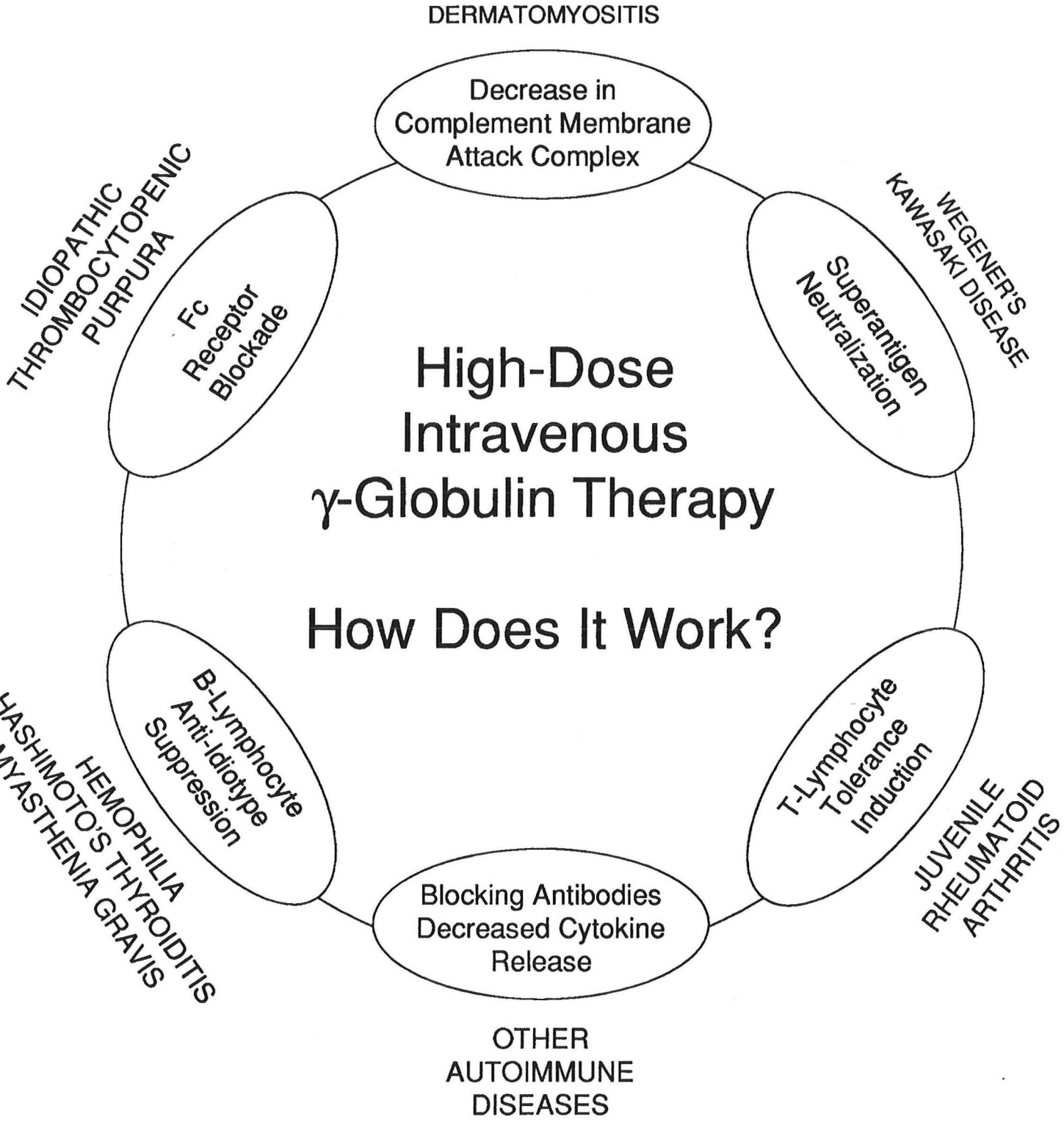

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HIGH-DOSE INTRAVENOUS GAMMA-GLOBULIN THERAPY : HOW DOES IT WORK?

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(I would like to thank Ms. Ruth Simon and Mr. Adrien Jump for their valuable assistance in preparing the figures and tables used in this presentation.)

"The best can be accomplished only if it is pleasing and involves a creator's joy." Arne Tiselius, 1969.

HIGH-DOSE IVIg: HOW DOES IT WORK?

Gamma-globulin, the legacy of Svedberg, Tiselius, Kabat and Cohn

Not every breakthrough in science or medicine has been appreciated by the generation or even the peers of the one making the discovery. Few redder faces have resulted than those of the editors of Biochemical Journal in 1931 after they rejected the paper of Arne Tiselius providing the original description of the separation of proteins into separate components using a new technique, electrophoresis. When the paper was finally published six years later in Transactions of the Faraday Society, it was recognized by other scientists and physicians, including Dr. Harvey Cushing, as one of the "classics of medical literature" (1), and resulted in 1948 in Tiselius receiving the Nobel Prize in Chemistry.

Tiselius was a graduate student of The Svedberg, the inventor of the ultracentrifuge, and both quickly recognized the heterogeneity of serum proteins in terms of weight. Tiselius correctly predicted that each separate protein component would also have a different electrical charge, and thus move at a different rate in an electrical field. He was able to devise refrigeration to dissipate the heat in such a field, and a U-shaped glass tube which allowed optical detection of different protein boundaries confirming his predictions. It was Elvin Kabat, a young colleague of Michael Heidelberger at College of Physicians and Surgeons, Columbia University in New York, who read Tiselius paper, and persuaded Tiselius to allow him to come to Sweden with a large collection of hyperimmune human and rabbit sera to find out where antibodies moved in the cumbersome electrophoresis apparatus. After serum albumin, there were three broad bands of protein which Tiselius and Kabat named alpha, beta and gamma-globulin and most of the antibodies migrated in the gamma-globulin band. Hence the term gamma-globulin was born.

In the early 1940's, Dr. E.J. Cohn, working with Oncley, Pillemer and others, developed the alcohol fractionation procedure, modifications of which are used even today to process large amounts of blood plasma or serum to produce a relatively pure fraction of gamma-globulin. It was the modification of paper and starch-block electrophoresis which reduced the size and technical difficulties of Tiselius' original device to produce a modern laboratory tool, and which allowed the development of the alcohol fractionation technique to the successful level which Cohn and his coworkers achieved. The availability of large amounts of human IVIg for therapeutic purposes today owes much to the legacy of these early pioneers in Uppsala, New York and Boston (1).

The Development of Usable IVIg

The lyophilized human gamma-globulin (Cohn Fraction II = FII) produced by the cold ethanol fractionation procedure from pooled plasma (often from >10,000 normal donors) contains mostly IgG, but also minor contaminants of IgA, and other proteins (2,3), including prekallikrein activator. The latter induces severe hypotension in most individuals when given intravenously. Some of the IgG in FII is aggregated into dimers, and trimers (which can activate serum complement), and in addition, small amounts of anti-erythrocyte antibodies also capable of fixing complement are present. This results in a variety of relatively serious reactions to FII which has precluded its administration intravenously, and even made its intramuscular administration painful and occasionally dangerous. In particular, the total amount of IgG which can be given in a finite period is sharply limited. For the above reasons, many laboratories sought ways to further purify FII to produce a product which could be used intravenously (4).

It was found that pepsin digestion produced a divalent fragment of IgG which could be given intravenously, but the F(ab')2 had a very short serum half-life, and lacked several necessary properties to make it adequate as a native IgG replacement. Ultimately several methods were developed, the technical details of which are covered in the references (5,6,7), which produced a satisfactory product for high-dose intravenous administration. The most successful of these were low pH (pH=4.0) associated with very minimum digestion with pepsin (1 part pepsin to 100,000 parts of FII); low pH (pH=4.0) alone followed by addition of maltose to discourage reaggregation; and reduction with dithiothreitol, or sulfonation followed by reduction, and alkylation of the free SH-groups with iodoacetamide. A comparison of five currently available commercial IVIg preparations with the immunoglobulin available for intramuscular use is shown in Fig. 1 (8). This figure presents gel filtration separation analyses (based on the size of proteins or aggregates) done by Dr. Donald Tankersley, Division of Blood and Blood Products, U.S. Food and Drug Administration.

