Intravenous Immunoglobulins in the Third Millennium

Edited by

Marinos C. Dalakas and Peter J. Späth

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Forword

This book of proceedings is the most recent in the series of proceedings of international symposia on the use of intravenous immunoglobulin (IVIG). The first of these symposia was held in 1981 and the most recent one from 25 to 27 September, 2003. The venue of all symposia has traditionally been Interlaken, Switzerland, a beautiful location in the heart of the Swiss Alps.

The 2003 Interlaken Symposium continued the unparalleled tradition of offering the most important forum in communicating the innovative uses of IVIG in various diseases and exchanging scientific data among clinicians/scientists from all disciplines. The meeting also coincided with the 60th anniversary of industrial plasma fractionation. It was in the first quarter of 1943 that fractionation of human albumin was transferred from Cohn's laboratory in Boston to various manufacturers in the USA.

The Scientific Committee of the Interlaken Symposium proudly offers this book to the ever-growing community of colleagues inter ested in IVIG. This community is spreading further around the globe, as is evident from the countries of the scientists who travelled to Interlaken.

This volume reviews the major challenges and achievements in manufacturing, pathogen safety, clinical use and mechanisms of action of IVIG and highlights the major recent and ongoing research in the field. As a result, this book is a comprehensive update that we anticipate will become a valuable source of information for all those interested in IVIG.

A tremendous progress in understanding primary immune deficiencies (PID) has been made since the last Interlaken Symposium. Developments in molecular biology have strengthened our understanding of differentiation and maturation of B cells and the bio-synthesis of immunoglobulins, and have identified a number of genetic defects as the basis of primary immune deficiencies. Although such progress in molecular medicine seems to over-shadow the spectrum of therapeutic options, IVIG remains a fundamental therapy. In the IVIG remains the fundamental therapy. In the present meeting, the discussions on the doses applied and the pros and cons of subcutaneous or intravenous administration of immunoglobulins continued the lively debate that began in the first symposium, in 1981.

The clinical use of IVIG based on its immunomodulatory and anti-inflammatory potential, remains an ongoing challenge. The role of Fcγ receptors or the receptors responsible for the long half-life of immunoglobulin G (IgG) in immunomodulation and anti-inflammatory effects were one of the main topics of the symposium.

The progress made since 1996 in establishing efficacy based on controlled clinical trials on the treatment of various autoimmune and inflammatory neuromuscular and central nervous system diseases was extensively discussed and included in the book. In neurology, probably more than in any other specialty, IVIG has changed the way autoimmune neuromuscular disorders can be treated.

The new methodologies applied in conducting clinical trials in a 'bench-to-bedside' fashion, and the emerging field of pharmacoeconomics have also been addressed and the respective reports are included in the volume.

The 5th International Symposium on the use of IVIG was the first held at the dawn of a new century and a new millennium. An outlook to the near future has indicated a number of new indications emerging, in particular in dermatology. Also of huge interest is the possible role of IVIG in chronic inflammatory conditions, particularly neurodegenerative disorders, such as macular degeneration, atherosclerosis, cardiomyopathy or even Alzheimer's disease.

We would like to thank the members of the faculty for their contribution to the success of the 5th International Symposium on IVIG and to the fifth volume of the 'white books of Interlaken'. We hope this book will be received as a valuable update on IVIG therapy like the former books. We wish to express our gratitude to the management of ZLB Bioplasma AG, Bern for the generous support of the Symposium. Our particular thanks go to Pam Lancaster from Parthenon Publishing, whose effort and support made it possible to publish this Book of Proceedings in such a short time.

Marinos C.Dalakas, MD
and Peter J.Späth, PhD

Two decades of experience with intravenous immunoglobulin

1
Manufacturing of intravenous immunoglobulin: challenges, achievements and opportunities

A.morell

INTRODUCTION

In many respects, intravenous immunoglobulins (IVIGs) are special products. In addition to their original indication, i.e. antibody replacement therapy in patients with immune deficiencies, a range of applications have been identified during the past 20 years. Nobody could have dreamed of this development and of the growing importance of IVIG in autoimmune and chronic inflammatory conditions. The observation of immunomodulating effects of IVIG in these diseases has significantly contributed to our understanding of immune mechanisms. Concomitantly, the increasing knowledge of basic immunology has had an influence on the development of IVIG products. This chapter highlights some of the challenges, achievements and opportunities that have been met by manufacturers of IVIG.

PATHOGEN SAFETY

A challenge of the uppermost importance is pathogen safety of IVIG. In addition to the classic viruses associated with plasma products, Creutzfeld—Jakob disease (CJD), variant CJD (vCJD), West Nile virus and severe acute respiratory syndrome (SARS) have recently emerged as infectious pathogens, and others are likely to follow.

The deferral of blood donors with infection risks and the continuously improved testing of blood or plasma donations for viral markers have greatly increased the safety of plasma products including IVIG[1]. In addition, traditional purification technologies based on cold ethanol fractionation methods have been shown to reduce a viral load still potentially present in the plasma pool[2,3]. However, in the early 1990s, there was an outbreak of hepatitis C caused by IVIG which involved more than 200 patients in the USA and in European countries[4,5]. There is evidence that elimination of anti-hepatitis C virus (HCV)-positive plasma donations, after the introduction of anti-HCV screening, may have contributed to this outbreak. Importantly, however, there were no appropriate virus-inactivating steps included in the manufacturing process of the IVIG products involved in HCV transmission. In the meantime, the integration of validated pathogen inactivation and elimination steps has resulted in plasma-derived products with excellent safety records with respect to the major blood-borne viral pathogens, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and HCV[6]. Validation of

manufacturing processes for their capacity to inactivate and/or remove a representative assortment of other enveloped and non-enveloped viruses has provided evidence that plasma products would also very likely be safe from many other potential viral pathogens with similar etiologies such as the West Nile virus and the SARS virus.

Indeed, three fundamental mechanisms can be applied to eliminating pathogens in the manufacturing steps of plasma products[6]:

(1) Partitioning during the purification process of IVIG (e.g. precipitation during fractionation and elimination from fractions used for product);
(2) Inactivation (e.g. solvent/detergent treatment, caprylic acid treatment, low pH incubation, pasteurization);
(3) Nanofiltration (specifically designed to filter out virus particles).

Manufacturers aim to build in redundancy into processes, and ideally at least two virus elimination steps with at least one of these effective against both lipid and non-lipid enveloped viruses should be included in processes.

The emergence of viral filtration technology adds another dimension of safety to plasma products. Just as sterile filtration technology revolutionized the manufacture of pharmaceuticals, viral filtration using filter pores three orders of magnitude smaller than sterile filters, provides a simple and non-invasive method for removing viruses and helps to ensure safety from both known and emerging pathogens[7]. The technology has been widely applied to low-volume plasma products with molecular weights of less than 100 kDa, such as factor IX concentrates. Immunoglobulin G (IgG) by contrast is a larger molecule (150 kDa) and is produced at large volumes, which add complexity to the application of this nanofiltration technology[8,9]. Nevertheless, it has been successfully introduced at ZLB Bioplasma AG, Bern, Switzerland in the past 3 years and now all ZLB intravenous immunoglobulins utilize nanofiltration technology.

Nanofiltration has also been shown to be effective at removing infectious transmissible spongiform encephalopathy agents in animal models which are thought to be related to the causative agent of vCJD[10]. There, however, still remains no evidence of transmission of vCJD by whole blood transfusion, cellular components or plasma products in humans.

In conclusion, IVIG products on the market today can be regarded as virally safe. This is without doubt a major achievement.

TOLERABILITY AND FUNCTIONAL ASPECTS OF INTRAVENOUS IMMUNOGLOBULIN

Another crucial topic is the tolerability of IVIG products. In the 1960s, clinicians knew that, to stay free of infections, patients with primary immune deficiencies needed regular immunoglobulin G (IgG) replacement therapy. However, the available IgG preparations had to be given as intramuscular injections, a procedure that was painful and often accompanied by severe reactions. Thus, manufacturers of plasma products faced the challenge of developing products for intravenous use that could be administered in the amounts needed without causing problems. A way to achieve this goal was modification of the IgG molecule. Protagonist products that appeared in the 1960s and 1970s were

partially digested by proteolytic enzymes, or chemically modified, e.g. β-propiolacton-treated, S-sulfonated or reduced/alkylated. However, with increasing knowledge about structure—function relationships of the IgG molecule, immunologists realized that intravenous tolerance of IVIG achieved by structural modifications of the IgG molecule was accompanied by a substantial loss of biological activity. Consequently, the new challenge was to design a well-tolerated IVIG that retained the full activity of the IgG molecule, in terms of both antigen binding and triggering of biological amplification mechanisms. To the best of the author's knowledge, this was first achieved in 1979 by ZLB's IVIG. As a consequence of a World Health Organization (WHO) meeting on IVIG in Geneva in 1982, a new generation of products consisting of functionally intact IgG molecules were developed[11].

Today, most IVIG products are well tolerated and meet functional requirements. However, adverse experiences, often due to inherent properties of IgG molecules and their interactions with host cells and biological structures, may still occur. In general, these reactions are associated with high-dose IVIG therapy. Twenty years ago, adult patients with primary immune deficiencies were treated with monthly infusions of 6–12 g of a 3–6% IVIG product, corresponding to approximately 100–150mg/kg. Subsequently, IVIG regimens have increased up to 800mg/kg in these patients, and up to 2g/kg or even more for immune modulation in patients with autoimmune or chronic inflammatory disorders. Usually, such high doses of IVIG are administered within a few hours by rapid infusion of concentrated products. Not surprisingly, some adverse experiences that were unknown in the early days of IVIG have been noted[12,13]. Although these events are generally rare, they remain a challenge for clinicians and manufacturers.

