*From the Department of Child Health, Medical School,
University of Indonesia, Jakarta.*

Uses and Limitations of Gammaglobulin Therapy
(Literature Review).

by

S U M A R M O

In the last 20 years gammaglobulin products have been used more and more in clinical medicine for both the prevention and treatment of infectious diseases. With the development of routine laboratory methods for the assessment of cellular and humoral immune competence, more patients with severe immunological deficiencies will be recognised, as will those with all shades of deficiency between complete absence of a functional immune system and the normal state (Simons, 1971). A major objective in the management of patients with hypo- or agammaglobulinaemia and other antibody deficiency states is to reduce their abnormality in susceptibility to infection. A survey carried out at the Hospital for Sick Children, London, revealed, that severe deficiency of cellular and humoral immunity was responsible for two per cent of all deaths (Berry, 1968). Clinical application of the advances made in basic immunological research is not restricted merely to the recognition and diagnosis of patients with immunological deficiency diseases, but also in rational immunological therapy.

This paper discusses the enormous advances in gammaglobulin therapy, its uses and limitations.

Replacement of immunoglobulins

One aspect of gammaglobulin therapy is the replacement of the deficient end products in those individuals in whom there is impaired function of immunoglobulin producing lymphoid tissues. Immunoglobulin replacement will continue until means become available for overcoming the central cellular defect. The second aspect is the inhibitions of immune responses by immunoglobulin therapy.

The clinical application of the gammaglobulin products for the prophylaxis and treatment of disease was regulated according to empiric experience. Several authors with a large experience in gammaglobulin therapy have come to the conclusion that a definite, critical examination of the prophylactic and therapeutic actions of gammaglobulin preparations is not yet possible (Barandun, 1964).

Certainly in normal, the use of gammaglobulins to prevent viral infections may be valuable, but more interesting today are viral infections which cannot be managed by immunization procedures. In agammaglobulinaemic patients gammaglobulin is so far the most important prophylactic therapeutic agent to prevent repeated bacterial infections.

The dose necessary for the prevention of several viral diseases is quite low (Rosen and Janeway, 1968), it increases, however, as the incubation period advances. After onset of the disease and particularly in cases of serious or prolonged infection, high doses of gammaglobulin are necessary to support the body efficaciously in its resistance to the pathogen. As a preventive measure, gammaglobulin is given at regular intervals in doses calculated to provide IgG levels of 200 mg per 100 ml i.e. levels suggested as being necessary for protection (Soothill, 1962). To achieve and maintain this serum IgG concentration, it is necessary to administer large volumes at frequent intervals. The intramuscular injections are painful, and the effective dose achieved is less than that administered due to local tissue proteolysis of some of the gammaglobulins. Gammaglobulin cannot be injected intravenously, even in small quantities. Even if the product is diluted and all precautionary measures are taken, a large percentage of patients react in a range from subjective feelings of anxiety and oppression, pain in the back, to more severe reactions of fever, respiratory distress and circulatory collapse may even occur in rare casses. As all of us know, gammaglobulin and in particular aggregated gammaglobulin, is capable of binding and activating complement. Histamine and similar substances are thus released which cause the side effects described.

Recent advances in immunoglobulin chemistry have enabled the preparation of specific immunoglobulin solutions, some of which may be administered intravenously. The investigation of these preparations gave the following theoretical results (Barandun et al., 1962; Barandun, 1964): The intolerance toward native gammaglobulins is dependent upon an endogenous individual factor. This is demonstrated by the fact that only certain individuals react to the intravenous application of gammaglobulin. The intolerance toward native gammaglobulins is also dependent upon an exogenous general fac-

TABLE 1 : *Comparison of 7 preparations of intravenous gammaglobulin labeled with* I [131]