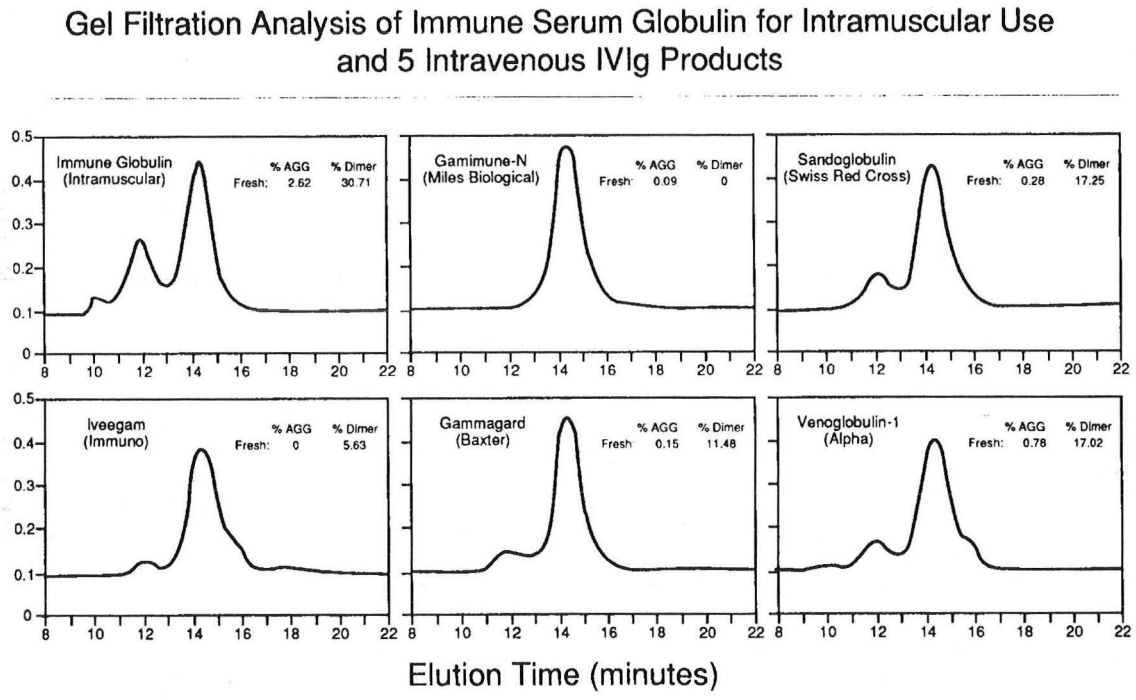

Fig. 1

The IgG dimers found in all of these preparations are <u>not</u> usually present if the IgG is prepared from a single donor, but increase progressively to the above levels as more and more blood units are pooled for the cold alcohol fractionation procedure. Roux and Tankersley (9) concluded that the dimers represented idiotype:anti-idiotype interactions (antibodies against the hypervariable regions of other antibodies), and they supported this conclusion by showing that pepsin-digested pooled normal IgG containing no Fc chain [only F(ab')2] also interacted to form dimers in about the same proportion as in the intact pooled IgG.

This increase in the fraction of total IgG forming dimers as more and more blood sera are pooled suggests that IgG idiotypes generate anti-idiotype responses in some patients which shut off the stimulating idiotype (antibody) synthesis, leaving only monomeric anti-idiotype antibodies. In other persons, the mechanism for initiation of the anti-idiotype response is less sensitive, and low levels of idiotype (antibody) exist without turning on an anti-idiotype response.

If these idiotypes are autoantibodies, their concentration may be low enough that no clinically significant disease results. We all have seen such patients with a variety of autoantibodies, but without obvious illness. Variations of each person's immune response may make these two different responses more or less random. However, when the serum from multiple blood donors is mixed, the 10-25% dimerization of IgG occurs due to idiotypes from one serum interacting with anti-idiotype antibodies in the serum of other donors.

The slightly modified commercial IVIg products have a normal serum half-life, retain most of the complement-fixing and other biological properties of native IgG, and most of the time can be given in high doses intravenously (4,7,8,10). Interestingly, it is the patient who is agammaglobulinemic who had the most frequent major reactions, and then only with the initial dose given with each replacement cycle (4), suggesting that unsaturated cell surface receptors for IgG are perturbed when initially exposed to the infused IgG and cause cytokine release, but once saturated, no longer cause systemic symptoms. Already saturated cell-IgG receptors in non-agammaglobulinemic recipients produce no reactions. By very slow initial intravenous administration, the intensity of these initial reactions in agammaglobulinemic patients can be made tolerable (5).

Perhaps because of the large amount of infused protein, alloimmunization to genetic differences in IgG from one person to another generally does not occur after IVIg therapy. Also, in follow-up studies in chimpanzees given human IVIg, no accelerated immune elimination of the human IVIg occurred (11). True anaphylactic reactions in non-agammaglobulinemic recipients of IVIg (12,13,14) are usually shown to result from an undetected absence of IgA in the recipient which may be encountered in from 1 in 300 to 1 in 3,000 persons as an isolated immunodeficiency (15). No history of an increase in infections is elicited from 50% of these IgA-deficient persons. This absence of IgA causes these individuals to not be immunologically tolerant to IgA, and when exposed to the tiny amount of IgA in IVIg, they develop anti-IgA antibodies. These may cause anaphylactic reactions to the small amount of IgA in IVIg. Any patient in whom administration of IVIg is planned should be screened for the presence of anti-IgA antibodies (14). Non-transfusion exposure to IgA, such as pregnancy, also may have sensitized a patient with selective IgA deficiency.

Concern for infection from hepatitis viruses A, B, or C; HIV; CMV or other infectious agents has led to extensive testing of commercial preparations of IVIg by pharmaceutical companies and by the FDA. All preparations have detectible antibody levels to all of the above, but the alcohol fractionation used to prepare the starting FII eliminates infectious particles, and so far, no documented infections have been transmitted by IVIg (16,17). Most patients receiving high-dose IVIg have some side effects. The most frequently encountered reactions are summarized in Table 1 (18). These are rarely severe enough to force discontinuation of IVIg therapy.

When independent laboratory analyses are made of commercial IVIg preparations, there are significant differences in IgG subclass content, particularly IgG4 (7). These differences for five commercial IVIg products are compared to the World Health Organization (WHO) reference standard serum IgG subclass content in Table 2. It is antigen activation of selective T-cell clones which regulates the serum levels of the various IgG subclasses (19). Studies (20) of patients with selective IgG subclass deficiencies have generally shown compensatory increases in the other subclasses, usually with no clear association with any clinical illness with the exception of IgG4. Even though this subclass makes up only about 4% of normal serum IgG, when absent, patients show recurrent sinopulmonary infections (20). In addition, persons with allergic states have increased levels of IgG4.

Table 1.

INCIDENCE (%) OF SIDE-EFFECTS ASSOCIATED WITH IVIg

SIDE-EFFECT	MILD	MODERATE	SEVERE	TOTAL
Headache	43	31	6	80
Nausea	23	16	0	39
Lethargy	29	10	0	39
Fever	16	12	0	28
Vomiting	14	9	1	24
Edema	21	0	0	21
Myalgias	17	0	0	17
Dyspnea	13	0	0	13
Anorexia	4	1	0	5
Rash	2	0	0	2
Peri-orbital swelling	0	1	0	1

From GM Leong, Immunotherapy with Intravenous Immunoglobulins, P Imbach, ed, 1991, p. 278.

Table 2.