INTRAVENOUS IMMUNOGLOBULIN FORMULATIONS

IVIG products on the market today come in two formats, i.e. lyophilized or liquid. The benefits of a lyophilized product are the long-term room temperature stability and the flexibility of the product concentration after reconstitution. The benefit of a liquid product is ease of use.

In general, liquid products are formulated with stabilizers and low pH (4.2–5.5) to limit or prevent formation of IgG dimers and aggregates on storage. Low pH formulations (around pH 4) are very stable with respect to polymer content, but more vulnerable to fragmentation by acid hydrolysis, particularly at room temperature storage. Stability in relation to dimers and larger polymers becomes more difficult the higher the protein concentration and the higher the pH are. The selection of stabilizers to prevent polymer formation becomes very important for liquid products with higher pH and high protein concentration.

Liquid products currently come as 5% or 10% solutions. In 2004, ZLB will be introducing a 12%, nanofiltered, liquid product (M. Borte, submitted for publication). The benefits of higher protein concentration are a reduced fluid intake and the potential to shorten infusion times. Infusion rates need to be controlled with due consideration of safety as adverse event rates are known to increase in all products with higher infusion rates

DEVELOPMENTS IN MANUFACTURING PROCESSES

The increasing number of patients treated with escalating doses of IVIG, the restricted availability of product and rising costs of plasma, pose new challenges to the manufacturers. IVIG has become the most important plasma product and drives the demand for plasma within the fractionation industry. The quantity of IVIG distributed per year worldwide grew to 47 tons by the year 2000, and is increasing today at an annual rate of 5% in Europe and 11 % in the USA. This corresponds to a plasma supply of more than 12 million liters. For manufacturers it is vital to get the highest possible yield of IVIG out of this expensive and valuable raw material. The classical cold ethanol fractionation method developed by E. Cohn in the 1940s is a relatively low-yielding process and captures about 30% of the total IgG in plasma. The addition of further purification steps results in further yield reductions. The yield obtained with the Kistler—Nitschmann procedure used at ZLB is in the order of 50% of total IgG. Newly developed chromatographic procedures, such as the process at CSL Melbourne, and the recent Bayer process, result in further yield improvements. However, the importance of yield should not be overlooked in terms of having a full antibody spectrum, as it is possible that by chromatography selective removal of some antibody specificities occurs.

ANTIBODY CONTENT OF INTRAVENOUS IMMUNOGLOBULIN

A last point relates to the antibody content of IVIG products. As a derivative from plasma of thousands of healthy donors, IVIG probably contains more than 10^6 different antibody specificities covering the entire range of infectious pathogens to which the donor population has been exposed. Clinical studies showed that IVIG conveys humoral immunity to patients with immune deficiencies who cannot mount an appropriate antibody response to invading pathogens. A number of clinically relevant antibodies against bacterial toxins, common pyogenic micro-organisms and viral pathogens have been identified, and most manufacturers provide some data on the content of these antibodies in their products[14]. This information is useful, although a comparison between products is hampered by differences in analytical methods and by batch-to-batch variability. However, well over 50% of all IVIG recipients suffer from autoimmune or chronic inflammatory disorders and need IVIG for immunomodulation, e.g. antibodies against inflammatory cytokines (antitumor necrosis factor-α), cellular receptors (anti-Fas), human leukocyte antigens (anti-HLA), as well as anti-idiotypic antibodies against a repertoire of common idiotypes[15,16]. We still know very little about the representation of such antibodies in IVIG, and there exist no validated methods for their determination. It remains a challenge for scientists and manu facturers to develop and validate appropriate techniques. The concept of immune modulation by IVIG could gain further credibility by a better definition of relevant antibodies and their contribution to the clinical benefit in debilitating diseases including Guillain-Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy and multiple sclerosis.

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2
Challenges and achievements in pathogen safety of intravenous immunoglobulin

P.J.Späth and C.Kempf

CHALLENGES

Today, intravenous immunoglobulin (IVIG) preparations produced under strict adherence to 'good manufacturing practice' (GMP) can be considered to provide very low risk for transmission of blood-borne pathogens. However, the above statement can only be made today because of considerable efforts by industry and authorities over the past 20 years. The pathogens that are relevant in the context of IVIG safety are those that can—at least theoretically—be transmitted by transfusions (Table 1).

Emergence of new (or previously unrecognized) viruses, or the spreading of known viruses to continents not affected before, has been a threat to the safety of IVIG over the past 20 years. These viruses include human immunodeficiency virus (HIV; acquired immune deficiency syndrome (AIDS)), Ebola (-like), Lassa, the Hanta family (Hantaan), avian flu H5N1, West Nile and, most recently, the severe acute respiratory syndrome corona virus (SARS-CoV) and monkey pox. There is evidence that the majority, if not all of them, were transmitted to humans from animals such as great apes or macaques (Ebola, HIV), rodents (Lassa, Hanta, monkey pox), poultry (H5N1), birds (West Nile) and animal food stock (SARS-CoV)[1–18]. For some of these pathogens mosquitoes are the vectors of transmittance[19].

Probably the most unexpected transmission of an animal pathogen to humans occurred when the agent of bovine spongiform encephalopathy (BSE, mad cow disease) crossed the species barrier and mediated human variant Creutzfeldt-Jakob disease (vCJD). vCJD belongs to a group of neurological diseases collectively called transmissible spongiform encephalopathies (TSEs) or prion diseases[20,21]. The suspected agents of TSEs, which have non-virus and non-bacterial characteristics, are prions. The prion protein 'scrapie' (prp^{sc}) is an isoform of the ubiquitous cellular PrP (PrP^{c})[22]. The basis for the crossing of the species barrier was the BSE epidemic in

Table 1 Some human pathogens posing a confirmed or theoretical risk of transmission by blood and plasma products

Transfusion an ascertained route of transmission	Transfusion an ascertained route of transmission	No indication for transmission by transfusion	Transfusion might represent a theoretical risk for

with possible severe clinical consequences	with no known clinical consequences		transmission
Hepatitis B virus	Hepatitis D virus	TSE agent of classical/sporadic CJD	TSE agent of the variant form of CJD
Hepatitis C virus	Hepatitis E virus		
HIV 1/2	Hepatitis F virus		
HTLV I/II	GBV-C/hepatitis G virus		
West Nile virus	SEN virus		
Hepatitis A virus	TT virus		
Parvovirus B19			

CJD, Creutzfeldt—Jakob disease; TSE, transmissible spongiform encephalopathy; HIV, human immunodeficiency virus; HTLV, human T-cell leukemia virus

cattle[23], which most likely had its roots in the widespread use of contaminated meat and bone meal[24]. It is still uncertain how the BSE agent crossed the species barrier ruminants/humans[25–27] and whether PrP^{Sc} is the (sole) cause of the clinical manifestation of vCJD[28–30].

Until the early 1980s cold-ethanol fractionation was believed to be sufficient to guarantee the viral safety of IVIG. Several reports on transmission of non-A, non-B hepatitis by licensed products challenged this belief[31–33]. It was a particular shock to patients, clinicians and manufacturers of IVIG when there were confirmed transmissions of hepatitis C virus (HCV) to a considerable number of primary immune deficient (PID) patients by two different IVIG preparations in the early 1990s[34–37].

Manufacturers were further challenged by reports of transmission by IVIG of other than non-A, non-B or HCV viruses. Most of these reports could, however, not be confirmed. For confirmation of a pathogen transmission by IVIG the following points must be fulfilled: (1) several patients infected by (2) the same lot(s) in (3) hospital having infused the corresponding lot(s), and (4) in the accused lots, the same pathogen as found in the diseased patients, is demonstrated.

In this short review on pathogen safety of intravenous immune globulins we will describe the measures taken to overcome these challenges and to achieve blood and plasma products with a maximized safety with regard to disease transmission. Furthermore, we will try to evaluate how these measures may affect the safety of IVIG with regard to the potential transmission of new emerging pathogens.

ACHIEVEMENTS IN REDUCTION OF POSSIBLE VIRUS LOAD OF IVIG

The various problems with pathogen safety of blood and plasma products have led the authorities to adapt over time, and rigidly enforce, safety recommendations. In addition, industry has voluntarily improved some of their standards. These achievements resulted in IVIGs with a high safety profile. According to these recommendations and standards, the current mainstays of pathogen safety of plasma products are:

(1) Donor selection in order to prevent donations by individuals at risk;
(2) Screening of donations in order to exclude potentially infectious donations;
(3) In-process controls in order to withhold a positive pool from fractionation;
(4) Validated steps for elimination and/or inactivation of potentially present infectious agents;
(5) Equipment cleaning and batch-to-batch segregation;
(6) Traceability of lots;
(7) Strict compliance with current GMP and quality assurance.