Preparation	Method	Ultracentrifuge sedimentation values	Anti-complement activity	Reactions in hypogammaglobulinaemia	Plasma T ½ in controls days	References
Standard HGG	Cold ethanol precipitation	6-7 S (93%)	marked	frequent serious	17-30	KOBLET et al., 1967
pH-4-HGG	Incubation with HCl, pH 4	6-7 S (94%)	nil	nil	14	BARANDUN et al., 1962 KOBLET et al., 1967
'Gamma-venin'	Incubation with pepsin (1 in 100)	5.6 S (60-70%)	nil	nil	9	SCHULTZ & SCHWICK, 1962 KOBLET et al., 1967
Plasmin-HGG	Plasmin digestion	6.5 S (45%)	nil	nil	16.1 (6.5 S)	SGOURIS, 1967 HINNMAN et al., 1967 MERLER et al., 1967
'IV-HGG'	Charcoal absorption	—	nil	very rare	—	STEINBUCH et al., 1967
HGG (CSL)	Incubation with pepsin (1 in 10,000)	6-7 S (90%)	nil	very rare	14.9 11.4	SCHIFF et al., 1968 SIMONS et al., 1968
Hyland Intravenous ISG	Polymer precipitation	6.4-7.2 S (79.5%)	low	nil	17-19	SHANBROM et al., 1970

After HAINSKI, M. et al., (1971).

tor, which is carried by the gammaglobulin itself. It is connected to its anticomplement activity and tissue binding capacity. Previous attempts to provide an immune serum globulin safe for intravenous use (I.V.-G.G.) have utilized Cohn fraction-II derived material. Using acid treatment, enzyme hydrolysis and other procedures, investigators have attacked the Fc portion of the IgG molecule to dissociate the aggregates formed during fractionation and retard their subsequent formation. These aggregated molecules of IgG have been implicated in previous reports of vasomotor reactions to I.V.G.G.

The advantages and characteristics of the "ideal" intravenous immune serum globulin:

Advantages

1. Larger doses are safer and more comfortable.
2. Losses due to local proteolysis and tissue fixation are reduced.
3. Level of circulating antibody is immediately increased.
4. Greater efficiency increases probability of succesful treatment at lower cost.

Characteristics

1. Free of high-molecular weight aggregates.
2. Low in anticomplementary activity.

3. In vivo survival comparable to intramuscular immune serum globulin.

One of the advantages over the regular intramuscular preparation is the dose to be given. For example, 400 mg./Kg. of I.V.G.G. has been given in a single infusion. This is equivalent to 2½ ml./Kg. of 16% intramuscular gammaglobulin, or 50 ml. for a patient weighing 20 Kg., an excessively large volume to be given intramuscularly to a child as part of routine management (Simons, 1968). There are different ways to treat standard gammaglobulin to obtain preparations free of anticomplement activity.

Table 1 compares the 7 preparations of I.V.G.G.

The acid treatment seems to work well initially, although it has been noted that the reaction may be incomplete (Schiff et al., 1968). Moreover, the subsequent reaggregation which tends to occur may render the preparation unpredictable chemically as well as clinically. The specific goal of enzyme hydrolysis has been to impair the IgG molecule's ability to aggregate, fix complement, and thus triger vasomotor reactions. In doing so, however, its clinical efficacy may also have been impaired. Material digested with pepsin has shown marked reduction in its in vivo survival (Janeway et al., 1968; Merler et al., 1967; Wells et al., 1969). This would

certainly reduce its prophylactic, if not its therapeutic usefulness. Sgouris and Matz (1967) reported that treatment with plasmin resulted in little or no change in antibody activity (Schiff et al., 1968). Painter (1965) has also reported that in vitro titres of fragmented material may be deceptive in light of their shortened in vivo survival. Merler et al. (1967) reported studies on plasmin digested material, and found that 55% of it was fragmented and rapidly catabolyzed. In the case of Gamma-Venin, the hydrolysis with pepsin and low pH, it seems apparently that the duration of action of Gamma-Venin in the organism is relatively short, because the small fragments cannot be retained (Diggelmann et al., 1964).

