

Mechanism of Action of Immunoglobulin: Sialylated IgG

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Abstract Glycosylation of IgG, including sialylation of the Fc region, influences binding of IgG to receptors. In addition to the classical Fc receptor members, we now know of several sugar-binding lectins that recognize sialylated oligosaccharides of IgG. These lectins, particularly human dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), may regulate immune reactions and are thus candidate molecules in the initiation of the sequence of IVIG-mediated anti-inflammatory events. This chapter reviews the emerging role of sialylated IgG Fc in the IVIG-mediated therapeutic effect, in particular the importance of DC-SIGN-initiated events.

Keywords DC-SIGN • Lectin • Fc receptor • Dendritic cell • Oligosaccharide

Introduction

The mechanisms underlying the action of IVIG involve blocking or neutralizing the effects of IgG Fc receptors (FcγRs), complements, pathogenic antibodies, superantigens, and cytokines. The effects of receptors and complements are attributed to the Fc region of IgG, and the effects of pathogenic antibodies, superantigens, and cytokines are ascribed to the Fab region and induction of regulatory cells including regulatory T (Treg) cells. The pluripotential nature of IgG and related molecules with which IgG molecules interact (Fig. 1) suggests the presence of multiple mechanisms by which IVIG alleviates disease. A recent series of studies by Ravetch and colleagues showed that IVIG mechanisms involve recognition of sialylated IgG Fc by a carbohydrate receptor on sensor macrophages and dendritic cells (DCs), namely DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN or CD209), which indirectly leads to upregulation of a unique inhibitory FcγR, FcγRIIB, on effector macrophages [1].

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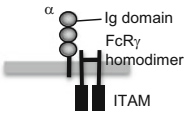
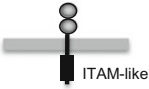
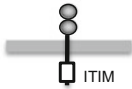
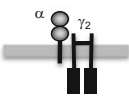

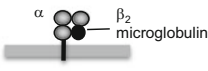
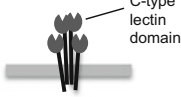

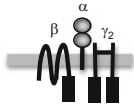
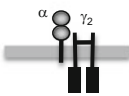


Ligand	Fc receptor (*present also in mice)	Structure	Expression
IgG	Fc γ RIA* (CD64)		Macrophages, monocytes, neutrophils, eosinophils, DCs
IgG	Fc γ RIIA, IIC (CD32A, C)		Macrophages, neutrophils, eosinophils, platelets, DCs, LCs
IgG	Fc γ RIIB* (CD32B)		B cells, mast cells, basophils, DCs, LCs, macrophages, eosinophils, neutrophils
IgG	Fc γ RIIIA* (CD16A)		Macrophages, monocytes, NK cells, mast cells, eosinophils, DCs, LCs, mouse neutrophils
IgG	Fc γ RIIIB (CD16B)		Neutrophils, eosinophils
IgG	FcRn*		Placenta, Intestinal epithelial cells, monocytes, DCs
(IgG)	DC-SIGN* (CD209)		DCs, macrophages
IgE (IgG)	FcεRII* (CD23)		Ubiquitous distribution, platelets, etc.
IgE	FcεRI*		Mast cells, basophils, eosinophils, human LCs, human DCs
IgA	FcαRI (CD89)		Macrophages, neutrophils, eosinophils
IgA, IgM	Fcα/μRI*		B cells, macrophages
IgM	FcμR*		B cells, human T cells

Fig. 1 Schematic view of Ig Fc-binding receptors

The schematic structures of human IgG Fc receptor proteins are shown with ligands and expression profiles. The structures of IgE-, IgA-, and IgM-binding Fc receptors are also shown. In the classical IgG Fc receptors Fc γ RI, RII, and RIII (and RIV in mice), Fc γ RI is a high-affinity receptor that

