

Review article

IVIG – mechanisms of action

Intravenous immunoglobulin (IVIG) preparations are fractionated from a plasma pool of several thousand donors. IVIG contain immune antibodies and physiologic autoantibodies. Immune antibodies reflect the immunologic experience of the donor population. This fraction of IVIG preparations is useful for replacement therapy and passive immunisation. Natural autoantibodies are able to react with the immune system of the recipient of IVIG and are suggested to help to correct immune deregulation. Immunomodulatory and anti-inflammatory properties are based on multiple mechanisms of action which are described. These mechanisms are effective concomitantly and synergistically at every occasion of use of IVIG in inflammatory and autoimmune disorders.

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Intravenous immunoglobulin (IVIG) preparations are standard in the treatment of primary and secondary immunodeficiency disorders associated with hypo- and agammaglobulinemia, subclass deficiencies or deficiency in one particular specificity. Intravenous immunoglobulin application is believed to deliver missing immune antibodies against pathogens, i.e. mainly affinity-matured antibodies, which are constituents of the humoral limb of the adaptive immune system. This treatment is largely evidence-based and is aimed at substitution or passive immunization against multiple bacteria and viruses.

In contrast, the use of IVIG in diseases associated with hyperreactive conditions of the immune system is not as widely accepted, although the efficacy of IVIG treatment has formally been demonstrated in several autoimmune diseases (1). One of the reasons for uncertainties is the lack of clear understanding of pathomechanisms of disease progression as well as of understanding of the mechanisms of how IVIG suppress hypersensitivity reactions of the immune system.

Here, we review some facts of immune antibodies in IVIGs as well as the potential immunologic mechanisms of actions initiated by natural antibodies also part of IVIG preparations that might be responsible for their immunomodulatory and anti-inflammatory properties in certain clinical conditions. Several excellent general reviews on IVIG have recently been published (1–4). This allows us to be brief in some parts and to review most recent developments.

Antibodies in IVIG for host protection

Efficacy in host defense of IVIG preparations rely on two general types of mechanism of action: antigen binding and effector functions. The effector functions include complement activation, complement binding, and binding to the various Fc receptors (Fig. 1). Antigen binding is mediated by the Fab part.

A polyclonal IVIG preparation is supposed to contain a wide variety of immune antibody (Ab) repertoires. Therapy with IVIG takes advantage of this fact and immune Ab titers in IVIGs are considered as being critical for efficacy (5–8). To reach some batch-to-batch consistency in protective Ab titers, the WHO defined the number of plasma units from individual donors in a pool of plasma for fractionation to be >1000 (9). Despite plasma pool sizes of >10 000, consistency of immune Ab titers in the final product remains variable (Fig. 2). Similar batch-to-batch variation in immune Ab titers has previously been reported (10, 11). Despite these batch-to-batch differences, the mean immune Ab titers in IVIG are fairly constant over years (Fig. 3).

The repertoire of immune antibodies is thought to reflect the immunologic experience of the donor population with pathogens. To cover best the needs of immunodeficient patients, it is believed that IVIG therapy is optimal when the recipient belongs to the same population as the donors. A general statement on particular suitability of IVIG derived from plasma of a given donor population seems however questionable (Fig. 3).