Achievements in reduction of pathogen load of the starting material

Donor selection

The main achievements in donor selection are donor education, introduction and continuous adaptation of donor questionnaires, physical examination of the donor and the confidential self-exclusion of donated blood/plasma from further processing. There are two types of plasma donors, those donating whole blood without remuneration (recovered plasma) and those being remunerated for donating plasma-pheresis plasma (source plasma). Safety concerns emerged for source plasma when studies performed in the 1980s and early 1990s showed that recovered plasma has less virus burden. The gap in pathogen load between recovered and source plasma was closed by the voluntary introduction of the International Qualified Plasma Program (IQPP) by the plasma collecting industry. This program comprises:

(1) The ‘Qualified donor standard’, which assures that plasma is processed only when the donor has donated again within 6 months and the second donation was negative for risk markers;
(2) The ‘Community-based donor population’ which assures the donor has a home and is living within a radius of 150 km to the plasmapheresis center (to avoid a high-risk donor ‘tourism’);
(3) The ‘Drug abuse screening’;
(4) The ‘National Donor Deferral Registry’ which assures that a donor rejected by one center is not able to give blood in another.

Mass screening of donations

Advances in screening are due to the application of new technologies and increasing scientific knowledge. The mandatory testing for liver enzymes in blood/plasma was complemented by the introduction of antibody testing for virus markers such as HIV 1/2 and HCV and screening for hepatitis B surface antigen (HBsAg). In the late 1990s nucleic acid testing (NAT) for HIV-RNA and HCV-RNA was introduced in order to minimize the so-called window period. This is the period after infection, when the agent is already present in the donors' plasma, but the screening assay is still negative, e.g. becuase anti-bodies have not yet been formed. Nowadays, voluntary introduction of NAT for HBV-DNA, HAV-RNA and parvovirus B19 DNA is in progress. Within the frame of the IQPP, remunerated source plasma is subject to the '60-day inventory hold', i.e. the plasma is withheld for 60 days and the donation is destroyed if, in a subsequent donation, the donor has turned positive for an infectious marker, suggesting the possibility of a window donation. Due to the above-mentioned measures, the quality of source plasma has reached the same level of safety as recovered, non-remunerated plasma[38,39]. This guarantees that large volumes of plasma with a high safety level are available for fractionation.

In-process controls

These are performed on a plasma pool ready for fractionation. The following tests are mandatory (in European countries): anti-HIV 1/2, anti-HCV, HBsAg, HCV-RNA. NAT for HIV-RNA and HBV-DNA are performed voluntarily by a number of manufacturers of IVIG.

Achievements in virus elimination and/or inactivation during the manufacturing process

The validation of virus elimination and/or inactivation steps in a manufacturing process is now required by health authorities. This has imposed a major effort on the plasma fractionation industry. In validation studies, the safety of a product has to be demonstrated on a laboratory scale by spiking starting materials and showing the elimination of the virus over the various steps of the process. The viruses used should resemble the virus potentially present in plasma and they should represent a wide range of physicochemical properties. However, the use of model viruses (as opposed to the actual virus infecting humans) is often necessary, as not all viruses can be cultured and assayed conveniently.

In addition, IVIG contains neutralizing antibodies to many human pathogens; therefore, it is often not possible to use the human virus. It is assumed by authorities that the application of different basic principles of virus removal and inactivation during fractionation and polishing makes a product pathogen safe. There are only three basic principles of virus elimination:

(1) Partitioning;
(2) Inactivation;

(3) Elimination based on size.

All manufacturers use partitioning to eliminate viruses, i.e. separation of viruses from the product during purification (Table 2). The methods of virus inactivation used are:

(1) Solvent/detergent treatment disrupting the viral envelope, a process which might

Table 2 Selected examples of published minimal logarithmic reduction factors (LRFs) obtained by partitioning during the production of six different intravenous immunoglobulin (IVIG) products in studies validating partitioning*

Product	HIV	HCV models	Large DNA	HAV models	B19 models
A	15.5	9.3	16.0	14.1	n.a.
B	14.6	1.3	8.2	6.7	7.4
C	11.2	3.2	7.9	8.3	9.2
D	10.1	3.5	5.3	n.a.	n.a.
E	13	3.8	4.5	8.5	8.4
F	3	6.4	3.3	3.6	6.4

*Differences in numbers are due to efficiency of methods and/or the number of steps validated
HIV, human immunodeficiency virus; HCV, hepatitis C virus; n.a., not analyzed

however fail with viruses having several layers of envelope (poxviridae);

(2) Caprylate-induced incorporation of a non-ionized molecule into the viral envelope and eventual disruption of the envelope;
(3) Disruption of the envelope by heat, i.e. pasteurization;
(4) Ionic disruption of the envelope and conformational changes of viral proteins needed for docking of virus to the target cell, e.g. by low pH treatment.

The primary target of all these inactivation methods is the viral envelope; therefore, they are mainly effective against enveloped viruses. Methods 3 and 4 also have potential for the inactivation of non-enveloped viruses. One of the key points of studies validating virus inactivation is to show kinetics of inactivation. All manufacturers of IVIG use one or more of the above virus inactivation methods (Table 3).

Nanofiltration was recently introduced into the large-scale manufacturing of IVIG, as a complement to partitioning and inactivation. The principle relies on virus size (Table 4) and is also able to remove small non-enveloped viruses under certain conditions[40].

ACHIEVEMENTS IN REDUCING THE THEORETICAL RISK OF TSE TRANSMISSION BY IVIG

TSEs of man are, among others, the classical or sporadic form of CJD and the variant form of CJD (vCJD). The agent of classical/sporadic CJD is localized predominantly in nerve tissues and transmission by blood or blood/plasma products could never been substantiated in humans[41–43]. There is no indication of hematological spread of PrP^{Sc} in humans[44]. The fear of a theoretical risk of transmission of vCJD is based on the fact that, in vCJD patients, PrP^{Sc} is found in abundance in nervous, as well as in

Table 3 Selected examples of published minimal logarithmic reduction factors (LFRs) obtained by different virus inactivation methods used in the production of six different intravenous immunoglobulin (IVIG) products in validation studies for virus inactivation

Product	HIV	HCV models	Large DNA	HAV models	B19 models
A	6.1	4.4	5.3	n.a.	4.7
B	3.7	4.9	4.1	n.a.	n.a.
C	9.7	8.6	10.7	n.a.	n.a.
D	5.4	6.4	3.6	4.5	n.a.
E	6	5.5	2.5	n.a.	n.a.
F	11	8.0	8.9	n.a.	n.a.

HIV, human immunodeficiency virus; HCV, hepatitis C virus; n.a., not analyzed

Table 4 Average size of various viruses

Virus family	Size (nm)	Members (examples)
Parvoviridae	18–25	parvovirus B19, bovine parvovirus, canine parvovirus, minute virus of mice
Picornaviridae	~30	hepatitis A, polio, bovine enterovirus
Flaviviridae	40–60	bovine viral diarrhea, hepatitis C, West Nile, dengue
Togaviridae	50–70	Venezuelan equine encephalitis, sindbis, rubella
Retrovirdae	80–130	HIV-1, HIV-2,

		HTLV-1
Herpesviridae	100–180	herpes type 1 and 2, HHV-8, varicella, pseudorabies
Coronaviridae	120–160	human coronavirus 229E, SARS
Poxviridae	~250×350	vaccinia, cow pox, smallpox, monkey pox

HIV, human immunodeficiency virus; HTLV, human T-cell leukemia virus; HHV, human herpes virus; SARS, severe acute respiratory syndrome

Table 5 Reduction of virus load by nanofiltration. Published minimal logarithmic reduction factors (LRFs) are given

Product	HIV	HCV models	Large DNA	HAV models	B19 models
A	4.9	4.5	4.4	5.1	n.a.

lymphatic tissues[27,45–51]. However, with sensitive techniques, some PrP^{Sc} outside the nervous tissues is also eventually found in sporadic CJD patients[52].

Reduction of the theoretical risk of transmission of the TSE agent begins with the appropriate questions in the donor questionnaire. Inactivation of the TSE agent is not possible without destruction of the biological activity of the product and only the principles of partitioning and size exclusion can be applied to eliminate TSE agents during manufacturing of IVIG. According to the prion hypothesis, PrP^{Sc} is the infectious agent of TSE[53]. In the laboratory PrP^{Sc} can be distinguished from PrP^{c} by proteinase K (PK) digestion[54]. PrP^{Sc} becomes truncated to the PK-resistant form PrP^{res}, while PrP^{c} is completely degraded. PrP^{res} can be used as a marker for TSE infectivity[55–58], although this is not universally accepted[59–61]. Therefore, in studies on the removal of TSE agents during manufacturing of IVIG, the not very sensitive detection of PrP^{res} is often supplemented by assessment of infectivity titers in various animal models. Studies by various groups have shown that the cold-ethonol fractionation methods according to Cohn and according to Kistler—Nitschmann have a similar potential for reductions of PrP^{res} and TSE infectivity[57,62–65]. The overall reduction during fractionation and filtration processes can be as high as >9 log. Nanofiltration might further reduce the risk of transmission of the vCJD agent by IVIG. Furthermore, nanofiltration has the potential to eliminate TSE agents as demonstrated by model experiments utilizing brain-derived infectivity[65].

ACHIEVEMENTS IN CLEANING AND BATCH-TO-BATCH SEGREGATION

Cleaning the fractionation plant equipment which has contact with plasma is mandatory to avoid cross-contamination of batches by potentially present pathogens. Non-enveloped viruses and, in particular, TSE agents are the main challenge for manufacturers. Cleaning usually encompasses extensive rinsing with water, sanitizing with, for example, sodium hydroxide and subsequent extensive rinsing with water. Sodium hydroxide was shown to inactivate minute virus of mice (MVM), a model for human parvovirus B19[66]. In endpoint measurements, the loss of infectivity of TSE agents after treatment by sodium hydroxide or hypochlorite paralleled the loss of PK resistance[67–69]. The kinetics of inactivation have been studied only recently. It was shown that sodium hydroxide even at concentrations as low as 0.1mol/l is highly efficient in rendering PrP^{res} PK-sensitive in solution and also when it is adhered to steel[70].