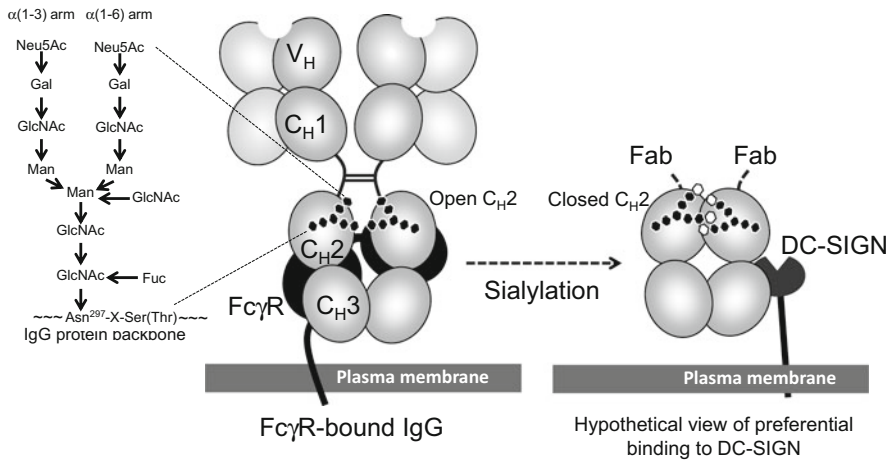


Fig. 2 Binding of glycosylated IgG to a canonical Fcγ receptor

A schematic view of an IgG molecule bound to a classical FcγR. Note that low-affinity FcγR receptors bind IgG molecules as multivalent immune complexes (not shown in figure). The N-linked oligosaccharides (*black hexagons*) are located in the Fc portions, as biantennary chains bound to Asn²⁹⁷ of C_H2 domains (for composition of the residues, *left*) [2], and in variable regions of the L and H chains (not shown), depending on the N-glycosylation consensus sequence, Asn-X-Ser/Thr. It has been proposed that sialylation (*white hexagons*) at the termini of oligosaccharides induces conformational change of the Fc C_H2-C_H3 domains, from “open” to “closed”, and reduces IgG binding to the Fc receptor [3], although this has not been verified by X-ray crystallography [4]. Man, mannose; GlcNAc, N-acetylglucosamine; Gal, galactose; Fuc, fucose; Neu5Ac, N-acetylneuraminic acid (sialic acid)

Glycosylated IgG

N-linked oligosaccharides of human serum IgG are present in the Fc portion (Fig. 2) and sometimes in the V_H and V_L regions, depending on the consensus sequence, Asn-X-Ser/Thr. Sialyl residues may be located at the termini of biantennary carbohydrate chains. Studies of the relation of IgG glycosylation to physiology and pathology began over 50 years ago [5]. An analysis of the composition of carbohydrate residues in glycosylated IgG [6] suggested the presence of one sialic acid residue per IgG molecule on average. It was also shown that the carbohydrates

← **Fig. 1** (continued) binds monomeric IgG. FcγRII and RIII (and RIV) show low affinities to IgG, so they bind IgG as immune complexes. FcγRI and RIIIA associate with a homodimeric Fc receptor common γ chain harboring an immunoreceptor tyrosine-based activation motif (ITAM), and FcγRIIA harbors an endogenous ITAM-like motif. Upon ligand binding, these receptors deliver activating signaling within cells. FcγRIIB has an immunoreceptor tyrosine-based inhibitory motif (ITIM) and delivers inhibitory signals upon ligand binding. DC-SIGN (CD209) and FcγRII (CD23) are C-type lectin family members and are proposed sialyl Fc IgG receptors. LCs, Langerhans cells

of IgG Fc determine the half-life of IgG in plasma and its deposition in tissues [7]. The serum level of IgG with no terminal galactose residues is associated with the rheumatoid arthritis (RA) pathology, eg, in the production of IgG rheumatoid factor (RF) [8]. This suggests that *N*-glycosylation of Fc may be linked to immune response and disease states in general. Fucose residues in oligosaccharides of IgG may also modulate immune responses, because fucosylation of IgG reduces binding to Fc γ Rs, and a fucose-less anti-CD20 monoclonal antibody, rituximab, exhibits more active antibody-dependent cell-mediated cytotoxicity toward Raji human B cells of human NK cells *in vitro* [9].

Sialylation of IgG Fc

Findings from earlier studies suggest that terminal sialylation of Fc is important in IgG turnover [5, 6]. In addition, the sialic acid content of RF isolated from an RA patient was lower than that of normal IgG [7], suggesting that sialylation is related to disease. However, the potential significance of sialyl IgG in health and disease was not well recognized until recent studies by Ravetch and colleagues, which highlighted sialyl IgG Fc and its relation to immune regulation. Their research suggests that Fc γ RIIB has a role in feedback regulation of B cells and in inflammatory responses by effector cells, a notion already widely accepted. Several lines of evidence indicate that the therapeutic activity of IVIG is mediated through its Fc portion and Fc γ RIIB in mouse models of immune thrombocytopenic purpura (ITP), RA, and nephrotoxic nephritis [10], and it was associated with upregulated expression of Fc γ RIIB on splenic macrophages in an ITP model.