Furthermore, a part of the gammaglobulin molecule (fragment Fc, III) is completely destroyed by the treatment mentioned above. This is just the part of the molecule which has no antibody activities, but is carrier of antigenic properties and of groups which bind in a non-specific way to complement and to various tissue structures.

The treatment with pepsin and low pH results in a gammaglobulin preparation without non-specific properties but leaving intact the antibody activities in quantitative and qualitative terms. The commercially I.V.G.G. made by Hyland Division Travenol Laboratories is resuspen-

ding the fraction III paste in normal saline and is processed using a polymeric fractionation agent. This is a preparation free of aggregates and low in anticomplementary activity. The in vivo half life was found to be comparable to intramuscular preparations.

The gammaglobulin products which have been available consist almost exclusively of IgG and contain mere traces of IgA and IgM. The evaluation of the numerous antibody assays show that antibodies against antigens are located mainly in the IgG, antibodies against carbohydrate antigens are found in IgM, and certain viral antibodies mainly in IgA. In the course of the immunization process, IgM antibodies appear sooner than IgG, they have more intense agglutinating phage-neutralizing, complement binding, and hemolysing properties than IgG (Janeway et al., 1968). It is known that IgA is detected not only in the serum, but also in the secretions of the mucous membranes and in the glands producing external secretions; it occurs in monomeric and polymeryc form. The level of the IgA fractions in the secretions of the mucous membranes can be higher than that in the serum. All these findings promoted the manufacture of IgA and IgM preparations for clinical applications.

Results obtained by Hobbs (1968) and other investigators (Stiehm and Fudenberg, 1966) have also proved

TABLE 2 : *Types of dysgammaglobulinaemia*

Deficiency	Normal or raised level	Dysgammaglobulinaemia
IgA and IgM	IgG	Type I
IgG and IgA	IgM	Type II
IgG	IgA and IgM	Type III
IgA	IgG and IgM	Type IV
IgM	IgG and IgA	Type V
IgG and IgM	IgA	Type VI

After: Hobbs (1968) and Stoelinga (1968).

that besides the classical agamma-globulinemia-Bruton, dysgammaglo-bulinemic conditions also exist, whe-re not all three, but merely one or two immunoglobulins are decreased or completely lacking.

Table 2 shows the types of dys-gammaglobulinaemia detected so far. Antibody deficiency disease of types I, V and VI is clinically most impor-tant. Patients often suffer from serious bacterial infections (Marder, 1971).

Clinical applications

Besides for both prevention and treatment of infections diseases, of hypo/agammaglobulinemic patients, IgM enriched immunoglobulins were given to 1500 gram underweight newborn and premature infants to prevent them against Gram negative pathogens, particularly Meningococ-cus, Klebsiella, Pyocyaneus, Coli and Proteus (Cohen and Norins, 1968). Further clinical experiments are be-ng carried out in the prophylaxis of nfections in patients treated with

cytotoxic agents, and in post-opera-tive prophylaxis of wound infections next to disturbances of the wound healing process in old people. Besi-des immunoglobulins, which are ob-tained from the pooled plasma of healthy donors, hyperimmunoglobu-lin is now produced for the treatment of numerous specific diseases. Teta-nus immunoglobulin obtained from vaccinated donors is indicated witho-ut precautionary measure as homolo-gous serum both for the prophylaxis and treatment of tetanus. The do-sage of human gammaglobulin is considerably lower than that of animal serum, the half life, and thus the duration of effect, is prolonged. Pertussis hyperimmune-globulin is used for the pre-vention and treatment of whooping cough, rubella immunoglobulin is used for the prevention of German measles in early pregnancy.

Parotitis immunoglobulin is being used for the prevention of orchitis in young males and adult men. There

has been repeated corfirmation of the effectiveness of immunoglobulin in preventing infectious hepatitis or at least preventing overt manifestations of liver infection (Krugman, 1963).