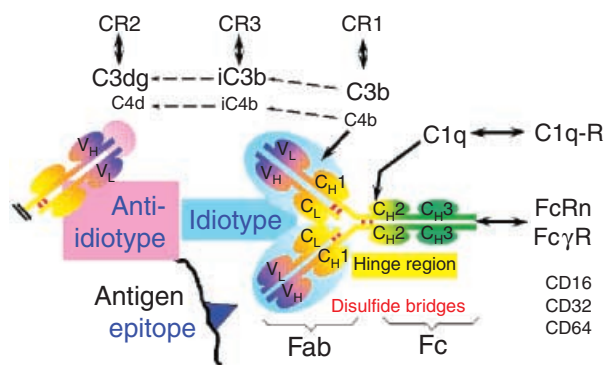


Figure 1. Schematic representation of an immunoglobulin G antibody (IgG Ab), the mechanisms contributing to the elimination of immune complexes, the mechanisms, which may inhibit the binding of pathogenic autoantibodies to the corresponding autoantigens, and of the mechanisms of recirculation of IgG by endothelial cells. The density of the Fc parts, generated by the immune complex upon binding, induces capping of the various activating Fc γ receptors. The generated density of receptors is the signal for activation of e.g. phagocytes. The same aggregated arrangements of IgG Fc parts form the complement-activating structures. Multimeric binding of C1q to the C γ 2 domain of the IgG Fc leads to activation of C1, C4, followed by C3. Activated C3 and C4 (C3b and C4b, respectively) bind to the Fab part of the immunoglobulins. Covalently bound C3b/C4b is converted to iC3b/iC4b, and finally degraded to C3dg/C4dg. Immune aggregates with bound complement proteins can accelerate phagocytosis considerably by binding to the respective complement receptors. CR1, CR2, and CR3 stand for complement receptor 1 (CD35), complement receptor 2 (CD21), and complement receptor 3 (CD11b/CD18), respectively. C1q-R = C1q receptor. The mentioned mechanisms become also effective when pathogenic antibodies recognize self structures. Anti-idiotypic antibodies may prevent the binding of pathogenic autoantibodies to the corresponding autoantigens. Binding of Fc-parts to 'neonatal Fc receptor' (FcRn) can help elimination of antigens and recycling of IgG.

Although several immune Ab titers in IVIG prepared from plasma of US or European origin (Germany + Switzerland) showed significant differences (Table 1), relevance of observed differences is not proven.

Based on the data presented in Table 1 and with exception of anti-hepatitis A virus (HAV) Ab titers, it seems to be difficult to approve a better quality of IVIG prepared from plasma from either continent because some of the titers were higher in European and others in United States (US)-derived IVIG. Similar observations have previously been reported (12). However, superiority of IVIG prepared from local plasma enriched with more particular immune antibodies to locally endemic pathogens might indeed exist. Some IVIG preparations have a declared minimal titer of a given Ab, e.g. anti-cytomegalovirus (CMV). A declared high Ab titer does not necessarily result in elevated titers in other antibodies (Fig. 4).

In conclusion, variations in immune Ab titers from batch-to-batch appear inherent to all IVIG preparations. Indication of Ab titers in IVIG without indication of a range is questionable. Without the indication of a range an immune Ab titer should only be accepted if given for a particular batch. Comparing Ab titers between different brands should be carried out with extreme care: the concentration of the IgG solution tested should be indicated and should be taken into consideration. The test system used can vary considerably, and standardization is still a problem.

Natural autoantibodies

Natural antibodies occur under physiologic conditions, are beneficial, and have partially been characterized (1, 13). These antibodies do not appear to be the result of an immune response, because they are also present in embryos and in animals, which were grown up under sterile conditions (14, 15). In agreement with these observations, rearrangement of the immunoglobulin genes and affinity maturation are mostly not evident in natural antibodies (16), i.e. affinity and specificity of the natural antibodies are often low (17, 18). It has been shown that natural antibodies prevent the dissemination of pathogens into the brain and the kidneys (19). They can therefore be considered as 'first-line defense' antibodies, which are elements of the innate immune system. Natural antibodies are mostly IgM antibodies, but can also be of the IgG or IgA class (14, 17).

Natural antibodies can be autoreactive but are not autospecific. These natural autoantibodies break the immune tolerance, however, in turn, control physiologic autoreactivity. The peripheral control of natural autoantibody production apparently occurs through their Fab parts (idiotypes), which recognize molecules with variable regions. In turn, these Fab parts are recognized by other natural autoantibodies. Natural autoantibodies can also react with self-antigens (1), particularly with those conserved over the evolution. Such natural autoantibodies can show tissue homeostatic functions.

