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Dissecting the Molecular Mechanism of IVIg Therapy: The Interaction between Serum IgG and DC-SIGN is Independent of Antibody Glycoform or Fc Domain

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Abstract

Intravenous immunoglobulin (IVIg) therapy is used to treat a wide range of autoimmune conditions and consists of pooled immunoglobulin G (IgG) from healthy donors. The immunosuppressive effects of IVIg are, in part, attributed to terminal α2,6-linked sialic acid residues on the N-linked glycans of the IgG Fc (*f*ragment *c*rystallizable) domain. This α2,6-sialylated Fc (sFc) has been reported to bind to the carbohydrate recognition domain (CRD) of the cell-surface lectin DC-SIGN (*d*endritic *c*ell-*s*pecific *i*ntercellular adhesion molecule-3-*g*rabbing *n*on-integrin) and its murine orthologue SIGN-R1 (*s*pecific *i*ntracellular adhesion molecule-*g*rabbing *n*on-integrin *R1*) and, via this interaction, to signal the downstream expression of immunosuppressive cytokines and receptors. Consistent with this model, the antiinflammatory effect of IVIg treatment is abolished in a murine knock-out of SIGN-R1 and can be restored by a knock-in with human DC-SIGN. In contrast, however, existing glycan array and X-ray crystallographic studies indicate that the CRDs of both SIGN-R1 and DC-SIGN bind to a restricted set of primarily oligomannose-type glycans that does not include the glycans found on sFc. We attempted to reconcile these immunological and biophysical observations. We first generated hypersialylated, desialylated, deglycosylated and untreated serum IgG and found that the affinity for the complete extracellular region of the DC-SIGN tetramer was similar for all antibody glycoforms. Moreover, the binding could be attributed to cross-reactive, polyclonal Fab (*f*ragment *a*ntigen-*b*inding) specificities in serum as neither recombinant Fc nor sFc bound to DC-SIGN. In addition, serum IgG exhibited no competition against known ligands of the DC-SIGN CRD. These findings lead us to suggest that IVIg therapy does not involve binding of IgG Fc to DC-SIGN and that alternative cell-surface lectins are required for the antiinflammatory activity of sFc.

Intravenous immunoglobulin (IVIg) therapy is effective in treating a wide range of autoimmune diseases and inflammatory conditions including multiple sclerosis, rheumatoid arthritis, chronic inflammatory demyelinating polyneuropathies, immune thrombocytopenia (ITP) and Kawasaki syndrome.[1–5] Recombinant alternatives to this human serum-derived product promise advances in the treatment of autoimmune pathologies. However, despite continuous clinical application over several decades, the molecular mechanisms of IVIg remain elusive and somewhat controversial.

IVIg itself comprises serum immunoglobulin G (IgG) pooled from multiple healthy donors[6] in which both variable Fab (*f*ragment *a*ntigen-*b*inding) and constant Fc (*f*ragment *c*rystallizable) regions of IgG may exert antiinflammatory effects.[5,7,8] For example, Fab-dependent immune complexes are able to alleviate the pathology of mouse ITP[9–11] although contrasting data suggest the minimal involvement of these immune complexes in the effect of IVIg on ITP.[12] Also, anti-idiotype antibodies appear to modulate the effector functions of pathogenic antibodies by neutralizing autoantibodies—though the contribution of this phenomenon to IVIg *in vivo* remains to be determined.[7,13] However, Fc fragments isolated from IVIg have also been shown to be similarly effective as whole IVIg preparations in

inducing immune suppression in both human and animal models of IVIg[14,15] although this Fc-mediated antiinflammatory effect was not recapitulated in an *ex vivo* assay using human whole blood.[16] A role for the Fc is further supported by a requirement for Fcγ receptors (FcγRs) in IVIg.[5,10,17,18]

Recently, it has been reported that the terminal α2,6-linked sialic acid moieties on the IgG Fc N-linked glycan are indispensable for the antiinflammatory property of IVIg.[15,19] The cellular receptor for this α2,6-sialylated Fc (sFc) was subsequently identified as the DC-SIGN (*d*endritic *c*ell-*s*pecific *i*ntercellular adhesion molecule-3-*g*rabbing *n*on-integrin). Similarly, SIGN-R1 (*s*pecific *i*ntracellular adhesion molecule-*g*rabbing *n*on-integrin *R1*), the mouse orthologue of DC-SIGN, acts as the murine sFc receptor.[20] In transgenic mouse model of IVIg wherein SIGN-R1 was replaced by DC-SIGN, the introduction of sFc leads to the subsequent expression of IL-4 and IL-33 (IL, interleukin) and induced the expression of antiinflammatory factors such as the inhibitory Fc receptor, FcγRIIb, on macrophages.[21]

