



Knockout Reporter Cells

The smart way to study nucleic acid sensing



The Emerging Importance of IgG Fab Glycosylation in Immunity

Fleur S. van de Bovenkamp, Lise Hafkenscheid, Theo Rispens and Yoann Rombouts

This information is current as of February 11, 2016.

J Immunol 2016; 196:1435-1441; ;
doi: 10.4049/jimmunol.1502136
<http://www.jimmunol.org/content/196/4/1435>

-
- | | |
|----------------------|--|
| References | This article cites 82 articles , 27 of which you can access for free at:
http://www.jimmunol.org/content/196/4/1435.full#ref-list-1 |
| Subscriptions | Information about subscribing to <i>The Journal of Immunology</i> is online at:
http://jimmunol.org/subscriptions |
| Permissions | Submit copyright permission requests at:
http://www.aai.org/ji/copyright.html |
| Email Alerts | Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/cgi/alerts/etoc |



The Emerging Importance of IgG Fab Glycosylation in Immunity

Fleur S. van de Boyenkamp,^{*,†,1} Lise Hafkenscheid,^{‡,1} Theo Rispens,^{*,†,2} and Yoann Rombouts^{‡,§,¶,2}

Human IgG is the most abundant glycoprotein in serum and is crucial for protective immunity. In addition to conserved IgG Fc glycans, ~15–25% of serum IgG contains glycans within the variable domains. These so-called “Fab glycans” are primarily highly processed complex-type biantennary *N*-glycans linked to *N*-glycosylation sites that emerge during somatic hypermutation. Specific patterns of Fab glycosylation are concurrent with physiological and pathological conditions, such as pregnancy and rheumatoid arthritis. With respect to function, Fab glycosylation can significantly affect stability, half-life, and binding characteristics of Abs and BCRs. Moreover, Fab glycans are associated with the anti-inflammatory activity of IVIGs. Consequently, IgG Fab glycosylation appears to be an important, yet poorly understood, process that modulates immunity. *The Journal of Immunology*, 2016, 196: 1435–1441.

Immunoglobulins are glycoproteins produced by B cells that play a crucial role in protective immunity. Both the Fc tail, responsible for triggering effector functions, and the Fab arms, responsible for Ag binding, may contain glycans (Fig. 1). In addition to a conserved *N*-linked glycan in the Fc of all Ig classes, *N*-linked glycans are found in the C_H1 domains of IgM and IgE, and *O*-linked glycans are found in the hinge regions of IgD, IgA (1), and IgG3 (2). Furthermore, the variable domains of Igs may also contain *N*-linked glycans. For IgG, the most abundant class of serum Igs, Fab glycosylation is restricted to the variable domains.

Although the existence of IgG Fab glycans has been known for quite some time, their emergence, regulation, and function remain poorly understood. Nevertheless, recent evidence shows that Fab glycans exhibit distinct patterns according to

the pathophysiological conditions and have immunomodulatory effects, thus highlighting their potential role in regulating immunity. In this article, we summarize the literature on the structural features of IgG Fab glycans, Fab glycosylation during physiological and pathological conditions, the influence of Fab glycosylation on IgG function, and immune modulation.

Structural features of IgG Fab glycosylation

Generation and occupancy of Fab glycosylation sites. *N*-linked glycosylation of IgG Fab is contingent on the presence of consensus amino acid motifs making up the so-called “*N*-linked glycosylation sites.” These sites consist of asparagine, followed by any amino acid but proline, and either serine or threonine (N-X-S/T). *N*-linked glycosylation sites are largely absent in the naive human B cell repertoire because few germline-encoded alleles (IGHV1-8, IGHV4-34, IGHV5-10-1, IGLV3-12, and IGLV5-37) contain such sites (3). Therefore, the relatively high frequency of IgG Fab glycans is primarily the result of somatic hypermutation during Ag-specific immune responses (4). Fab glycosylation sites can appear in both H and L chains, in CDRs, and in framework regions (5).

Although a glycosylation site is required, it is not sufficient for the addition of a glycan. For instance, for IGHV4-34, one of the more frequently used human V_H alleles, the germline-encoded glycosylation site is usually unoccupied (6). Similarly, the mouse IGKV5-45 allele contains a germline-encoded glycosylation site that remains unoccupied in both the therapeutic chimeric Abs infliximab (7) and cetuximab (8, 9). In contrast, the mouse IGHV2-2 germline-encoded glycosylation site in cetuximab carries glycans (8–10).

Estimates of the percentage of Fab-glycosylated IgGs in healthy individuals range from ~15 to 25%, depending on the experimental approach used. About 20% of IgG variable domains from Ig sequence database entries contain *N*-linked

*Department of Immunopathology, Sanquin Research, 1066 CX Amsterdam, the Netherlands; [†]Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, 1105 AZ Amsterdam, the Netherlands; [‡]Department of Rheumatology, Leiden University Medical Center, 2333 ZA Leiden, the Netherlands; [§]Center for Proteomics and Metabolomics, Leiden University Medical Center, 2333 ZA Leiden, the Netherlands; and [¶]Université Lille, CNRS, UMR 8576, Unité de Glycobiologie Structurale et Fonctionnelle, F 59 000 Lille, France

¹F.S.v.d.B. and L.H. contributed equally to this work.

²T.R. and Y.R. contributed equally to this work.

ORCIDs: 0000-0003-0507-268X (F.S.v.d.B.); 0000-0003-1622-8483 (L.H.); 0000-0003-4482-2199 (Y.R.).

Received for publication September 30, 2015. Accepted for publication December 8, 2015.

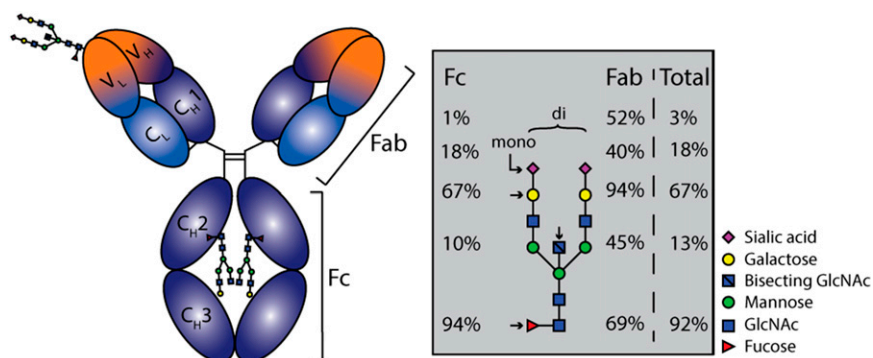
This work was supported by the Dutch Arthritis Foundation, the Netherlands Organization for Scientific Research (Project 435000033), and the Innovative Medicines Initiative-funded project Be the Cure (Contract 115142-2). L.H. was supported by the Dutch Arthritis Foundation (NR 12-2-403), and Y.R. was supported by a Boehringer Ingelheim-funded project within Be the Cure.

