

Dimeric IVIG contains natural anti-Siglec-9 autoantibodies and their anti-idiotypes

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Abstract

Background: Intravenous immunoglobulin (IVIG) preparations are increasingly used for the treatment of autoimmune and chronic inflammatory diseases. Naturally occurring autoantibodies against Siglec-9 and Fas are thought to contribute to the anti-inflammatory effects of IVIG via cell death regulation of leukocytes and tissue cells. Dimeric IVIG fractions are suspected to contain idiotypic (Id)-anti-idiotypic complexes of antibodies, which might also include anti-Siglec-9 and anti-Fas autoantibodies.

Methods: Dimeric IVIG fractions were separated from monomeric IVIG by size-exclusion chromatography and remonomerized by low pH treatment. Binding studies of total, monomeric, and dimeric IVIG were performed using surface plasmon resonance and flow cytometry on primary human neutrophils.

Results: Anti-Siglec-9 and anti-Fas autoantibodies were contained in both monomeric and dimeric IVIG fractions, but anti-Siglec-9 antibodies were highly enriched in dimeric IVIG. The propensity to engage in dimer formation was paratope dependent. IVIG binding to Siglec-9 was specific and sialylation independent. Interestingly, we detected anti-idiotypic antibodies (anti-Ids) against anti-Siglec-9 autoantibodies in dimeric, but not in monomeric fractions of IVIG.

Conclusions: Our study supports the concept that idiotype-anti-idiotype (Id-anti-Id) interactions contribute to the dimer formation in IVIG preparations. To our knowledge, this is the first description of Id-anti-Id dimers of death receptor-specific antibodies in IVIG. Such Id-anti-Id interactions might determine the activity of immunomodulatory antibodies present both in IVIG and the patient.

Originally used as a replacement therapy for patients with primary or secondary antibody deficiencies, intravenous immunoglobulin (IVIG) is increasingly being used as an anti-inflammatory agent for the treatment of chronic inflammatory and autoimmune diseases (1–3). Despite its widespread use, the understanding of its immunoregulatory mechanisms of action remains incomplete (3).

We and others have previously shown that IVIG has the capacity to modulate the survival of leukocytes or tissue cells by means of naturally occurring autoantibodies against Fas, sialic acid immunoglobulin-like lectin (Siglec)-8, and Siglec-9 (4–9). Siglecs are a novel family of immunoregulatory receptors that recognize sialic acid (Sia) (10, 11), and whose cytotoxic efficacy and potency are significantly enhanced by a

pro-inflammatory cytokine environment (12, 13). Siglec-9 is expressed on neutrophils, monocytes, dendritic cells, and on a subpopulation of lymphocytes (10). Ligation of Siglec-9 by monoclonal antibodies or natural autoantibodies in IVIG leads to neutrophil apoptosis (8, 14, 15). Interestingly, Siglec-9-mediated death is dramatically enhanced in the presence of proinflammatory cytokines, such as granulocyte/macrophage colony-stimulating factor, interferon-alpha (IFN-alpha), and IFN-gamma (8, 14), and in inflammatory cells *ex vivo* from inflammatory diseases, such as sepsis and rheumatoid arthritis (8, 14, 15). Recent evidence suggests that Siglec-9 may also act as an inhibitory receptor: sialylated capsular polysaccharide of group B *Streptococcus* may engage neutrophil Siglec-9, dampening neutrophil responses in a Sia- and

Siglec-9-dependent manner, including oxidative burst, formation of neutrophil extracellular DNA traps, and bacterial killing (16). Siglec-9-mediated inhibition or clearance of immune cells by cell death might provide an important immunoregulatory mechanism (10), which might eventually be exploited by pathogens as molecular mimicry (16).

Agonistic anti-Fas antibodies present in IVIG have been shown to induce apoptosis of primary leukocytes and different B and T cell lines *in vitro* (5, 6). In patients with toxic epidermal necrolysis (TEN), IVIG treatment dramatically improves the clinical situation, which has been linked to antagonistic anti-Fas autoantibodies (4). Intravenous immunoglobulin contains concurrent agonistic and antagonistic anti-Fas antibodies, and their effect on Fas is concentration dependent (6).

The regulation of the bioactivity of natural anti-Fas and anti-Siglec autoantibodies in IVIG may have several important clinical implications for the treatment of inflammatory diseases (9, 17). Anti-idiotypic regulation (18) might present one potential mechanism to control the activity of natural autoantibodies in IVIG. Intravenous immunoglobulin contains antibodies so-called anti-idiotypes (Ids) that are capable of interacting with variable regions of other antibody molecules to form Id–anti-Id complexes. Indeed, a broad range of anti-Ids have been identified in IVIG that after intravenous application bind to disease-associated and natural autoantibodies of the patient as well as to B cell receptors (3, 19, 20).

