The Disease-Specific Arm of the Therapeutic Effect of Intravenous Immunoglobulin in Autoimmune Diseases: Experimental Autoimmune Myasthenia Gravis as a Model

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Abstract

Backgraound: Intravenous immunoglobulin administration has been beneficially used for the treatment of a variety of autoimmune diseases including myasthenia gravis, although its mode of action and active components have not yet been fully identified.

Objectives: To isolate from IVIg a disease-specific fraction involved in the therapeutic activity in myasthenia and to identify its properties and function.

Results: IVIg administration in experimental autoimmune MG results in suppression of disease that is accompanied by decreased Th1 cell and B cell proliferation. Chromatography of IVIg on columns of IgG from rats with EAMG or from MG patients resulted in depletion of the suppressive activity that IVIg has on rat EAMG. Moreover, the minute amounts of IgG fractions eluted from the EAMG or MG-specific columns retained the immunosuppressive activity of IVIg.

Conclusions: Our study supports the notion that the therapeutic effect of IVIg is mediated by a minor disease-specific immunoglobulin fraction that is present in IVIg and is essential for its therapeutic activity.

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Intravenous immunoglobulin administration has been beneficially used in recent years in a variety of autoimmune diseases although its mode of action is still not clear. Multiple mechanisms of action, not necessarily mutually exclusive, have been proposed for explaining the therapeutic effect of IVIg [1,2].

Myasthenia gravis and experimental autoimmune myasthenia gravis are T cell-dependent, antibody-mediated autoimmune diseases in which the acetylcholine receptor at the neuromuscular junction is the major autoantigen. IVIg has been used to treat MG for about two decades. We employed the well-characterized experimental model for MG, i.e., rat EAMG, to investigate whether IVIg is effective in treating an ongoing experimental disease and to explore the immunological mode of action of this treatment [3]. We demonstrated the potential of IVIg treatment not only to prevent the induction of EAMG but also to immunosuppress an ongoing disease when treatment is initiated at either the acute or chronic stage of disease. As such, this is an excellent model to delineate the mode of action and the involvement of various immunological and molecular aspects of IVIg therapy in MG in particular and in other human autoimmune diseases in general. Our analysis of various immune-related molecules suggests that the mechanism by which IVIg modulates EAMG is by suppression of Th1-type cells and B cell proliferation but probably not via the induction of regulatory T cells) [3].

The drawbacks of IVIg treatment include its high cost and the fact that patients are given huge amounts of protein of which only a minor portion may be responsible for the therapeutic effect. In an attempt to isolate a specific fraction from pooled normal human immunoglobulin and to analyze its immunosuppressive activity in an autoimmune disease we used the above described IVIg-treated rat EAMG model.

Results and Discussion

In order to determine whether a disease-specific component of pooled human IVIg contributes to its immunomodulatory therapeutic activity we fractionated IVIg on rat EAMG-specific or on human MG-specific IgG columns, and studied the immunosuppressive activity of the resulting fractions on EAMG [4]. As controls for the EAMG and MG-specific columns we used columns prepared from IgG isolated from complete Freund's adjuvant-immunized rats and from healthy patients, respectively. For specific fractionation IVIg was first passed through the control column (rat or human) in order to remove non-specific anti-antibodies and then on the disease-specific column (rat EAMG-specific or human MG-specific, respectively). For a control, IVIg was passed on the respective rat or human control column alone [Figure 1]. Very minute amounts of IVIg were depleted from the IVIg preparations following these chromatographies and there were no detectable changes in their protein concentration. The adsorbed IgG were eluted from the EAMG and MG-specific columns, and their content corresponded to a 70,000–100,000-fold enrichment of the disease-specific fractions [4]. These

IVIg = inravenous immunoglobulin
MG = myasthenia gravis
EAMG = experimental autoimmune myasthenia gravis

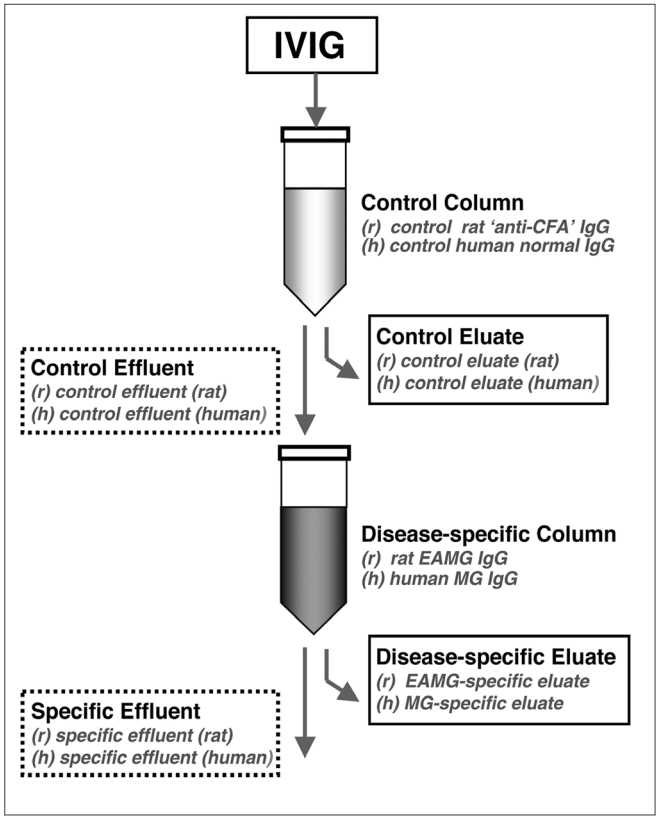

Figure 1. Schematic presentation of IVIg fractionation on rat (r) and human (h) IgG columns. [From ref. 4].

eluates were designated EAMG-specific and MG-specific eluates, respectively.