Address correspondence and reprint requests to Dr. Theo Rispens or Dr. Yoann Rombouts, Department of Immunopathology, Sanquin Research, Plesmanlaan 125, 1066 CX Amsterdam, the Netherlands (T.R.) or Leiden University Medical Center, 2333 ZA Leiden, the Netherlands (Y.R.). E-mail addresses: T.Rispens@sanquin.nl (T.R.) or Yoann.Rombouts@gmail.com (Y.R.)

Abbreviation used in this article: GlcNAc, *N*-acetylglucosamine.

Copyright © 2016 by The American Association of Immunologists, Inc. 0022-1767/16/\$30.00

FIGURE 1. Igs and glycosylation. Fc glycans are linked to asparagine 297 in the C_H2 domain, whereas Fab glycans are linked to *N*-glycosylation sites in V_H and V_L (left panel). Contrast of glycoprofiles found in Fab and Fc (right panel). Adapted from (15).



glycosylation sites (11). Using sialic acid-binding lectin-affinity chromatography, 25% of human serum IgGs were reported to contain Fab glycans (12), calculated as the percentage of sialylated Fab arms (10–12%) (13) divided by the percentage of Fab glycans containing sialic acid residues (46%) (12, 14). However, high-resolution analytical techniques yield a higher degree of sialylation (79–93%) (15–17), which equates to ~15% IgG Fab glycosylation. Furthermore, using high-resolution HPLC with 2-aminobenzoic acid labeling, ~14% Fab glycosylation was found (17).

Structure of IgG Fab glycans. *N*-linked glycans at both the Fc and Fab of polyclonal serum IgG are mainly complex-type biantennary *N*-linked glycans (Fig. 1). The core heptasaccharide consists of two *N*-acetylglucosamine (GlcNAc) residues, three mannose residues, and two more GlcNAc residues. In addition, fucose, bisecting GlcNAc, galactose, and sialic acid residues can be present in the glycan. Compared with IgG Fc glycans, IgG Fab glycans contain high percentages of bisecting GlcNAc, galactose, and sialic acid and low percentages of core fucose (15–17) (Fig. 1). Furthermore, although only 4% of polyclonal IgG Fab glycans are high-mannose glycoforms (versus 0% for Fc glycans), these structures can predominate on certain mAbs, depending on their precise position (see next section) (18). The high degree of sialylation of Fab glycans of serum IgG might stem, in part, from selective removal of nonsialylated structures via the hepatic asialoglycoprotein receptor (see “Ab Half-Life” below). Furthermore, Fab glycans are presumably more accessible for glycosyltransferases, resulting in more processing compared with Fc glycans that are spatially localized at the inner face of the C_H2 domains. Indeed, lectin- and Ab-binding data indicate that Fab glycans are highly exposed, whereas Fc glycans are (partially) shielded (19, 20). Nonetheless, enzymatic-digestion experiments suggested that Fc glycans are more accessible, at least to some enzymes, than Fab glycans (17, 21).

Modulation of IgG Fab glycan presence and structure. Several factors modulate the presence and structure of IgG Fab glycans. First, the glycoprofile varies, depending on the position of the glycosylation site (22, 23) and amino acid residues surrounding it (24–27). For instance, introducing glycosylation sites in the CDR2 of otherwise identical Abs resulted in complex-type biantennary glycans at asparagines 54 and 58 but high-mannose structures at asparagine 60 (22). Furthermore, Fab glycosylation may be regulated by the mode of B cell activation. For instance, IL-6 and progesterone can enhance the expression of oligosaccharyltransferase, which catalyzes attachment of the *N*-linked glycan precursor to the polypeptide chain in the lumen of the endoplasmic reticulum, resulting in

increased IgG Fab glycosylation with a proportional increase in high-mannose structures up to 30% (28–30). T cell signaling, known to influence the structure of Fc glycans (31), may also affect Fab glycosylation, but this has not been studied. In addition, endogenous lectins may serve as surrogate B cell Ags via binding to Fab glycans, as described for certain B cell lymphomas (see “Malignancies” below).

Fab glycosylation during physiological and pathological conditions

The amounts and types of Fab glycans can vary during certain physiological and pathological conditions, suggesting that they might contribute to immune suppression or pathophysiology or potentially serve as a biomarker, as discussed below.

Physiological changes. Margni and Binaghi (32) investigated the properties of IgG Abs that are retained on a ConA affinity resin (5–15%) and concluded that their Fab arms contain high-mannose glycans. However, ConA also retains complex-type biantennary *N*-linked glycans without bisecting GlcNAc; most likely, the retained IgG Abs contain both types of Fab glycans (33, 34). Interestingly, levels of ConA-binding Abs are increased during the second trimester of pregnancy. Moreover, reduced serum levels of ConA-binding Abs were found during the first and second trimester of pregnancy in women who subsequently suffered from spontaneous abortions, thus serving as a potential early marker for diagnosing a threatened pregnancy (35–37). Consistent with these results (given that ConA interacts preferentially with nonbisected complex-type biantennary *N*-linked glycans), mass spectrometry data show that IgG Fab glycans contain more sialic acid and less bisecting GlcNAc during pregnancy compared with after delivery (15). Similar shifts in sialic acid content are observed for Fc glycans.

Intriguingly, it was proposed that only one Fab arm of the ConA-binding Abs carries a glycan, which might minimize elimination of paternal (fetal) Ags by maternal Abs during pregnancy (see “Ab Aggregation and Immune Complex Formation” below) (32). In line with this, the majority of Fab-glycosylated IgG Abs from the mother is directed against paternal Ags (38). Although firm evidence is lacking, the hypothesis that Fab glycosylation can modulate the Ab repertoire to minimize unwanted reactivity toward the fetus is worth exploring.

Autoimmune diseases. In contrast to the supposedly protective role of Fab glycosylation during pregnancy, enhanced IgG Fab glycosylation is associated with several autoimmune diseases. Using a small number of individuals/patients ($n = 7$), Youings et al. (39) reported that the Fab of IgGs from rheumatoid arthritis (RA) patients carry three times more oligosaccharides

than do those from healthy individuals. Moreover, they observed an increase in monogalactosylated glycoforms containing bisecting GlcNAc and fucose residues in the RA patients (39). Furthermore, we recently found that anti-citrullinated protein Abs, a diagnostic and prognostic biomarker in RA, have a higher m.w. compared with other IgG (auto)antibodies as the result of an increase in IgG Fab glycans (40). Nevertheless, although IgG Fc-glycosylation patterns were associated with disease activity/severity and outcome (41, 42), such associations for Fab glycans still need to be established. Other studies suggested that differences in Fab glycosylation may also characterize other types of autoantibodies. For instance, enhanced and reduced levels of Fab glycosylation were described for anti-neutrophil cytoplasmic Abs and anti-glomerular basement membrane Abs, respectively (43). Furthermore, primary Sjögren's syndrome patients show a higher prevalence of IgG B cell sequences with *N*-linked glycosylation sites than do healthy controls (44). It was suggested that B cell hyperproliferation and selection within the parotid glands may result from Ag-independent interactions of glycosylated BCRs with microbial lectins, as described for lymphoma B cells (see below).