Evidence of both theoretical and experimental research suggests that Id–anti-Id complexes contribute to dimer formation in IVIG (21–26). Dimer formation has been linked to both beneficial effects and transient early adverse reactions of IVIG (3, 27). We have recently shown that the recognition of human cellular structures is higher in dimeric than in monomeric IVIG, and that the tissue reactivity of the dimeric fraction is further increased after dissociation by pH treatment (28, 29). The increased self-reactivity of dimeric IVIG after remonomerization supports the concept that the dimeric fraction contains Id–anti-Id complexes and that anti-Ids may efficiently neutralize natural autoantibodies by blocking their binding sites. Here, we show that naturally occurring anti-Ids to anti-Siglec-9 autoantibodies exist and that they are localized in the dimeric fraction of IVIG. These novel observations together with previous work are summarized in a model where cytotoxic autoantibodies found in IVIG are regulated by three mechanisms: soluble receptors, antagonistic autoantibodies, and Id–anti-Id interactions.

Materials and methods

Cell isolation

Neutrophils were isolated from the peripheral blood of healthy donors by Ficoll-Hypaque centrifugation as previously described (8, 14, 15). Briefly, peripheral blood mononuclear cells were separated by centrifugation on Ficoll-Hypaque (Seromed-Fakola, Basel, Switzerland). The lower phase, mainly granulocytes and erythrocytes, was treated with erythrocyte lysis solution (155 mM NH_4Cl , 10 mM KHCO_3 , and 0.1 mM EDTA, pH 7.3). Resulting cell populations contained

greater than 95% neutrophils. Written consent was obtained from all donors, and the study was approved by the Medical Ethics Committee of the Canton Bern.

IVIG preparations

Intravenous immunoglobulin fractions were prepared as described previously (28, 29). In brief, 12% (w/v) liquid IVIG was produced by reconstituting 12 g of lyophilized Sando-globulin (CSL Behring AG, Bern, Switzerland) with 0.9% NaCl. This preparation consisted of approximately 13% dimeric IgG and 86% monomeric IgG and IgG aggregates (<1%). To separate monomeric from dimeric IgG, size-exclusion chromatography (ReproSil 300 $\times$ 20 column; Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) was performed. The purity of monomeric and dimeric IgG was 98.5% and 95%, respectively. All IVIG fractions were monomerized by dialysis against 10 mM acetic acid at pH 4.0. To exclude rheumatoid factor activity, all IVIG fractions were tested by routine diagnostic nephelometry and were below detectable levels (data not shown).

Intravenous immunoglobulin-derived anti-Siglec-9 antibodies (Abs) were isolated by batch affinity purification as previously described (8). Briefly, a recombinant Siglec-9/Fc fusion protein (R&D Systems, distributed by Buehlmann, Basel, Switzerland) was coupled to Affi-Gel beads (Bio-Rad, Reinach, Switzerland). Intravenous immunoglobulin was coincubated (overnight, 4°C, on a rotor) with the beads, allowing for binding of anti-Siglec-9-specific Abs to the coupled antigen. Following several washing steps, the anti-Siglec-9-specific Abs were eluted with 0.1 M glycine–HCl, pH 2.7, (Riedel-DeHäen, Seelze, Germany) and neutralized with 1 M NaOH. Following centrifugation, the supernatant containing the Siglec-9-specific Abs was immediately subjected to buffer exchange employing Vivaspin concentrator tubes (10 000 Da cutoff; Vivascience, Göttingen, Germany).

Inhibition of binding to Siglec-9 and Fas

Freshly isolated neutrophils were preincubated with the indicated concentrations of IVIG for 30 min, washed once with PBS (4°C), and subsequently incubated for 15 min with fluorescein isothiocyanate (FITC)-coupled anti-Siglec-9 monoclonal antibody (mAb) (1 : 25) (clone E10-286; BD Biosciences, Basel, Switzerland), anti-Fas mAb (clone DX2; BD Biosciences) (1 : 25), anti-CD16 mAb (Beckman Coulter International SA, Nyon, Switzerland) (1 : 50), or a control IgG1 mAb (BD Biosciences) in PBS at 4°C, followed by a single washing step with PBS (4°C). Neutrophil staining with anti-Siglec-9 and anti-Fas mAb was measured by flow cytometry (FACS Calibur, BD Biosciences).