The therapeutic effect of the various fractions was tested in rats in which EAMG had been induced by immunization with AChR. Treatments were initiated one week following disease induction, when signs of acute EAMG are usually observed. Rats were followed for disease development, and their clinical score and body weight were recorded twice a week. The results of fractionation on either the rat or human IgG columns yielded similar results. There was a clear difference between the suppressive effects of the control effluent fractions and the effluents from the EAMG or MG-specific columns. The 'specific effluent' [Figure 1] obtained following chromatograpy of IVIg, first through the control column and then through the disease-specific columns, completely lost its therapeutic effect. In contrast, the control effluent, obtained following chromatography through only the control columns, retained its full suppressive effect on EAMG [4].

To examine whether the suppressive activity in the IVIg preparation was retained by the EAMG and MG-specific columns, the effluent from these columns ['specific effluent (rat)' or 'specific effluent (human)'] [Figure 1], which were devoid of the suppressive activity of IVIG, were reconstituted each with its relevant eluate from these columns ('EAMG-specific eluate' and 'MG-specific eluate', respectively) and the resulting reconstituted mixtures were applied for treatment. The therapeutic effect that was lost in the groups treated with 'specific effluents' was indeed recovered in the groups treated by the reconstituted samples ('specific effluent (rat)' + 'EAMG-specific eluate' or 'specific effluent (human)' + 'MG-specific eluate') as reflected also in the rise in the mean body weight [4]. Recovery varied from 60% to 100% of the therapeutic effect of the unfractionated IVIg in the various experiments. It should be noted that reconstitution of the 'specific effluents' (rat or human, Figure 1) with the respective 'control eluate' fractions did not recover any of the therapeutic activity.

It should be noted that in this study we used *human* IVIg and its fractions for investigating the therapeutic effect on *rat* EAMG [4]. This is similar to previously reported studies on the application of allogeneic pooled *human* immunoglobulin (IVIg) for treatment of experimental autoimmune diseases in *mice* and *rats* [3,5-13]. The reason for using human immunoglobulins in allogeneic systems stems from technical difficulties of obtaining rat (autologous) immunoglobulins in the amounts needed for the treatment experiments. The effectiveness of experiments performed in murines (rats or mice) with human IVIg implies that there is an interspecies cross-reactivity in the active components of IVIg. Indeed, cross-species idiotypes in the anti-AChR response were previously reported by us [14,15]. We assume that pooled human immunoglobulins, obtained from thousands of healthy donors, contain a vast repertoire of anti-antibodies (anti-idiotypes), some of which may have interspecies cross-reactivity.

The most striking observation in this study is the ability to specifically eliminate the therapeutic activity on EAMG from the IVIg preparation by its chromatography through an EAMG or MG-specific column. Such a chromatography resulted in adsorption of a very small amount of IgG from the IVIg preparation and eliminated its immunosuppressive activity on EAMG, whereas chromatography of the IVIg through the non-specific control columns did not reduce its therapeutic capability. This is a strong indication that only the disease-specific columns were able to adsorb and remove the disease-specific immunosuppressive fraction from the IVIg preparation. These observations indicate that the fraction removed from IVIg by the EAMG or MG-specific column is essential for the therapeutic activity of the IVIg on EAMG. Whether it may also be sufficient is still not clear.

We cannot exclude the possibility that additional components in the non-active IVIg effluent fractions are required for reconstituting the full suppressive activity. Such components could include factors modulating the expression and function of Fc receptor and of complement activation, immunoglobulin molecules interacting with the specific anti-antibodies to form dimmers, and other factors that have been proposed to account for some of the clinical effects of IVIg [1,16,17]. Although needed for the immunosuppressive activity of IVIg, such components that are not necessarily disease specific may require the presence of the disease-specific anti-antibodies (anti-idiotypes) that are essential for the observed clinical effect on EAMG.

We assume that the specificity of the isolated anti-antibody

AChR = acetylcholine receptor

active fraction is determined by the disease-specific immunoglobulin employed to fractionate the IVIg preparation. In our case it was only the anti-antibody fraction isolated on immunoglobulin columns from rats with EAMG or from MG patients, and not on immunoglobulin columns from control animals immunized with CFA or from normal patients, that had a therapeutic clinical effect on EAMG. Anti-antibodies with specificity for other autoimmune diseases can possibly be isolated by their selective binding to the respective disease-specific IgG. In another reported study DNA-specific antibodies from patients with systemic lupus erythematosus were used to isolate from IVIg a fraction of anti-antibodies (anti-idiotypes) that suppressed experimental SLE [13]. It is possible that the same IVIg preparation could serve for several serial fractionations of anti-antibodies – *each* specific for a different disease – and could thus make this therapeutic approach more specific and possibly less costly. Moreover, a personalized fractionation of IVIg on the patient's own autoreactive immunoglobulins might in the future be considered as the treatment of choice.

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CFA = complete Freund's adjuvant
SLE = systemic lupus erythematosus

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