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Reply to — IVIG pluripotency and the concept of Fc-sialylation: challenges to the scientist

Inessa Schwab, Anja Lux and Falk Nimmerjahn

In their response to our recent Review article (Intravenous immunoglobulin therapy: how does IgG modulate the immune system? *Nature Rev. Immunol.* **13**, 176–189 (2013))[1], von Gunten and colleagues (IVIG pluripotency and the concept of Fc-sialylation: challenges to the scientist. *Nature Rev. Immunol.* (2014)[2]) raise the concern that the Review may be too focused on the role of IgG glycovariants as an important component of the anti-inflammatory activity of intravenous immunoglobulin (IVIG) therapy. Although we share the opinion of our colleagues that IVIG may work via many independent and non-mutually exclusive mechanisms (most of which are depicted in Figure 2 of our Review[1]) depending on the type of autoimmune or inflammatory disease being treated, it is impossible to cover all of these mechanisms in detail within a single article. This would have resulted in a mere list of possible mechanisms without an in-depth and detailed discussion, which would be necessary for each type of different inflammatory and autoimmune disease. Instead, we chose to focus on results that have been obtained in humans or from *in vivo* model systems that closely reflect human disease. This covers major clinically relevant autoimmune diseases such as idiopathic thrombocytopenic purpura (ITP), chronic inflammatory demyelinating polyneuropathy (CIDP), Kawasaki's disease, Guillain–Barré syndrome, inflammatory arthritis and autoimmune skin-blistering diseases, including epidermolysis bullosa acquisita (EBA). It was not possible to discuss the wide range of *in vitro* experiments that have used cell lines, and naive or cytokine-primed cell subsets, but that have no available *in vivo* correlates due to the absence of clinical data or well-defined animal model systems, but this topic has been covered by other reviews in depth[3]. Furthermore, we decided not to cover IVIG-mediated effects on T cells (and regulatory T cells) in detail but rather to make the interested reader aware of this pathway, which has been reviewed superbly by Kaveri and Bayry[4], for example. In light of the recent finding that sialic acid-rich glycoforms are crucial for the induction of regulatory T cells via dendritic cells, this topic would have fitted very well without any question[5]. Similar arguments apply for the exciting work of Shoenfeld and colleagues on the use of specific subfractions of IVIG (sIVIG)[6]. We need to reply in detail, however, with respect to several statements that von Gunten and colleagues have made in their Correspondence article.

Sialic acid and IVIG activity

As an argument against a role for sialic acid-rich IgG glycovariants, the authors cite studies that were either done purely *in vitro* or in no autoimmune setting *in vivo*. For example, the study of Käsermann and colleagues reported that the $F(ab')_2$ fragment of IVIG is important for suppressing CC-chemokine ligand 2 (CCL2; also known as MCP1) release in a whole blood lipopolysaccharide (LPS) stimulation assay[7]. However, a study by Guhr and colleagues[8], showed that IVIG that was enriched for sialylated $F(ab')_2$ was less effective than standard IVIG in inhibiting disease activity in a model of ITP, thereby contradicting the *in vitro* results of Käsermann *et al.*[7]. The same applies for the final study by Leontyev *et al.*[9], who again did not enrich for sialylated Fc and therefore quite expectedly could not detect an enhanced anti-inflammatory activity. Thus, in contrast to what von Gunten *et al.* state, neither of these studies challenges the concept that enriching IVIG for the sialic acid-rich IgG fraction enhances its therapeutic activity[10]. The interesting result of the Leontyev *et al.* study is, however, that neuraminidase-treated IVIG does retain its activity under their experimental conditions, which we refer to in our Review[1]. As this study uses a chronic ITP model system with a daily escalating dose of a rat-derived anti-mouse CD41 antibody, this difference in the experimental model system may be a straightforward explanation of their results. By contrast, a very recent study using the same rat anti-mouse CD41 antibody in addition to three other autoimmune model systems — including EBA, inflammatory arthritis and ITP — under preventive and therapeutic treatment conditions fully validated the important role of sialic acid-rich IgG glycoforms for IVIG activity *in vivo*[11].