Malignancies. B cell malignancies are sometimes characterized by aberrant Fab glycosylation. Increased frequencies of *N*-linked Fab-glycosylation sites in IgG and/or BCR were described for follicular lymphoma, diffuse large B cell lymphoma, and Burkitt's lymphoma B cells, whereas mutated chronic lymphocytic leukemia, multiple myeloma, and MALT lymphoma B cells were comparable with normal B cells (5, 45, 46). These tumor-associated Fab glycans are high-mannose structures, whereas the corresponding Fc glycans are complex-type biantennary glycans, confirming that the normal *N*-linked glycan-processing pathway is intact (6, 47). Signaling through interactions between *N*-linked glycans on follicular lymphoma BCRs and lectins, such as DC-SIGN or mannose-binding lectin, at the surface of macrophages and dendritic cells may free these cells from dependence on Ag and may contribute to tumor cell persistence or growth (48, 49). Some lectins from opportunistic pathogens, such as *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, can also interact with high-mannose BCR Fab glycans (50) (Fig. 2A). Increased IgG Fab glycosylation also was observed in myeloma patients, accompanied by an increased proportion of sialic acid, bisecting GlcNAc, and fucose (51). Furthermore, variable domain glycosylation of Bence Jones proteins (free Ig L chains) was 4-fold higher in multiple myeloma patients with amyloidosis than those without amyloidosis, suggesting a role for variable domain glycans in pathogenesis (see "Ab Aggregation and Immune Complex Formation" below) (52).

In addition to B cell malignancies, it was shown that, during the progression of malignant melanoma, autoantibodies to GRP78 are expressed, and changes in their oligosaccharide chains (putative increases in Fab glycosylation) stimulate melanoma cell growth and survival (53).

Influence of Fab glycosylation on IgG function

Ag binding. Several studies showed that the presence of *N*-linked glycosylation sites in the variable domains can increase (18, 23, 54–56) or decrease (18, 23, 50) Ag-binding affinity. For instance, the anti- $\alpha(1\rightarrow6)$ -dextran Ab 14.6b.1 has a 10-fold higher affinity compared with a single amino acid mutant that lacks the *N*-linked glycosylation site (54), presumably

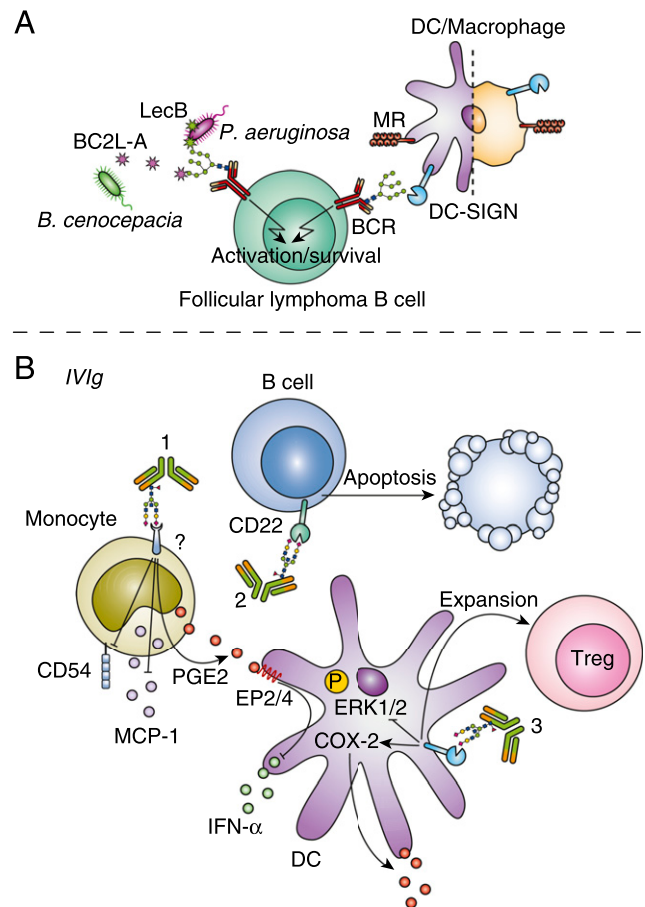
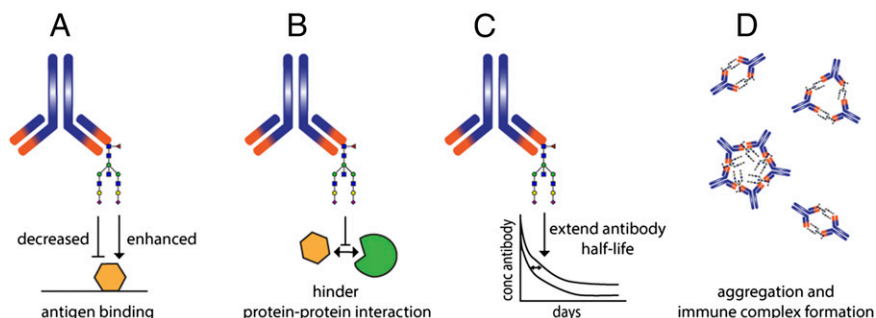


FIGURE 2. Effect of Fab glycosylation on immunity. **(A)** Follicular lymphomas may interact with MR, DC-SIGN, or bacterial lectins via BCR-linked high-mannose glycans, presumably enhancing tumor survival. **(B)** SNA-purified IVIg may (1) downregulate CD54 and MCP-1 and upregulate PGE₂ on monocytes, (2) induce B cell apoptosis via CD22, and (3) inhibit ERK1/2 phosphorylation and IFN- α and upregulate COX-2 and PGE₂, which stimulates regulatory T cell expansion, via DC-SIGN on dendritic cells.

due to additional hydrophilic interactions of the Fab glycans with the carbohydrate Ag (Fig. 3). Interestingly, insertion of alternative *N*-linked glycosylation sites by mutagenesis around the pre-existing *N*-linked glycosylation site differentially affects the Ag binding, depending on the location of Fab glycans. Furthermore, a 100-fold reduction in binding to tetanus toxoid, diphtheria toxoid, and dsDNA was observed for the polyreactive human mAb CBGA1 upon blocking *N*-linked glycosylation during production by tunicamycin (56). Decreased binding was explained by a conformational change in the variable domains in the absence of Fab glycans. In addition, the removal of sialic acid residues from Fab glycans can decrease Ag-binding affinity (57). In contrast, insertion of follicular lymphoma-specific *N*-linked glycosylation sites into the model BCRs B1-8 and HyHEL10 strongly impairs the binding to their respective Ags (50), and the presence of *N*-linked glycans in the Fab of an anti-CD33 Ab decreases its binding to CD33 by 3–8-fold (58). Fab glycans may also leave Ag-binding affinity essentially unaffected (23).