TRACEABILITY OF BATCHES AND QUALITY ASSURANCE

IVIG manufacturers assure traceability from each blood donor and his donation to a given batch of IVIG and to the hospital where it is delivered. It is the responsibility of the hospital to document the traceability of each IVIG batch to the individual patients. The basis of traceability is a bar code identification system which was developed during the past 20 years. Traceability includes donor identification, identification of the donated blood and plasma and the link of the donor and his donation to mass-screening test results. A proper traceability system ensures that each lot can be recalled, should this be necessary. It further allows the handling of post-donation information and pharmacovigilance quickly.

Quality of manufacturing is enforced by strict adherence to current GMP rules, GMP being a system of quality measures introduced in the past 20 years[38]. Finally, the quality of a product in its daily application is ascertained by post-marketing surveillance and proper pharamcovigilance.

CONCLUSIONS

Over the past 20 years a tremendous effort by the authorities and manufacturers has improved the safety of IVIG with regard to potential disease transmission to a very high level; the risks of transmission of pathogens is low and this is acknowledged by the authorities. A condition is, however, that manufacturers strictly adhere to the rules and recommendations elaborated by the authorities.

The question emerges as to how well we are prepared for new emerging viruses. On the one hand it is not possible to answer this question definitively. On the other hand, experience with West Nile virus showed almost identical results as obtained with BVDV thus confirming the model virus approach. However, in some cases, e.g. for inactivation

or partitioning processes, it might be difficult to draw firm conclusions from model viruses. Parvovirus B19, for example, turned out to be much more sensitive to heat treatment compared with previously used model viruses such as porcine parvovirus or minute virus of mice. In contrast, extrapolation from model virus results obtained with nanofiltration will most probably be reliable since virus elimination is solely based on size and not on physicochemical parameters that govern partitioning and inactivation.

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3
Treatments modalities and clinical efficacy of replacement therapies in primary immunodeficiency

A.D.B.Webster

HISTORICAL PERSPECTIVE

Following the pioneering work by Barandun at the ZLB Central Laboratory in Berne on the formulation of immunoglobulin suitable for intravenous infusion (IVIG)[1], the first substantial clinical studies of IVIG were reported in the early 1980s. Patients with severe anti-body deficiency had previously relied on regular plasma infusions or intramuscular injections of pooled immunoglobulin (IMIG), with a strong clinical impression that this replacement therapy reduced the incidence of infection. Many immunologists had assumed that only small amounts of specific antibody were necessary to protect against infection, but it soon became clear that higher doses reduced the infection rate. The first indication of this came from the Medical Research Council (MRC) trial in the late 1960s where two doses of intramuscular immunoglobulin were compared, with a significant reduction in signs of infections with the higher dose (50mg/kg/week)[2]. It was not practicable to increase the amount of IMIG any further because of the discomfort and frequency of reactions after injection. Nevertheless, there was a growing consensus that higher doses given intravenously would be beneficial, and subsequent trials comparing IVIG with IMIG supported this view (reviewed in reference [3]).

The main purpose of immunoglobulin replacement therapy is to prevent infections. Patients with primary antibody deficiency are prone to infection with a limited number of organisms, in contrast to patients with severe combined immunodeficiency whose lives are constantly threatened by many human pathogens and opportunistic infections. The study of patients with X-linked agammaglobulinemia (XLA), who have normal T-cell and innate immune function, has provided useful information on the role of antibodies in the protection against infection[4].

Table 1 Typical infections in primary antibody deficiency

Microbe	Clinical presentation
Pneumococci	pneumonia/septicemia/meningitis*
Haemophilus influenzae type B	pharyngitis/septicemia/meningitis*
Meningococci	meningitis/septicemia*

H. influenzae (non-typeable)	bronchitis/sinusitis/otitis media†
Streptococci	bronchitis/sinusitis/otitis media†
Moraxella catarrhalis	bronchitis†
Mycoplasmas	arthritis/urethritis/otitis media**
Enteroviruses	meningoencephalitis/myositis **
Campylobacter pylori	enteritis**
Giardia lamblia	enteritis**

Maintaining trough IgG level at ~7g/l: *prevents systemic spread of infection; **provides partial protection; †provides poor protection—many patients require prophylactic antibiotics

INFECTIONS IN PATIENTS WITH PRIMARY ANTIBODY DEFICIENCY

Antibodies are important for protection against encapsulated bacteria, with pneumococcal septicemia a common cause of death in primary antibody deficiency (PAD) patients before immunoglobulin prophylaxis was available. However, the majority of PAD patients suffer from more insidious infection in the respiratory tract, most patients presenting with symptoms of chronic sinusitis and bronchitis, the latter often leading to bronchiectasis. The organisms most commonly involved are non-encapsulated *Haemophilus influenzae, Moraxella catarrhalis* and pneumococci. Patients are also susceptible to *Giardia* and *Campylobacter* infection in the bowel, particularly those living in unhygienic conditions. A minority of patients (about 10%) develop chronic enteroviral or mycoplasmal infection, the former causing chronic meningoencephalitis and the latter chronic arthritis, cystitis/urethritis and occasionally deep abscesses. This susceptibility to a relatively limited group of pathogens is characteristic of patients with XLA (Table 1). Other more complex conditions (e.g. X-linked hyper-Ig M syndrome) have additional cellular defects, which explain the predisposition to opportunistic infections such as cryptosporidia.

EFFICACY OF IMMUNOGLOBULIN PROPHYLAXIS

This discussion focuses on the use of IVIG prophylaxis, since with this method the serum levels of IgG can be increased to within or above the normal range. There are no published properly conducted trials comparing patients with or without immunoglobulin prophylaxis, the evidence level for efficacy probably only reaching 4 (i.e. based on expert committee reports and/or respected authorities)[2,3,5]. However, the evidence level in favor of higher doses offering more protection against infection reaches 1b (i.e. one good-quality randomized adequately powered controlled trial[2]). There is no published meta-analysis or Cochrane Review of trials. Although it may seem self-evident that replacing antibodies in PAD patients is beneficial, it is doubtful whether the Food and Drug Administration (FDA) would have granted a license for IVIG if presented today with the available evidence of efficacy.

Table 2 Serum immunoglobulin G (IgG) at diagnosis of enteroviral or mycoplasma disease. The patients with primary antibody deficiency were either on intravenous immunoglobulins or intramuscular immunoglobulin prophylaxis. Values shown are mean plus range[7,8]

Infection	Number of patients	IgG *(mg/dl)*
Enterovirus	23	303 (7–930)
Mycoplasma	12	370 (50–780)

Nevertheless, many immunologists were impressed during the 1980s by the reduction in morbidity and mortality after the introduction of IVIG prophylaxis. The numbers of PAD patients referred to a tertiary UK center for the diagnosis and management of either enteroviral or mycoplasmal infection have declined rapidly over the past 15 years (Webster, unpublished data), during a period when most UK clinics were developing protocols to maintain IgG trough levels above 7.0g/l. In addition, manufacturers have been increasing the numbers of donors for individual batches of immunoglobulin, with current numbers being about 10 000 compared with <5000 during the 1980s. There is evidence of a wide variation in levels of antibodies to specific enteroviruses in smaller donor pools[6], so in the past it was a matter of chance whether a patient had recently received a batch with a high enough level of antibody to protect against enteroviral strains prevalent in the community. Furthermore, surveys of enteroviral and mycoplasmal infection in PAD patients show that affected patients usually have low IgG levels at diagnosis of infection (Table 2), implying that their immunoglobulin prophylaxis has not been adequate[7,8].

Although maintaining IgG levels within the normal range appears to protect against some infections, the effect on the frequency of *Haemophilus mfluenzae*-associated bronchitis is limited. Our experience at the Royal Free clinic in London shows that at least 30% of PAD patients remain susceptible to *H. influenzae* colonization of the upper

and/or lower respiratory tract, despite maintaining trough IgG levels above 7g/l. Penetration into the bronchi of specific IgG antibodies in IVIG to outer membrane proteins (OMPs) of *H. influenzae* seems to be very poor. Preliminary work in our laboratory shows that specific antibodies to *H. influenzae* OMPs in pooled immunoglobulin remain in the plasma for weeks after infusion into patients with chronic *H. influenzae* bronchitis. This suggests that these antibodies, which are considered to be protective in animal models[9], do not penetrate the bronchi and bind to organisms on the mucosa. It may be possible to overcome this problem by using very high doses of IVIG, since respiratory function improved during high-dose therapy in one study[10]. Further studies are needed to test whether *H. influenzae* is eradicated by such regimens, although the expense and practicalities of long-term high-dose prophylaxis need to be considered.

The failure of standard doses of IVIG to protect against *H. influenzae* bronchitis in PAD patients has led to the greater use of prophylactic antibiotics. The quinolones are particularly active against this organism and have good tissue penetration[11], the usual minimal inhibitory concentration (MIC) being <0.05μg/ml. Furthermore, there has been little evidence of emerging resistance after prolonged use of ciprofloxacin in about 40 patients in the Royal Free (London) clinic. Although the use of long-term single-antibiotic prophylaxis is not encouraged by microbiologists, there is a strong impression that this therapy has improved the quality of life for many PAD patients.

