

I FUNDAMENTALS

Immunomodulating effects of intravenous immunoglobulin in autoimmune and inflammatory diseases

Srinivas V Kaveri, Luc Mouthon, Michel D Kazatchkine

Intravenous immunoglobulin (IVIg) therapy is increasingly used for its immunomodulating properties in a wide range of pathological conditions including autoimmune diseases, systemic inflammatory diseases and allotransplantation. Intravenous immunoglobulins are obtained from a pool of several thousand healthy blood donors. They are composed of intact IgG with a distribution of subclasses corresponding to that of normal serum and a half life of three weeks. IVIg represent a wide spectrum of natural and induced antibody activities which are present in normal human serum, including antibodies directed against external antigens, autoantibodies and anti-idiotypes.¹ This review will focus on the mechanisms by which infusion of normal human polyspecific IgG may be beneficial in patients with autoimmune disorders.

Immunomodulating functions of IVIg

Immunomodulatory effects of IVIg may depend on the interaction of infused IgG with inflammatory cells and lymphocytes through Fc portions and/or interactions of IVIg with circulating immunoglobulins or antigen receptors on lymphocytes through variable (V) regions. To facilitate understanding of the mechanisms of action of IVIg, we have divided them into three groups: a) V-region dependent mechanisms, b) Fc-dependent mechanisms, and c) other mechanisms of action which include modulation of synthesis and release of cytokines, interactions with surface molecules and modification lymphocyte population (table). The group may depend on both V region and Fc fraction of infused Ig.

FC DEPENDENT MECHANISMS OF ACTION OF IVIg

Evidence for Fc-receptor blockade came from the following findings: a) administration of IVIg is followed by a decrease in the clearance of autologous erythrocytes coated with anti-D antibodies *in vivo*²; b) peripheral blood monocytes from IVIg-treated patients with ITP exhibit a decreased ability to form rosettes with IgG-coated erythrocytes *in vitro*³; c) antibodies against Fc γ RIII show similar effects to those of IVIg in ITP *in vivo*⁴;

and d) anti-D IgG induces an increase in platelet counts in RhD-positive ITP patients.⁵ Saturation of Fc receptors on splenic macrophages is likely to play a critical role in the beneficial effect of IVIg in peripheral immune cytopenias. Another factor contributing to the beneficial effect of IVIg in such diseases is idiotype-anti-idiotypic interacting, as shown in peripheral immune thrombocytopenia with anti-GP IIb/IIIa.⁶

Basta *et al* observed a binding of IVIg to activated complement components C3b and C4b, preventing them from binding to antibody-coated endothelial cells.⁷ Such binding results in solubilisation and dissociation of circulating immune complexes (CIC) following IVIg therapy⁸ and protects guinea pigs from shock induced by lethal doses of anti-Forsmann antibodies.⁹ The effects of IVIg on NK cell activity have been assessed in patients with peripheral immune cytopenias before and after treatment with IVIg. Clinical response and elevation of peripheral cell counts were associated with a decrease in the natural killer (NK) cell activity.¹⁰ IVIg also diminishes NK cell activity of healthy donors *in vitro* in a dose-dependent pattern.¹⁰

V REGION-DEPENDENT MECHANISMS OF ACTION OF IVIg

Idiotypic interactions between IVIg and autoantibodies

The presence in IVIg of an anti-idiotypic activity against disease-associated autoantibodies was first suggested by the rapid fall in the titre of anti-factor VIII autoantibodies in the plasma of patients with anti-factor VIII autoimmune disease treated with IVIg. We have now collected five lines of evidence to show that IVIg interacts through V regions with autoimmune disease-associated and with natural autoantibodies:

- 1) F(ab')₂ fragments of IVIg neutralise the functional activity of autoantibodies and/or inhibit the binding of autoantibodies to autoantigens.
- 2) Autoantibodies are specifically retained on affinity chromatography columns of F(ab')₂ fragments of IVIg coupled to Sepharose.¹¹
- 3) Selective retention of autoantibodies on affinity columns of IVIg.¹²
- 4) IVIg do not contain detectable antibodies

INSERM U28, Hôpital
Broussais, Rue Didot,
Paris, France

S V Kaveri
L Mouthon
M D Kazatchkine

Correspondence to:
Dr S V Kaveri, INSERM
U 28, Hôpital Broussais,
96 rue Didot, 75014 Paris,
France.

*Immunomodulating effects of IVIg***1 Early effects of IVIg infusion**

Neutralisation of circulating autoantibodies
 Functional blockade of FcR on splenic macrophages
 Inhibition of complement-mediated damage
 Changes in solubility and clearance of immune complexes
 Modulation of production of proinflammatory cytokines
 Phenotype changes on circulating leukocytes

2 Long term effects of IVIg infusion

Down regulation of IVIg-reactive B cell clones and suppression of antibody synthesis
 Stimulation of IVIg-reactive B cell clones
 Alterations in spontaneous fluctuations of autoantibody titres in serum
 Selection of T cell repertoires (?)
 Selection of functional T and B cell repertoires through changes in patterns of cytokines produced (?)
 Suppression of GVHD; suppression of alloantibodies

against the Gm1 (3), Gm1 (4), Gm1 (17), Gm1 (1) and Km (1) allotypes that are most commonly expressed in the F(ab')₂ region of human IgG.¹³

5) IVIg share anti-idiotypic reactivity towards idiotypes of autoantibodies with heterologous anti-idiotypic antibodies.