Real-time binding to Fas, Siglec-9, and IVIG-derived anti-Siglec-9 Ab

Recombinant Fas/Fc (Alexis Corporation, Lausen, Switzerland), Siglec-9/Fc proteins (R&D Systems), and anti-Siglec-9 Abs affinity-purified from IVIG were covalently coupled to a

CM5 sensor chip according to manufacturer's instructions (Biacore AB, Uppsala, Sweden) and as previously described (8). In brief, coating was performed by repeated manual injections of Fas/Fc, Siglec-9/Fc, and IVIG-derived anti-Siglec-9 Abs in 10 mM sodium acetate at a concentration of 20 µg/ml and followed by blocking with 1 M ethanolamine-HCl, pH 8.5. For the analysis, all Abs were diluted in HBS-EP buffer (Biacore) to the concentrations indicated in the results and the real-time binding was measured on a Biacore X machine (Biacore).

Desialylation of IVIG

Intravenous immunoglobulin was coincubated with neuraminidase (New England BioLabs Ltd., distributed by Bioconcept, Allschwil, Switzerland) at a concentration of 20 000 U/mg in a 0.05 M calcium citrate, pH 6.0, overnight at 37°C. The desialylation was verified by SDS-PAGE and subsequent lectin blot, using the NuPage system (Invitrogen AG, Basel, Switzerland).

In brief, a 10% Bis-Tris gel was loaded with 1 µg IVIG and neuraminidase-treated IVIG and run in MOPS SDS running buffer at 200 V, 120 mA, for 45 min. The gel was stained by GelCode Blue Stain Reagent (Pierce; distributed by Thermo Fisher Scientific, Wohlen, Switzerland). Subsequently, the proteins were blotted for 1 h at 25 V, 160 mA, to a nitrocellulose membrane (Invitrogen AG), blocked in 5.0% low-fat milk powder, and detected with a combination of biotinylated *Sambucus nigra* agglutinin (SNA) (Vector Laboratories, Burlingame, CA, USA) and alkaline phosphatase-labeled streptavidin (Invitrogen AG), using an alkaline phosphate conjugate substrate kit (BioRad, Hercules, CA, USA).

Results

Dimeric IVIG is enriched with anti-Siglec-9 autoantibodies

We and others have previously shown that IVIG contains functional autoantibodies against the death receptors Siglec-9

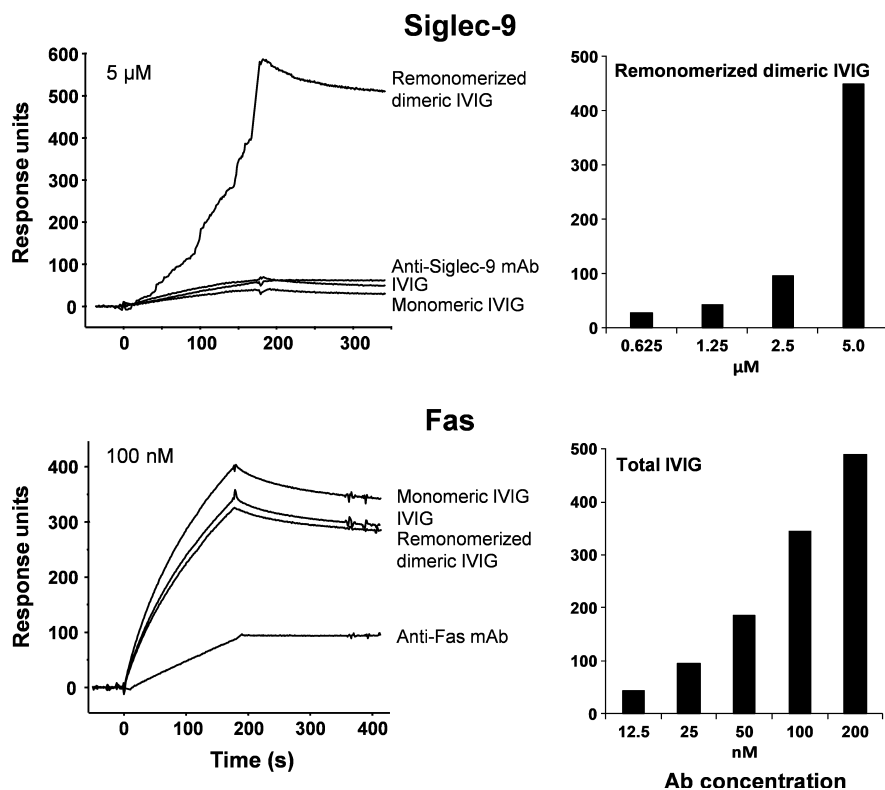


Figure 1 Anti-Siglec-9 autoantibodies are prevalent in dimeric intravenous immunoglobulin (IVIG), whereas similar proportions of anti-Fas autoantibodies reside in dimeric and monomeric IVIG, as assessed by surface plasma resonance (SPR). Sensorgrams of the real-time association (0–180 s) and dissociation (181–360 s) of different fractions of IVIG and control antibodies to immobilized recombinant Siglec-9/Fc and Fas/Fc fusion proteins are shown. Binding of all fractions of IVIG to Siglec-9 (all at 5 µM) and to Fas (all at 100 nM) was detected. A marked increase in the association curve for dimeric IVIG to Siglec-9 was observed, confirming the