Importance of the IVIG Fc fragment

The most central point made by the authors is that mouse model systems may not reflect the human system and that more studies in humans are required. This is actually the underlying theme of our Review, in which we describe how initial observations in human patients with ITP led to experimental work in *in vivo* mouse model systems, and not the other way around. Most importantly, we would like to point out that the notion put forward by the authors that IVIG may work via $F(ab')_2$-dependent mechanisms and only "probably" via Fc-mediated mechanisms ignores the fact that it was actually demonstrated in humans for the first time that the IVIG Fc fragment is sufficient for its therapeutic activity in ITP[12]. Along the same lines, it was demonstrated in human trials that blocking FcγRIIIA[13] or signalling pathways that initiated via these receptors is crucial for ITP development, and this is fully consistent with studies in mice. In summary, we very carefully selected model systems in which a high level of overlap between data obtained in mice and humans *in vivo* is already evident from the literature. We fully agree, however, that mice and humans differ and that more human clinical trials in other autoimmune and inflammatory diseases would be ideal to further corroborate the importance of the IVIG Fc and $F(ab')_2$ fragments. Alternatively, different types of humanized mouse models may be an attractive preclinical surrogate to further strengthen data obtained *in vitro* or in classical mouse models *in vivo*.

Role of FcγRIIB in IVIG activity

The authors make the point that "in patients, upregulation of the expression of FcγRIIB by IVIG could not be confirmed by gene expression profiling even in a pathological condition, Kawasaki's disease, where IVIG has a proven efficacy". This statement ignores the study by Tackenberg *et al.*, who have shown that FcγRIIB expression is upregulated on monocytes and B cells in patients with CIDP after IVIG therapy[14]. Moreover, the study by Abe and colleagues, which von Gunten and colleagues refer to as negative evidence, did not investigate FcγRIIB protein expression but rather used an antibody that binds both FcγRIIA and FcγRIIB (clone CIKM5) to demonstrate

IVIG-mediated effects on the expression of FcγRIIA/B family members by monocytes[15]. As monocytes express both FcγRIIA and FcγRIIB, this study cannot distinguish between these two receptors and therefore no conclusion can be drawn. In fact, CIKM5 dominantly recognizes FcγRIIA and the downmodulation of activating FcγRs observed in this study is fully consistent with data obtained in mouse model systems and with human natural killer cells. Moreover, the observed lack of an effect of IVIG on gene expression may merely reflect a mechanism of regulation that is independent of transcription, but rather depends on translation and/or subcellular protein localization.

With respect to the role of FcγRIIB in mice, the authors refer to two studies that supposedly claim that FcγRIIB is not required for IVIG activity in a model of ITP. When looking at the study of Bazin *et al.*[16], however, IVIG activity is abrogated in FcγRIIB-deficient mice on the C57BL/6 background, which is fully consistent with our interpretation and the work of other independent groups. The authors do note, however, that on the BALB/c background IVIG might be less dependent on FcγRIIB, which has been confirmed in a later study by Leontyev *et al.* (also cited in our Review). In general, care should be taken when using antibodies that are derived from other species (such as rat IgG1) in mice from different genetic backgrounds in which the capacity of binding to the corresponding mouse FcγRs, and therefore the capacity to be inhibited by FcγRIIB is not known. Thus, the finding that IVIG may not work via FcγRIIB on the C57BL/6 background remains a single observation by Leontyev *et al.* that contrasts with the literature published over the last 12 years by several independent groups using the same model system.

As a matter of fact, sialic acid residues were recently demonstrated to be crucial for IVIG-mediated amelioration of ITP in models on both the C57BL/6 and the BALB/c backgrounds, and therefore this effect is strain independent[11]. Moreover, FcγRIIB was identified as essential for IVIG activity under therapeutic treatment conditions in models of inflammatory arthritis and EBA[11]. Combined with previous results showing an FcγRIIB dependence of the amelioration of chronic ITP, we would argue that the conclusion of von Gunten and colleagues that the role for FcγRIIB in the immunomodulatory activity of IVIG may be of minor importance is lacking convincing evidence.

Taken together, our Review covers several well-defined pathways of IVIG activity. As we state at several places throughout the Review, these pathways may not be relevant to all types of disease in which IVIG is used but we tried to provide a view on a set of autoimmune diseases for which human and mouse *in vivo* data are at hand to ensure a clinical relevance. In the end, well-planned clinical trials and more defined animal model systems will provide definitive answers about which pathways will or will not be clinically relevant. For example, reports claiming that the IVIG concentration determines the outcome of agonistic and antagonistic tumour necrosis factor (TNF)-specific antibodies within the IVIG preparation on neutrophil death *in vitro* may also need to show that these different concentrations do indeed act on neutrophils differentially depending on their activation state and localization *in vivo*. All of these studies will provide exciting new insights into IVIG activity *in vivo* and may lead to novel therapeutic avenues. Finally, we would like to thank our colleagues for this stimulating discussion which may reflect our enthusiasm about this exciting field of research.

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Competing interests statement
The authors declare no competing interests.