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Role of sialylation in the anti-inflammatory activity of intravenous immunoglobulin – F(ab')$_2$ versus Fc sialylation

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Immunoglobulin (Ig)G antibodies play an important role in the defence against pathogenic microorganisms, but are also responsible for tissue destruction and inflammation during autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Paradoxically, pooled IgG preparations from thousands of donors [intravenous immunoglobulin (IVIg)] are also an efficient treatment to suppress several autoimmune diseases and chronic inflammation. Research has highlighted the role of the sugar domain attached to the IgG Fc-fragment as a molecular switch, which can enhance or block the pro- and anti-inflammatory effector functions of the antibody molecule. Indeed, deglycosylation of serum IgG in mice with active autoimmune diseases results in an amelioration of autoantibody-dependent inflammation [1]. Of note, altered IgG glycosylation patterns, containing low levels of terminal galactose and sialic acid residues, and single nucleotide polymorphisms in glycosyltransferase genes have been associated with active autoimmune disease in RA, SLE and Crohn's disease, for example [2–5]. Furthermore, IVIg infusion was shown to induce sialylated IgG glycovariants in patients with Kawasaki disease and correlated with a prolonged treatment response [6]. Together with the results of several studies showing that sialylated IgG glycovariants are critical for the anti-inflammatory activity of IVIg in several *in vivo* model systems, this has led to proposing a model in which one possible mechanism of IVIg therapy may be to replenish these active anti-inflammatory and immunomodulatory IgG glycovariants, thereby re-establishing immune homeostasis. However, sialic acid-containing sugar moieties can attach to the IgG Fc- or F(ab′)$_2$-fragment, and evidence showing the relevance of both these sialylated sugar domains for IVIg activity has been reported recently.

Role of Fc-sialylation

In humans, infusion of Fc fragments can ameliorate idiopathic thrombocytopenic purpura/immune thrombocytopenia (ITP) [7]. Similar short-term Fc-dependent effects of IVIg administration which block autoantibody-dependent effector functions have been observed in mouse models of ITP, RA, nephrotoxic nephritis and transfusion-related acute lung injury (TRALI). Investigations into the role of Fc glycosylation for IVIg activity in models of inflammatory arthritis, ITP and epidermolysis bullosa acquisita (EBA) showed that cleavage of the intact sugar moiety or selectively the terminal sialic acid residues of the Fc fragment of IVIg can reduce its anti-inflammatory activity [8–10]. Moreover, the anti-inflammatory activity can be enhanced following enrichment of terminal sialic acid residues linked to the Fc fragment. F(ab′)$_2$ sialylation does not appear to increase therapeutic activity and IVIg cannot be replaced with other glycosylated serum proteins, suggesting that it is not the sugar moiety itself, but rather a conformational change induced by a high content of sialic acid residues in the amino acid backbone of IgG which is responsible for this effect [8,11–13]. More recently, the requirement of IVIg sialylation for the treatment of established autoimmune diseases was investigated and Schwab and colleagues demonstrated that IVIg sialylation is essential for its activity in two model systems of ITP, inflammatory arthritis and EBA [9]; however, IVIg sialylation was not essential in other model systems or under different experimental setups, requiring further studies to clarify these discrepancies between different groups [14].

Role of F(ab')$_2$-sialylation

With respect to a role of F(ab′)$_2$ sialylation, different effects have been noted depending on the model system under investigation. Käsermann and colleagues reported that cleaving the sialic acid residues of the F(ab′)$_2$ fragment led to a loss of anti-inflammatory activity in an *in vitro* model of inflammation using whole blood stimulated with lipopolysaccharide (LPS) or phytohaemagglutinin (PHA) [15]. In contrast, Guhr *et al.* reported the opposite effect, where F(ab′)$_2$ sialylation enrichment resulted in a reduction of the anti-inflammatory activity in a murine model of ITP

[16], although Leontyev *et al.* [17] found that neither sialylated $F(ab')_2$ or Fc were necessary for amelioration of ITP in a similar mouse model. In yet another study, Wiedemann *et al.* suggest a role for $F(ab')_2$ sialylation in IVIg-mediated modulation of cytokine release by plasmacytoid dendritic cells (pDCs). In this study, the release of interferon (IFN)-α by human pDCs upon stimulation with Toll-like receptor (TLR) agonists could be blocked indirectly by IVIg. In detail, sialic acid enriched fragments of $F(ab')_2$ promoted the production of prostaglandin E_2 by monocytes, which ultimately suppressed IFN-α secretion [18].