The effect of IVIG prophylaxis on chronic sinusitis is less clear. This is a common complication of PAD[12], with *H. influenzae* often being present in aspirates from sinus cavities[13]. Severe persistent sinusitis has become a rarity in the Royal Free clinic over the past decade, during a time when many patients have been maintained on both higher doses of IVIG and ciprofloxacin. However, there is evidence of significant transfer of immunoglobulin from the plasma to the sinuses during acute infection[14], so the transfer of IgG across the mucosa in the sinuses may be more efficient than transfer across the bronchi.

KINETICS, DOSE AND FREQUENCY OF INTRAVENOUS IMMUNOGLOBULIN PROPHYLAXIS IN PRIMARY ANTIBODY DEFICIENCY

Various dose regimens for IVIG have been proposed, but most patients are currently advised to have monthly infusions of 400mg/kg body weight. Most adult UK patients are on home therapy[15], with most large cities having a clinic equipped to train patients and assistants in this procedure. A small minority of PAD patients feel better on more frequent infusions (e.g. 200mg/kg every 2 weeks) despite adequate trough IgG levels, and many patients maintain that they feel generally better for a few days after infusion. There is no scientific explanation for these effects, although IVIG has been shown to reduce the level of some inflammatory cytokines *in vitro* and *in vivo*[16].

There is variation between patients in the time it takes to reach a target level of serum IgG, determined by the speed of equilibrium between plasma and tissues and the catabolic rate of IgG[17]. Reaching equilibrium is usually achieved after four infusions over 2–4 months, but a few patients, particularly those with common variable immunodeficiency and granulomatous disease, catabolize IgG rapidly and may require

higher doses. Furthermore, a falling trough level of IgG in patients established on immunoglobulin replacement is a useful marker of chronic inflammation and should prompt a search for infection or granulomatous complications. Dose regimens should therefore be individualized to maintain a level of serum IgG in the normal range; this should be checked at least every 4 months.

SAFETY OF IMMUNOGLOBULIN PROPHYLAXIS

There were many reports of hepatitis C infection from contaminated batches of IVIG until the early 1990s, but this problem now seems to be resolved with meticulous screening of donors and eradication of these viruses during the manufacturing process. There are still concerns about transmission of prion diseases, but to date there is no evidence that this has occurred. Immediate reactions following infusion of IVIG are common, but usually mild (e.g. transient headache). A UK survey in 1995 showed only 19 reactions out of a total of 2031 infusions, 16 of these being mild[18]. This safety record has been maintained over recent years with only one cluster of moderately severe urticarial reactions associated with one UK product, apparently due to trace contaminants in the product following a temporary failure of quality control. The rarity of reactions during home therapy has opened a debate on the necessity for patients to be trained to use adrenalin for severe reactions.

Severe reactions caused by anti-IgA anti-bodies are very rare, and there is poor correlation between the level of these antibodies and susceptibility to reactions[19]. Nevertheless, most UK centers routinely measure anti-IgA antibodies in all patients prior to the first infusion, and if present in high titers (>1000 by hemagglutination), a product with the lowest levels of IgA is chosen. However, further work is needed to reach a consensus on this issue. It is well known that PAD patients tend to have mild/moderate reactions during their first infusion, although the mechanism is not clear. For this reason many centers routinely give intravenous hydrocortisone, sometimes together with an antihistamine, prior to the first few infusions. We have found that this prevents reactions and helps the patient to feel more confident.

SUBCUTANEOUS IMMUNOGLOBULIN THERAPY

During the past decade there has been a growing enthusiasm for using regular injections of subcutaneous immunoglobulin (SCIG)[20]. There is evidence that this provides a more consistent plasma level of IgG[20,21], although it is more inconvenient in that the patient usually has to infuse every week, rather than every 4 weeks with IVIG. Nevertheless, this is now the preferred route of administration in babies and young children, and for all ages in some Scandinavian countries[22,23]. There is some evidence that patients who have repeated reactions to IVIG, whether or not associated with anti-IgA antibodies, can tolerate subcutaneous infusion, and that this method may lead to some form of ‘tolerance’ to IgA[24]. There are no clear cost savings between IVIG and SCIG, and there are additional expenses for the pumps required for SCIG, with adults often preferring to use two pumps simultaneously to reduce the infusion time. Nevertheless, SCIG is a simpler

procedure for home therapy, and could reduce the numbers of patients having infusions in hospital. We estimate in the UK that about 20% of adult PAD patients will prefer SCIG to IVIG, leaving the majority on monthly IVIG (Table 3).

Table 3 Immunoglobulin G (IgG) replacement therapy—routes of administration

	Advantages/problems
Plasma infusions	donors require regular viral screening, limited antibody repertoire, obsolete in Western countries
Intramuscular (IMIG)	maximum tolerated dose ~25mg/kg/week; difficult to maintain trough level >7g/l; high incidence of reactions; painful injections
Intravenous (IVIG)	easy to maintain trough level >7g/l with monthly infusions; low incidence of reactions; suitable for home therapy
Subcutaneous	easy to maintain trough level >7g/l, but need weekly infusions; reactions rare; preferred route in infants and young children; IgG blood level more constant than with IVIG; suitable for home therapy; contraindicated in bleeding disorders
Inhaled/topical	experimental

LOCAL IMMUNOGLOBULIN G THERAPY

There are anecdotal reports of using immunoglobulin as eye drops for conjunctivitis and orally for gastrointestinal infections, but no trials supporting efficacy. Research now needs to focus on better ways of preventing respiratory disease in PAD patients, thus reducing dependence on antibiotics. Regular inhalation of IgG concentrate is a theoretical possibility now that hand-held machines are available to aerosol proteins. Preliminary studies with one product at the Royal Free clinic (London) have shown that this is feasible and safe when using an 8% IgG concentrate; formal trials are being planned to test whether regular inhalation prevents colonization with *H. influenzae*. If this is confirmed, then patients with PAD may look forward to a better quality of life with more independence, on a combination of daily inhalations and 2–4-weekly subcutaneous infusions.

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4
Immunomodulation by intravenous immunoglobulin: idiopathic thrombocytopenic purpura as a model

P.Imbach

DEVELOPMENT OF INTRAVENOUS IMMUNOGLOBULIN: 1962–85

Prior to 1980 the only registered indication for intravenous immunoglobulin (IVIG) was primary and secondary immunodeficiency. Since 1980, however, the treatment of idiopathic thrombocytopenic purpura (ITP) has become a model for a new biological and immunomodulatory approach in the intravenous application of human immunoglobulin G (IgG) concentrate, produced from healthy blood donors.

Early on, Silvio Barandun and his colleagues at the Institute for Clinical and Experimental Cancer Research of the University of Berne identified IgG aggregates as the main cause of side-effects of (intravenous) gammaglobulin in patients with hypogammaglobulinemia[1]. Subsequently, at the Central Laboratory of the Swiss Red Cross (CL-SRC) in Berne, the chance observation that acidification of human plasma to pH 4 during IVIG production eliminated the aggregation effects of the new product without impairing its function[2] allowed development of an IVIG product that was safe for intravenous administration. At this time, IVIG was produced by a non-profit organization without experience in professional marketing. Under the guidance of Charles Studer of Sandoz/Novartis Basel, in co-operation with CL-SRC, the professional marketing, distribution and registration of IVIG advanced over the next 20 years.

The role of IVIG as a new immunomodulatory treatment of autoimmune disorders, particularly for patients with ITP, was the result of an astute clinical observation. In 1980, at the University Children's Hospital, a boy with severe hemorrhagic chronic ITP and secondary hypogammaglobulinemia due to long-term immunosuppressive treatment received 0.4g IVIG/kg body weight and showed a dramatic increase in his thrombocyte count within 24 hours, which continued with the administration of an additional four doses of IVIG. Immediately after that anecdotal observation, a pilot study in 12 consecutive children with acute or chronic ITP and without hypogammaglobulinemia showed similar dramatic effects in response to IVIG[3], and a randomized, controlled multicenter study comparing corticosteroid with IVIG treatment confirmed the role of IVIG in ITP[4] and showed convincing evidence of efficacy of this biological treatment.

The mechanism of action of IVIG in this setting was of great interest. In addition to the increase of serum IgG levels, there was a significant increase in serum IgM, which was not present in the transfused IVIG preparation (Figure 1). This IgM increase after

IVIG infusion was felt to provide evidence of the immunomodulatory effect in patients with ITP

Over the next 4 years, IVIG was shown to have similar dramatic clinical effects in other disorders with an immunological basis, and disturbances on different levels of the immune response (Figure 2). IVIG was used in a boy with acquired hemophilia[6], for herpes zoster infection in immunocompromised children[7], in a girl with Guillain—Barré syndrome, in a patient with relapsing—remitting multiple sclerosis, in a boy with severe Stevens—Johnson syndrome and in children with Kawasaki disease in Japan[8]. Interest in the mechanisms of action of IVIG was stimulated, and intense clinical and laboratory studies resulted worldwide.