These physiologic autoantibodies are believed to provide the anti-inflammatory effects of IVIG preparations. Table 2 shows a list of autoantigens to which natural autoantibodies have been shown to bind to. They are generated by B cells, which have not been recognized as autoreactive, and, consequently, have not been deleted during the differentiation process. Moreover, as these B cells generate autoantibodies, mechanisms leading to 'clonal anergy' can also be excluded. Whether the production of the natural autoantibodies is controlled by mechanisms which are additional to that of the V-region connectivity, is unclear. The B cells producing natural antibodies are found in the marginal

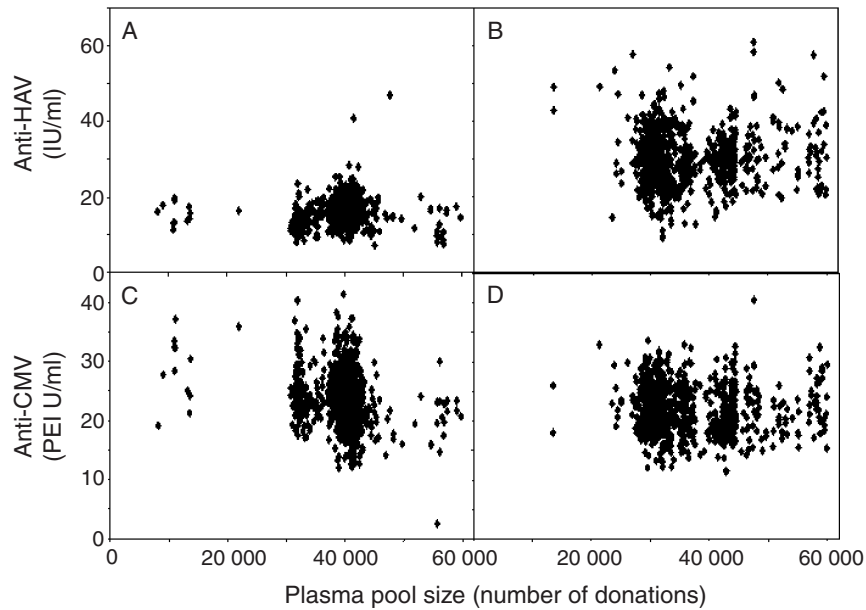


Figure 2. Dependence of antibody (Ab) titers in intravenous immunoglobulin (IVIG) on the number of plasma donations in a pool used to manufacture the individual lots. Nonremunerated donations served to prepare IVIG. The two upper panels compare anti-hepatitis A virus Ab titers in IVIG prepared from plasma originating from USA (A; $n = 850$) and from Europe (B; Germany + Switzerland; $n = 777$). The two lower panels compare anti-cytomegalovirus Ab titers in IVIG from USA (C) or European origin (D). PEI U = Paul-Ehrlich-Institute units. Immunoglobulin solutions of 6% were analyzed.