Kaneko et al. [11] demonstrated the significance of sialyl Fc in the anti-inflammatory action of IVIG. Measurement of the affinities of the sialyl and non-sialyl forms of IgG to Fc γ Rs indicated that sialylation reduces binding affinities by five- to ten-fold. In addition, sialyl Fc, but not non-sialyl IVIG, mediated anti-inflammatory activity in an arthritis model, suggesting that the sialic acids of Fc are responsible for the effect. They also found that the sialic acid content of IgG was reduced upon induction of an antigen-specific immune response, which suggests that differential sialylation constitutes a switch from steady-state anti-inflammatory activity to pro-inflammatory effects upon antigenic challenge (Fig. 3).

Anthony et al. [12] developed a fully recombinant sialyl IgG1 Fc fragment with greatly enhanced potency, as compared with IVIG, in arthritis and ITP models. A specific receptor for sialyl Fc was believed to be involved in this pathway. This receptor was later identified as a C-type lectin, SIGNR1 (specific ICAM-3-grabbing non-integrin-related 1), which is expressed in the splenic marginal zone in mice [13]. Genetic deletion of SIGNR1 in mice abrogated the anti-inflammatory activity of IVIG and sialylated Fc. The study authors also suggested that human DC-SIGN, a homolog of murine SIGNR1, could be a receptor for sialyl Fc and a mediator of anti-inflammation because of the similarity to SIGNR1 in its binding to sialyl Fc *in vitro*.

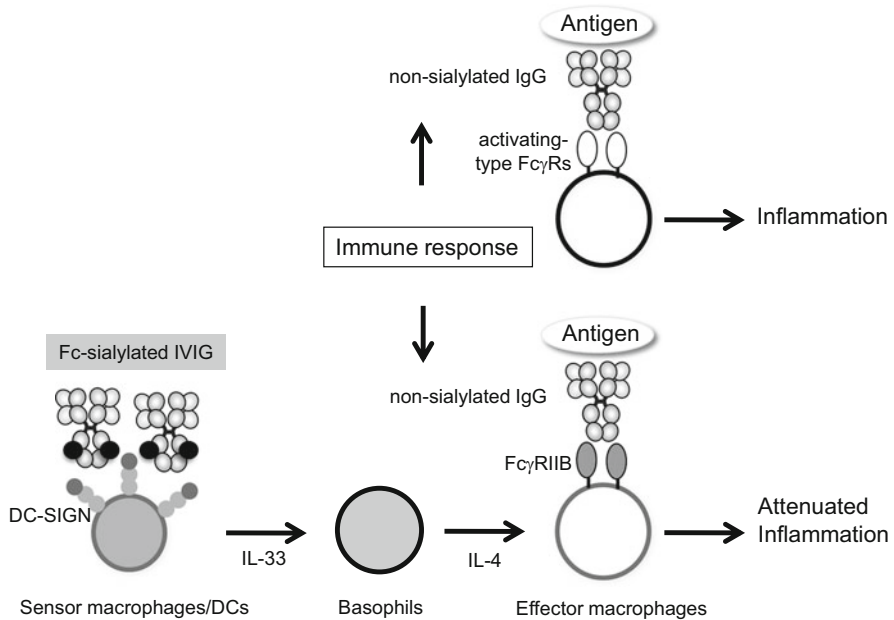


Fig. 3 Proposed mechanism of sialylated IgG Fc-DC-SIGN-FcγRIIB-mediated anti-inflammation

On the basis of data from animal models, a model for DC-SIGN-mediated anti-inflammation has been proposed in which sialylated Fc binds to DC-SIGN on sensor macrophages and DCs, thereby inducing production of IL-33, which then induces basophils to produce IL-4. These basophils then promote increased expression of FcγRIIB on effector macrophages [1]