A number of *in vivo* observations directly support the sFc/DC-SIGN model: antibodies that down-regulate or block the carbohydrate recognition domain (CRD) of SIGN-R1 abolish the effect of IVIg in ITP and serum-induced arthritis models[20,22]; removal of SIGN-R1 removes the effect of IVIg in serum-induced arthritis model, whereas subsequent knock-in with DC-SIGN restores efficacy[20,21]; recombinant sFc with α2,6-linked, but not α2,3-linked, sialic acid is able to recapitulate the effect of IVIg in the murine rheumatoid arthritis model[15]; similarly, sFc induces the expression of immunoregulatory cytokines, IL-4 and IL-33, and exogenous addition of either of these cytokines can offer protection similar to that seen for IVIg[21]; macrophages or dendritic cells from human DC-SIGN transgenic mice pulsed with either α2,6-sFc or IVIg can reverse serum arthritis. Notably, the efficacy of these introduced cells required that the recipient mouse was competent for FcγRIIb, IL-4 and IL-4R.[21]

However, the sFc/DC-SIGN hypothesis does not appear to provide a complete explanation for all aspects of IVIg. For example, desialylated IVIg efficiently protected mice against fatal herpes simplex virus-induced encephalitis and ITP.[23,24] Similarly, IVIg fractions either enriched or depleted for the sialylated antibody fraction (by the α2,6-Neu5Ac-specific lectin, *Sambucus nigra*) equally protected mice from both herpes simplex virus-induced encephalitis and antibody-mediated platelet depletion.[23,25] Some of these apparently contradictory results may be explained by the fact that different mouse strains respond differentially to IVIg treatment, as is the case for the ITP model. These results may therefore simply reflect the fact that the sFc/DC-SIGN effect is only one of multiple immunosuppressive IVIg pathways, which are differentially revealed by various animal disease models. Alternatively, they may suggest that a more general re-evaluation of the sFc/DC-SIGN model of IVIg is warranted.

Although significant immunological data support a central role for DC-SIGN/SIGN-R1 in many of the antiinflammatory effects of IVIg,[20–22] current biophysical data of DC-SIGN, from glycan array studies, X-ray crystal structures and solution-phase binding assays, do not suggest an obvious binding mechanism between sFc and DC-SIGN.[26–28] The well-resolved CRD of DC-SIGN readily explains the selective, calcium-dependent binding to D-mannose, L-fucose and *N*-acetyl-D-glucosamine residues but does not reveal a likely binding site for sialic acid.[26] Similarly, glycan array binding data indicate that sialylated biantennary N-linked glycan structures found on IgG Fc are not ligands for DC-SIGN or SIGN-R1.[29,30] In contrast to these existing biophysical data, a direct interaction between DC-SIGN and sFc has nonetheless been inferred from the ability of DC-SIGN-transfected cells to deplete α2,6-sFc (but not α2,3-sFc) from cell culture supernatant. This depletion could be inhibited by known ligands of the DC-SIGN CRD[20] and would thus appear to provide evidence in support of the sFc-mediated, DC-SIGN-dependent IVIg model. The interaction between DC-SIGN and sialylated IgG lies at the nexus of the current model of the IVIg pathways and represents a key target for therapeutic exploitation. To date, however, no direct binding measurements of this interaction have been reported.

To investigate the role of Fc glycosylation in the putative interaction between DC-SIGN and IgG, we determined, by ELISA, the relative affinities of extracellular, tetrameric DC-SIGN for natural and engineered IgG glycoforms (Fig. 1). Hyper-α2,6-sialylated serum IgG was prepared by sequential treatment with β-1,4-galactosyltransferase (B4GALT1) and α2,6-sialyltransferase; desialylated and deglycosylated serum IgG were generated using nonspecific sialidase and protein *N*-glycanase F, respectively (Fig. 1). The carbohydrate binding capacity of DC-SIGN in this assay was confirmed by its interaction for the heavily mannosylated surface glycoprotein from human immunodeficiency virus type 1 (HIV-1), gp120 (Fig. 2a). In contrast, serum IgG bound to DC-SIGN with an apparent affinity (1 μM) approximately 1000-fold less than that for gp120, consistent with previous reports for both these glycoproteins.[20,32] Notably, both hypersialylated and desialylated serum IgG bound to DC-SIGN with very similar affinities to that of naturally glycosylated material. In addition, even fully deglycosylated serum IgG exhibited an unchanged affinity for DC-SIGN. Moreover, natively glycosylated recombinant Fc, hypersialylated Fc (sFc, generated by sequential treatment with B4GALT1 and α2,6-