Inferential evidence is presented that attenuation of hepatitis-B-antigen (H.B.Ag)-positive hepatitis may favour the progression of acute to chronic liver disease and the development of a persistent H.B.Ag carrier state. These considerations may be relevant to the use of antibody to the hepatitis-B-antigen (anti-H.B.Ag) in the prophylaxis of hepatitis B. Controlled trials to test the efficacy of anti-H.B.Ag are being planned or are in progress. It is urged that these trials go beyond questions relevant to acute, overt hepatitis and specifically examine the possible induction of chronic liver disease and a persistent viral carrier state (Alter et al., 1972). It is particularly worthy of note that anti-rhesus serum applied shortly after delivery can prevent morbus hemolyticus neonatorum in the forthcoming siblings.

Inhibition of humoral immune responses

The rationale for this procedure is based on experimental finding that the passive administration of antibody to an animal previously chalenged with antigen can suppress the production of endogenous antibody (UHR and Muller, 1968). This prin-

ciple has recently been applied in the prevention of Rh haemolytic disease. The intramuscular injection of a very small volume of high titre anti-Rh (D) antibody is sufficient to suppress the production of red cell damaging antibody. The mechanism of this phenomenon has not been elucidated.

The formation of antigen-antibody complexes are being incriminated in the pathogenesis of an increasing number of disease states. These include diseases as apparently unrelated as acute Glomerulonephritis, serum sickness, systemic Lupus Erythematosus, and renal allograft rejection. With the progressive use of immunoglobulin and antibody solutions the number of these disorders may even increase. Another area where suppression of immunoglobulin production by specific antiserum may find an application is in the control of IgE dependent reactions that are unresponsive to conventional therapy. Reaginic antibodies of the IgE class have been shown to be involved in allergic disorders including hay fever, asthma and eczema (Ishizaka et al., 1966). It is conceivable that the infusion of anti IgE antisera may be of therapeutic benefit in intractable cases.

In the preparation of immune antisera which cannot be obtained from human sera and so have to be raised in experimental animals, a major problem is to raise antibodies to a

restricted range of antigenic deter-
minants. In the production of anti-
lymphocyte serum (ALS) for exam-
ple, it is well recognised that the
injection of human lymphocytes from
any of several sources results in the
appearance not only of antilympho-
cyte antibodies but also of antibodies
directed against red cells, platelets
and other cell types. Elimination of
these antibodies from the final pre-
paration is a major practical problem.
Absorption procedures are time con-
suming and pose the difficulty of
obtaining red cells and other cells in
numbers sufficient for complete ab-
sorption of the crude ALS (Simons,
1971). Inhibition of haemagglutinins
and other undesired antibodies by
the passive administration of anti-
sera at the time of lymphocyte im-
munisation would be a much more
satisfactory alternative.

Limitations

The ideal preparation for gamma-
globulin therapy would be a gamma-
globulin without severe alteration of
the molecules, but only slight rear-
rangements of the intramolecular
structure. It could be injected intra-
venously without risk and would be
retained in the organism because of
intact molecular size and intact tis-
sue-binding capacity.

Simons et al., (1968) reported that
even though the anticomplementary
activity of the I.V.G.G. has been ra-
duced by acid pepsin treatment, a

number of untoward reactions to
infusion of this material were ob-
served. These included pallor, res-
tlessness, tachycardia, drowsiness,
headache, nausea and abdominal dis-
comfort. It was found that the oc-
currance and severity of these side
effects were dependent on the rate
of infusion, as they appeared whene-
ver the rate exceeded a certain criti-
cal value. They were of the opinion
that these reactions might be partly
due to temporary occlusion of small
blood vessels by agglutinated cells,
in accordance with their findings
that I.V.G.G. agglutinated erythro-
cytes and leucocytes in vitro. All
these findings of limitations indicate
that clinically modified gammaglobu-
lin preparations show various degrees
of aggregation, reaggregation and
fragmentation which make them less
than desirable for clinical use.

