors (by glycosylation modifications, for example) or modulating the absolute cell surface levels of these receptors will change the IgG concentration required to trigger cellular activation. In models of ITP, AHA, RA, and nephrotoxic nephritis, the ability of IVIG to protect mice from pathogenic IgG antibody–driven responses depends on FcγRIIB (7–9, 25, 31, 32). The only other mouse strain in which IgG-mediated inflammation occurs but IVIG protection is lost is the *op/op* mouse, in which loss of the hematopoietic growth factor colony-stimulating factor (CSF)-1 results in a depletion of CSF-1–dependent macrophage populations (7). IVIG administration to normal mice, but not *op/op* mice, increases the surface expression of FcγRIIB on a population of CSF-1–independent, "effector" macrophages in the spleen (Fig. 2). These results imply that two different macrophage subsets are involved in the mechanism of IVIG action—a subset that is dependent on CSF-1 (and thus absent in *op/op* mice) that may act as a "regulator" of IVIG, and a CSF-1–independent, effector subset, which mediates the inflammatory responses to IgG–FcγR cross-linking. By engaging the regulatory macrophage, IVIG triggers a suppressive pathway that attenuates the ability of the effector macrophages to respond to cytotoxic or immune-complexed IgG that cross-link activating FcγRs. This inhibition involves increasing the surface expression of the inhibitory FcγRs, thereby raising the threshold required for triggering activating FcγRs. The mechanism by which IVIG engaged regulatory macrophages, which then triggered the up-regulation of inhibitory FcγRs expression on the effector population, remained unknown. In other words, we did not yet know what was distinctive about the IgG in IVIG that promoted this antiinflammatory activity.

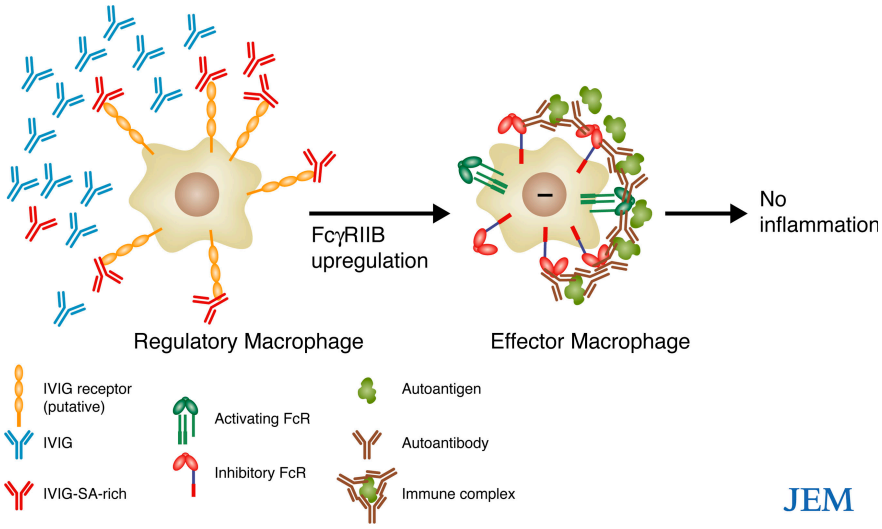

Figure 2. Two-cell model for the mechanism of IVIG activity. Sialic acid–rich antibodies (red) in the IVIG preparation bind to an as yet unknown IVIG receptor on CSF-1–dependent regulatory macrophages. This induces the up-regulation of the inhibitory FcγRIIB on effector macrophages, thereby increasing the threshold for cell activation in response to the binding of immune complexes and inhibiting the release of destructive inflammatory and cytotoxic mediators.

Why are high doses of IVIG results required?

If IVIG does not work by blocking FcγRs or FcRn, why are such high doses required? Recently, we explored the hypothesis that the antiinflammatory activity of IVIG depends on a specialized subset of IgG that is present at limiting concentrations in the IVIG preparation. The glycosylation requirement for IVIG activity suggested that a specific glycoform of the Asn297-linked glycan may be involved in the antiinflammatory activity. Because over 30 glycoforms are found at this position in human serum, the pooled serum used to prepare IVIG may contain limiting amounts of the antiinflammatory IgG glycoform. We tested this hypothesis by fractionating IVIG and its Fc fragments by sialic acid–specific lectin (SNA) affinity chromatography and found that 1–2% of unfractionated IVIG has sialic acid at the Asn297-linked glycan. Enrichment by SNA binding increased the sialic acid content to 20% with a concomitant 10-fold increase in its antiinflammatory activity. In a mouse model of RA, 100 mg/kg of sialic acid–enriched IVIG protected as well as 1 g/kg of unfractionated IVIG. Further enrichment for the active glycoform of IgG should reduce this concentration requirement to 20 mg/kg or below. No difference was observed in the serum half-life of the SNA-enriched IVIG compared with total IVIG, or in its requirement for FcγRIIB expression (31). These results are consistent with the model proposed in Fig. 2, in which the sialylated IgG in normal serum engages a regulatory macrophage receptor with specificity for the sialylated form of IgG, triggering an inhibitory pathway that ultimately results in the up-regulation of inhibitory FcγR expression on effector macrophages. One candidate for the regulatory macrophage receptors are the siglec family of receptors (33), which bind sialic acid and have been suggested to mediate inhibitory signals via ITIM sequences found in their cytoplasmic domains.

Others have proposed that IVIG cross-linking of activating FcγRs on dendritic cells may contribute to the antiinflammatory effects of IVIG in a model of ITP (24). These findings suggest that under some experimental conditions, ex vivo stimulation of dendritic cell populations through activating FcγRs may trigger antiinflammatory responses, perhaps by promoting the differentiation or survival of regulatory T cells. It is unlikely, however, that this mechanism is relevant to IVIG activity, as antibodies enriched in sialic acid show reduced binding to activating FcγRs yet display enhanced antiinflammatory activity (27). The reduced binding of

the active glycoforms of IgG in IVIG to activating FcγRs bolsters the argument against the simple competition mechanism of IVIG action proposed in model 1.

Concluding remarks

The observation that IVIG activity is caused by a limiting concentration of a sialic acid–bearing IgG glycoform provides the rationale for the preparation of a sialic acid–enriched IVIG product that would confer greater antiinflammatory activity at doses 1/10 to 1/100 currently required. It also suggests that a fully recombinant IVIG composed of hypersialylated IgG would be a potent antiinflammatory agent for use in autoimmune diseases. The identity of the macrophage receptor involved in the binding of sialylated IgG and the characterization of the inhibitory pathways induced by sialylated IgG will provide insights into the normal biological functions of sialylated IgG in vivo and the homeostatic pathways that regulate IgG effector activity.

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