IDIOPATHIC THROMBOCYTOPENIC PURPURA AS A MODEL FOR RESEARCH OF AUTOIMMUNITY

In a recent expert meeting, results of clinical and laboratory research in childhood ITP have been discussed and will be published in a supplement to the *Journal of Pediatric*

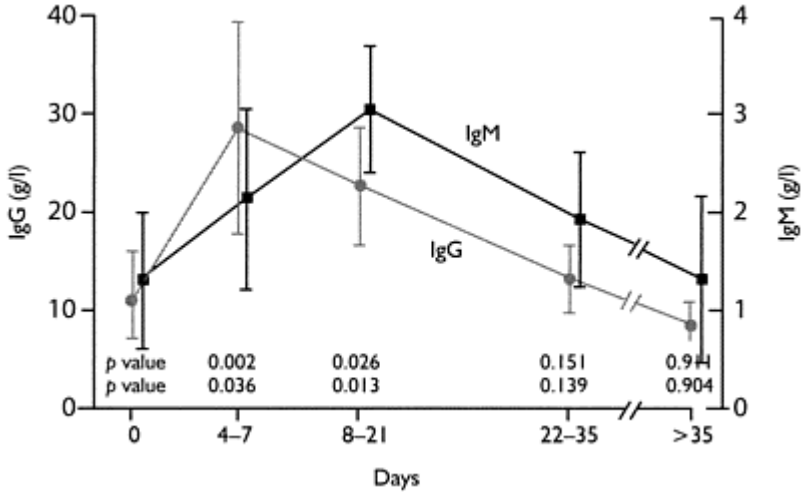
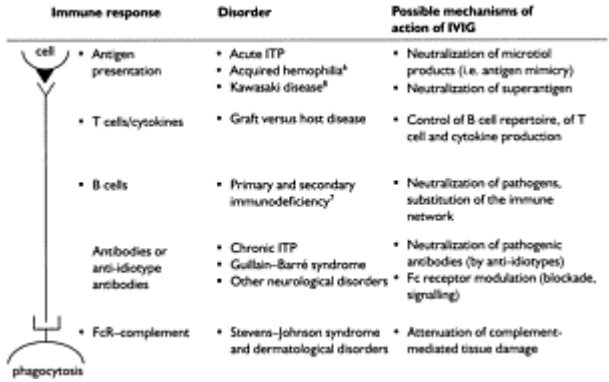


Figure 1 Variation of serum immunoglobulins (IgG and IgM) after 5×0.4g IVIG/kg body weight. Values are shown as mean±SD. *p* Values in relation to the initial value are indicated[5]

Figure 2 Cascade of immune response and possible mechanisms of action of intravenous immunoglobulin (IVIG) in different disorders. FcR, Fc receptor; ITP, idiopathic thrombocytopenic purpura



Hematology/Oncology (December 2003). The findings of the expert meeting are summarized below, with names in parentheses to indicate the first author of the respective article.

Platelet function balances between coagulant and inflammatory properties. Platelets are a heterogeneous cell population; they react for different agonists and trigger the immune response (Kekomäki).

There is much interest in the underlying cause of ITP in children, since an understanding of the pathophysiology may lead to the development of new treatments for autoimmune disorders. In ITP, the immune response and the self-tolerance mechanisms may be altered in the presence of an antigen stimulus. In acute, post-infectious ITP, for example, antigen mimicry may occur with pathogenassociated antigens which ultimately lead to tolerance to self-antigens. Acute transient ITP differs from chronic ITP in many ways, but probably including the underlying pathophysiology and immunological changes. In acute ITP, there appears to be a correlation with T-helper cells Th0 and Th2 expression and with transforming growth factor-β (TGF-β) as a potent immunsuppressive modulator among the different cytokines. Genetic factors that predispose to ITP or predict response to treatment are also of recent interest. Inherited mutations in key genes in the host response might lead to loss of tolerance or sustained production of autoantibodies in individuals with ITP. In a pilot study of children with chronic ITP, common genetic polymorphisms in two related cytokine pathways have been identified as possible genetic factors contributing to chronic disease[9] (Chanock).

In chronic ITP, antibodies are under the control of T-helper cells and related cytokines[10]. Antigen-driven clones of T cells and interleukins direct autoreactive B cells to secrete autoantibodies (Semple). Antibody targets on platelets are most commonly glycoprotein GPIIb/IIIa complex, with a variety of epitope[11] (McMillan); GPIb/IX and

rarely GPIa/IIa, IV and V have also been implicated as antibody targets. A Th1 pattern and abnormal activation of autoreactive B cells characterize the autoimmunity in chronic ITP Circulating and platelet-associated autoantibodies can be detected by capture assays (immunoblot assay and monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay). The sensitivity (positive results in patients with ITP) of the plateletassociated antibody assay is 49–66%, and the specificity (negative results in patients with non-immune thrombocytopenia) is 78–95%. Platelet-associated autoantibodies typically have a hypervariable region, with specific amino acid sequences for each individual anti-body which are foreign to the host, who starts to produce neutralizing, regulatory antibody. The patient's own anti-idiotypic antibodies or those in IVIG preparations seem to down-regulate or modulate the production of platelet antibodies[12,13].

Phagocytosis and Fcγ receptors (FcγR) may also play an important pathogenic role in ITP The low-affinity FcγIIA with the capacity for IgG1 and IgG_3 binding, and FcγIIIA which binds IgG_2, are important mediators of platelet clearance in ITP[14]. Immune complexes of anti-bodies bound to platelets are further bound to phagocytes and eliminated together with the platelet. High-affinity FcγRIII, which binds monomeric IgG, seems to play a minor role in ITP Several genetic polymorphisms exist for FcγRII and FcγRIII in humans, which may result in altered affinities for antibodies (Chanock).

The inhibitory FcγRIIB leads to co-crosslinking with B cell receptors resulting in down-modulation of phagocytosis (in Crow and Lazarus)[15].

Additional investigation of these mechanisms of antibody-mediated platelet clearance may provide opportunities for therapeutic developments in the future. Monoclonal anti-bodies against various steps of the immune response such as anti-T cell antibodies, CD20 antibody (rituximab), which binds to antibody-producing B cells, and anti-Fc receptor anti-bodies may provide specific therapies for ITP and other immune-related disorders. Rituximab has been used with some success in anecdotal reports of patients with chronic, refractory ITP or autoimmune hemolytic anemia (AIHA). Whether other monoclonal antibody treatments would need to be combined with IVIG and would have an acceptable side-effect profile remains to be tested.

CLINICAL RESEARCH IN ITP: THE INTERCONTINENTAL CHILDHOOD ITP STUDY GROUP (ICIS)

Many questions about ITP remain unanswered. The etiology, pathogenesis, clinical presentation (Kühne, Blanchette, Provan), natural history (Buchanan, Bolton-Maggs), management (Cines), quality of life and economical aspects (Klaassen, Bullinger) are not satisfactorily defined. Only a few prospective controlled clinical studies have been done[4,16–18]. Since 1992, approaches to the definition and guidelines for treatment have been published by various groups[19,20]. Ongoing surveys[21,22] and assessments[23,24] emphasize the diversity in clinical management strategies. Validation of scoring systems and guidelines would be helpful to characterize better this heterogeneous disorder.

In 1997, the Intercontinental Childhood ITP Study Group (ICIS) was organized with funds from an unrestricted grant to the author to support the formation of a network of

investigators and centers for prospective studies in childhood ITP The aim was to achieve more evidence-based data regarding the different aspects of ITR

After 6 years of experience, ICIS is well-established worldwide. The first project, Registry I, included 2031 children with newly diagnosed ITP and has been published in *The Lancet*[25]. Registry I provided important confirmatory evidence of the presenting features of ITP, and demonstrated the significant variability in initial management of children with thrombocytopenia. New findings from Registry I included the higher rate of boys versus girls (54.8% vs. 45.2%) with newly diagnosed ITP in all continents. Chronic ITP has been observed in 31 % of children overall, and in equal numbers of girls and boys. Current projects include the prospective Splenectomy Registry, which was designed to evaluate the appropriate timing and the perioperative management of children with ITP who undergo splenectomy. To date, 132 evaluable patients are enrolled, and preliminary analysis of the first 5 years of follow-up has been done[26]. Long-term follow-up after splenectomy is needed in this group of children. ICIS Registry II is an ongoing investiga tion of the frequency, location, timing and severity of bleeding in children with newly diagnosed ITR A first analysis of 531 children enrolled as of July 2003 was recently performed[27].

The current goals for ICIS include defining a long-term concept of its structure and ongoing projects. Plans include organization of an expert panel to develop new definitions of the important aspects of ITR An example that is controversial and could be considered by such an expert panel is the classification of bleeding symptoms and recommendations for management of ITP

Other issues, provided by an international expert group (see ‘Local and cultural aspects in ITP’, Imbach and colleagues) are:

(1) An accurate definition of chronic ITP, 6 months versus 1 year;
(2) The exact rate of secondary ITP and of treatment-refractory ITP;
(3) The true risk of severe bleeding;
(4) Prognostic factors (e.g. genetic polymorphisms);
(5) Cultural, environmental and economic aspects worldwide;
(6) Quality of life for patients and families;
(7) Evaluation of new treatments.

Another aim is the co-operation of pediatric and adult hematologists concerning chronic ITP Plans for a prospective database on both pediatric and adult patients with chronic ITP with long-term follow-up, the Pediatric and Adult Intercontinental Registry of Chronic ITP (PARC-ITP), are being formulated. The hypothesis of the PARC study is to validate definitions and guidelines of ITP and to find new selection criteria for future clinical trials in chronic ITP. The PARC study is designed for worldwide co-operation of investigators willing to register patients anonymously and to report data by the registry procedure similar to Registry I and Registry II[25].