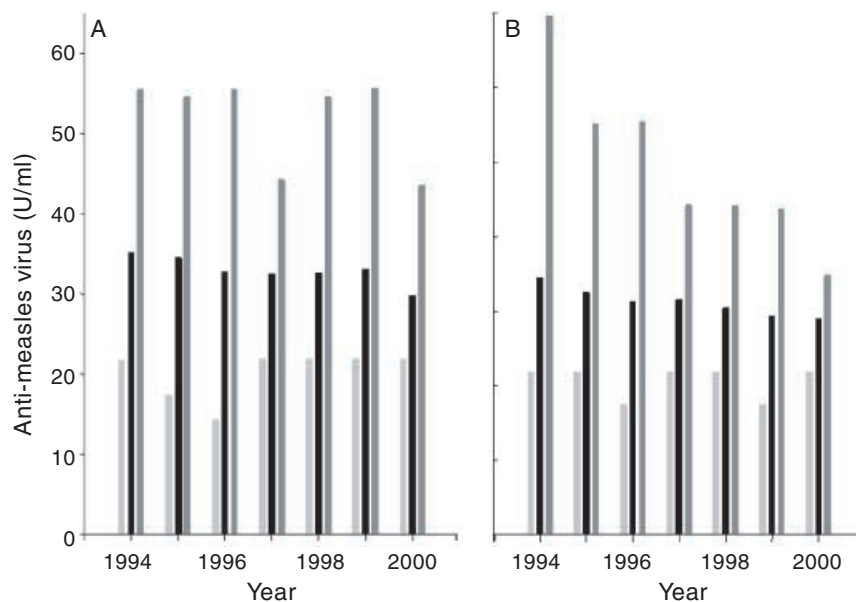


Figure 3. Variation in anti-measles virus antibody (Ab) titers in intravenous immunoglobulin (IVIG) batches manufactured in the same calendar year. The left bar indicates the lowest titer measured for that year. The middle bar indicates mean values of titers of all lots produced in one calendar year. The right bar gives the highest titer measured in that year. Panel A depicts anti-measles virus Ab titers in IVIG prepared from nonremunerated plasma of European origin (Germany + Switzerland) and panel B in IVIG prepared from nonremunerated plasma of US origin. Immunoglobulin solutions of 60 mg/ml were analyzed.

zone of the spleen, but not in germinal centers of lymph nodes (20). T cells do not appear to play a role in maturation of marginal zone B cells (21).

How can natural autoantibodies be part of the 'first-line defense' and, at the same time, do not cause autoimmune diseases? This question appears to be crucial

Table 1. Antibody titers in intravenous immunoglobulin (IVIG) of various plasma origins

Antigen	Titer in IVIG prepared from EU plasma	Titer in IVIG prepared from US plasma	P-value
CMV (PEI U/ml)	21.9	24.2	<0.0001
HbsAg (U/ml)	0.95	0.95	0.867
HAV (U/ml)	30.2	15.8	<0.0001
Measles (U/ml)	32.8	31.1	<0.0001
Varicella (U/ml)	12.1	14.7	<0.0001
Polio 1 (U/ml)	17.4	13.8	<0.0001
Parvo B19 (U/ml)	120.3	190.9	0.144
Diphth. Tox. (U/ml)	3.09	3.46	<0.0001
Streptol. (U/ml)	549.6	612.6	<0.0001

HAV, hepatitis A virus; HbsAg, hepatitis B surface antigen; PEI, Paul-Ehrlich-Institute; CMV, cytomegalo virus.

when we consider IVIG, which has been demonstrated to contain physiologic autoantibodies (1, 13), as a therapeutic option in immune dysregulation. By binding of pathogens and complement factors, it is possible that natural autoantibodies contribute to the initiation of an effective adaptive immune response because of efficient uptake of pathogens by antigen-presenting cells. In this case, the immune response against pathogen-specific epitopes, which do not cross-react with self-antigens, is supported, and the development of autoimmunity prevented (22). Moreover, it has been speculated that natural autoantibodies cover epitopes, which prevent the immune response against self-antigens derived from damaged or aged cells (23, 24). However, additional mechanisms controlling peripheral physiologic autoimmunity may play a role and are discussed on the following pages.

Potential immunosuppressive activities mediated by IVIG

Immune and natural first-line defense antibodies

Autoimmune diseases have often been associated with bacterial or viral infections (25, 26), which may trigger, sustain or accelerate such conditions. Intravenous immunoglobulin may help to efficiently eliminate such pathogens either by providing pathogen-specific immune antibodies and/or by natural first-line defense antibodies. A particular therapeutic role can be attributed to anti-superantigen antibodies. Superantigens can trigger the activation of autoreactive cells in an MHC restriction-bypassing manner. Intravenous immunoglobulin contains antibodies to superantigens such as TSST-1 and staphylococcal enterotoxins and these anti-microbial antibodies can be considered as immunomodulatory.

Autoantibodies against antigen-binding structures

Antigen-binding structures are variable regions within antibodies and antigen receptors present on T and B cells. Intravenous immunoglobulin contain natural autoantibodies directed against the idiotype, the hinge region, as well as against the constant heavy chain 1 and the constant light chain domains of antibodies (1, 13) (Fig. 1). The anti-idiotypic antibodies have been shown to bind pathogenic autoantibodies *in vitro*, suggesting that they might also be able to prevent binding to autoantigens under *in vivo* conditions (1, 27). Indeed, evidence for the therapeutic value of natural autoantibodies in IVIG has been obtained initially in patients with acquired hemophilia (28) and later in Guillain-Barré