CONTENT OF VARIOUS IgG SUBCLASSES IN COMMERCIAL IMMUNOGLOBULIN PRODUCTS

PRODUCT (Manufacturer)	PERCENTAGE OF IgG PRESENT AS:			
	IgG_1	IgG_2	IgG_3	IgG_4*
WHO Reference	60.0	29.4	6.5	4.1
Gammagard/ARC (Hyland)	66.8	25.4	7.4	0.3 - 2.6
Gammimmune-N (Cutter)	58.7	29.3	6.3	0.9 - 5.3
Sandoglobulin (Sandoz)	60.5	30.2	6.6	2.6 - 7.4
Venoglobulin-I (Alpha)	60.9	29.4	5.3	2.0
Iveegam (Immuno)	66.6	32.0	0.0	1.4

*IgG_4 values are independent laboratory assays. Other values are from manufacturers' published data. From BH Greenbaum, Am J Ped Hematology/Oncology 1990.

For example, bee keepers frequently stung by their bees with inocuous result have high levels of IgG4, and this subclass has been shown to be univalent (rather than divalent as one would ordinarily expect from its structure) suggesting that it may be playing the role of "blocking antibody", modifying the host's response to a specific antigen. Also IgG4 is the only IgG subclass that does not activate the classical complement pathway. IgG2 and IgG4 (but not IgG1 and IgG3) are the IgG molecules most often displayed on receptors on cell surfaces (not including B-lymphocytes which are synthesizing a selected IgG subclass on their surface specific for an antigenic determinant). If IgG4 is monovalent, it would be less capable of "capping" groups of surface receptors to activate a given cell to differentiate or to release cytokines.

A recent paper has provided one explanation for the mechanism by which an IgG molecule can become univalent. Miranda, et al (21), have studied commercial hyperimmune anti-tetanus IgG, and shown the presence of asymmetric (non-precipitating or co-precipitating) antibodies in three preparations of anti-tetanus gamma-globulin. They found an average of 27% of the specific anti-tetanus antibodies were of the asymmetric type, a value two to three times that found in normal IgG (Table 3).

Table 3 CON-A BINDING BY INTACT AND F(AB) FRAGMENTS OF NORMAL IgG AND HYPERIMMUNE ANTI - TETANUS

ORIGIN	% OF TOTAL FRACTION BOUND TO CON-A COLUMN		
	INTACT IgG	$F(ab')_2$	F(ab)
Normal IgG	13	9.6	51
Anti-Tetanus IgG	33	27.0	48
IgG not adsorbed by tetanus toxoid affinity column	12	8.5	45

From Miranda, et al. Immunology 1992.

The cause of this asymmetry was the glycosylation of one arm of the molecule near the antigen combining site, rendering that arm unable to combine with antigen. They used endo-beta-N-acetylglucosaminidase H (Miles Laboratories) to split the oligosaccharide attached to an asparagine residue of the asymmetrical IgG to restore bivalent activity. This effect of idiotope glycosylation is shown in Fig 2. These asymmetrical antibodies were 2-7 times less effective in neutralizing tetanus toxin in toxicity tests in mice. Clearly, this production of univalent antibodies satisfies the "blocking antibody" concept well known to allergists, but once enough antibody response has occurred, this generation of univalent blocking antibodies also provides a damping down effect on cell-mediated immune responses by binding antigen without allowing that antigen to cap cell surface receptors to drive further immune response. The fact that IVIg would contain a certain proportion of these univalent antibodies, including perhaps most of the IgG4 present, provides a hypothetical explanation of the suppression of immune responses by high-dose IVIg.

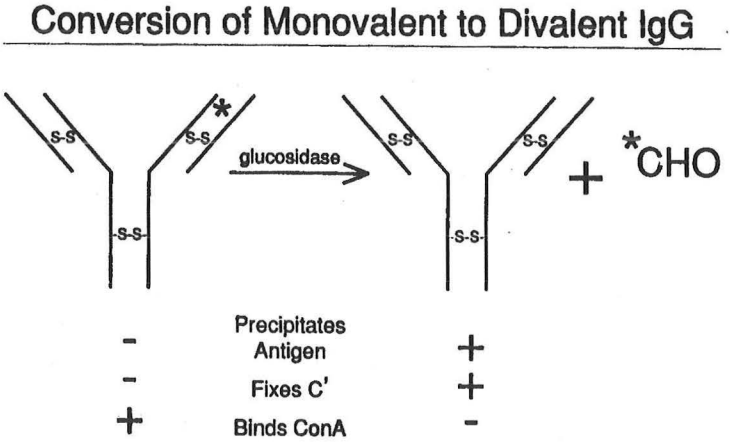

Fig. 2

Traditionally, the primary use of replacement IgG is to raise the normal IgG level above 500 mg/DL (a level sufficient to prevent most common bacterial infections) in patients with agammaglobulinemia or hypogammaglobulinemia (15,22,23), and in patients with functional agammaglobulinemia such as those with chronic lymphocytic leukemia, multiple myeloma, or children with AIDS (24,25,26). It also is known that about 30% of children with agammaglobulinemia or hypogammaglobulinemia develop a rheumatoid-like arthritis, and that this will respond to treatment with intramuscular injections of gamma-globulin (22,23). Other than its effect on potential infections, other actions of gamma-globulin replacement in immunodeficient patients are unknown. IVIg use has made therapy in these patients easier within the limitations discussed above regarding the need for very gradual infusion of the initial IVIg at the beginning of each cycle of replacement. This aspect of IVIg use is discussed in detail in the references provided and will not be emphasized further in this discussion. However, with the Communicable Diseases Center reporting more and more antibiotic-resistent microorganisms, the near future may demand much more frequent use of specific hyperimmune gamma-globulin for control of antibiotic resistant Staphylococci and other bacterial and viral infections (27).

Other Mechanisms of Action of High-dose IVIg

It is easy to explain the impact of replacement therapy in patients lacking sufficient IgG to defend themselves from infections. However, for several of the conditions which we will discuss, the mechanism of action of IVIg is much less obvious.

Table 4.

PATHWAYS BY WHICH HIGH-DOSE IVIg MODULATES DISEASE

SUGGESTED ACTION	MECHANISM	DISEASE	REFS
1. Down regulation of immune system or inflammatory cells	Blocks or binds IgG-Fc receptor(s)	ITP Other thrombocytopenias	28,29 30,31
2. Neutralization of microbial antigen or superantigen	Provides Ab not available to patient	Kawasaki disease Wegener's granulomatosis	32,33 34
3. Reduces complement mediated injury Accelerated immune complex removal	Modifies Ab:Ag ratio (fixes complement) Solubilizes I.C.'s Blocks C3 uptake	Dermatomyositis Forssman shock	35,36 37
4. Anti-idiotypic direct binding & down-regulation	Binds pathogenic autoantibodies Binds B-cell Ig	Hashimoto's ITP Hemophilia	38,39 40,41 42
5. Alteration of T-helper & T-supressor functions	Binds to IgG receptor on $CD8^{+}$-T-lymphocytes	Myasthenia gravis Polymyositis	43,44 45,46

When you realize that the amount of IVIg given will double or treble the level of IgG in the recipient, and that trace amounts of non-IgG proteins (IgA, soluble cell surface proteins, oxidized or aggregated IgG components, and unknown cytokines) are also being infused in biologically significant amounts, then functions of IVIg, other than as specific antibody, need to be considered to explain its therapeutic effects. Much is known of these potential functions, much reasonable speculation about immune mechanisms has been accumulated, and much remains unknown. This presentation will attempt to sort out fact from fiction regarding IVIg's therapeutic functions, and to focus these functions on a limited number of well-studied disease situations. Table 4 lists several suggested modes of action for high-dose IVIg.