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5
Witnessing the introduction and use of intravenous immunoglobulin in neurology

M.C.Dalakas

Intravenous immunoglobulin (IVIG) has had a major impact on the treatment of neurological disorders in the years since 1990. It was around that time that I became interested in IVIG but discovered that the industry had no interest in supporting clinical trials with IVIG in the field of neurology in the USA, despite the fact that a few reports had already been published from Europe on the treatment of myasthenia gravis[1,2], Guillain—Barré syndrome[3] and chronic inflammatory polyneuropathy[4]. Although IVIG was unknown to the neurology field in the USA and, in spite of difficulties, we managed to launch the first controlled clinical trials at the National Institutes of Health (NIH) in 1990. Our first report on dermatomyositis was published in 1993[5].

The situation has changed since then and we have been able to carry out a number of clinical trials. In fact, the highest number of controlled clinical trials conducted with IVIG has been with IVIG in neurology.

IVIG has been shown in controlled trials to be as good as other agents, such as plasma exchange or steroids, in the treatment of many neurological disorders. In some circumstances, IVIG has been superior to other agents and in other cases, has been shown to be better than anything else currently available. As a result, we have been able to treat patients who were untreatable before. Among the disorders on which IVIG has made a dramatic impact are dermatomyositis, Guillain—Barré syndrome, chronic inflammatory demyelinating polyneuropathy (CIDP), multifocal motor neuropathy (MMN), myasthenia gravis and stiff person syndrome.

For example, patients with dermatomyositis who had been unresponsive to other treatments and had been confined to a wheelchair have shown marked improvement in ability to walk after IVIG treatment. Improvement in skin condition has also been seen which can have a major impact on a patient's quality of life.

Other diseases poorly responding to conventional therapies in which impressive results have been obtained with IVIG treat-ment have been MMN, CIDP and stiff person syndrome.

In addition to improving patients' lifestyles and strength, much has been learned about the mechanisms of action of IVIG from these studies of neurological disorders.

Examples of the mechanisms of action of IVIG in autoimmune neuropathies can be used as a paradigm for other disorders. For example, we believe that demyelinating diseases, like many of the other autoimmune disorders, begin when tolerance is broken probably by an infection. The putative antigen is presented to the T-cell receptor by the

antigen-presenting cells which lead, in the presence of co-stimulating molecules, to clonal expansion of T cells and cytokine production of autoantibodies. Cytotoxic T cells transmigrate across the endothelial cell wall and invade the target organ (nerve axon, myelin, muscle, etc.). Macrophages and antibodies invade the myelin via the Fc receptors, where antibodies recognize antigens by fixing complement. Cytokines and chemokines facilitate this process[6]. There is evidence from various laboratories that IVIG works at a number of these levels in the hierarchy of the immune dysregulation. It can act on co-stimulatory molecules and antibodies; there is clear evidence that IVIG can modulate Fc receptors and that it inhibits complement activation in a number of conditions. Finally, it is known that a number of chemokines and cytokines are down-regulated. It has become clear that IVIG works in treating neuropathies not by one mechanism but via all the above. This is the reason why dramatic results are obtained in such a heterogeneous group of disorders. To put in the simplest way, IVIG works by restoring the immune balance.

In spite of the progress made, a number of issues remain unsettled. First, there is a clear need for clinical trials with combination therapies, which have been used successfully in other areas such as oncology, but have not so far been tried to any extent with neurological disorders.

Second, more research is needed in the field of pharmacogenomics to answer the question as to why IVIG works in some patients but not in others. We provide some evidence in this meeting (see Chapter 23) based on gene profile analysis that chemokines such as MIG were up-regulated in patients with dermatomyositis who improved after IVIG treatment but were down-regulated in those who showed no improvement. This was particularly noticeable in patients with inclusion body myositis (IBM). Another example is of intercellular adhesion molecule (ICAM-1), which was down-regulated in IBM patients whose condition improved with IVIG but remained the same in patients whose condition did not improve.

Third, it is important to find the optimal dose of IVIG for induction of a response and for maintenance of the response. Although we begin treatment with the arbitrary dose of 2g/kg, there is evidence that this may not be the ideal dose. For example, it has been reported that 2g IVIG was no better than 1g for the treatment of myasthenia gravis. There are data showing that low-dose IVIG may be effective for treatment of multiple sclerosis.

Fourth, we hope that the industry will supply us with a new generation of IVIGs that enable self-administration, and are better tolerated and have minimal side-effects. There is also a need for products tailored specifically for particular disorders.

Fifth, in the field of pharmacoeconomics, although neurology has been at the forefront, it is critical to gain information by comparing the cost of IVIG and its long-term benefits and improvements in quality of life against the cost of traditional treatments. Plasmapheresis, dialysis, immunosuppressants, long-term steroid use, the frequency of infections, the need for hospitalization or days lost from work need to be considered in comparing long-term cost. New data compiling all aspects are needed to convince regulatory bodies of the value of IVIG compared to other less expensive therapies.

Finally, there are a number of new indications emerging, in particular in dermatology and ophthalmology. Also, huge interest is emerging in the possible role of IVIG in chronic inflammatory conditions, particularly neurodegenerative disorders. Four new

such conditions being considered for treatment with IVIG are macular degeneration, atherosclerosis, cardiomyopathy and Alzheimer's disease. Consequently, I view the future of immune therapies to be very promising and envision IVIG will continue to play a basic role in our therapeutic armamentarium for patients with autoimmune neurological disorders.

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6
Pharmacoeconomics of intravenous immunoglobulin administration in neurological disorders: the US experience

P.D.Donofrio

Intravenous immunoglobulin (IVIG) has been shown in controlled studies to be useful in treating numerous neurological conditions (Table 1). Despite verification by published data, reimbursement for treatment is not guaranteed. Insurance companies consider many variables before rendering payment: Food and Drug Administration (FDA) approval, accuracy of International Classification of Diseases (ICD) coding, documentation of the diagnosis, pre-certification and site of infusion. Coverage varies widely among private insurance companies, and Medicare and Medicaid carriers.

Table 1 Neurological disorders responding to intravenous immunoglobulin (IVIG): controlled studies

Guillain—Barré syndrome
Chronic inflammatory demyelinating polyneuropathy
Myasthenia gravis
Dermatomyositis
Polymyositis
Multifocal motor neuropathy
Stiff person syndrome
Lambert—Eaton myasthenic syndrome
Relapsing and remitting multiple sclerosis

Table 2 Intravenous immunoglobulin: commonly used brands

Baxter Hyland/Immuno	Gammagard® S/D
	Iveegam®
Bayer Pharmaceutical	Gamimune® N 5%
	Gamimune® N 10%
Aventis Behring	Gammar®-P IV
ZLB Bioplasma Inc.	Carimune® (formerly

(formerly Novartis)	Sandoglobulin®)
Alpha Therapeutics	Venoglobulin®-S 5%
	Venoglobulin®-S 10%

S/D, solvent detergent

Table 3 Food and Drug Administration (FDA)-approved indications for intravenous immunoglobulin (IVIG)

Primary agammaglobulinemia
Common variable immunodeficiency
X-linked agammaglobulinemia
Severe combined immunodeficiency
X-linked hyper-IgM deficiency
IgG subclass deficiency
Chronic lymphocytic leukemia
Children with HIV
Bone marrow transplantation (allogeneic)
Idiopathic thrombocytopenic purpura
Kawasaki syndrome

HIV, human immunodeficiency syndrome

IVIG was first licensed in the USA in 1981. Table 2 lists the five manufacturers and commonly used brands of IVIG. Table 3 lists the FDA-approved indications for IVIG. The FDA does not regulate or dictate coverage; however, FDA approval usually guarantees coverage. All local Medicare carriers must cover FDA-approved indications for drugs and biologicals. Most other payers cover FDA-approved indications for biologicals, but do not carry all products in their formularies.

Once a biological has been approved for marketing for a specific disorder, treatment experience often shows usefulness in other medical conditions. An unlabelled use is one not listed in the biological's product labelling. Presently, IVIG is not FDA-approved for any neurological condition, yet IVIG is frequently administered for neurological disorders. A 1999 study by the University Health System Consortium reported that 58% of in-patient IVIG use was off-label[1].

Medicare covers the 'off-label' use of IVIG in compliance either with a national coverage determination (NCD) or a local medical review policy (LMRP). Other carriers often follow Medicare policies, sometimes with modifications. Some carriers cover IVIG as a 'standard of care' for a specific market or geographic region. Others cover what is deemed 'medically necessary'.

To determine Medicare 'off-label' coverage, carriers use 'reasonable and necessary' criteria. 'Off-label' uses of FDA-approved biologicals may be covered under Medicare if the carrier determines the indication to be medically accepted in the community, taking into consideration information from the major drug compendia, authoritative medical literature and accepted standards of medical practice.

Medicare sometimes issues an NCD binding on all contractors. Currently, a new NCD covers IVIG infusions for the treatment of certain autoimmune mucocutaneous blistering

diseases, but allows discretion among local carriers[2]. No NCD exists for using IVIG for neurological conditions.

Managed care companies vary in their policies towards the prescription of IVIG for neurological illnesses. Some companies accept the diagnosis of the ordering physician without question. If the indication for IVIG is retrospectively questioned, the medical director may seek the advice of a neurologist in the community to help determine the appropriateness of treatment. If the indication for IVIG remains controversial, the medical director may request 3–4 articles from the neurological literature to support its use.