prevalence of anti-Siglec-9 autoantibodies in this fraction. In contrast, binding of monomeric IVIG to Siglec-9 was low. Binding to Fas was similar for all IVIG fractions, although the association of monomeric IVIG with Fas was slightly enhanced (left panels). Binding was concentration dependent as shown here for the interaction between remonomerized dimeric IVIG and total IVIG with Siglec-9 and Fas, respectively, at near maximal RU (170 s, right panels). The control mAbs, used at a low concentration (1 nM), showed stable binding with constant association and dissociation curves.

and Fas (4–8). In the present study, we decided to analyze the distribution of anti-Siglec-9 and anti-Fas autoantibodies in monomeric and dimeric IVIG fractions and assessed their binding capacity to immobilized recombinant Siglec-9/Fc and Fas/Fc fusion proteins by surface plasmon resonance (SPR), as previously described (8). Monomeric and dimeric IVIG fractions were separated by size-exclusion chromatography and acid treated (pH 4) for monomerization of the dimeric fraction, as previously described (29). Reactivity with the human IgG1 Fc was excluded by nephelometry (8). We then performed kinetic SPR experiments with monomeric IVIG, remonomerized dimeric IVIG, and total IVIG. To exclude nonspecific binding effects, parallel signals produced by an uncoated but otherwise identical flow cell were continuously subtracted from the primary curves. The anti-Siglec-9 mAb (E10-286) and anti-Fas (DX-2) mAbs were employed as positive controls (both at 1 nM). They exhibited stable binding and constant 'on-rate' and undetectable 'off-rate', reflecting their high-affinity interactions with Siglec-9 and Fas.

Figure 1 depicts the real-time association (0–180 s) and dissociation (181–360 s) curves of the IVIG fractions at 5 μ M to Siglec-9 and at 100 nM to Fas (left panels). For the detection of Fas, lower quantities of IVIG were required than for the detection of Siglec-9. Remonomerized dimeric IVIG demonstrated a markedly increased association with Siglec-9, which appeared to be concentration dependent (right panel). Interestingly, this association curve (of remonomerized dimeric IVIG) reproducibly exhibited an exponential binding pattern followed by readily visible dissociation. Binding of monomeric IVIG to Siglec-9 was similar compared to total IVIG, but slightly reduced. In contrast to Siglec-9 recognition, no major differences in binding to Fas were found among the IVIG fractions, which all exhibited similar association and dissociation curves (left panel). However, the monomeric fraction of IVIG showed a slightly higher rate of Fas association. Fas binding of total IVIG was concentration dependent (right panel).

Taken together, these data show that dimeric IVIG is enriched in anti-Siglec-9 autoantibodies, whereas anti-Fas autoantibodies are similarly distributed between monomeric and dimeric IVIG.

Paratope-dependent distribution of antibodies in dimeric and monomeric IVIG

Previously, we reported that agonistic and antagonistic anti-Fas autoantibodies recognize different epitopes (6). We wondered about similarities and differences in terms of epitope specificity between autoantibodies contained in monomeric and dimeric IVIG. Therefore, we decided to perform flow cytometric blocking assays using anti-Siglec-9 monoclonal antibody (mAb) clone E10-286 and anti-Fas mAb clone DX-2 to compete with natural anti-Siglec-9 and anti-Fas autoantibodies in monomeric and dimeric IVIG fractions for binding to their specific receptor epitope.

Preincubation of human neutrophils with total IVIG inhibited flow cytometric staining of Siglec-9 with FITC-conjugated mAb clone E10-286 and of Fas by FITC-conjugated

mAb clone DX-2 in a concentration-dependent manner (Fig. 2A). This observation confirmed the occurrence of natural autoantibodies in IVIG with specificity for the E10-286 and DX-2 epitopes. A concentration of 5 mg/ml of total IVIG was sufficient to completely block staining with clone E10-286, whereas staining with clone DX-2 was blocked at 8 mg/ml.