The interaction with IgG-Fc receptors (Fc-gamma-RI, -RIIa, -RIII) has been one of the mechanisms of action of IVIg most widely accepted (28,39) and may account for more than interference with the uptake of autoantibody sensitized platelets and various body cells. At least three IgG-Fc receptors have been identified, and their principal functions characterized (47). Table 5 briefly summarizes the cellular distribution binding characteristics, and possible functions of these three IgG-Fc receptors.

Table 5. CELL DISTRIBUTION AND VARIABLE FUNCTIONS OF Fc-γ RECEPTORS

RECEPTOR	CELL TYPES	FUNCTIONAL FEATURES
Fc-γ RI	Macrophages IFN-γ stimulated PMNs	High avidity Binds monomeric IgG: $IgG_1 > IgG_3 > IgG_4 >> IgG_2$ Promotes ADCC
Fc-γ RIIa	Macrophages, PMNs, Eos, platelets, B-cells	Low avidity Binds aggregated IgG Triggers oxidative burst of PMNs
Fc-γ RIII	NK cells, PMNs, Eos Minor population of T-cells Not monocytes, But macrophages, Kuppfer and littoral cells	Low avidity Major mechanism of removal of sensitized RBCs and platelets by spleen and liver Selectively binds IgG_1 & IgG_3

From JC Unkeless, J Clin Invest 83:356(1989).

Use of High-dose IVIG in Thrombocytopenic Purpuras

In 1964 a Swiss pediatrician, Dr. E. Gugler, was the first to treat childhood thrombocytopenia successfully with IVIg (48). That same year, Barandun and Isliker (5) observed a child with hypogammaglobulinemia and hemolytic anemia associated with a strongly positive Coombs' test. When the patient was treated with 10 g of IVIg, the erythrocyte hemolysis abruptly stopped, and they found that the Coombs' test had reverted to negative. They then treated three other similar patients, all also hypogammaglobulinemic with hemolytic anemia, with similar prompt responses. F(ab')2 fragments of the same IVIg was not effective. Also, non-hypogammaglobulinemic patients with hemolytic anemia did not respond.

The success with the immunodeficient patients with hemolytic anemia, (and Gugler's result in thrombocytopenic purpura) encouraged them to treat two children with hypogammaglobulinemia and ITP with IVIg and, in both patients they also obtained good responses (5). IVIg was then used to treat ITP in non-hypogammaglobulinemic children in Bern and elsewhere, and IVIg therapy also was given to adults with ITPwith similar results (49).

A recent analysis of 28 published reports of immune thrombocytopenic purpura in 282 children treated with IVIg (39) revealed an increase within two to five days in the platelet count to more than 100,000/cu mm in 64% of patients and to more than 50,000/cu mm in 83% of patients. Long-term remissions were more likely to occur in patients with thrombocytopenia of less than 6 months' duration. Nevertheless, children with chronic thrombocytopenic purpura often had a good response. After reviewing all of the published data, the FDA added acute and chronic thrombocytopenic purpura in children to to immune deficiency diseases as approved indications for IVIg therapy. The extensive literature on the use of high-dose IVIg therapy in both children and adults is covered in the references (50-53). In patients with chronic thrombocytopenic purpura, it has been possible to follow the kinetics of platelet survival using Indium-111-labeled platelets, and to predict those patients who will respond to IVIg (54).

In addition, several closely related immune thrombocytopenias associated with alloimmunization (30), drugs (55), leukemia (56), sarcoidosis (57), systemic lupus erythematosus (31), and infections (58) also have been shown to respond promptly to high-dose IVIg therapy. In all cases, if response is to occur, the platelet count rises to more than 30,000/cu mm within two to five days of the initial IVIg infusion. Treatment of thrombotic thrombocytopenic purpura (TTP) with high-dose IVIg has not produced as consistently beneficial responses. Some reports of small numbers of patients have indicated beneficial response (59,60), and others have not (61).

Mechanisms of Action of High-dose IVIg in Immune Thrombocytopenias

Two mechanisms of action of IVIg in the treatment of immune thrombocytopenias have been suggested. Blockade of the Fc-gamma-RIII (See Table 4) on phagocytic cells in the liver and spleen which are active in removal of antibody-sensitized platelets is thought by most investigators to be the primary mechanism by which IVIg benefits immune thrombocytopenias (8,51). Two studies indirectly support this view. Clarkson, et al (62), successfully treated a patient with refractory immune thrombocytopenic purpura with murine monoclonal antibody (3G8) to a low-affinity IgG-Fc receptor (Fc-gamma-RIII), and obtained the same beneficial effect on ITP as IVIg therapy. See Fig. 3. Also, if the patient with ITP has Rh-positive erythrocytes, a small amount of anti-D(Rh) antibody can be given. The antibody-coated red blood cells then clog up the Fc-receptors in the spleen and liver, and temporarily halt antibody-sensitized platelet destruction (8). The response to anti-D antiserum in patients with ITP differs from the beneficial effects of IVIg in several ways, however. The platelets rise after IVIg within 48 hours, whereas anti-D therapy shows no increase until 72 hours. Patients who have been splenectomized do not respond to anti-D, but show no difference from non-splenectomized patients in their response to IVIg (51).

These observations have led to a second suggested mechanism for the action of IVIg in immune thrombocytopenias. It has been shown that commercial IVIg contains anti-idiotype antibodies which selectively bind to the antigen-combining site (idiotype) of the anti-platelet antibodies. After IVIg infusion, the levels of autoantibodies to platelets fall (63). There is also a decrease in platelet-associated IgG (64) which improves responses in patients otherwise refractory to platelet transfusions.

Anti-idiotype antibodies in IVIg also could bind platelet autoantibodies which are expressed on the surface of B-cells (precursors of plasma cells producing anti-platelet antibodies).

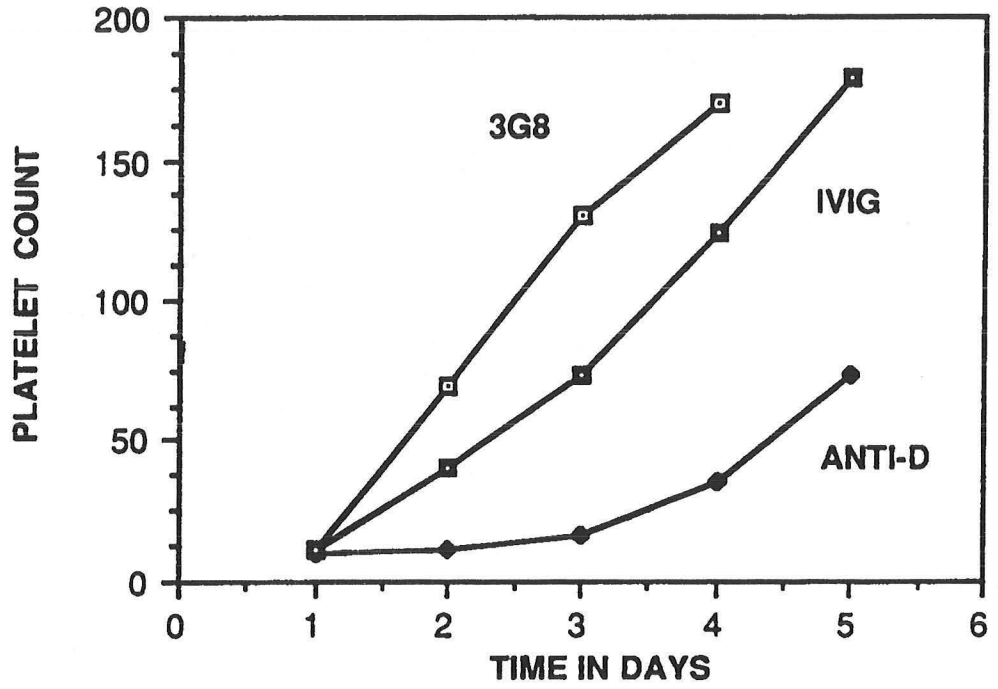

Fig. 3 Responses of patients with ITP to IVIg, 3G8 and Anti-D (Ref. 51)

This would allow these B-cells to be targeted for complement-mediated lysis or antibody dependent cellular cytotoxicity (ADCC), and be eliminated. When the anti-platelet antibody is bound to the platelet surface antigen, it may become unavailable for anti-idiotype binding, explaining why thrombocytopenia is not made worse by the addition of exogenous anti-idiotypic antibodies in IVIg.