Complete removal of the Fc fra-
gment and its associated anticom-
plementary activity can be accompli-
shed only at the expense of splitting
off this fragment, with the conse-
quent diminution in tissue binding
affinity, and reduction of molecular
size. This in turn is accompanied by
increasingly rapid elimination in the
urine with a reduction of the in vivo
half-life to a level impracticable for
long term therapy. In Simons' serial
patients using Gamma-Venin, 80%
of the infused I^{125} labeled material
were recovered in the urine during
the first 24 hours.

Another limitation of I.V.G.G. is its use for the routine management of children with immunoglobulin deficiency states which is restricted by the age of the child, since small infants are unlikely to co-operate with monthly intravenous infusions. It may also be inapplicable to those children who require unusually large maintenance doses or who have low threshold for side effects, because the prolonged duration of infusion required would then preclude the practicability of regular therapy on an outpatient basis.

I.V.G.G. is probably less suited than fresh plasma to the emergency treatment of life threatening infection in patients known to be agammaglobulinaemic or in whom with a strong suspicion of deficient antibody formation, since it contains negligible amounts of IgA and IgM. Thus in the emergency therapy of agammaglobulinaemic children who have deficiency of all 3 major immunoglobulin classes, it may be preferable to administer fresh plasma (Stiehm et al., 1966) followed by prolonged maintenance of IgG levels by repeated I.V.G.G. infusion. Fresh plasma would be impractical in routine management because of the prolonged infusion periods that would be necessary in order to provide adequate replacement. The price of the gammaglobulin preparations, is also one of the limitation of its application.

Another limitation especially in developing countries where infectious diseases are still overwhelming, is that the gammaglobulin preparations available are made from healthy donors in affluent countries who never have had infections still existing in the developing countries.

Besides all the limitations mentioned above, there is reason to hope that future improved preparative methods for the modification of gammaglobulin may yet result in a lower anticomplementary activity, a conveniently long biological half-life, enriched with IgA and IgM, and a reasonable price.

Summary

An enormous progress in gammaglobulin preparation, which can be administrated intravenously has been described.

Gammaglobulin therapy is now indicated in: 1. The prevention and treatment of infectious diseases in hypo/agammaglobulinemic patients. 2. IgM enriched immunoglobulins are given to less than 1500 gram underweight newborns to prevent infection by Gram negative pathogens. 3. Hyperimmuneglobulin is used for the treatment of numerous infectious diseases. 4. Studies in the use of antibody against hepatitis-B antigen in the prophylaxis of hepatitis B are in progress. 5. Anti-rhesus serum applied shortly after delivery can prevent morbus hemolyticus neonatorum in forthcoming siblings.

However, limitations to the widespread use of immunoglobulins still exist.

REFERENCES

1. ALTER, H.J., HOLLAND, P.V., SCHMIDT, P.J. and PLOTZ, P.H. : Gamma-globulin for Hepatitis-Virus B. Prevention or extension? Lancet i : 1110 (1972).

2. BARANDUN, S., KISTLER, P., JEUNET, F. and ISLIKER, H. : Intravenous administration of human gammaglobulin. Vox. Sang. 7 : 157 (1962).

3. BARANDUN, S.: Die Gammaglobulin. Therapie (Karger, Basel/New York 1964).

4. BERRY, C.L.: The neonatal thymus and immune paresis. Proc. R. Soc. Med. 61 : 867 (1968).

5. COHEN, I.R. and NORINS, L.C. : Antibodies of the IgG, IgM and IgA classes in newborn and adult sera reactive with Gram-Negative Bacteria. J. Clin. Invest. 47 : 1053 (1968).

6. DIGGELMANN, H., KOBLET, H., BARANDUN, S. and GERBER, H. : Determination of the total clearance of different preparations of gammaglobulins. Panel meeting on radioisotope techniques in the study of protein metabolism. Vienna 1964.

