



Intravenous immunoglobulin and Anti-D in Idiopathic Thrombocytopenic Purpura (ITP): Mechanisms of Action

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■ Infusion of large amounts of intravenous immunoglobulin (IVIg) or anti-D can reverse the low platelet count in patients with ITP within hours of the initiation of treatment. In some cases, the effects of IVIg appear to far outlast several half-lives of the product. Several mechanisms have been proposed to explain these rapid and long term effects and these will be discussed in this review. © 1998 Elsevier Science Ltd. All rights reserved ■

to the D antigen, intentionally or by prior pregnancy. The first use of IVIg in treating idiopathic thrombocytopenic purpura (ITP) was by Imbach in 1981 who reported that high doses of IVIg promoted fast recovery of ITP in children.¹ The first use of anti-D in ITP was by Salama and co-workers who postulated that the success of IVIg in treating ITP was due to competitive inhibition of the reticuloendothelial system (RES) by sensitized erythrocytes.^{2,3} In this review we will discuss several potential mechanisms of how IVIg and anti-D ameliorate ITP.

INTRODUCTION

IVIg is prepared from large pools of plasma from more than 10 000 "normal healthy" donors. Most preparations only contain IgG with minimal levels of "contaminants" such as IgA. The IgG exists in a predominantly monomeric form with a subclass distribution characteristic of the subclass distribution in normal serum. Anti-D, on the other hand, consists of IgG selectively taken from the plasma of donors immunized

RETICULOENDOTHELIAL SYSTEM (RES) BLOCKADE

The major site of platelet destruction is well known to involve the spleen. The spleen contains large numbers of Fc receptor-bearing phagocytic cells, such as monocytes and macrophages, which can bind and destroy opsonized platelets. Although the spleen is not the only site of platelet destruction, splenectomy is a successful treatment for some individuals with ITP. Perhaps the most direct early evidence that RES blockade by IVIg can prolong the half-life of antibody-sensitized cells were experiments by Fehr and co-workers.⁴ These authors studied four patients with ITP who had not undergone splenectomy; infusion of IVIg prolonged the *in vivo* clearance of radiolabelled

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antibody-sensitized erythrocytes. These results have been confirmed by other investigators using both erythrocytes and platelets as target cells.⁵⁻⁸ Several studies comparing intact IVIG to F(ab')₂ fragments from IVIG (the latter do not bind to Fc receptors) have clearly indicated that the intact preparations are more efficacious in reversing the thrombocytopenia.^{9,10} Studies in animals have further shown that administration of a monoclonal antibody which is specifically known to functionally block Fc receptors can prevent clearance of IgG sensitized erythrocytes.¹¹

The most compelling evidence to suggest that a major mechanism of action of anti-D is via RES blockade, is that anti-D appears to be almost completely ineffective in patients who are Rh D antigen negative,^{12,13} who would thus not have antibody-sensitized red cells to effect RES blockade. In one study, three patients who were Rh D antigen negative, but c antigen positive, and were refractory to treatment with anti-D, were then successfully treated with anti-c.¹³ Evidence that anti-D exerts its effect in an RES- and spleen-dependent fashion is that splenectomized patients with ITP do not respond well to anti-D treatment.¹⁴⁻¹⁶ In a recent study with 272 patients with immune forms of thrombocytopenia, in which higher doses of anti-D were employed to treat 11 splenectomized patients, the authors found that even a four fold increase in anti-D gave a limited response.¹⁷ Since the world-wide supply of anti-D is limited, a small prospective study on seven Rh positive patients with chronic ITP was initiated to test a human (IgG₁) monoclonal anti-D.¹⁸ Five patients showed signs of hemolysis and two became anemic; six patients did not respond to this therapy and one patient underwent a transient increase in platelet count. Thus while this monoclonal anti-D antibody (MONO-D) did not appear to be efficacious in ameliorating ITP, a larger prospective study, using anti-D monoclonal antibodies representing different

subclasses in cohorts of patients, is required.

An important point to be considered in view of the multiple observations suggesting that both IVIG and anti-D mediate short term increases in platelet count due to RES blockade is that blockade may occur via two independent mechanisms:

- (a) IVIG contains IgG dimers and multimers which are well known to be capable of binding to Fc receptors and block platelet clearance and prolong platelet survival.
- (b) IVIG contains IgG molecules which have reactivity with a variety of host antigens. These IgG molecules bind to host antigens, form immune complexes, and compete with antibody-sensitized platelets for Fc receptors in the RES resulting in prolonged platelet survival.

Since it is difficult to distinguish between these two possibilities, it may be that further "improvements" in IVIG resulting in lower concentrations of IgG multimers may give rise to a less effective therapeutic than the current IVIG preparation. Conversely, IVIG with a proportionately higher fraction of dimers or multimers may be more efficacious *in vivo* than the current commercially available preparations.