An analogy can be made between the recovery from autoimmune diseases using IVIg therapy and the spontaneous remission from autoimmune diseases which occurs in association with the generation of auto-anti-idiotypic antibodies against prerecovery autoantibodies. Anti-idiotypic antibodies against autoantibodies have been found in remission sera of patients with myasthenia gravis,¹⁴ Guillain-Barré syndrome,¹⁵ systemic vasculitis with ANCA autoantibodies,¹⁶ systemic lupus erythematosus,¹⁷ anti-factor VIII autoimmune disease,¹⁸ and anti-fibrinogen autoimmune disease.¹⁹ In patients who spontaneously recovered from anti-factor VIII autoimmune disease,¹⁸ Guillain-Barré syndrome¹⁵ and in patients in remission from systemic vasculitis with ANCA autoantibodies,¹⁶ we have observed that F(ab')₂ fragments of patients' post-recovery IgG inhibited autoantibody activity in F(ab')₂ fragments of autologous IgG obtained during the acute phase of the disease.

INTERACTION OF IVIg WITH T LYMPHOCYTES

The V region-dependent immunomodulatory effects of IVIg cannot be restricted to the idiotype-anti-idiotypic interactions between IVIg and autoantibodies and the B cell antigen receptor. There is evidence that IVIg contains antibodies directed against constant and variable regions of the β chain of the T-cell receptor²⁰ and our own recent results indicate the presence in IVIg of antibodies reacting with CD5 and CD4.^{21, 22} Preliminary evidence in vitro suggests that IVIg contains antibodies reactive with V regions (idiotypes) of anti-class I and class II antibodies.²³ Preliminary results using the synthetic peptides B2702. 60-84 and LB5-77 derived from regions of the molecules involved in binding to T cells²⁴ indicate that IVIg contains antibodies reactive with these regions of HLA molecules as assessed by ELISA (Kaveri and Glotz, unpublished observations).

Functional modulation of T lymphocytes by IVIg has been demonstrated as another possible mechanism of action of IVIg in an experimental model where infusion of IVIg resulted in the protection of F1 (Lewis Brown-Norway) rats against the T cell dependent experimental model of autoimmune uveoretinitis (EAU). Treatment with IVIg decreased lymphocyte proliferative and antibody responses to S-Ag and the proliferative response to Con A. Lymph node cells from IVIg-treated and S-Ag immunised animals neither proliferated nor secreted IL-2 in response to S-Ag but proliferated when cocultured with lymph node cells from rats immunised with S-Ag. These findings suggest peripheral anergy of T lymphocytes associated with infusions of IVIg.²⁵

MODULATION OF THE RELEASE AND FUNCTION OF PRO-INFLAMMATORY CYTOKINES BY IVIg

Involvement of cytokines in systemic inflammatory diseases and autoimmune disorders is dependent on their ability to mediate and regulate inflammatory responses (for example, interleukin-1 (IL-1), IL-6, tumour necrosis factor (TNF), IL-8, IL-10, IL-12 and IL-13), to regulate activation, growth and differentiation of lymphocytes (for example, IL-2, IL-4, IL-10, interferon (IFN) γ and tumour growth factor (TGF) β) and to stimulate growth and differentiation of leukocytes in the bone marrow (colony stimulating factors, CSFs). We have demonstrated that resolution of the inflammatory syndrome following infusion of IVIg is associated with a somewhat paradoxical dramatic decrease in the elevated serum levels of IL-1 receptor antagonist (IL-1Ra) that are present before treatment.²⁶ The protective effect of IVIg on coronary artery damage in Kawasaki syndrome could thus be related to decreased release and function of monocytic cytokines, decreased mononuclear cell infiltration together with decreased antibody-mediated cell lysis.

IVIg has also been shown to inhibit the release of IL-1 and TNF from LPS-activated monocytes in vitro (Carreno, unpublished results) and has recently been shown to stimulate the release of IL-1 Ra, from monocytes. Preliminary observations from our laboratory indicate that at least part of the effects of IVIg in inducing the natural IL-1 antagonist, IL-1Ra, from monocytes depends on the reactivity of V regions of IVIg with membrane molecules of monocytes.²⁷

We have recently analysed the changes that occur in the expressed autoreactive antibody repertoire and in network organisation following the infusion of normal polyspecific IgG in a patient with autoimmune thyroiditis.²⁸ The results have enabled us to collect evidence indicating that changes in serum antibody concentrations observed after infusion of IVIg do not merely reflect passive transfer of IgG into the patient. The dynamic behaviour of autoantibodies in the patient before infusion of immunoglobulin shows a clearly distinct pattern with marked rhythmicity suggesting disruptions of connectivity within the

immune network. Conversely, the kinetic pattern after the infusion of IVIg is similar to that seen in healthy individuals. Thus infusion of pooled normal immunoglobulin restored in the patient a network organisation of autoantibodies characteristic of the physiological conditions.

These observations support our hypothesis that the beneficial effect of IVIg in autoimmune diseases is not merely due to the passive transfer (transfusion) of neutralising anti-idiotypic antibodies against autoantibodies but that IVIg alters the structure and the dynamics of the idiotypic network in the autoimmune patient to regain physiological control of autoimmunity. Thus IVIg would clearly differ in its mode of action from the immunosuppressive approach to the treatment of autoimmune diseases.^{29 30}

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