Use of High-dose IVIg to Treat Kawasaki Disease

Kawasaki disease was first described in 1974 by Tomisaku Kawasaki as "mucocutaneous lymph node syndrome" (65). Although most frequently seen in children of less than five years of age of Oriental ancestry, epidemic outbreaks are occurring with increasing frequency in the United States with an incidence of 150/100,000 children in involved communities. This suggests an infectious etiology, but no person-to-person transmission or point source for an infection has yet been documented. The diagnosis is made on the basis of clinical criteria which are outlined in Table 6.

Table 6.

CLINICAL CRITERIA FOR KAWASAKI DISEASE

Fever of Unknown Origin for More than 5 Days
PLUS 4 OR MORE OF THE FOLLOWING SIGNS
Conjunctival Injection
Erythema Multiforme Rash
Cervical Lymphadenopathy
Erythema Of Mucosa Of Upper Respiratory Tract
Edema And/or Erythema Of The Palms And Soles Followed By Desquamation Of The Surface Skin

From Shackelford & Strauss, N Eng J Med 1991

Kawasaki disease is a moderately severe disease with over 20% of patients developing a systemic vasculitis, particularly of the coronary arteries, and occasionally a fatal myocarditis (65,66). In untreated patients, a mortality rate as high as 2% has been found (66). Although large doses of aspirin or prednisone lower fever and give symptomatic improvement, neither treatment prevents aneurysm formation and/or occlusion of coronary vessels. One study even showed the incidence of coronary artery aneurysms to be increased by giving prednisolone (65).

Role of a Microbial Superantigen in Kawasaki Disease

Although the etiology of Kawasaki disease is unknown, recent indirect evidence points to a microbial superantigen as a possible cause. All patients with Kawasaki disease show evidence of intense immune stimulation. Protein surface markers measured with monoclonal antibody reagents on T-cells, B-cells and macrophages are altered to indicate activation and clonal expansion. Abe, et al (32), analyzed the types of beta chain of the T-cell antigen receptor (TCR) present in the blood of patients with Kawasaki disease. They showed selective expansion of T cells expressing TCR variable regions, V beta 2 and V beta 8, as measured both by surface TCR protein, and by V beta 2 and V beta 8 gene expression. There was no increase in the expression of 20 other TCR V beta genes measured by polymerase chain reaction (PCR) DNA analyses. This data is shown in Fig. 4.

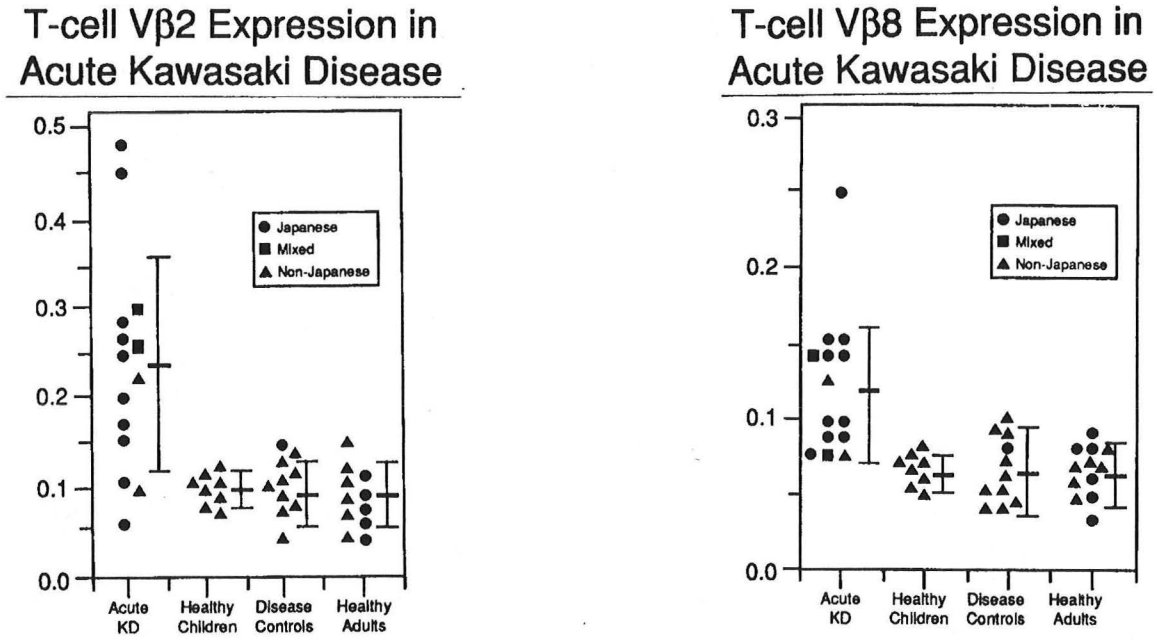

Fig 4.

Microbial superantigens are a class of bacterial or viral substances which stimulate a large fraction of the T cell population without requiring previous sensitization. This T cell stimulation is mediated by the dual affinity of superantigens for the Class II histocompatibility complex on activated accessory cells (macrophages, activated B-cells, dendritic cells, etc) and for the relatively invariant sequence of a variable (V) beta region of the TCR. Different superantigens selectively stimulate different V beta families (33). Exotoxins of Staphylococcus aureus species are among the best studied superantigens. For example, toxic shock toxin from Staphylococcus aureus selectively stimulates the TCR V beta 2 family (67).

Recently it has also been shown that staphylococcal enterotoxin A in the presence of T cells, selectively stimulates B cells expressing the heavy-chain variable regions 3 and 4 (VH3, VH4), indicating that under appropriate circumstances that bacterial antigens also may act as B cell superantigens (68).

Recently, IVIg has been shown to contain specific antibodies inhibitory to activation of T cells by staphylococcal superantigens (33). This would suggest an immunoregulatory role of normal IgG in down-regulating the disruptive action of microbial superantigens, either by direct interaction of IgG with the superantigen itself, or by modulating the T-cell response to these selective TCR V beta activators. Fig. 5 shows diagramatically the proposed role of IVIg in blocking the action of a putative microbial superantigen in Kawasaki disease.