There are two further interesting studies by Seite *et al.* and Massoud *et al.*, in which a role for sialic acid-containing IgG glycovariants was noted. However, these two studies did not differentiate between $F(ab')_2$ and Fc-sialylation. Both studies used *Sambucus nigra* agglutinin (SNA) chromatography to enrich for the IVIg fraction containing high levels of sialic acid. As it was demonstrated that this method preferentially enriches sialylation in the $F(ab')_2$ fragments rather than Fc fragments [19], it seems likely that the observed effects are $F(ab')_2$-dependent, but this needs further investigation. In the first study, Séité *et al.* investigated the capacity of IVIg to modulate B cell receptor signalling and consequently B cell fate. This study showed that B cell apoptosis is enhanced by the presence of IVIg and that sialic acid-rich IgG glycoforms within IVIg may bind to CD22 on the surface of B cells to modulate B cell survival and activation [20]. This suggests that IVIg can modulate the adaptive immune system in a sialic acid-dependent manner. Consistent with this notion, Tackenberg *et al.* noted that IVIg infusion in chronic inflammatory demyelinating polyneuropathy (CIDP) patients results in an up-regulation of FcγRIIB on B cells [21]. The second study, conducted by Massoud *et al.*, used SNA-enriched IVIg and demonstrated that the dendritic cell immunoreceptor (DCIR) may be a receptor on dendritic cells with the capacity to recognize sialic acid-rich glycoforms, and may be responsible for regulatory T cell induction following IVIg administration [22]. Sialic acid-enriched IVIg was found to increase the frequency of regulatory T cells in the lungs of mice with allergen-induced airway hyper-responsiveness. The release of proinflammatory cytokines is also controlled by sialic acid-enriched fraction of IVIg. Further evidence that sialylated antibodies generated upon vaccination under Th2-type polarization conditions can be generated *de novo in vivo* and are immunosuppressive was reported by Hess *et al.*, strongly supporting an important role of sialylated IgG glycoforms as potent immunomodulators [23].

Despite this convincing array of data, two recent studies have not been able to support a role of IgG sialylation in IVIg activity in the previously discussed model systems. Leontyev *et al.* suggest that sialylation is not critical for IVIg activity under therapeutic treatment conditions in a model of ITP, in which daily escalating autoantibody doses are injected into mice to achieve a chronic thrombocytopenia [17]. Campbell *et al.* found that IVIg sialylation is not involved in IVIg Fc-dependent suppression of arthritis [14]. However, these two studies did not use the same experimental protocols as the previous studies, which may be one plausible explanation for these results.

The most important proof of clinical relevance of this concept will be to show that IVIg products enriched for sialic acid-containing sugar moieties have an enhanced activity across different *in vivo* model systems, and ultimately in human clinical trials. Given the variety of autoimmune diseases responding to IVIg therapy, we would not expect that every type of disease will require IVIg sialylation. However, even if only select patient groups respond to therapy with sialic acid-enriched IVIg this would represent a major achievement and allow the introduction of fully recombinant IVIg replacements in the near future.

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Disclosure

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References

1 Albert H, Collin M, Dudziak D, Ravetch JV, Nimmerjahn F. *In vivo* enzymatic modulation of IgG glycosylation inhibits autoimmune disease in an IgG subclass-dependent manner. Proc Natl Acad Sci USA 2008; **105**:15005–9.

2 Lauc G, Huffman JE, Pucic M *et al.* Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. PLoS Genet 2013; **9**:e1003225.

3 Parekh RB, Dwek RA, Sutton BJ *et al.* Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature 1985; **316**:452–7.

4 Scherer HU, van der Woude D, Ioan-Facsinay A *et al.* Glycan profiling of anti-citrullinated protein antibodies isolated from human serum and synovial fluid. Arthritis Rheum 2010; **62**:1620–9.

5 Shinzaki S, Iijima H, Nakagawa T *et al.* IgG oligosaccharide alterations are a novel diagnostic marker for disease activity and the clinical course of inflammatory bowel disease. Am J Gastroenterol 2008; **103**:1173–81.