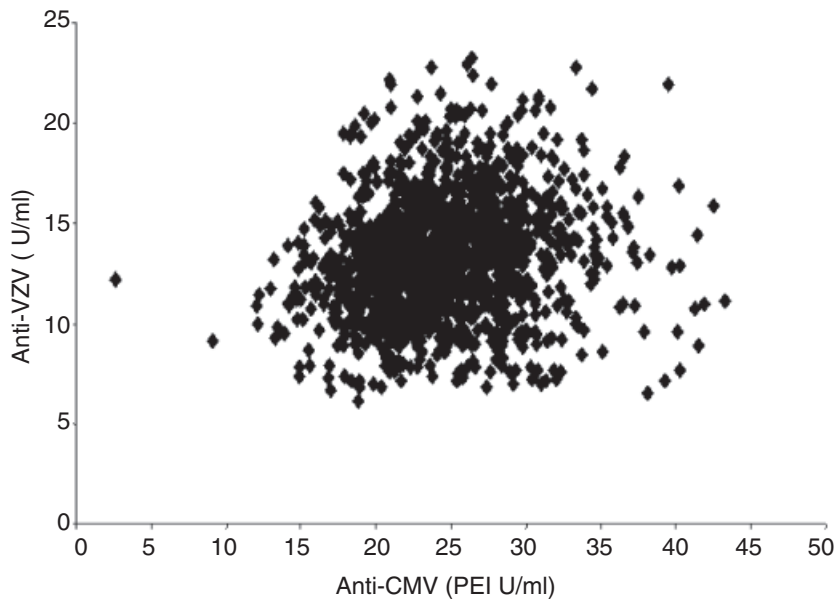


Figure 4. Antibody titers to two human herpesviridae in individual batches of IVIG. Batches were prepared from recovered plasma (whole blood nonremunerated donations). No distinction on geographic origin of plasma was made. Anti-cytomegalovirus and anti-varicella zoster virus titers were assessed in a 6% solution of 180 batches. No relationship among the two Ab titers was observed.

Table 2. Autoantigens recognized by natural autoantibodies

Intracellular proteins: DNA, myosin, actin, tubulin, spectrin, heat-shock proteins, etc.
Variable regions of immunoglobulins and antigen receptors
Hinge region of IgG
Cell surface molecules: CD4, HLA-DR molecules, Fas receptor
Cytokines
Complement factors

syndrome (29, 30), chronic inflammatory demyelinating polyradiculoneuropathy (31), myasthenia gravis (32), systemic ANCA positive vasculitis (33), autoimmune thyroiditis (34), and idiopathic thrombocytopenia (35).

In addition, it is also possible that anti-idiotypic autoantibodies bind to variable regions of antigen receptors, rendering cell activation by autoantigens impossible. For instance, physiologic autoantibodies against the variable region of the T-cell receptor block autoantigen-mediated T-cell activation *in vitro* (36, 37). Another potential mechanism is the concurrent activation of antigen and Fc γ RIIb receptors; the latter activates inhibitory pathways on B cells, resulting in a specific proliferation block of autoreactive B cells (38). Taken together, the result of the described mechanisms might be a long-term down-regulation of pathogenic autoantibodies as a consequence of IVIG therapy (27).

Autoantibodies against hinge regions of IgG

An indirect correlation between the levels of pathogenic and natural anti-hinge region autoantibodies has been observed in AIDS, vasculitis, and following transplantations. These observations are in agreement with *in vitro* findings, demonstrating that natural anti-hinge region autoantibodies block B-cell proliferation as long as the autoantigen is bound to the B-cell antigen receptor (13). It should be noted that natural anti-hinge region autoantibodies are not specific for autoreactive B-cell clones, suggesting that they are part of a physiologic negative feedback mechanism in each humoral immune response (13).

Autoantibodies against CD4 and MHC class I molecules

Natural anti-CD4 and anti-MHC class I autoantibodies have been affinity-purified from IVIG preparations. Natural anti-CD4 antibodies blocked T-cell proliferation in mixed lymphocyte cultures and the infection of CD4-positive T cells by human immunodeficiency virus *in vitro* (39). Similarly, the natural anti-MHC class I antibodies blocked the function of CD8 T-cells *in vitro* (40). Together with anti-idiotypic antibodies against components of the T-cell antigen receptor, these data suggest that IVIG might modulate T-cell-mediated autoimmune and perhaps allergic hypersensitivity reactions.