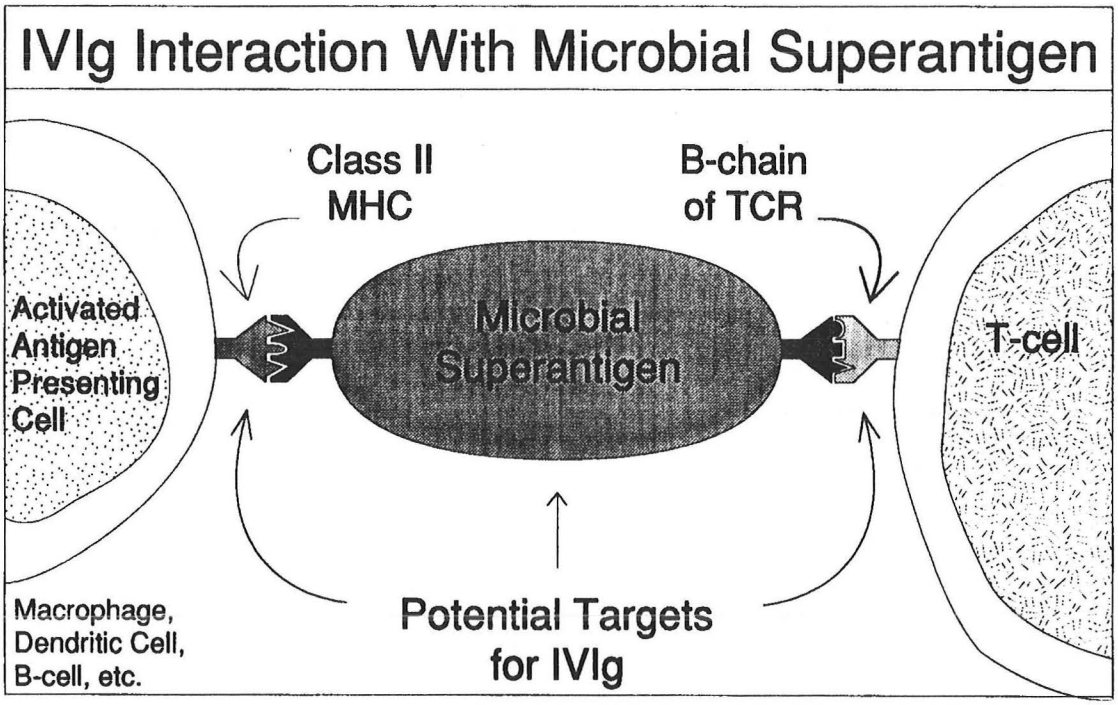

Fig. 5

Clinical Experience with High-dose IVIg in Kawasaki Disease

In 1984, Furusho et al (69) administered IVIg with impressive improvement in patients with Kawasaki disease. In particular, the incidence of coronary aneurysms or occlusion was decreased from 23% to 8% two weeks after IVIg therapy (70,71). Other clinical features of Kawasaki disease also responded promptly to IVIg, given as 2 g per kilogram over a 10-hour infusion period. These included defervescence in less than 2 days and prompt disappearence of the rash and erythematous mucosal inflammation, also within 2 days. However, the ESR often remained in the 70-100 mm/hr range for up to 2 weeks after therapy (72,73). In general, the use of high-dose IVIg in Kawasaki disease has been associated with remarkably few serious side effects, assuming that the recipient is not IgA-deficient. However, because IVIg contains antibodies to blood-type antigens, a rare patient may experience significant immune hemolysis, disseminated intravascular coagulation, and serum sickness after large doses of IVIg (74). Nevertheless, because of the substantially greater efficacy of IVIg in Kasawaki disease when compared with other available treatment, it has been established as the treatment of choice. IVIg also results in less total cost and in lower rates of permanent coronary artery dilation (75).

Use of High-dose IVIg in Wegener's Granulomatosis

Since the discovery of anti-neutrophil cytoplasmic antibodies (ANCA) in 1982, there has been growing evidence that they play a pathogenetic role in several forms of systemic vasculitis, including Wegener's granulomatosis and microscopic polyarteritis nodosa. In vitro studies have shown that ANCA-rich immunoglobulin has a direct toxic effect on intact neutrophils, and also promotes vascular endothelial cell cytotoxicity (34). This has been discussed at length by Dr. Alper in a recent Internal Medicine Grand Rounds. However, the isotypes of ANCA (c-ANCA and p-ANCA) are present in small amounts in pools of normal IgG (IVIg), suggesting that otherwise normal people are making small amounts of these autoantibodies. This must mean that an appropriate control mechanism converts these otherwise pathogenetic antibodies into harmless bystanders. This is now thought to occur because of the presence of anti-idiotypic antibodies to ANCA in normal serum which down-regulate ANCA production, and modulate their impact on target cells (34). Anti-idiotypic activity toward ANCA has been demonstrated in ANCA-negative remission sera from patients recovering from Wegener's granulomatosis (76). Two uncontrolled trials of IVIg in Wegener's (77,78) report improvement in disease activity, but whether or not IVIg is an alternative to conventional immunosuppression awaits controlled clinical studies. In my opinion, treatment with high-dose IVIg should be considered in patients with Wegener's who have failed to respond to cyclophosphamide and prednisone.

Use of High-dose IVIg in Steroid-refractory Dermatomyositis

O.N.J. is a 68 WF with diabetes mellitus and hypertension who was otherwise well until June 1993 when she noted progressive proximal muscle weakness, dysphagia, dysphonia, and a skin rash on her knuckles, forearms, face and on the flush area of the upper chest. She was hospitalized and found to have a creatine phosphokinase (CPK) of 23,000, and an ESR of 90. She was diagnosed as having dermatomyositis (DM) and discharged home on 80 mg of prednisone daily with no evidence of clinical response after 4 weeks. A neoplastic evaluation, including a mammogram, had been negative. She then became acutely ill, and was hospitalized with aspiration pneumonia and ARDS, and was treated with intubation and intravenous antibiotics with gradual resolution of the pneumonia, but persistence of the rash and worsening of her muscle weakness to the point that she was effectively quadraplegic. By October 1993, she had developed a sacral decubitus ulcer, and was becoming increasingly dyspneic due to respiratory muscle involvement.

At that time she was begun on high-dose IVIg given as 400mg/kg/day over a five-day period (Total 140g). Her CPK fell from 28,000 before IVIg to 39 on the third day of therapy! Her rash cleared within five days after IVIg therapy, and muscle strength began to gradually return. She was transferred to the Rehabilitation Unit for intensive physical therapy and management of the decubitus ulcer. She showed gradual improvement, becoming able to sit unassisted in a wheelchair, swallow food and feed herself. One month after the initial therapy, the CPK remained at 42, but her ESR was 79 and it was elected to repeat the IVIg therapy (2 g/kg over a 5-day period). After three days, she developed a generalized pruritic rash, thought possibly related to trimethoprim/sulfa which she had been taking for a urinary tract infection. Nevertheless, the IVIg was stopped until the rash cleared, then the last two days of infusion completed without incident.

Because of the concern about a malignancy (in view of the elevated ESR), a repeat mammogram was obtained, and a left breast mass detected. This was biopsied and shown to be a 2.4 cm, infiltrating ductal cell carcinoma with prominent lymphoplasmocytic infiltration, and vascular invasion. She underwent a left radical mastectomy which showed local lymph node involvement, but no evidence of pulmonary or distant metastases.

She did well after the surgery, continuing to gain muscle strength and weight to the point that she could speak normally, swallow normally, feed and dress herself, although she was still confined to a wheelchair. About 2 weeks after the mastectomy, and approximately 6 weeks after her second IVIg infusion, she again began to experience a florid return of the rash on her face, upper chest, arms and hands. However, her CPK was 31. The rash had a prominent photosensitive distribution with sharp delineation above the line of her brassiere, on the outer surfaces of her arms and on her face. Because of her overall improvement except for the skin rash, an attempt was made to treat her with topical steroid cream (Lidex) and a sun screen (zinc oxide ointment). This initially improved the rash, but after four weeks, it began to weep, crust and develop a burning sensation. At that point, she received her third 140 g IVIg infusion, given this time in one day over a 6-hour period with no adverse side effects. The rash improved dramatically within 5 days after she received the IVIg. She has shown no evidence of recurrence of the breast neoplasm at this time.

This patient illustrates a fairly typical response of DM to high-dose IVIg (35), with gradual relapse in about 6 weeks after treatment. Three of her clinical findings need to be stressed. The association with a neoplasm is a fairly frequent finding in adult DM. This is particularly likely in patients who have been unresponsive to high-dose prednisone therapy, and who have a significantly elevated sedimentation rate (ESR). If adult patients with polymyositis (PM) and DM are examined as a group, 65% will have a normal ESR. Of the remaining 35% who have an elevated ESR, about half will be found to have an associated neoplasm, and almost all of these will have clinical features of DM. Recently Dalakas and his colleagues at NIH have reviewed their experience with inflammatory myopathies, and have generated a new classification of these disorders (79), which is presented in Table 7.