Modification of Ab activity. Fab glycans may also alter Ab activity without affecting Ag binding. LE2E9, an anti-factor VIII (FVIII) Ab derived from a patient with mild hemophilia A,

FIGURE 3. Influence of Fab glycosylation on IgG function. Oligosaccharides linked to variable domains can enhance or decrease Ag binding (**A**), block binding between two proteins by steric hindrance (**B**), extend Ab half-life as a result of sialylation (**C**), and presumably affect Ab aggregation and immune complex formation (**D**).



neutralizes FVIII activity up to 90% and prevents FVIII binding to von Willebrand factor (vWF). Removal of the Fab glycans from the CDR1 by enzymatic digestion or mutagenesis restored FVIII–vWF complex formation and rendered the Ab 40% less efficient in inhibiting FVIII activity, without altering the binding affinity of LE2E9 to FVIII. It was hypothesized that, via steric hindrance, the oligosaccharide prevents binding of FVIII to vWF, as well as the assembly of activated FVIII, activated FIX, and FX protein complexes (59) (Fig. 3).

Ab half-life. The serum half-life of glycoproteins is modulated by the structure of the glycans. These can interact with glycoreceptors of liver cells, including the asialoglycoprotein receptor, which recognizes terminal galactose and *N*-acetylgalactosamine residues (60). Thus, the absence of terminal sialic acid residues can result in a decreased half-life of a glycoprotein (61).

Structural variation in conventional biantennary Fc glycans does not significantly influence the Ab half-life (62), probably because of the inaccessibility of these glycans to glycoreceptors (see “Structure of IgG Fab Glycans” above), although “nonnatural” high-mannose glycans can increase the clearance rate (63). In contrast, Fab glycan sialylation can enhance the serum half-life of mAbs, as shown for the anti-EGFR Ab cetuximab and for the anti-TA-MUC1 Ab PankoMab (64). Furthermore, depending on their position, Fab glycans enhanced or decreased the half-life of anti- $\alpha(1\rightarrow6)$ -dextran Abs in mice (7 d) by ± 3 d (23). Interestingly, the rapidly and slowly clearing fractions were found to accumulate predominantly in the liver and kidney or in the blood and spleen, respectively (23), indicating

that Fab glycans may also influence the accumulation of Abs in different organs.

Ab aggregation and immune complex formation. Variable domain glycosylation may affect the propensity of Abs and L chains to aggregate and/or precipitate. One example is cryoimmunoglobulins, which precipitate at low temperatures and are associated with human diseases like RA and systemic lupus erythematosus (65). Sialic acid residues in the variable domains of the cryoimmunoglobulin “Ger” were found to provide additional electrostatic contacts required for its cryoprecipitation (66) (Fig. 3). Furthermore, variable domain glycans might enhance aggregation of Bence Jones proteins, given that such glycans were reported more often in multiple myeloma patients with amyloidosis (52). It was proposed that glycosylation can induce a conformational change in these proteins and trigger fibril formation (67), thereby contributing to amyloidosis; however, this hypothesis needs further research to substantiate. In contrast, a recent study described a decreasing effect of Fab glycans on Ab aggregation (68).

Fab glycosylation also was implicated in the modulation of immune complex formation. IgG Abs that bind ConA exhibited distinct Ag-binding profiles, which were attributed to the presence of glycans in only one Fab arm (69). If true, the glycosylated Fab arms may not bind Ag, and the Ab would be effectively univalent (“asymmetrical”), precluding formation of precipitating immune complexes and triggering effector mechanisms, analogously to IgG4 (70). Of course, in case of partial occupancy of a glycosylation site, one would expect B cells to secrete asymmetrically glycosylated Abs, as well as Abs either without Fab glycans or with a glycan in both Fab

Table I. IgG Fab glycosylation: what is (un)known?

	References
Structural knowledge	
Found in 15–25% of IgG	(11, 12, 17)
Highly processed complex-type biantennary <i>N</i> -linked glycans	(15–17)
Occupancy and structure may vary with position	(22, 23)
Distinct patterns are associated with pathophysiological conditions	(5, 15, 39, 40, 43–46, 51)
Established activities	
Can influence Ag binding	(18, 23, 50, 54–56)
Can affect in vivo Ab half-life	(23)
Can alter Ab stability and aggregation	(52, 66, 68)
Possible activities	
Signaling through glycans may lead to malignant B cell survival	(48, 49)
Associated with anti-inflammatory activity of IVIg	(13, 74)
May diminish autoreactivity	(25, 71)
Unsolved questions	
Regulation of emergence, starting from largely absent, is unexplored	(3)
Main function(s) still unknown	

arms. However, it is unclear whether and how this asymmetry might be specifically imposed upon the Abs.

Immune modulation by Fab glycans

Elimination of autoreactivity. Instead of interfering with Ag binding (see “Ag Binding” above), the effect of introducing a Fab glycan may be more subtle, not affecting binding to cognate Ag but shaping the Ab reactivity in such a way that autoreactivity is minimized. One study described that, in nonimmunized rats ~10–20% of IgGs were ConA-binding Abs, of which 78% exhibited autoreactivity and 14% reactivity against intestinal bacterial Ags. In contrast, only 40% of the “conventional” rat IgG was found to be autoreactive, suggesting that at least part of the Abs containing a Fab glycan confers a higher autoreactivity (71). In another study, following immunizations in mice, N-linked glycosylation sites were introduced in the variable domains of the BCR of anergic B cells that presumably allowed these cells to move away from autoreactivity, while allowing them to react against cross-reacting foreign Ags (25). However, in another study, autoantigen binding of follicular lymphoma-derived Abs was unaffected upon expression in the presence of tunicamycin to suppress N-linked (Fab) glycosylation (46).