Next, we preincubated the cells with monomeric and remonomerized dimeric IVIG fractions. Surface staining of Siglec-9 on neutrophils with the FITC-conjugated E10-286 mAb was efficiently blocked by total and remonomerized dimeric IVIG at 5 mg/ml (Fig. 2B). In contrast, monomeric IVIG only partially inhibited E10-286 binding. DX2 binding was significantly blocked by preincubation with monomeric IVIG, but not with remonomerized dimeric IVIG. In agreement with

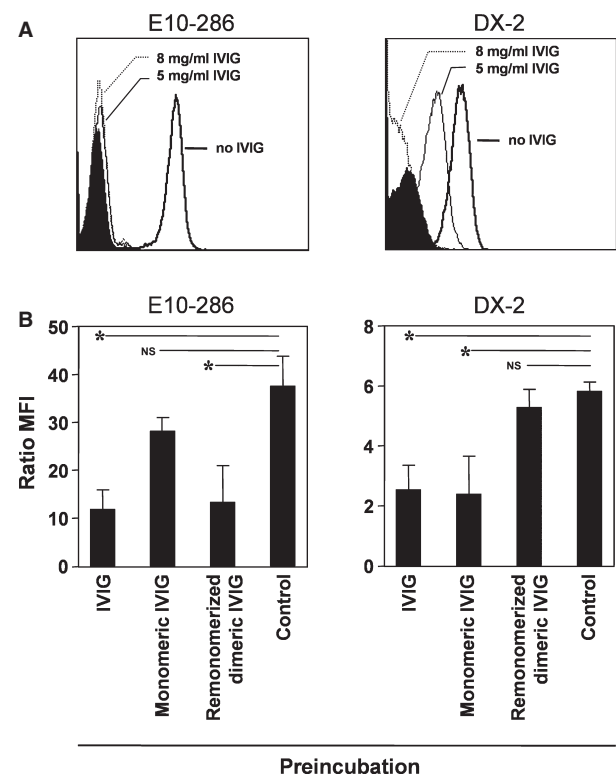


Figure 2 The distribution of natural autoantibodies in monomeric or dimeric intravenous immunoglobulin (IVIG) is paratope dependent, as assessed by flow cytometry. (A) Cells were preincubated with IVIG for 30 min before staining with FITC-conjugated anti-Siglec-9 mAb clone E10-286 and anti-Fas mAb clone DX2. Intravenous immunoglobulin concentration dependently inhibited staining with clone E10-286 and DX2 on human neutrophils. Data represent at least four independent experiments. (B) The capacity to inhibit Siglec-9- and Fas-staining on neutrophils varied between monomeric and dimeric IVIG. Cells were preincubated with IVIG at 5 mg/ml for 30 min. Dimeric IVIG blocked E10-286 staining. In contrast, DX2 binding was inhibited by monomeric IVIG, whereas dimeric IVIG had no blocking effect. Values indicate the mean fluorescence intensity (MFI) as ratio compared to an unspecific FITC-IgG1 isotype control ($n = 3$). * $P < 0.05$.

previous work (6), none of the IVIG fractions inhibited staining of a FITC-conjugated anti-CD16 mAb, used as a control (data not shown). These data show that autoantibodies recognizing the E10-289 epitope of Siglec-9 are predominantly contained in dimeric IVIG together with the majority of anti-Siglec-9 autoantibodies. In contrast, anti-Fas autoantibodies recognizing the DX2-epitope are enriched in monomeric IVIG. This is remarkable because DX2 epitope-specific antibodies appear to reside preferentially in monomeric IVIG, whereas overall Fas-specific autoantibodies are equally distributed within monomeric and dimeric fractions (Fig. 1). This observation suggests that the propensity of a given antibody to form dimers in IVIG is linked to its paratope.

Sialylation-independent binding of IVIG to Siglec-9

As Siglecs are known for binding to Sia (10, 11), they have been proposed as candidate receptors for sialylated IVIG glycoforms (30). To estimate the extent of Sia-mediated binding of IVIG to Siglec-9, we decided to compare the binding capacities of regular and desialylated total IVIG.

Intravenous immunoglobulin was treated with neuraminidase to remove Sia (14, 31). Lectin blotting of control and neuraminidase-treated IVIG (NA IVIG) with SNA lectin was used to assess the efficacy of the neuraminidase treatment in removing the lectin-accessible Sia. Desialylation with

neuraminidase completely abrogated the binding signal of the Sia-specific plant lectin SNA confirming the successful removal of Sia from NA IVIG by neuraminidase treatment (Fig. 3A).

Surface plasmon resonance was employed to compare the binding capacity of IVIG and NA IVIG to immobilized Siglec-9/Fc. No differences between IVIG and NA IVIG in terms of binding to Siglec-9 (Fig. 3B) were seen, suggesting that the association of IVIG with Siglec-9 is Fab dependent and not mediated by Sia.