Table 7

CLASSIFICATION OF INFLAMMATORY MYOPATHIES

CHARACTERISTIC	DERMATO-MYOSITIS	POLY-MYOSITIS	INCLUSION-BODY MYOSITIS
Age of onset	Adults & Children	>18 yr	>50 yr
Associated with:			
Scleroderma and MCTD	yes	yes	yes
Overlap syndr.(nucleolar ANA)	yes	no	no
Autoimmune gene (DR3/B8)	no	yes	no
Malignant neoplasms	yes	no	no
Viral infections	?	yes	?
Parasitic diseases	no	yes	no
Drugs (D-penicillamine)	yes	yes	no
Increased familial frequency	no	no	yes

From MC Dalakas, N Eng J Med 325:1487, 1991.

The rash, and some of the inflammatory injury to muscle in DM involves a subtle vasculitis of the microvasculature with deposition of the C5b-9 membranolytic attack complex of the complement system (79), while the muscle destruction in PM and inclusion-body myositis (IBM) is mediated by CD8+ cytotoxic T cells which are antigen specific for Type 1 (fast-twitch, white meat) muscle fibers. The T cells in PM and IBM release tumor necrosis factor which depolarizes the target muscle fibers causing electrolyte and enzyme leakage, then myolysis.

IBM differs in several ways from PM and DM. IBM often involves older (>50 yr) patients, it attacks both proximal and distal muscle groups, and it is usually refractory to steroid or immunosuppressive therapy, accounting for about one-third of treatment-refractory patients referred to NIH (79). Histologically IBM shows characteristic basophilic inclusions rimmed around slit-like vacuoles which, under electron microscopy, contain membranous whorls. Filamentous inclusions in the muscle fiber cytoplasm, prominent in the vicinity of the rimmed vacuoles, are pathognomonic of IBM (79).

A recently described variant of PM has been characterized by muscle infiltration with T-cells expressing gamma/delta chains in their TCR, but DR4(-), DR8(-), and CD3(+). This type of T-cell makes up about 5% of circulating T-cells, and is known to interact with bacterial heat shock proteins, but its function in this form of PM is not known. This variant of polymyositis responds readily to steroids (80).

Most of the T-cells infiltrating muscle in DM are CD4+ and lie in close proximity to B-cells and macrophages, suggesting local antigen-driven antibody synthesis as the mechanism for complement activation and vascular injury (79).

Table 8 summarizes the result of three clinical trials of IVIg in DM and PM.

Table 8.

THREE CLINICAL TRIALS OF IVIg IN REFRACTORY DM†

Type of Trial (Ref)		No. Pts T/C*	No. Responding to IVIg (%)	Adverse Reactions (%)
3 months, adults Double-blind Placebo Controlled (35)		8/7	7/8 (87.5)	
Crossover		7 + 5 = 12	9/12 (75)	2/12 (16) severe headaches
4 months, Adults Unblinded (81)	DM†	8	7/8 (87.5)	2/8 (25) fever, headaches
	PM†	12	11/12 (92)	2/12 (16) fever, sweating
9 months, Children Unblinded (82)	DM†	5	100	No side effects noted

* T/C = Treated patients/Control patients; †DM = dermatomyositis, PM = polymyositis

In addition to the 12 PM patients noted above (81), other isolated reports of one (83) or two (84) patients with steroid unresponsive PM, and one report of 4 patients with IBM (85) have been published. Usually, there was some improvement in muscle strength, and some patients normalized muscle enzyme levels. Three of the four patients with IBM had muscle strengh improvement which lasted for two to four months (85).

IVIg in Other Immunologically-mediated Diseases

Acquired and genetic hemophilia and von Willebrand's disease: Acquired autoantibodies to the blood clotting factors VIII:C and the von Willebrand factor may cause life threatening hemorrhage which is very difficult to manage, yet responds to IVIg therapy (86,87). Fig. 6 illustrates the remarkably quick drop in the anti-VIII:C antibody activity following IVIg therapy in two patients with spontaneously acquired hemophilia in whom high levels of autoantibody to factor VIII:C were detected (one patient had a titer of 25,000 Bethesda units, and the other, a titer of 10,500 Bethesda units) (88). In vitro testing of purified IgG derived from the two sera containing these high levels of autoantibody showed that it required four to 12 times as much (weight/weight) of normal IVIg to produce complete neutralization of the anti-coagulant activity in the IgG from the test sera.

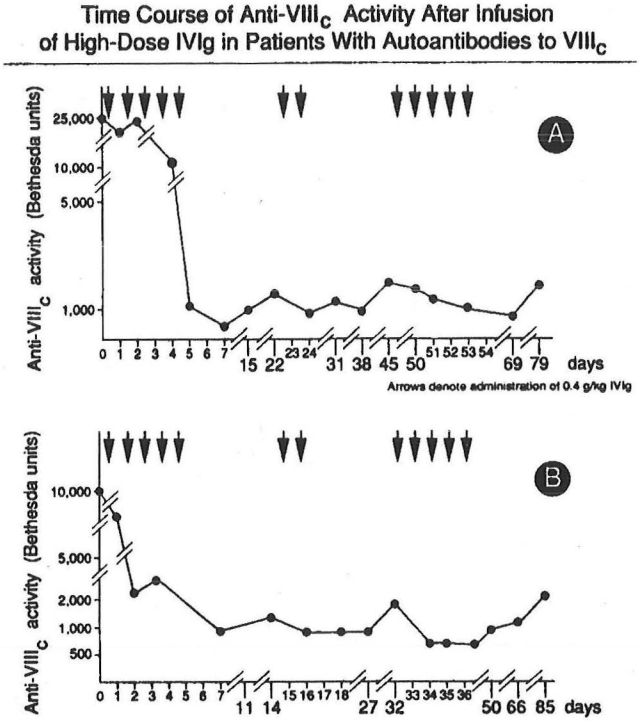

Fig. 6 [From Sultan, et al, Lancet 2:765-768 (1987)]

IVIg was also shown to have neutralizing anti-idiotypic antibodies against alloantibodies produced in patients with genetic hemophilia A who had been treated with fresh frozen plasma from normal factor VIII donors, but this response was less impressive or consistent than in patients with autoimmune-acquired disease (86,88). Life-threatening bleeding in patients with acquired von Willebrand's disease also responds promptly to IVIg therapy (87). And finally, in patients with acquired, autoimmune anti-VIII:C in whom spontaneous recovery has occurred, post-recovery serum contains anti-idiotypic antibodies which neutralize the anti-VIII:C in stored serum from the same patient's active-disease phase (89). Occasionally, autoantibodies have been found which are neutralized by IVIg derived from one supplier, and not by the IVIg from another supplier (86), suggesting variations in different sources (placenta versus pooled out-dated blood bank units versus fresh plasma from plasmapheresis donor centers) of the pooled normal IVIg in terms of anti-idiotypic antibodies and non-IgG proteins present.

Myasthenia gravis (MG) and Guillain-Barré (G-B) syndrome: Exchange transfusions or plasmapheresis are occasionally required to prevent death in MG or G-B syndrome (90,91,92). Polyclonal autoantibodies, usually of the IgG type, directed against the main immunogenic region of the two alpha subunits of the acetylcholine receptor (AChR) on muscle fibers are found in 85 to 90% of patients with MG. Furthermore, if the lymphocytes of anti-AChR-negative patients are cultured in vitro, over 90% can be shown to be synthesizing anti-AChR autoantibodies (90), suggesting that essentially all patients with MG are making anti-AChR autoantibodies, but that some patients absorb out circulating antibodies on muscle receptors, making anti-AChR undetectible in their serum.

Theoretically the removal of anti-AChR autoantibodies should uniformly lead to clinical improvement. Unfortunately, removal of serum from these patients gives only a partial and transient drop in autoantibody titer, and does not remove tissue-absorbed antibodies. The plasmapheresis is quickly followed by a rebound phenomenon which carries the autoantibody titer to even higher levels than before treatment. To suppress this rebound, immunosuppression is required, and with it the danger of infection, neoplasia, and non-specific drug-induced injury unrelated to the MG. For this reason, the selective impact of IVIg in these two conditions has attracted much recent attention (92,93).