Potential role for Fab glycosylation in IVIg therapy. IVIg treatment successfully ameliorates the symptoms of a number of autoimmune diseases, like systemic lupus erythematosus, RA, and idiopathic thrombocytopenic purpura. A variety of possible mechanisms was suggested (72). Recent studies emphasized the importance of glycosylation in the therapeutic effects of IVIg (73). In certain mouse models (74, 75), but not in others (13, 76–78), the anti-inflammatory activity of IVIg depends mainly on a fraction of sialylated Abs. These sialylated Abs may interact with C-type lectins (like DC-SIGN, DCIR) and sialic acid-binding Ig-type lectins, such as CD22, at the surface of immune cells (79, 80). It was suggested that these lectins interact with Fc-bound sialic acid (74, 75), which was further supported by a study that showed that IVIg with fully sialylated Fc glycans has enhanced anti-inflammatory activity compared with IVIg (81). However, a number of studies [including (74)] point to a role for Fab glycosylation of IVIg in modulating the immune system (Fig. 2B). These studies used *Sambucus nigra* agglutinin (SNA) lectin-affinity chromatography to fractionate IVIg, thereby predominantly enriching for Fab sialylation rather than Fc sialylation (13, 76). Fab-sialylated IVIg downregulated CD54 expression and the MCP-1 secretion of monocytes, whereas neither IVIg without sialylated Fab nor a highly sialylated Fc could achieve these effects (13). SNA-enriched IVIg also was shown to induce upregulation of PGE₂ by monocytes, thereby enabling inhibition of IFN- α production by plasmacytoid dendritic cells (82). In addition, the treatment of dendritic cells with IVIg Fab downregulated ERK1/2 phosphorylation after TLR4 stimulation (83). Furthermore, the interaction of IVIg with DC-SIGN also induced COX-2 expression and PGE₂ production, as well as the expansion of regulatory T cells (84). Silencing of human B cells via apoptosis could be induced by SNA-enriched IVIg through binding to CD22 (80, 85). Altogether, the available literature suggests that the anti-inflammatory effects of IVIg are mediated, at least in part, by its Fab arms, with a potential role for Ig variable domain sialylation (73). Differential effects

by SNA-enriched and -depleted fractions might reflect, in part, differences in their respective Ab specificities and not be a direct result of the presence or absence of a glycan (13). However, the fact that removal of sialic acid using neuraminidase can ameliorate the effects of IVIg suggests otherwise (75). It would be interesting to investigate whether Fab-glycosylated IgG from pregnant women (see “Physiological Changes” above) shares similar, possibly immunomodulatory, properties with SNA-enriched IVIg.

Conclusions

IgG Fab glycosylation appears to be an important process in immunity, with demonstrated effects on stability, half-life, and binding characteristics of Abs and BCRs. Moreover, in addition to the potential to affect Ag binding, an increasing number of studies hint at the involvement of (endogenous or exogenous) lectins that can interact with these glycans, thereby potentially exerting immunomodulatory effects. At the same time, many unanswered questions remain (Table I). For instance, it is unclear why increased Fab glycosylation is observed during situations of diminished immunity (pregnancy), as well as in certain autoimmune settings, and what the consequences are. Also, why are glycosylation sites largely absent in the naive B cell repertoire? And how is their emergence and structure regulated during B cell activation? All in all, the exact role of Fab glycosylation in immunity remains poorly understood.

Disclosures

The authors have no financial conflicts of interest.

References

1. Arnold, J. N., M. R. Wormald, R. B. Sim, P. M. Rudd, and R. A. Dwek. 2007. The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.* 25: 21–50.
2. Plomp, R., G. Dekkers, Y. Rombouts, R. Visser, C. A. Koeleman, G. S. Kammeijer, B. C. Jansen, T. Rispens, P. J. Hensbergen, G. Vidarsson, and M. Wuhrer. 2015. Hinge-Region O-Glycosylation of Human Immunoglobulin G3 (IgG3). *Mol. Cell. Proteomics* 14: 1373–1384.
3. Lefranc, M. P. 2011. IMGT, the International ImMunoGeneTics Information System. *Cold Spring Harb. Protoc.* 2011: 595–603.
4. Dunn-Walters, D., L. Boursier, and J. Spencer. 2000. Effect of somatic hypermutation on potential N-glycosylation sites in human immunoglobulin heavy chain variable regions. *Mol. Immunol.* 37: 107–113.
5. Zhu, D., H. McCarthy, C. H. Ottensmeier, P. Johnson, T. J. Hamblin, and F. K. Stevenson. 2002. Acquisition of potential N-glycosylation sites in the immunoglobulin variable region by somatic mutation is a distinctive feature of follicular lymphoma. *Blood* 99: 2562–2568.
6. McCann, K. J., C. H. Ottensmeier, A. Callard, C. M. Radcliffe, D. J. Harvey, R. A. Dwek, P. M. Rudd, B. J. Sutton, P. Hobby, and F. K. Stevenson. 2008. Remarkable selective glycosylation of the immunoglobulin variable region in follicular lymphoma. *Mol. Immunol.* 45: 1567–1572.
7. Liang, S., J. Dai, S. Hou, L. Su, D. Zhang, H. Guo, S. Hu, H. Wang, Z. Rao, Y. Guo, and Z. Lou. 2013. Structural basis for treating tumor necrosis factor α (TNF α)-associated diseases with the therapeutic antibody infliximab. *J. Biol. Chem.* 288: 13799–13807.
8. Li, S., K. R. Schmitz, P. D. Jeffrey, J. J. Wiltzius, P. Kussie, and K. M. Ferguson. 2005. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. *Cancer Cell* 7: 301–311.
9. Magdelaine-Beuzelin, C., Q. Kaas, V. Wehbi, M. Ohresser, R. Jefferis, M. P. Lefranc, and H. Watier. 2007. Structure-function relationships of the variable domains of monoclonal antibodies approved for cancer treatment. *Crit. Rev. Oncol. Hematol.* 64: 210–225.
10. Qian, J., T. Liu, L. Yang, A. Daus, R. Crowley, and Q. Zhou. 2007. Structural characterization of N-linked oligosaccharides on monoclonal antibody cetuximab by the combination of orthogonal matrix-assisted laser desorption/ionization hybrid quadrupole-quadrupole time-of-flight tandem mass spectrometry and sequential enzymatic digestion. *Anal. Biochem.* 364: 8–18.
11. Jefferis, R. 2007. Antibody therapeutics: isotype and glycoform selection. *Expert Opin. Biol. Ther.* 7: 1401–1413.
12. Stadlmann, J., M. Pabst, and F. Altmann. 2010. Analytical and Functional Aspects of Antibody Sialylation. *J. Clin. Immunol.* 30(Suppl. 1): S15–S19.