Presence of naturally occurring anti-idiotypes of anti-Siglec-9 autoantibodies in dimeric but not monomeric IVIG

It has been postulated that dimers in IVIG are formed by Id-anti-Id interactions (21–26). Our observation that the propensity of dimer formation is paratope related, is very well compatible with this assumption because Id-anti-Id interactions depend on characteristics of the variable region (antigen-binding site) of the idiotype. Furthermore, we hypothesized that the exponentially shaped SPR association curve of remonomerized dimeric IVIG to Siglec-9 in Fig. 1 might originate from reformation of Id-anti-Id complexes alongside the binding of the immobilized antigen. To test this hypothesis, we measured anti-Ids in the IVIG-derived fractions binding to covalently immobilized anti-Siglec-9 Ids, which were affinity-purified from IVIG as previously described (8).

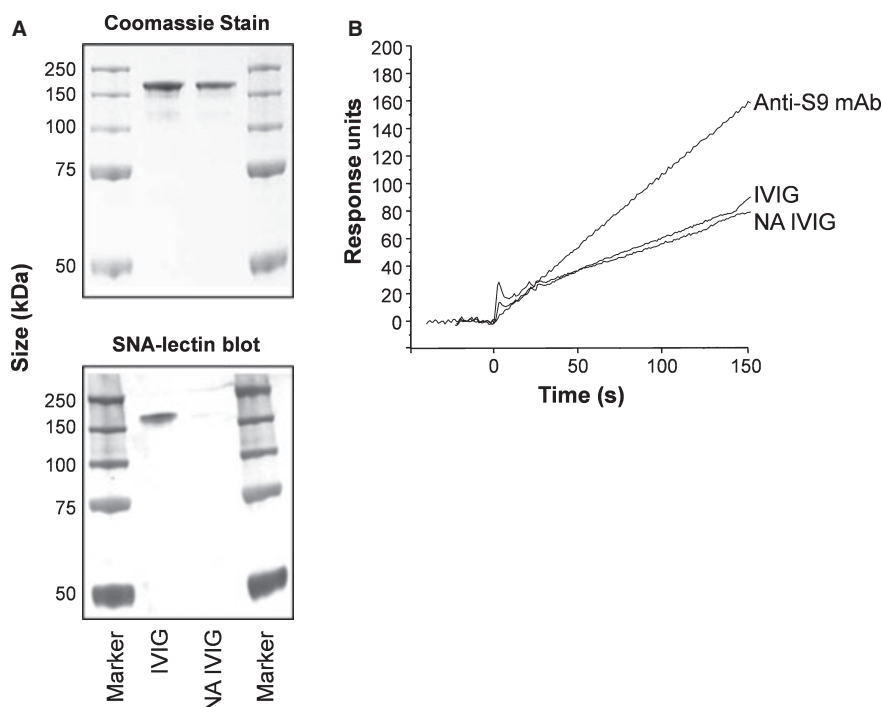


Figure 3 Binding of anti-Siglec 9 autoantibodies in intravenous immunoglobulin (IVIG) is sialic acid (Sia) independent. (A) Coomassie-stained gel (upper panel) and *Sambucus nigra* agglutinin (SNA) lectin blot (lower panel) of control and neuraminidase-treated IVIG (NA IVIG). Neuraminidase treatment resulted in loss of SNA binding, confirming the successful desialylation of NA IVIG. Lectin blots

are representative of three independent experiments. (B) Sensorgram of the binding activity of control IVIG and NA IVIG (both at 5.7 μ M) and anti-Siglec-9 mAb (5 nM) to immobilized recombinant Siglec-9/Fc. Desialylation of IVIG had no effect on its binding capacity to Siglec-9.

The remonomerized dimer fraction, used at concentrations ranging from 0.3 to 5 μM , bound to the immobilized anti-Siglec-9 Ids (Fig. 4) in a concentration-dependent manner, confirming the presence of natural anti-Ids to anti-Siglec-9 autoantibodies in IVIG. In contrast, no binding of the monomeric IVIG to the purified anti-Siglec-9 Ids was detected. These findings suggest that anti-Ids do bind their counterparts in IVIG, i.e., Siglec-9-specific Ids, with sufficient affinity to form relatively stable dimeric Id–anti-Id complexes.