General Characteristics of DC-SIGN

DC-SIGN is preferentially expressed on human peripheral and lymph node DCs and some macrophages [14] (Fig. 1) and was initially characterized as a receptor for the HIV envelope glycoprotein gp120. *In vitro* analyses of DC-SIGN have revealed that this receptor recognizes a broad range of pathogen-derived ligands and self-glycoproteins as a pattern recognition receptor and is capable of mediating different aspects of DC biology, including intercellular communication, migration, pathogen recognition, signaling, and antigen presentation [14]. However, the *in vivo* function of DC-SIGN is difficult to ascertain, in part because there are multiple genetic homologs (SIGNR1–8) in mice with no clear DC-SIGN ortholog. While human DC-SIGN is expressed on DCs and some macrophages, mouse SIGNR1 is expressed exclusively on marginal zone macrophages, not on DCs. SIGNR1 and SIGNR3 knockout and human DC-SIGN transgenic mouse models have failed to reproduce the predicted *in vitro* physiological functions of DC-SIGN. Thus, available mouse models may be limited in their capacity to reveal the physiological role of DC-SIGN in *in vivo* models [14].

The Potential DC-SIGN-Mediated IVIG Mechanism

Anthony et al. [1] proposed a cascade by which sialylated IVIG Fc and DC-SIGN induce anti-inflammatory activity in an arthritis model expressing human DC-SIGN as a transgene. They found that IVIG action was attained by transfer of macrophages or DCs treated with sialylated Fc into naïve recipient mice. Sialylated Fc administration to mice induced a T helper (Th)₂ cytokine, IL-33, which then induced basophils to produce another Th₂ cytokine, IL-4. These basophils ultimately increased expression of FcγRIIB on inflammatory effector macrophages (Fig. 3). To better understand the structural basis of sialylated Fc binding to DC-SIGN, Sondermann et al. [3] used circular dichroism spectrometry to examine alterations in Fc structure and found that sialylation induced significant structural alterations, from an “open” to “closed” state in the C_H2 domain of Fc (Fig. 2). CD23, a low-affinity receptor for IgE (Fig. 1), bound IgG upon Fc sialylation in a cell-based binding assay. On the basis of these results, they hypothesized that Fc domains undergo a shift between an “open” activating conformation and a “closed” anti-inflammatory state, thereby regulating Fc binding to FcγRs or DC-SIGN, which could be a general immunoregulatory mechanism for maintaining homeostasis. Crispin et al. [4] tested this hypothesis by means of X-ray crystallography but observed no conformational changes upon sialylation of Fc, whose structure was strikingly similar to that of a previously reported IgG-bearing nonsialylated Fc. Thus, the mechanism by which sialyl IgG Fc mediates anti-inflammatory activity remains unclear.

Massoud et al. [15] observed that DC immunoreceptor, or DCIR, which is also a C-type lectin on DCs, mediates the effect of sialylated IVIG. In this case, the anti-inflammatory effect was associated with induction of Treg cells in an airway hyperresponsiveness model. This suggests that sialyl IgG can also be bound by DCIR, which induces inhibitory signaling and makes the DC tolerogenic. This mechanism differs from that of the DC-SIGN–FcγRIIB. Käsermann et al. [16] found that the action of IVIG on human monocytes *in vitro* depends on sialylated Fab rather than on the Fc portion. Additionally, a mechanism in human DCs for IVIG-mediated reciprocal regulation of Th17 and Th1 cells, and Treg cell induction is Fab-dependent [17], and DC-SIGN on DCs directly interacts with Fab of IVIG, thus inducing expansion of Treg cells [18].

Taken together, these observations caution against oversimplification of the IVIG action of DC-SIGN and sialyl Fc and against overestimation of the sialyl Fc–DC-SIGN–FcγRIIB axis in different mouse disease models and its extrapolation to IVIG therapy in humans [19]. The mechanisms of action of IVIG involve a wide spectrum of Fab-mediated and, probably, distinct Fc-mediated mechanisms, which may or may not depend on IVIG sialylation. For example, upregulation of FcγRIIB expression by IVIG could not be confirmed by gene expression profiling, even in Kawasaki disease [20]. von Gunten et al. [19] stressed that many of the disease-specific mechanisms of IVIG observed in mouse models must be validated in humans, as animal models offer only limited insight into human disease and might be biased due to the xenogeneic or species-specific properties of IVIG.

Conclusion

DC-SIGN expressed on macrophages and DCs may be responsible for initiating IVIG Fc-mediated anti-inflammatory events leading to upregulation of Fc γ RIIB on effector macrophages. Because IVIG is a pluripotent drug, its mode of action allows for multiple scenarios, potentially including the sialyl Fc–DC-SIGN–Fc γ RIIB cascade.

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