13. Käsermann, F., D. J. Boerema, M. Rügsegger, A. Hofmann, S. Wymann, A. W. Zuercher, and S. Miescher. 2012. Analysis and functional consequences of increased Fab-sialylation of intravenous immunoglobulin (IVIg) after lectin fractionation. *PLoS One* 7: e37243.
14. Stadlmann, J., A. Weber, M. Pabst, H. Anderle, R. Kunert, H. J. Ehrlich, H. Peter Schwarz, and F. Altmann. 2009. A close look at human IgG sialylation and subclass distribution after lectin fractionation. *Proteomics* 9: 4143–4153.
15. Bondt, A., Y. Rombouts, M. H. Selman, P. J. Hensbergen, K. R. Reidig, J. M. Hazes, R. J. Dolhain, and M. Wührer. 2014. Immunoglobulin G (IgG) Fab glycosylation analysis using a new mass spectrometric high-throughput profiling method reveals pregnancy-associated changes. *Mol. Cell. Proteomics* 13: 3029–3039.
16. Holland, M., H. Yagi, N. Takahashi, K. Kato, C. O. Savage, D. M. Goodall, and R. Jefferis. 2006. Differential glycosylation of polyclonal IgG, IgG-Fc and IgG-Fab isolated from the sera of patients with ANCA-associated systemic vasculitis. *Biochim. Biophys. Acta* 1760: 669–677.
17. Anumula, K. R. 2012. Quantitative glycan profiling of normal human plasma derived immunoglobulin and its fragments Fab and Fc. *J. Immunol. Methods* 382: 167–176.
18. Wright, A., M. H. Tao, E. A. Kabat, and S. L. Morrison. 1991. Antibody variable region glycosylation: position effects on antigen binding and carbohydrate structure. *EMBO J.* 10: 2717–2723.
19. Dalziel, M., I. McFarlane, and J. S. Axford. 1999. Lectin analysis of human immunoglobulin G N-glycan sialylation. *Glycoconj. J.* 16: 801–807.
20. Lammerts van Bueren, J. J., T. Rispen, S. Verploegen, T. van der Palen-Merkus, S. Stapel, L. J. Workman, H. James, P. H. van Berkel, J. G. van de Winkel, T. A. Platts-Mills, and P. W. Parren. 2011. Anti-galactose- α -1,3-galactose IgE from allergic patients does not bind α -galactosylated glycans on intact therapeutic antibody Fc domains. *Nat. Biotechnol.* 29: 574–576.
21. Mimura, Y., P. R. Ashton, N. Takahashi, D. J. Harvey, and R. Jefferis. 2007. Contrasting glycosylation profiles between Fab and Fc of a human IgG protein studied by electrospray ionization mass spectrometry. *J. Immunol. Methods* 326: 116–126.
22. Endo, T., A. Wright, S. L. Morrison, and A. Kobata. 1995. Glycosylation of the variable region of immunoglobulin G—site specific maturation of the sugar chains. *Mol. Immunol.* 32: 931–940.
23. Coloma, M. J., R. K. Trinh, A. R. Martinez, and S. L. Morrison. 1999. Position effects of variable region carbohydrate on the affinity and in vivo behavior of an anti-(1–6) dextran antibody. *J. Immunol.* 162: 2162–2170.
24. Petrescu, A. J., A. L. Milac, S. M. Petrescu, R. A. Dwek, and M. R. Wormald. 2004. Statistical analysis of the protein environment of N-glycosylation sites: implications for occupancy, structure, and folding. *Glycobiology* 14: 103–114.
25. Sabouri, Z., P. Schofield, K. Horikawa, E. Spierings, D. Kipling, K. L. Randall, D. Langley, B. Roome, R. Vazquez-Lombardi, R. Rouet, et al. 2014. Redemption of autoantibodies on anergic B cells by variable-region glycosylation and mutation away from self-reactivity. *Proc. Natl. Acad. Sci. USA* 111: E2567–E2575.
26. Valliere-Douglass, J. F., P. Kodama, M. Mujacic, L. J. Brady, W. Wang, A. Wallace, B. Yan, P. Reddy, M. J. Treuheit, and A. Balland. 2009. Asparagine-linked oligosaccharides present on a non-consensus amino acid sequence in the CH1 domain of human antibodies. *J. Biol. Chem.* 284: 32493–32506.
27. Valliere-Douglass, J. F., C. M. Eakin, A. Wallace, R. R. Ketchum, W. Wang, M. J. Treuheit, and A. Balland. 2010. Glutamine-linked and non-consensus asparagine-linked oligosaccharides present in human recombinant antibodies define novel protein glycosylation motifs. *J. Biol. Chem.* 285: 16012–16022.
28. Canellada, A., S. Blois, T. Gentile, and R. A. Margni. 2002. In vitro modulation of protective antibody responses by estrogen, progesterone and interleukin-6. *Am. J. Reprod. Immunol.* 48: 334–343.
29. Gutiérrez, G., I. Malan Borel, and R. A. Margni. 2001. The placental regulatory factor involved in the asymmetric IgG antibody synthesis responds to IL-6 features. *J. Reprod. Immunol.* 49: 21–32.
30. Miranda, S., A. Canellada, T. Gentile, and R. Margni. 2005. Interleukin-6 and dexamethasone modulate in vitro asymmetric antibody synthesis and UDP-Glc glycoprotein glycosyltransferase activity. *J. Reprod. Immunol.* 66: 141–150.
31. Oefner, C. M., A. Winkler, C. Hess, A. K. Lorenz, V. Holeccka, M. Huxdorf, T. Schommartz, D. Petzold, J. Bitterling, A. L. Schoen, et al. 2012. Tolerance induction with T cell-dependent protein antigens induces regulatory sialylated IgGs. *J. Allergy Clin. Immunol.* 129: 1647–55.e13.
32. Margni, R. A., and R. A. Binaghi. 1988. Nonprecipitating asymmetric antibodies. *Annu. Rev. Immunol.* 6: 535–554.
33. Bhattacharyya, L., M. Haraldsson, and C. F. Brewer. 1987. Concanavalin A interactions with asparagine-linked glycopeptides. Bivalency of bisected complex type oligosaccharides. *J. Biol. Chem.* 262: 1294–1299.
34. Taniguchi, T., T. Mizuchi, M. Beale, R. A. Dwek, T. W. Rademacher, and A. Kobata. 1985. Structures of the sugar chains of rabbit immunoglobulin G: occurrence of asparagine-linked sugar chains in Fab fragment. *Biochemistry* 24: 5551–5557.
35. Barrientos, G., D. Fuchs, K. Schröcksnadel, M. Ruecke, M. G. Garcia, B. F. Klapp, R. Raghupathy, S. Miranda, P. C. Arck, and S. M. Blois. 2009. Low levels of serum asymmetric antibodies as a marker of threatened pregnancy. *J. Reprod. Immunol.* 79: 201–210.
36. Zenclussen, A. C., T. Gentile, G. Kortebani, A. Mazzolli, and R. Margni. 2001. Asymmetric antibodies and pregnancy. *Am. J. Reprod. Immunol.* 45: 289–294.
37. Blois, S., A. C. Zenclussen, M. E. Roux, S. Olmos, J. di Conza, P. C. Arck, and R. A. Margni. 2004. Asymmetric antibodies (AAb) in the female reproductive tract. *J. Reprod. Immunol.* 64: 31–43.
38. Malan Borel, I., T. Gentile, J. Angelucci, J. Pivadori, M. C. Guala, R. A. Binaghi, and R. A. Margni. 1991. IgG asymmetric molecules with antipaternal activity isolated from sera and placenta of pregnant human. *J. Reprod. Immunol.* 20: 129–140.