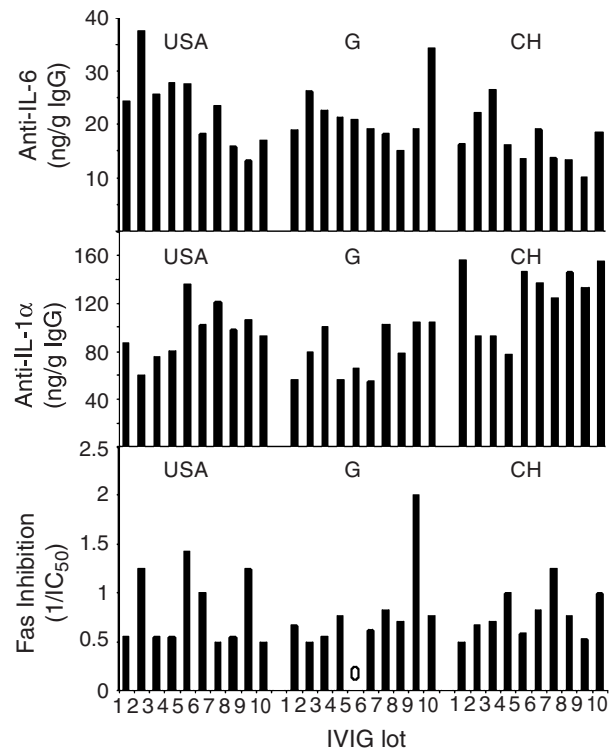


Figure 5. Physiologic autoantibodies to three autoantigens in IVIG prepared from plasma of various geographic origin: USA, G = Germany and CH = Switzerland. Randomly selected batches of the same IVIG were analyzed for their content in anti-IL-6 (upper panel), anti-IL-1 α (middle panel) and Fas inhibitory autoantibodies (lower panel). Physiologic anti-cytokine autoantibodies were measured by kits obtained from R&D. Fas inhibitory activity (1/IC₅₀) was assessed in frame of a contract research project of ZLB and Apotech SA, Switzerland, using a standardized *in vitro* cell death assay (46).

Autoantibodies against the Fas receptor (APO-1/CD95)

Patients with toxic epidermal necrolysis apparently benefit from the treatment with IVIG as it emerges from case reports (41–44) and from studies with larger number of patients (45, 46). In this disease, keratinocytes are highly sensitive toward Fas ligand-mediated apoptosis. Intravenous immunoglobulin contain natural anti-Fas receptor autoantibodies which are able to block molecular Fas ligand/Fas receptor interactions, and, consequently, keratinocyte apoptosis (45, 47). The titers in Fas inhibitory antibodies vary from batch to batch to a similar extent as the anti-microbial antibodies. The origin of the plasma does not appear to play a role for these autoantibody titers (Fig. 5, lower panel). Interestingly, agonistic anti-Fas receptor autoantibodies have also been reported as being present in IVIG preparations (48, 49). The reason(s) for these contrasting reports is still unclear. First data from our own laboratories suggest that the physiologic autoantibodies to Fas have various specificities

and that effect on cell death depends on the IVIG dose (F. Altnauer, S. von Gunten, P. Späth, H.-U. Simon; unpublished observation). Other dual effects of IVIG on proliferation of antigen-specific T cells were recently reviewed (4). It should be noted that agonistic anti-Fas receptor autoantibodies might also mediate anti-inflammatory effects by inducing apoptosis in inflammatory cells, such as activated T cells (50), neutrophils (51), or eosinophils (52).