Although RES blockade appears to be the most highly accepted mechanism to account for the effects of IVIG and anti-D, several lines of evidence suggest that other mechanisms may also be involved in reversing the thrombocytopenia. These include a report of a patient receiving anti-D who was Rh negative and nevertheless had a good clinical response,¹⁹ the observations that some beneficial effects of IVIG therapy extend considerably beyond the half-life of IVIG *in vivo*, the ability of IVIG to affect the immune system in an Fc receptor-independent fashion, and the ability of some patients to respond

to F(ab')₂ fragments of IVIG, which have virtually no Fc receptor binding activity.^{9,10}

ANTI-IDIOTYPIC ANTIBODIES

One of the better supported alternative mechanisms involves the regulatory properties of a subset of antibodies called anti-idiotypic antibodies, i.e. antibodies which bind to the antigen combining region of other antibodies. The antigen combining region of IgG contains the idiotypic region (hyper-variable region) and possesses amino acid sequences which encode the fine specificity of each antibody. When an individual is immunized and produces antibodies against an antigen, for example ovalbumin, the resulting anti-ovalbumin antibody will possess an idiotypic region which has never before been seen by the host. In effect, exposure of a host to a foreign antigen results in the production of a new IgM or IgG molecule which in turn possesses an idiotypic region foreign to the host. In the above example, the host responds by making IgM and IgG antibodies to the anti-ovalbumin antibody, these two antibodies interact and the final effect is the neutralisation of both antibodies. These idiotypic anti-idiotypic interactions have been postulated to be intimately involved in regulating the immune system, a hypothesis which was put forward by Niels Jerne who received the Nobel Prize for this contribution to science.

One of the major targets of the autoantibodies in ITP is the platelet membrane glycoprotein (GP) IIb/IIIa. Berchtold and co-workers demonstrated that IVIG contains antibodies which can interact and neutralize the effects of anti-GPIIb/IIIa.²⁰ This effect would prevent new platelets from encountering anti-GPIIb/IIIa autoantibodies, which would be evidenced by a decrease in platelet-associated IgG and reversal of thrombocytopenia. Boughton and co-workers showed that five patients with

ITP who had anti-GPIIb/IIIa autoantibodies demonstrable by a direct monoclonal antibody immobilized platelet antigen (MAIPA) assay, who were treated with anti-D, underwent an increase in platelet counts and a concurrent decrease in platelet-associated antibody.²¹ The latter observation could not be accounted for by RES blockade but likely demonstrates that anti-D also possesses significant levels of anti-idiotypic antibodies which blocked anti-platelet antibody. Since the concentration of anti-D administered is much lower than IVIG, it may be that anti-D preparations possess more anti-idiotypic antibodies than do IVIG preparations. An increased level of anti-idiotypes in anti-D preparations might be a consequence of the types of donors who are used to prepare anti-D, i.e. a pool of donors who respond easily to immunization with Rh positive cells. In an analogous model, Atlas and co-workers demonstrated increased anti-idiotypic antibodies to HLA antigens in previously sensitized multiparous women.²² In addition to the direct effects of antibody neutralization, anti-idiotypic antibodies may combine with surface immunoglobulin on B lymphocytes and downregulate or inactivate these B cells.

OTHER MECHANISMS

IVIG has been demonstrated in a number of studies to have effects on the cellular immune response itself. Specifically, long term responses following IVIG administration have been shown to be associated with enhanced suppressor T lymphocyte function and decreased autoantibody production.²³⁻²⁵ In one study, IVIG was shown to reduce the number of CD4⁺ T helper cells *in vivo* in some patients.²⁶ IVIG prepared as monomeric or aggregated human gamma globulin has been shown to be capable of inducing immune tolerance in both B cells and T cells.²⁷ The mechanism(s) of how IVIG exerts its

regulatory functions on T cells has not yet been definitively established, but IVIG has been shown to affect both cytokine and cytokine receptor levels both *in vitro* and *in vivo*.²⁸⁻³⁰ A key cytokine in the immune response, interleukin (IL)-2, is inhibited by IVIG and this inhibition appears to be upstream of inhibition of IL-2 secretion and regulated at the posttranscriptional level³¹. The described IVIG-and/or anti-D-induced changes in cellular immunity may result in modulation of B and T cell functions and may affect selection of B and T cell repertoires, resulting in suppression of autoantibody production.

One of the *in vitro* effects of IVIG is the growth arrest of fibroblasts, hematopoietic cell lines and lymphocytes. Since this anti-proliferative effect of IVIG may be related to *in vivo* efficacy, Vuist and co-workers³² recently sought to understand how IVIG can inhibit cell growth. IVIG was demonstrated to bind to the surface of cells whose proliferation was inhibited. A human B cell line termed JY whose growth is inhibited by IVIG was used as an affinity matrix to purify JY-binding antibodies present in IVIG. Affinity-purified anti-JY antibodies were tested for functionality and specificity. They were shown to be capable of inhibiting the mixed lymphocyte reaction at 1000 fold lower concentration than unfractionated IVIG. This JY-purified IVIG bound glycolipids expressed by JY cells and lymphocytes. Thus some of the anti-proliferative effects of IVIG-induced growth arrest may be mediated by anti-glycolipid antibodies. Further study is needed to ascertain whether the growth-arresting effect of IVIG relates to reduction in autoantibody production.

While the precise mechanism(s) of action of IVIG and anti-D may be difficult to define, it is likely that many of the above discussed mechanisms are not mutually exclusive and may contribute differentially or additively to the success of IVIG and anti-D therapy in different cohorts of patients. It should

also be recognized that, in addition to the above mechanisms, there are other activities of IVIG described, such as the ability of IVIG to inhibit complement-dependent *in vivo* clearance of appropriately sensitized cells.³³ In addition, IVIG represents the antibody repertoire of a large number of individuals, all of whom would have been exposed to a variety of pathogens: this may have particular relevance for its success in acute childhood ITP, often associated with viral infection. Finally, it is possible that some immune modulatory effects of IVIG are not due to the immunoglobulin fraction itself but are due to "contaminating" products present in the product. Kekow and co-workers recently determined that commercially available IVIG from all of three manufacturers tested, contained "high levels" of the T cell growth factor, TGF- β .³⁴ In the same study, the authors showed that 15 patients with rheumatoid arthritis and infused with IVIG displayed small but significantly increased levels of this immunosuppressive cytokine after IVIG infusion.

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