39. Youings, A., S. C. Chang, R. A. Dwek, and I. G. Scragg. 1996. Site-specific glycosylation of human immunoglobulin G is altered in four rheumatoid arthritis patients. *Biochem. J.* 314: 621–630.
40. Rombouts, Y., A. Willemze, J. J. van Beers, J. Shi, P. F. Kerkman, L. van Toom, G. M. Janssen, A. Zaldumbide, R. C. Hoeben, G. J. Puijn, et al. 2015. Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. *Ann. Rheum. Dis.* DOI: 10.1136/annrheumdis-2014-206598.
41. Ercan, A., J. Cui, D. E. Chatterton, K. D. Deane, M. M. Hazen, W. Brintnell, C. I. O'Donnell, L. A. Derber, M. E. Weinblatt, N. A. Shadick, et al. 2010. Aberrant IgG galactosylation precedes disease onset, correlates with disease activity, and is prevalent in autoantibodies in rheumatoid arthritis. *Arthritis Rheum.* 62: 2239–2248.
42. Rombouts, Y., E. Ewing, L. A. van de Stadt, M. H. Selman, L. A. Trouw, A. M. Deelder, T. W. Huizinga, M. Wührer, D. van Schaardenburg, R. E. Toes, and H. U. Scherer. 2015. Anti-citrullinated protein antibodies acquire a pro-inflammatory Fc glycosylation phenotype prior to the onset of rheumatoid arthritis. *Ann. Rheum. Dis.* 74: 234–241.
43. Xu, P. C., S. J. Gou, X. W. Yang, Z. Cui, X. Y. Jia, M. Chen, and M. H. Zhao. 2012. Influence of variable domain glycosylation on anti-neutrophil cytoplasmic autoantibodies and anti-glomerular basement membrane autoantibodies. *BMC Immunol.* 13: 10.
44. Hamza, N., U. Hersherberg, C. G. Kallenberg, A. Vissink, F. K. Spijkervet, H. Bootsma, F. G. Kroese, and N. A. Bos. 2015. Ig gene analysis reveals altered selective pressures on Ig-producing cells in parotid glands of primary Sjögren's syndrome patients. *J. Immunol.* 194: 514–521.
45. Zhu, D., C. H. Ottensmeyer, M. Q. Du, H. McCarthy, and F. K. Stevenson. 2003. Incidence of potential glycosylation sites in immunoglobulin variable regions distinguishes between subsets of Burkitt's lymphoma and mucosa-associated lymphoid tissue lymphoma. *Br. J. Haematol.* 120: 217–222.
46. Sachen, K. L., M. J. Strohmman, J. Singletary, A. A. Alizadeh, N. H. Kattah, C. Lossos, E. D. Mellins, S. Levy, and R. Levy. 2012. Self-antigen recognition by follicular lymphoma B-cell receptors. *Blood* 120: 4182–4190.
47. Radcliffe, C. M., J. N. Arnold, D. M. Suter, M. R. Wormald, D. J. Harvey, L. Royle, Y. Mimura, Y. Kimura, R. B. Sim, S. Inogès, et al. 2007. Human follicular lymphoma cells contain oligomannose glycans in the antigen-binding site of the B-cell receptor. *J. Biol. Chem.* 282: 7405–7415.
48. Amin, R., F. Mourcin, F. Uhel, C. Pangault, P. Ruminy, L. Dupré, M. Guirriec, T. Marchand, T. Fest, T. Lamy, and K. Tarte. 2015. DC-SIGN-expressing macrophages trigger activation of mannoseylated IgM B-cell receptor in follicular lymphoma. *Blood* 126: 1911–1920.
49. Coelho, V., S. Krysov, A. M. Ghaemmaghami, M. Emara, K. N. Potter, P. Johnson, G. Packham, L. Martinez-Pomares, and F. K. Stevenson. 2010. Glycosylation of surface Ig creates a functional bridge between human follicular lymphoma and microenvironmental lectins. *Proc. Natl. Acad. Sci. USA* 107: 18587–18592.
50. Schneider, D., M. Dühren-von Minden, A. Alkhatib, C. Setz, C. A. van Bergen, M. Benkiser-Petersen, I. Wilhelm, S. Villringer, S. Krysov, G. Packham, et al. 2015. Lectins from opportunistic bacteria interact with acquired variable-region glycans of surface immunoglobulin in follicular lymphoma. *Blood* 125: 3287–3296.
51. Kinoshita, N., M. Ohno, T. Nishiura, S. Fujii, A. Nishikawa, Y. Kawakami, N. Uozumi, and N. Taniguchi. 1991. Glycosylation at the Fab portion of myeloma immunoglobulin G and increased fucosylated biantennary sugar chains: structural analysis by high-performance liquid chromatography and antibody-lysin enzyme immunoassay using Lens culinaris agglutinin. *Cancer Res.* 51: 5888–5892.
52. Holm, E., K. Sletten, and G. Husby. 1986. Structural studies of a carbohydrate-containing immunoglobulin-lambda-light-chain amyloid-fibril protein (AL) of variable subgroup III. *Biochem. J.* 239: 545–551.
53. Selim, M. A., J. L. Burchette, E. V. Bowers, G. G. de Ridder, L. Mo, S. V. Pizzo, and M. Gonzalez-Gronow. 2011. Changes in oligosaccharide chains of autoantibodies to GRP78 expressed during progression of malignant melanoma stimulate melanoma cell growth and survival. *Melanoma Res.* 21: 323–334.
54. Wallick, S. C., E. A. Kabat, and S. L. Morrison. 1988. Glycosylation of a VH residue of a monoclonal antibody against alpha (1—6) dextran increases its affinity for antigen. *J. Exp. Med.* 168: 1099–1109.
55. Tachibana, H., J. Y. Kim, and S. Shirahata. 1997. Building high affinity human antibodies by altering the glycosylation on the light chain variable region in N-acetylglucosamine-supplemented hybridoma cultures. *Cytotechnology* 23: 151–159.
56. Leibiger, H., D. Wüster, R. D. Stigler, and U. Marx. 1999. Variable domain-linked oligosaccharides of a human monoclonal IgG: structure and influence on antigen binding. *Biochem. J.* 338: 529–538.
57. Khurana, S., V. Raghunathan, and D. M. Salunke. 1997. The variable domain glycosylation in a monoclonal antibody specific to GnRH modulates antigen binding. *Biochem. Biophys. Res. Commun.* 234: 465–469.
58. Co, M. S., D. A. Scheinberg, N. M. Avdalovic, K. McGraw, M. Vasquez, P. C. Caron, and C. Queen. 1993. Genetically engineered deglycosylation of the variable domain increases the affinity of an anti-CD33 monoclonal antibody. *Mol. Immunol.* 30: 1361–1367.
59. Jacquemin, M., C. M. Radcliffe, R. Lavend'homme, M. R. Wormald, L. Vanderelst, G. Wallays, J. Dewaele, D. Collen, J. Vermynen, R. A. Dwek, et al. 2006. Variable region heavy chain glycosylation determines the anticoagulant activity of a factor VIII antibody. *J. Thromb. Haemost.* 4: 1047–1055.
60. Ashwell, G., and A. G. Morell. 1974. The role of surface carbohydrates in the hepatic recognition and transport of circulating glycoproteins. *Adv. Enzymol. Relat. Areas Mol. Biol.* 41: 99–128.
61. Bork, K., R. Horstkorte, and W. Weidemann. 2009. Increasing the sialylation of therapeutic glycoproteins: the potential of the sialic acid biosynthetic pathway. *J. Pharm. Sci.* 98: 3499–3508.