Discussion

In the present study, we provide evidence for the existence of anti-Id antibodies against anti-Siglec-9 autoantibodies in IVIG. Whereas dimer formation in IVIG was initially thought of as unspecific complex formation depending on chemical properties of the preparation, indirect evidence has accumulated suggesting that Id–anti-Id interactions contribute significantly to the dimer formation (21–26). Our findings confirm this concept and suggest that the binding activity of natural autoantibodies may be affected by anti-idiotypic

regulation. Anti-idiotypic regulation of self-reactive immunoregulatory antibodies may be important for normal immunological homeostasis (28, 29) and is likely to account for at least some of the clinical effects of total IVIG (3).

Anti-Siglec-9 and anti-Fas antibodies in IVIG have previously been described (9), and the presence of such antibodies in our preparations was confirmed by SPR analysis and flow cytometric blocking experiments on primary human neutrophils. Anti-Fas autoantibodies were similarly distributed in monomeric and dimeric IVIG. Because there was a striking prevalence of anti-Siglec-9 autoantibodies in dimeric IVIG, we hypothesized that the majority of anti-Siglec-9 autoantibodies in IVIG are bound as dimeric Id–anti-Id complexes. Indeed, SPR experiments using affinity-purified IVIG-derived anti-Siglec-9 autoantibodies revealed the presence of anti-Id antibodies in dimeric, but not monomeric IVIG. Our results indicate distribution differences for epitope-specific autoantibodies within monomeric and dimeric IVIG and suggest that dimer formation in IVIG is not a stochastic process, but depends on characteristics of the paratope, the variable region of the idiotype, probably determining the stability of the Id–anti-Id complex. Notably, agonistic and antagonistic antibodies may recognize different epitopes as shown for Fas (6). Whether Siglec-9 anti-Id antibodies exhibit a protective role in autoimmunity remains to be determined. In such future studies, it would be interesting to quantify and compare anti-Id antibodies to anti-Siglec-9 autoantibodies in single sera of healthy individuals and patients with autoimmune disorders.

Recent evidence suggests an anti-inflammatory role of sialylated glycoforms of IVIG (31, 32). As distinct Sia-containing carbohydrates are known ligands of Siglecs (10, 11), these lectins have been proposed as candidate receptors for sialylated IVIG (30). In our study, desialylation of IVIG had no apparent effect on Siglec-9 binding in SPR assays, suggesting that Fab-dependant recognition by specific autoantibodies provides the main mechanism of IVIG interaction with Siglec-9. However, our study does not entirely exclude a role of Sia-mediated binding, because such protein-glycan interactions are usually of low affinity and may not be detectable in our assays.

We and others have described the presence of agonistic autoantibodies against Fas, Siglec-8, and Siglec-9 in IVIG, with the capacity to regulate immune cells by induction of cell death (5–9). The clearance of immune cells by death is important in the resolution phase of an inflammation and might significantly contribute to the anti-inflammatory action of IVIG (2, 3, 10, 17, 33). On the other hand, regulatory mechanisms must exist that control the activity of these potentially harmful autoantibodies in IVIG. We propose that death-inducing autoantibodies in IVIG are controlled by at least the following three mechanisms: soluble receptors, antagonistic autoantibodies, or Id–anti-Id interactions (Fig. 5). Soluble forms of the Fas receptor exist that protect Fas-bearing sensitive cells from apoptosis induced by agonistic antibodies, as well as by the Fas ligand (34, 35). Second, we have previously reported that both agonistic and antagonistic anti-Fas autoantibodies are present in IVIG, and that