Natural autoantibodies against cytokines

Physiologic autoantibodies to IL-6, IL-1 α TNF- α , IL-8, GM-CSF, and others have been found in healthy individuals. Consequently IVIG contain natural anti-cytokine autoantibodies (53–55). The anti-cytokine autoantibody content of IVIG shows similar batch-to-batch variations as immune antibodies and the origin of plasma used for fractionation apparently has no influence on these titers (Fig. 5). It is possible that anti-inflammatory activities of IVIG are mediated by neutralizing overshooting pro-inflammatory cytokines. These natural autoantibodies together with the other anti-inflammatory effects of IVIG might have been responsible for the immediate relief of life-threatening condition associated with the rapid drop of excessive serum ferritin and considerable elevated cytokine levels observed in a patient with reactive macrophage activation syndrome with no obvious microbial background (56).

Fc receptor-mediated effects

Fc γ receptors with intracellular 'immune receptor tyrosine-based activation motifs' mediate inflammatory effector functions. In hypogammaglobulinemic patients with reduced occupation of Fc γ receptors, infusion of IVIG can result in the release of pro-inflammatory and subsequently anti-inflammatory cytokines (57, 58). In more than 100 healthy and diseased individuals receiving various IVIG preparations with very low or undetectable amounts of polymers and no spontaneous complement activation, evidence was obtained that IgG–IgG dimers present in IVIG might largely be responsible for such cytokine release and that a measurable induction of cytokine release can also occur in individuals with normal IgG levels (59). Furthermore, there is evidence that dimers might not only contribute to clinical efficacy but also to adverse events associated with IVIG treatment (60–63). This dual effect heralds the physiologic frontier, which might set an upper limit for the IVIG dose applied for immunomodulation.

In contrast to the above mentioned Fc γ receptors, Fc γ RIIb contains within its intracellular part an 'immune receptor tyrosine-based inhibition motif' and can therefore transduce inhibitory signals (38). Interferon-gamma has been shown to increase the expression of Fc γ RIIb on

monocytes and neutrophils, whereas IL-4 has the opposite effect (64). This suggests that in Th1-mediated hypersensitivity reactions IVIG can activate Fc γ RIIb, a mechanism that may result in decreased release of pro-inflammatory cytokines (65). The possible importance of Fc γ RIIb for the therapeutic effect of IVIG has directly been demonstrated in a mouse model of idiopathic thrombocytopenia (66). The results of this study, combined with the emerging role of dimers in cytokine modulation, and finally the observation by Pricop et al. (64) on cytokine milieu-dependent expression of activating and inhibiting receptors may explain many aspects of anti-inflammatory effects of IVIG.

In addition, we would like to speculate at this point that IVIG may also influence the differentiation of new monocytes/macrophages by IgG–IgG dimers, which usually do not exist in the circulation, as the dimers may shift the balance between inhibitory and activating receptor on monocytes/macrophages. Taken together, there are multiple possible mechanisms in which activation or blocking of Fc receptors may be involved. The actual contribution, however, needs to be clarified by relevant models. Moreover, the importance of the recently identified family of Fc receptor homologs expressed by mature B cells (67, 68) in relation to IVIG therapy requires additional experimental work.

Intravenous immunoglobulin have also been reported to accelerate autoantibody catabolism by binding to a Fc receptor called 'FcRn' on endothelial cells (69). If IgG is pinocytosed and bind in a low pH milieu to FcRn, the immunoglobulin is protected from lysosomal degradation. Intravenous immunoglobulin treatment may result in the saturation of FcRn receptors and through competition preventing pathogenic autoantibodies from binding, with the consequence of their accelerated degradation. This hypothesis was supported when, recent in an animal model, FcRn knockout mice IVIG did not slower down the clearance of a monoclonal anti-platelet Ab, while in the wild-type animals it did (70).

Binding and inhibition of complement factors

Immunoglobulin G binds the active complement factors C4b and C3b (Fig. 1). High concentrations of soluble monomeric IgG may therefore prevent the damage of tissue by deviation of the complement cascade from target tissue to the (exogenous) IgG in the circulation. The *in vivo* importance of this potential anti-inflammatory mechanism has been demonstrated in an experimental model of complement-mediated acute inflammation (71). In addition, natural anti-C3b autoantibodies have been identified, that inhibited C3 convertase activity *in vitro* (72). Both potential anti-complement mechanisms might play a role in the IVIG treatment of patients with dermatomyositis (73, 74).