6 Ogata S, Shimizu C, Franco A *et al.* Treatment response in Kawasaki disease is associated with sialylation levels of endogenous but not therapeutic intravenous immunoglobulin G. PLoS One 2013; **8**:e81448.

7 Debre M, Bonnet MC, Fridman WH *et al*. Infusion of Fc gamma fragments for treatment of children with acute immune thrombocytopenic purpura. Lancet 1993; **342**:945–9.

8 Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 2006; **313**:670–3.

9 Schwab I, Mihai S, Seeling M, Kasperkiewicz M, Ludwig RJ, Nimmerjahn F. Broad requirement for terminal sialic acid residues and FcgammaRIIB for the preventive and therapeutic activity of intravenous immunoglobulins *in vivo*. Eur J Immunol 2014; **44**:1444–53.

10 Schwab I, Seeling M, Biburger M, Aschermann S, Nitschke L, Nimmerjahn F. B cells and CD22 are dispensable for the immediate antiinflammatory activity of intravenous immunoglobulins *in vivo*. Eur J Immunol 2012; **42**:3302–9.

11 Ahmed AA, Giddens J, Pincetic A *et al*. Structural characterization of anti-inflammatory immunoglobulin G Fc proteins. J Mol Biol 2014; **426**:3166–79.

12 Schwab I, Nimmerjahn F. Intravenous immunoglobulin therapy: how does IgG modulate the immune system? Nat Rev Immunol 2013; **13**:176–89.

13 Sondermann P, Pincetic A, Maamary J, Lammens K, Ravetch JV. Reply to Crispin *et al*.: molecular model that accounts for the biological and physical properties of sialylated Fc. Proc Natl Acad Sci USA 2013; **110**:E3547.

14 Campbell IK, Miescher S, Branch DR *et al*. Therapeutic effect of IVIG on inflammatory arthritis in mice is dependent on the Fc portion and independent of sialylation or basophils. J Immunol 2014; **192**:5031–8.

15 Käsermann F, Boerema DJ, Ruegsegger M *et al*. Analysis and functional consequences of increased Fab-sialylation of intravenous immunoglobulin (IVIG) after lectin fractionation. PLoS One 2012; **7**:e37243.

16 Guhr T, Bloem J, Derksen NI *et al*. Enrichment of sialylated IgG by lectin fractionation does not enhance the efficacy of immunoglobulin G in a murine model of immune thrombocytopenia. PLoS One 2011; **6**:e21246.

17 Leontyev D, Katsman Y, Ma XZ, Miescher S, Käsermann F, Branch DR. Sialylation-independent mechanism involved in the amelioration of murine immune thrombocytopenia using intravenous gammaglobulin. Transfusion 2012; **52**:1799–805.

18 Wiedeman AE, Santer DM, Yan W, Miescher S, Käsermann F, Elkon KB. Contrasting mechanisms of interferon-alpha inhibition by intravenous immunoglobulin after induction by immune complexes versus Toll-like receptor agonists. Arthritis Rheum 2013; **65**:2713–23.

19 Stadlmann J, Weber A, Pabst M *et al*. A close look at human IgG sialylation and subclass distribution after lectin fractionation. Proteomics 2009; **9**:4143–53.

20 Seite JF, Cornec D, Renaudineau Y, Youinou P, Mageed RA, Hillion S. IVIg modulates BCR signaling through CD22 and promotes apoptosis in mature human B lymphocytes. Blood 2010; **116**:1698–704.

21 Tackenberg B, Jelcic I, Baerenwaldt A *et al*. Impaired inhibitory Fcgamma receptor IIB expression on B cells in chronic inflammatory demyelinating polyneuropathy. Proc Natl Acad Sci USA 2009; **106**:4788–92.

22 Massoud AH, Yona M, Xue D *et al*. Dendritic cell immunoreceptor: a novel receptor for intravenous immunoglobulin mediates induction of regulatory T cells. J Allergy Clin Immunol 2014; **133**:853–63 e5.

23 Hess C, Winkler A, Lorenz AK *et al*. T cell-independent B cell activation induces immunosuppressive sialylated IgG antibodies. J Clin Invest 2013; **123**:3788–96.