Ansura (94) studied the use of IVIg in 12 patients with MG, and compared his results to 7 other published series containing 48 additional patients. Eleven of Ansura's 12 patients showed a beneficial response beginning an average of 3.6 days after starting the IVIg infusion. Overall, in the 60 patients with MG given IVIg, 73% responded favorably to IVIg treatment. Most received approximately 2 g/kg of IVIg over a five-day period, and their response lasted for 6 to 13 weeks. In all but five of the 60 patients, simultaneous treatment with prednisone and/or azathioprine was also being given, but the patients had not responded to prednisone and/or azathioprine before receiving IVIg. Less than half of the 60 IVIg-treated patients with MG showed a decrease in the level of their serum anti-AChR, casting doubt on the role of the anti-idiotype antibodies (to anti-AChR) in the IVIg used to treat MG (94).

Cook and her colleagues (95) examined the cellular immunity of five patients with MG who had been treated successfully (one in temporary remission, three markedly improved, one improved) with high-dose IVIg, and showed that mononuclear cells positive for surface IgM and IgG markers doubled (from 13% to 26%) by the fifth day of IVIg therapy. In addition, mononuclear cells positive for CD16 (Leu 11), the surface protein representing the IgG-Fc receptor on NK cells, rose from 11% to 24%. Also, CD4/CD8 T cell ratios fell slowly from 2.9 to 2.2 after 7 days, and remained low for several weeks. These findings provide alternative mechanisms by which IVIg may alter the immune system in MG.

Severe, steroid-dependent asthma: Mazer, Giclas and Gelfand (96) at the National Jewish Center for Immunology and Respiratory Medicine in Denver described a patient who had intractable, high-dose steroid-dependent asthma who was treated with monthly IVIg (2g/kg) for 6 months. Prior to treatment, the patient had unremitting asthma, and had developed severe steroid side effects. During treatment, she had only one asthma attack, her FEV-1 doubled and she was able to reduce her steroid dose by 75%. Testing showed her to have had a 3-log decrease in skin-prick sensitivity to antigens to which she was known to be highly sensitive prior to the IVIg treatment. In addition, her specific IgE (RAST) levels to known antigens decreased markedly, and IgG1 and IgG2 antibodies to two of the antigens (Alternaria, Cladosporium) increased 2.5-fold, presumably representing blocking antibodies in the infused IVIg.

In most asthma patients, allergen-specific immunotherapy, when successful, may achieve the same immunomodulatory influence on their allergic responses (97) that the above patient achieved "passively" with the IVIg infusions.

IVIg in Severe RA, JRA, SLE and Related Diseases

In 1969, Morris Ziff, Hugo Jasin, Jerome Herman and I read of the success of Drs. Good and Gabrielsen (23) in treating children with arthritis and agammaglobulinemia or hypogammaglobulinemia by replacing gamma-globulin. This caused the 30% of hypogammaglobulinemic children who had developed rheumatoid-like arthritis to improve. We decided to try intramuscular immunoglobulin injections in adult patients with rheumatoid arthritis. We set up a blinded, controlled study in which I gave the patients either a placebo or intramuscular gamma-globulin once each week, and Drs. Jasin and Herman counted involved joints and measured laboratory parameters of arthritis activity.

Dr. Ziff reviewed the data without knowledge of the treatment of each patient. Each of the 10 treated patients received 17.5 grams of Gamma-gee (Merck) intramuscularly over a 12-week period, and a matched control group received a similar volume of saline. Both groups received 2 ml of 1% xylocaine with each intramuscular injection to prevent the patients from recognizing the otherwise painful intramuscular gamma-globulin injection. There were no detectible differences in response between the treated and untreated patients (98). This low-dose (17.5 g total over 12 weeks) gamma-globulin therapy was similar on a weight basis to the replacement dose which Good and Gabrielsen (23) had used to treat hypogammaglobulinemic children in whom remission of joint complaints had been observed within 12 weeks.

Later studies by Combe, et al (99), using human placenta-eluted gamma globulin showed statistically significant improvement in patients with RA following intramuscular IgG treatment. The gamma globulin which we had used (Merck Gamma-gee) had been prepared from pooled, out-dated blood plasma. Recent studies (100,101) of high-dose IVIg in adults with rheumatoid arthritis have used up to 840 g of total IgG over 17 weeks, yet still have produced only "limited efficacy" in one study (100) and a 50% improvement of the Ritchie Index with no change in the ESR in six of 7 patients in a second study (101). Because of the very high cost, it is unlikely that IVIg has any significant place in the treatment of adult rheumatoid arthritis, based on presently available data. Felty's syndrome had been shown earlier also to be unresponsive to high-dose IVIg (29).

In contrast to adult RA, a recent carefully performed study of eight patients with juvenile rheumatoid arthritis (JRA) treated with IVIg (2 g/kg/mo) for six months (102) showed substantial improvement in both clinical and laboratory features of the disease in seven of the eight patients. Hemoglobin and albumin increased, ESR and platelet count decreased, and steroid dosage requirements were reduced by 80%.

Four studies of systemic lupus (SLE) and related autoimmune diseases have provided preliminary data suggesting benefit of IVIg therapy: One in thrombocytopenia linked to SLE (31), one in hypocomplementemic glomerulonephritis with the IgG autoantibody directed against the C3-convertase of the alternative complement pathway (C3NeF) (103), and two in the anti-phospholipid syndrome (104,105). In the anti-phospholipid syndrome, it is suggested that treatment of the severely involved patient during pregnancy or prior to surgery to reduce anti-phospholipid antibodies will decrease fetal loss and reduce the complications of major surgery. Another study of IVIg in a lupus patient with SLE diffuse proliferative glomerulonephritis showed substantial worsening of the renal disease coincidentally with IVIg therapy (106).

How much does IVIg cost? Can the costs be justified? What improvements can be made in the future?

IVIg costs $70/g in the average hospital pharmacy. This makes treatment of a 70 kg adult receiving 2g/kg of IVIg cost about $9,800 exclusive of other hospital and physician expenses. IVIg is a valuable medical resource which requires all of the IgG found in approximately 50 units of blood! Except for Kawasaki disease where a single treatment suffices, most of the above conditions relapse and require retreatment in about 6 weeks, meaning a need for about 8 treatments/year or about $80,000/year. Some investigators have described longer or more permanent remissions after a few treatments in some patients (8), and others have argued that IVIg should only be bridge-treatment allowing splenectomy in thrombocytopenia (28), or allowing introduction of slower onset immunosuppressive therapy (42).

The high costs of IVIg may be justifiable in some patients, depending upon particular circumstances. Currently, the FDA has approved the use of IVIg in Kawasaki disease, acute and chronic immune thrombocytopenias in children, and in agammaglobulinemic or hypogammaglobulinemic patients.

I feel the approval of this treatment should be extended for treatment of patients with steroid/immunosuppressive-refractory dermatomyositis, and for very carefully selected patients with acquired hemophilia, intractable asthma, and myasthenia gravis. Most other diseases should be further evaluated with experimental trials to justify the high cost and limited duration of efficacy, and perhaps to elucidate mechanisms of IVIg action which would lead to less expensive therapy.

Future Improvements in IVIg Therapy

Just as fractionation of blood donation units into cell and plasma fractions have improved the usefulness of blood banking, current technology is available to fractionate the IgG in serum into antibody fractions with specific antigen targets (including anti-idiotypic antibodies) for use in immunomodulation of autoimmune diseases, and to treat specific microbial antigens. In that manner, a single batch of serum from approximately 50 donors, now producing enough IVIg to treat one patient one cycle, might be used to treat 50 recipients with substantially less cost and waste of a valuable medical resource. Fractionation using affinity chromatography would be particularly necessary if more and more bacteria acquire antibiotic resistance, and require alternate treatment with hyperimmune gamma-globulin (27).

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