62. Naso, M. F., S. H. Tam, B. J. Scallon, and T. S. Raju. 2010. Engineering host cell lines to reduce terminal sialylation of secreted antibodies. *MAbs* 2: 519–527.
63. Alessandri, L., D. Ouellette, A. Acquah, M. Rieser, D. Leblond, M. Saltarelli, C. Radziejewski, T. Fujimori, and I. Correia. 2012. Increased serum clearance of oligomannose species present on a human IgG1 molecule. *MAbs* 4: 509–520.
64. Goletz, S., A. Danielczyk, and L. Stoeckl. 2012. Fab-glycosylated antibodies. Patent WO 2012/020065.
65. Retamozo, S., P. Brito-Zerón, X. Bosch, J. H. Stone, and M. Ramos-Casals. 2013. Cryoglobulinemic disease. *Oncology (Huntingt.)* 27: 1098–1105, 1110–1116.
66. Middaugh, C. R., and G. W. Litman. 1987. Atypical glycosylation of an IgG monoclonal cryoimmunoglobulin. *J. Biol. Chem.* 262: 3671–3673.
67. Karimi, M., K. Sletten, and P. Westermark. 2003. Biclinal systemic AL-amyloidosis with one glycosylated and one nonglycosylated AL-protein. *Scand. J. Immunol.* 57: 319–323.
68. Courtois, F., N. J. Agrawal, T. M. Lauer, and B. L. Trout. 2016. Rational design of therapeutic mAbs against aggregation through protein engineering and incorporation of glycosylation motifs applied to bevacizumab. *MAbs* 8: 99–112.
69. Borel, I. M., T. Gentile, J. Angelucci, R. A. Margni, and R. A. Binaghi. 1989. Asymmetrically glycosylated IgG isolated from non-immune human sera. *Biochim. Biophys. Acta* 990: 162–164.
70. Aalberse, R. C., and J. Schuurman. 2002. IgG4 breaking the rules. *Immunology* 105: 9–19.
71. Canellada, A., T. Gentile, J. Dokmetjian, and R. A. Margni. 2002. Occurrence, properties, and function of asymmetric IgG molecules isolated from non-immune sera. *Immunol. Invest.* 31: 107–120.
72. Clynes, R. 2007. Protective mechanisms of IVIG. *Curr. Opin. Immunol.* 19: 646–651.
73. Schwab, I., and F. Nimmerjahn. 2014. Role of sialylation in the anti-inflammatory activity of intravenous immunoglobulin - F(ab')₂ versus Fc sialylation. *Clin. Exp. Immunol.* 178(Suppl. 1): 97–99.
74. Kaneko, Y., F. Nimmerjahn, and J. V. Ravetch. 2006. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 313: 670–673.
75. Schwab, I., S. Mihai, M. Seeling, M. Kasperkiewicz, R. J. Ludwig, and F. Nimmerjahn. 2014. Broad requirement for terminal sialic acid residues and FcγRIIB for the preventive and therapeutic activity of intravenous immunoglobulins in vivo. *Eur. J. Immunol.* 44: 1444–1453.
76. Guhr, T., J. Bloem, N. I. Derksen, M. Wuhrer, A. H. Koenderman, R. C. Aalberse, and T. Rispen. 2011. Enrichment of sialylated IgG by lectin fractionation does not enhance the efficacy of immunoglobulin G in a murine model of immune thrombocytopenia. *PLoS One* 6: e21246.
77. Leontyev, D., Y. Katsman, X. Z. Ma, S. Miescher, F. Käsermann, and D. R. Branch. 2012. Sialylation-independent mechanism involved in the amelioration of murine immune thrombocytopenia using intravenous gammaglobulin. *Transfusion* 52: 1799–1805.
78. Othy, S., S. Topçu, C. Saha, P. Kothapalli, S. Lacroix-Desmazes, F. Käsermann, S. Miescher, J. Bayry, and S. V. Kaveri. 2014. Sialylation may be dispensable for reciprocal modulation of helper T cells by intravenous immunoglobulin. *Eur. J. Immunol.* 44: 2059–2063.
79. Massoud, A. H., M. Yona, D. Xue, F. Chouiali, H. Alturaihi, A. Ablona, W. Mourad, C. A. Piccirillo, and B. D. Mazer. 2014. Dendritic cell immunoreceptor: a novel receptor for intravenous immunoglobulin mediates induction of regulatory T cells. *J. Allergy Clin. Immunol.* 133: 853–63.e5.
80. Sêité, J. F., D. Cornec, Y. Renaudineau, P. Youinou, R. A. Mageed, and S. Hillion. 2010. IVIg modulates BCR signaling through CD22 and promotes apoptosis in mature human B lymphocytes. *Blood* 116: 1698–1704.
81. Washburn, N., I. Schwab, D. Ortiz, N. Bhatnagar, J. C. Lansing, A. Medeiros, S. Tyler, D. Mekala, E. Cochran, H. Sarvaia, et al. 2015. Controlled tetra-Fc sialylation of IVIg results in a drug candidate with consistent enhanced anti-inflammatory activity. [Published erratum appears in 2015 *Proc. Natl. Acad. Sci. USA* 112: E4339.] *Proc. Natl. Acad. Sci. USA* 112: E1297–E1306.
82. Wiedeman, A. E., D. M. Santer, W. Yan, S. Miescher, F. Käsermann, and K. B. Elkon. 2013. Contrasting mechanisms of interferon-α inhibition by intravenous immunoglobulin after induction by immune complexes versus Toll-like receptor agonists. *Arthritis Rheum.* 65: 2713–2723.
83. Bayry, J., K. Bansal, M. D. Kazatchkine, and S. V. Kaveri. 2009. DC-SIGN and alpha2,6-sialylated IgG Fc interaction is dispensable for the anti-inflammatory activity of IVIg on human dendritic cells. *Proc. Natl. Acad. Sci. USA* 106: E24–, author reply E25.
84. Trinath, J., P. Hegde, M. Sharma, M. S. Maddur, M. Rabin, J. M. Vallat, L. Magy, K. N. Balaji, S. V. Kaveri, and J. Bayry. 2013. Intravenous immunoglobulin expands regulatory T cells via induction of cyclooxygenase-2-dependent prostaglandin E2 in human dendritic cells. *Blood* 122: 1419–1427.
85. Sêité, J. F., C. Goutsmet, P. Youinou, J. O. Pers, and S. Hillion. 2014. Intravenous immunoglobulin induces a functional silencing program similar to anergy in human B cells. *J. Allergy Clin. Immunol.* 133: 181–8.e1, 9.