7. DIGGELMANN, H., KOBLET, H., GERBER, H. and BARANDUN, S. : Bestimmung der totalen Clearance von Standard-Gammaglobulin, pH-4-Gammaglobulin and Gamma-Venin. Path. Microbiol. 27 : 572 (1964).

8. HAINSKI, M., PAYNE, J.H., ORDONEZ, G.A. and SHANBROM, E. : A new preparation of immune serum globulin for intravenous administration.Vox. Sang. 20 : 469 (1971).

9. HOBBS, J.R. : Immune imbalance in Dysgammaglobulinemia Type IV. Lancet i : 110 (1968).

10. ISHIZAKA, K., ISHIZAKA, T. and HORNBROOK, M.M.: Physicochemical properties of reaginic antibody V. Correlation of reaginic activity with gamma-E-globulin antibody. J. Immunol. 97 : 840 (1966).

11. JANEWAY, C.A., MERLER, E. and ROSEN, F.S. : Intravenous gammaglobulin. Metabolism of gammaglobulin fragments in normal and agammaglobulinemic persons. New Engl. J. Med. 278 : 919 (1968).

12. KRUGMAN, S. : The clinical use of gammaglobulin. New Engl. J. Med. 269 : 195 (1963).

13. MARDER, E. : Immunoglobulin and hyperimmuneglobulin therapy. Ann. Gen. Pract. Submit. 29-35 (1971).

14. MERLER, E., ROSEN, F.S. and SALMON, S. : Studies with intravenous gammaglobulin. Vox. Sang. 13 : 102 (1967).

15. PAINTER, R.H., WALCROFT, M.J. and WEBER, J.C.W. : The efficacy of fragmented immune serum globulin in passive immunization. Canad. J. Biochem 44 : 381 (1965).

16. ROSEN, F.S. and JANEWAY, C.A. : Diagnosis and treatment of antibody deficiency syndromes. Posgrad. Med. 43 : 188 (1968).

17. SCHIFF, P., SUTHERLAND, S.K. and LANE, W.R. : The preparation, testing and properties of human gammaglobulin for intravenous administration. Aust. paediatr. J. 4 : 121 (1968).

18. SGOURIS, J.T. and MATZ, M.J. : Observations on the fragmentation of venous and placental immune serum globulin. Vox. Sang. 13 : 59 (1967).

19. SIMONS, M.J., SCHUMACHER, M.J and FOWLER, R. : Intravenous gam-

maglobulin therapy of immunoglobulin deficiency diseases. Aust. paediatr. J. 4 : 127 (1968).

20. SIMONS, M.J. : Trends in immunological therapy. Ann. Gen. Pract. Submit. 36-41 (1971).

21. SOOTHILL, J.F. : The concentration of gammaglobulin and gammamacroglobulin in the serum of patients with hypogammaglobulinaemia. Proc. roy. Soc. Med. 55 : 395 (1962).

22. STIEHM, E.R., VAERMAN, J.P. and FUDENBERG, H.H. : Plasma infusions in immunologic deficiency states: metabolic and therapeutic studies. Blood 28 : 918 (1966).

23. STIEHM, E.R. and FUDENBERG, H.H. : Clinical and immunologic features of dysgammaglobulinemia type I. Report of a case diagnosed in the first year of life. Am. J. Med. 40 : 805 (1966).

24. STOELINGA, G.B.A. Päd. u. Pädologi 4 : 77 (1968), cited by Marder, E.

25. UHR, J.W. and MULLER, G.: Regulatory effect of antibiody on the immuneresponse. Advances in Immunol. 8 : 81 (1968).

26. WELLS, J.V. and PENNY, R. : Survival studies on a commercial preparation of intravenous human gammaglobulin labelled with I^{131}. Austr. Ann. Med. 18 : 271 (1969).