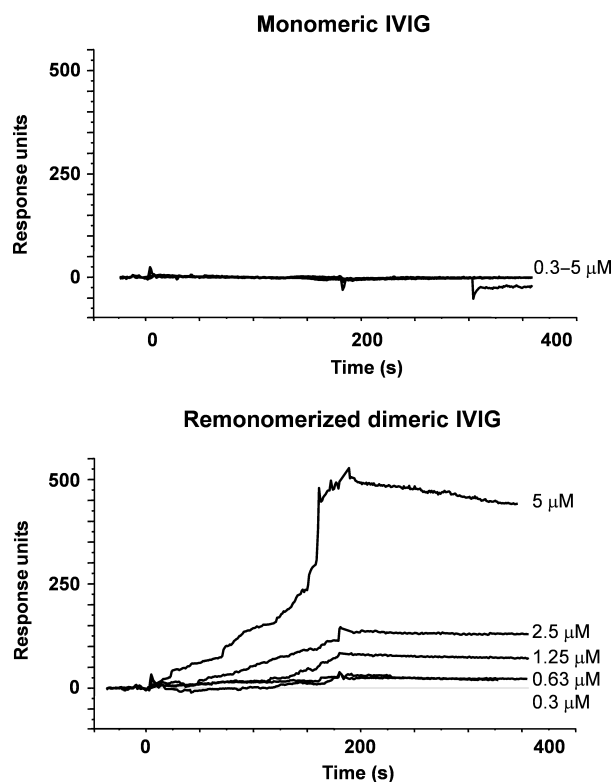


Figure 4 Dimeric, but not monomeric, intravenous immunoglobulin (IVIG) contains anti-idiotypic anti-Siglec-9 antibodies. Sensorgrams of the binding activity of monomeric and remonomerized dimeric IVIG fractions (at 0.3–5 μM) to immobilized anti-Siglec-9 idiotype Abs, which were affinity-purified from whole IVIG. Remonomerized dimeric IVIG showed a concentration-dependent increase in the association curve revealing the presence of anti-idiotypic Abs in dimeric IVIG. No binding of the monomeric fraction was detectable.

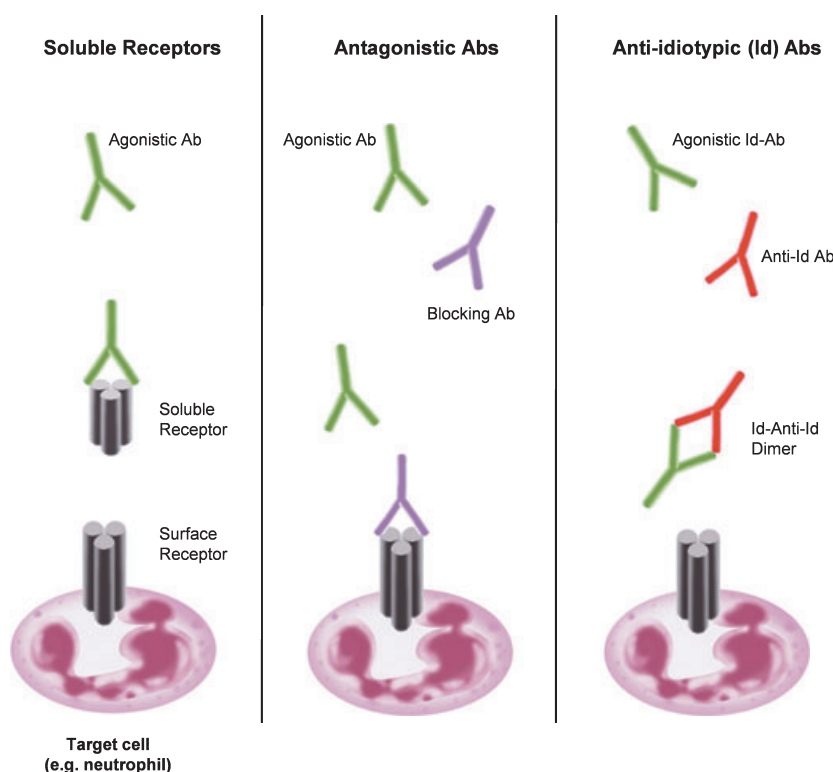


Figure 5 Proposed model of the regulation of death-inducing autoantibodies in intravenous immunoglobulin (IVIG). Potentially harmful cytotoxic autoantibodies in IVIG may be controlled by the following three mechanisms: soluble receptors, antagonistic autoantibodies, or Id–anti-Id interactions. (1) Soluble receptors bind to agonistic autoantibodies and prevent targeting of the cell surface receptor, as known for soluble Fas receptor. (2) Antagonistic autoantibodies

compete with cytotoxic (agonistic) antibodies for receptor engagement, e.g., antagonistic anti-Fas autoantibodies protected neutrophils from apoptosis induced by agonistic anti-Fas autoantibodies. (3) Anti-idiotypic (anti-Id) autoantibodies bind and neutralize agonistic, idiotypic (Id) autoantibodies resulting in formation of Id–anti-Id dimers, as shown for anti-Siglec-9 autoantibodies in the present study.

the effect on Fas is concentration dependent (6). Indeed, the beneficial effect of IVIG in TEN is believed to be because of the presence of naturally occurring blocking antibodies to Fas (4). Third, in the present study, we provide evidence for the occurrence of anti-Id antibodies that bind anti-Siglec-9 autoantibodies in IVIG. Similarly, the presence of anti-Fas autoantibodies in dimeric IVIG suggests that a proportion of these autoantibodies might be bound by anti-Ids. Taken together, these mechanisms might determine the therapeutic activity of functional autoantibodies after IVIG application. Because the levels of soluble receptors and antagonistic or anti-Id antibodies in both original pooled sera or in patients may differ considerably, the clinical response to IVIG therapy might be significantly influenced by these factors (9, 17).

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Conflict of interest

The authors have no conflicts of interest concerning the data reported in this study.

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