

II FROM FUNDAMENTALS TO CLINICAL APPLICATIONS

In vitro effects of polyvalent immunoglobulin for intravenous use

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High dose intravenous immunoglobulin (IVIg) therapy is used in the treatment of a wide range of auto-immune or presumed immune-mediated neurological disorders. A beneficial effect of IVIg has been reported in Guillain-Barré syndrome,¹ chronic inflammatory demyelinating polyneuropathy,²⁻⁶ myasthenia gravis,^{7,8} multiple sclerosis,⁹ multifocal motor neuropathy,¹⁰⁻¹² intractable childhood epilepsy,¹³ and dermato- and polymyositis.^{14,15}

The mechanisms of response to IVIg treatment in these disorders is not fully understood. Firstly, adequate models to study the effects of IVIg are lacking. Secondly, treatment with IVIg has probably multiple effects on induction and amplification, as well as the effector phase of the immune response.

Effects of IVIg on the effector phase of the immune response

Most studies on IVIg have dealt with the effector phase of the immune response. It has been demonstrated that IVIg has the following effects *in vivo*: inhibition of autoantibody production,¹⁶ neutralisation of circulating pathogenic antibodies,¹⁷⁻²¹ and decrease of antibody dependent cellular cytotoxicity (ADCC) by Fc receptor blockade.²² *In vitro* it was shown that IVIg inhibits pokeweed mitogen stimulated antibody production,²³⁻²⁶ contains anti-idiotypic antibodies against multiple auto-antibodies,^{18-21,27-29} suppresses ADCC,³⁰⁻³² decreases natural killer cell function,³³ and interferes with complement-activation.^{34,35} These mechanisms are dependent on the presence of the intact IgG molecule, which has a half-life of approximately three weeks *in vivo*.³⁶ However, the observed long term effect of IVIg treatment, in some diseases for up to three months, is not fully explained by the modes of action already mentioned. IVIg may therefore modulate auto-immunity at other levels of the immune response.

Effects of IVIg on the induction and amplification phase of the immune response**ANTIPROLIFERATIVE EFFECTS**

The effects of IVIg on the induction and amplification of the immune response in which the CD4⁺ helper T-cell interacts with

antigen presenting cells (in a way that costimulatory signals are activated) has not yet been explored. Interactions of IVIg with T-cells have been scarcely investigated.³⁷ An enhancement of CD8 positive suppressor T-cell function *in vivo* has been reported.³⁸⁻⁴⁰ Furthermore, IVIg appears to interfere with the cytokine network. *In vitro* administration of IVIg results in induction of the interleukin-1 receptor antagonist and downregulation of the production of Il-1 and tumour necrosis factor- α . Moreover, antibodies against Il-1 α have been demonstrated in IVIg.⁴¹⁻⁴³

We recently showed inhibitory effects of IVIg on antigen-specific and non-specific stimulated lymphocytes.⁴⁴ Subsequent experiments showed that IVIg added to cultures of various autonomously growing cell lines (including the haematopoietic cells HL-60, Daudi, Molt-4 and K562, B-cell hybridomas, EBV transformed B-cells and neuroblastoma cell lines of various human and non-human origin) also ceased with proliferation. We demonstrated that IVIg interfered with DNA-synthesis and protein metabolism. ³H-thymidine uptake of activated cells was measured, after incubation with IVIg for various intervals. IVIg interferes with ³H-thymidine uptake, in a dose-dependent fashion, regardless of the stimulus. To confirm these observations, cells cultured with and without IVIg, were pulse-labelled with bromodeoxyuridine (BrdU) and subsequently analysed in an immunofluorescence-assay. The BrdU labelling studies showed that cells were arrested in the G₀-/G₁- and no longer progressed into the S-phase. Similar results were obtained with normal human myoblasts and fibroblasts in culture. Parallel experiments with similar concentrations of human albumin did not show these effects. There was no apparent cytotoxic effect, as removal of IVIg within an interval of several days and reculturing in fresh medium, resulted in normal proliferation of K562, HL-60, EBV transformed B-cells, human myoblast and fibroblast. Further experiments are needed to exclude that a proportion of cells are induced to undergo apoptosis.

GROWTH FACTOR SIGNALLING

A possible explanation for this broad proliferative arrest could be the interference of IVIg with essential growth factors. Il-1 α , Il-2, Il-3

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Putative effects of IVIg on induction, amplification and effector phase of the immune response.

Induction and activation phase

Effect on signal transduction
Inhibitory effect on antigen-specific stimulated lymphocytes*
Inhibitory effect on antigen non-specific stimulated lymphocytes (PHA, PWM)*
Enhancement of CD8 positive suppressor T-cell function*
CD4 helper T-cell activation

Amplification phase

Effect on co-stimulatory cells
Effect on accessory cells*
Interference with the cytokine network*

Proliferation and maturation phase

Interference with growth factors (receptors and production)
Interference with DNA synthesis*
Interference with protein metabolism
Interference with antibody production*

Effector phase

Binding to Fc γ I,II,III receptors*
Neutralisation of circulating pathogenic antibodies*
Anti-idiotypic interactions*
Decrease of ADCC by Fc γ receptor blockade*
Decrease of natural killer cell function*
Interference with complement activation*

* published effects of IVIg, see text.

and supernatant of lymphokine (CD3 and Il-2) activated killer cells were therefore added in increasing doses to lymphocytes in various antigen specific and antigen non-specific stimulated models, as well as to various autonomously growing cell-lines. However, this could not abolish the inhibitory effect of IVIg. These results suggest that these interleukins do not interact with IVIg.

There are many similarities between IVIg and tumour growth factor- β . TGF- β has inhibitory effects on various T-cell and B-cell interactions and activities, on tumour cells and normal cell proliferation.^{45,46} To exclude the possibility that IVIg contains TGF- β activity we added anti-TGF- β neutralising antibody to IVIg. Despite increasing doses of this antibody we found no reduction of the anti-proliferative effect of IVIg.

Fc γ -RECEPTOR SIGNALLING

Another explanation for the anti-proliferative action of IVIg could be the binding to a receptor providing essential cofactors for growth of activated or autonomous proliferating cells. Fc γ -receptors might be the common denominator between the various cell types affected by IVIg. The expression of the distinct Fc γ -receptors was measured in an immunofluorescence assay using monoclonal antibodies against CD64 (Fc γ RI), CD32 (Fc γ RII), and CD16 (Fc γ RIII).⁴⁷ However, most cells are lacking Fc γ I, II, and III receptor expression. IVIg contains multiple antibodies against polymorphic (HLA class I or II) and non-polymorphic (CD5 receptor) structures.^{48,49} Binding of IVIg to HLA antigens is an unlikely explanation for this effect as some cell lines which were inhibited in a dose-dependent fashion by IVIg, for instance the Daudi cell-line, do not express HLA antigens on their surface.

Conclusion

Our results show that, in addition to the F(ab)₂-mediated anti-idiotypic neutralisation

of autoantibodies, the Fc-mediated immunoregulation of B-cell function and the depression of monocyte effector ADCC-function, IVIg interferes with cell proliferation in vitro and reversibly arrests growth of various cell populations. By which mechanism(s) this inhibitory effect of IVIg on cell proliferation is mediated is currently under study. Besides the interference on the effector phase of the immune response, IVIg profoundly modulates in vitro the induction and probably the amplification phase of the immune response.

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