The use of IVIG therapy in autoimmune and allergic diseases

As a consequence of the anti-inflammatory properties, IVIG preparations are currently not only used in hypo-reactive but also in hyperreactive conditions of the immune systems. In general and at least in the initial phase of treatment, high-dose IVIG treatments are recommended for hypersensitivity diseases. Hints from an animal model and the clinical outcome in Kawasaki disease indicate that few and (very) high doses of IVIG might be more efficient than the same dose divided into three or more infusions (71, 75). In autoimmune diseases, such as Kawasaki disease (76), Guillain-Barré syndrome (77), and immune thrombocytopenic purpura (78), IVIG preparations are an accepted or even standard therapy. In chronic inflammatory demyelinating polyneuropathies (79), multifocal motor neuropathy (80), and dermatomyositis (81), the efficacy of IVIG has formally been proven. In other diseases, such as myasthenia gravis (82), anti-neutrophil cytoplasmic autoantibody (ANCA) positive vasculitis (83), and systemic lupus erythematosus (84–86), IVIG are used. Moreover, IVIG appears to be also effective in patients with blistering diseases (87), autoimmune urticaria (88) and toxic epidermal necrolysis (41–46, 89–92).

The proposed anti-inflammatory mechanisms (Table 3) suggest that IVIG might also be beneficial for allergic

patients. As asthma is often associated with hypogammaglobulinemia secondary to steroid treatment and asthma exacerbations are often the consequence of infections (93), it is not surprising that studies have been performed evaluating the potential benefit of IVIG in asthma therapy. In addition, IVIG inhibit IgE production by human B cells *in vitro* (94). In long-term studies using high-dose IVIG, improved lung function and reduced steroid requirement were observed (95). Presently, this treatment is reserved for patients who have high systemic steroid requirement (96) and/or Churg–Strauss syndrome (97). Good clinical efficacy of IVIG has also been reported in severe cases of atopic dermatitis. Interestingly, discontinuation of the therapy was associated with symptom recurrence (98).

Conclusions

Intravenous immunoglobulin preparations have been shown to exhibit anti-inflammatory properties in patients with diseases associated with a hypersensitive immune system. Many *in vitro* and also *in vivo* data suggest a number of potential mechanisms of action. There is increasing understanding that all of these mechanisms can be effective concomitantly and synergistically. Additional investigations are required to understand which of these mechanisms are more prominent under given *in vivo* conditions. A better understanding of pathogenesis of many inflammatory diseases is also urgently needed to be able to study the effect of IVIG in the relevant clinical conditions. Clearly, the identification of markers, which help to more precisely select those patients who most likely benefit from IVIG, would be a great advantage.

In spite of many uncertainties, high-dose IVIG is presently a valuable treatment option for a number of autoimmune diseases. In addition, IVIG is often beneficial in patients with autoimmunity or allergy who do not respond to conventional therapies. In many of these diseases, however, placebo-controlled studies are necessary to confirm previously published encouraging results.

Acknowledgments

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Table 3. Potential anti-inflammatory mechanisms mediated by intravenous immunoglobulin (IVIG)

Immune antibodies
Elimination of pathogens
Enhanced expression of inhibitory Fcγ receptors
Release of anti-inflammatory cytokines
Increased catabolism of pathogenic autoantibodies
Binding of C4b and C3b by soluble IgG
Natural antibodies
Elimination of pathogens*
Activation of inhibitory Fc receptors*
Release of anti-inflammatory cytokines*
Protection of autoantibody and autoreactive lymphocyte targets by anti-idiotypic antibodies†
Inhibition of B-cell proliferation by anti-hinge region antibodies†
Inhibition of T-cell proliferation by anti-CD4 and anti-MHC class I antibodies†
Prevention of tissue cell apoptosis and induction of immune cell apoptosis by anti-Fas receptor antibodies†
Neutralization of pro-inflammatory cytokines by anti-cytokine antibodies†
Inhibition of the complement cascade by anti-C3b antibodies†

* Immune antibodies as well as natural autoantibodies.

† Natural autoantibodies.

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