Most insurance companies have published policies regarding payment for non-labelled uses of IVIG. They expect the submission of an appropriate ICD code and may request medical records to substantiate the diagnosis. Pre-certification of treatment is advised, but payment after infusion is possible if the indication for treatment meets the criterion of the insurance company.

Coverage of IVIG by Medicare is regulated by each state and is posted in the LMRP (http://www.draftmrp.net/) of the insurance carriers for each state. By law, pre-certification is not required. Medicare takes the position that the patient should be managed by the physician using the most appropriate treatments. Coverage among states is similar, but not identical. The need for IVIG is judged retrospectively using the LMRP of the Medicare carriers for the state. A proper ICD code is crucial, and must match an illness for which coverage of IVIG is accepted by the local carrier.

Most insurance companies will reimburse for IVIG infused in the hospital, a hospital out-patient clinic, or a private physician's office, or administered by a home infusion company. Medicare usually covers treatments in these same locations except for those provided by home infusion companies.

Some Medicare carriers base coverage of IVIG on the US Pharmacopeia *Drug Information for the Health Care Professional* (USP-DI), a compendium on drugs prescribed in the USA[3]. The publication lists both accepted and bracketed or off-label uses of biologicals. A bracketed indication is one lacking the US product labelling. The most recent edition of USP-DI (2003) lists Guillain—Barré syndrome, chronic inflammatory demyelinating polyneuropathy (CIDP) and Lambert—Eaton myasthenic syndrome as bracketed disorders for first-line IVIG coverage[3]. IVIG is considered a bracketed second-line treatment for dermatomyositis, multifocal motor neuropathy and relapsing and remitting multiple sclerosis[3]. Chronic inflammatory demyelinating polyneuropathy (CIDP) is not listed. A review of the LMRPs for IVIG treatment will highlight the wide variability of coverage among carriers.

Other organizations show a broader attitude towards approval of IVIG treatment. Medicaid of North Carolina pays for most requests for IVIG use and at all sites of infusion including home infusion. Reimbursement does not depend on the ICD code, FDA approval, documentation of diagnosis or pre-certification. Some private insurance carriers require that IVIG treatment satisfies their proprietary requirements for medical necessity and experimental/investigational definitions. In many cases, they consider any treatment to be experimental or investigational if it is not FDA-approved.

Correct coding and billing for IVIG treatment requires the use of the proper ICD-9 or diagnosis code and current procedural terminology (CPT) or procedure code. For example, the common peripheral neuropathy ICD-9 codes for which IVIG administration

might be prescribed include: 357, inflammatory and toxic neuropathy; 357.0, acute infective polyneuritis, Guillain—Barré syndrome, post-infectious; and 357.8, other chronic inflammatory demyelinating polyneuritis. Allowable codes vary by carrier. Two CPT codes exist for IVIG infusion: 90780, intravenous infusion for therapy/diagnosis, administered up to 1h; and 90781, each additional hour, up to 8h.

In addition, to be reimbursed for the cost of IVIG, the physician must use separate infusion codes (HCPCS codes, or Healthcare Common Procedure Coding System). Currently, two HCPCS codes exist for IVIG infusion: J1561, injection, immune globulin, intravenous for 500mg of IVIG; and J1563, injection, immune globulin, intravenous for 1g.

IVIG is priced as the average wholesale price (AWP) per gram. Little price variation exists among manufacturers, and volume discounts for large infusions of IVIG are rare. Reimbursement for IVIG varies among third-party payers. Medicare typically pays 5% less than the AWP of the least expensive product[4]. Private insurance companies commonly pay AWP minus 10%. Home infusion companies accept a range of discounts from the AWP of 5–15%, depending on the exclusivity of the contract with the insurance company.

Home infusion companies typically follow an organizational plan for processing requests for IVIG that are designed to shield the patient from receiving a large unexpected bill from his insurance company. Requests for IVIG infusion are first submitted to an internal reimbursement department that contacts the patient's insurance company for network compatibility, policy limitations and an authorization number. The patient is informed of co-payments and potential non-payment before signing a pre-infusion consent form. In the event of non-payment for IVIG infusion, all insurance companies and carriers including Medicare have organized appeal policies that utilize medical directors and two levels of independent reviewers.

In summary, it is important for neurologists prescribing IVIG to understand the policies of private insurance companies and Medicare and Medicaid towards unlabelled uses of IVIG and the array of ICD, CPT and HCPCS codes needed to ensure reimbursement.

ADDENDUM

Some of the material for this manuscript is taken from an article by Peter D. Donofrio, MD and Neil A. Buses, MD entitled: Regulatory and reimbursement issues in treating patients with immune-mediated neuropathies. *Neurology* 2002; 59(Suppl 6): S41–5

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7
Pharmacoeconomics of intravenous immunoglobulin for polyneuropathies

P.McCrone

INTRODUCTION

The direct costs of intravenous immunoglobulin (IVIG) are high for two main reasons: demand for the product exceeds its supply and the process by which IVIG is produced is complex. Demand is unlikely to be reduced because new indications for the use of IVIG are frequently being suggested. Consequently, unless there is either a substantial increase in the supply of plasma or a large reduction in the production costs of IVIG the price will remain relatively high. Health-care resources in most, if not all, countries are limited and there are competing uses for funds. It is therefore important to compare alternative ways of treating particular conditions in order to see which is most clinically and economically appropriate. IVIG's high price acts as a disincentive for its use. However, the acquisition cost of a product such as IVIG cannot on its own determine whether treatment with it is 'cost-effective'. Rather, information on costs needs to be combined with data on outcomes, to see what level of clinical change can be expected by spending money in one way rather than another or, similarly, to see what it costs to achieve a predefined level of change. This chapter describes methods that have been developed in the area of health economics by which costs and outcomes can be compared, and summarizes the health-economic evidence for the use of IVIG in treating poly neuropathies.

TYPES OF ECONOMIC EVALUATION

The objective of all methods of economic evaluation is to combine data on costs with data on outcomes. Costs should be measured comprehensively, so that in addition to the costs of IVIG the costs of other relevant services are also included. Ideally, patient-specific service use data should be collected alongside data on outcomes within a clinical trial. However, it is quite common for economists to use decision-tree models, where outcome data that have

Table 1 Cost-effectiveness grid. This table shows which treatment (intravenous immunoglobulin (IVIG) or a comparator) should be preferred, given different combinations of cost and outcome findings

	IVIG outcomes worse	Outcomes equal	IVIG outcomes better
IVIG costs higher	comparator	comparator	?
Costs equal	comparator	?	IVIG
IVIG costs lower	?	IVIG	IVIG

already been published are combined with costs data based on assumptions about the resource consequences of being in different health states. Each method described below measures service costs in monetary units, but outcomes are measured in quite distinct ways.

Cost-minimization analysis

This form of economic evaluation does not actually involve the measurement of patient outcomes because it assumes that these are identical for interventions that are being compared. If this is the case, the most efficient intervention is then the one that costs the least. This form of evaluation is limited because outcomes are not usually known before an evaluation has taken place. However, given the increase in the number of systematic reviews that are being conducted, this situation may change and 'stand-alone' cost studies linked to data from such reviews may in some cases be acceptable.

Cost-effectiveness analysis

This term is often used to describe all forms of economic evaluation, but it is in fact a quite specific method. Here, costs are usually linked with outcomes that are measured in clinically specific units (such as disability level or relapse rates) in the form of a cost-effectiveness ratio, which shows how much it costs to achieve a one-unit improvement in outcome.

Cost-benefit analysis

Here outcomes are measured in monetary units, and an intervention is deemed to be appropriate if these benefits exceed the costs. The problem with cost-benefit analysis is that few outcomes can easily be measured in this way.

Cost-utility analysis

This final method measures outcomes in generic units such as quality-adjusted life years. By using measures that are not illness-specific, comparisons can be made across different conditions. This is particularly useful for evaluations of IVIG given its versatility of use.

The most useful forms of evaluation are cost-effectiveness analysis and cost-utility analysis. With both of these, if IVIG were to produce better outcomes than a comparative intervention and to have lower costs then it would be described as dominant (lower right in Table 1). However, it is likely that better outcomes are achieved at a higher cost than comparative interventions (upper right in Table 1). In such circumstances it is a value judgement as to whether the extra cost is justified by the improved outcomes gained.

ECONOMIC EVALUATION OF INTRAVENOUS IMMUNOGLOBULIN FOR POLYNEUROPATHIES

There have been few health-economic studies of IVIG generally, and only two studies evaluating its use for polyneuropathies have been identified.

Chronic inflammatory demyelinating polyradiculoneuropathy

In this randomized controlled trial, prednisolone was compared with IVIG (2g/kg). There was little difference in clinical outcome[1]. For the economic evaluation, quality-adjusted life years (QALYs) were generated and these favored IVIG, although not significantly[2]. Health-care and informal care costs were substantially higher for IVIG, and the cost per QALY of IVIG treatment was €250000. Whether or not IVIG is appropriate for this condition depends on whether decision-makers would find this ratio acceptable or not. However, costs and outcomes were only measured over a 6-week period and therefore these results need to be treated with some caution.

Guillain—Barré syndrome

In this analysis of published data, Nagpal and colleagues[3] compared IVIG with plasma exchange. Outcomes were found to be very similar. Health-care costs were 39% less for plasma exchange. IVIG therefore appeared to be less cost-effective than plasma exchange, but the result was very sensitive to the price of IVIG.

Clearly, the evidence base for the cost-effectiveness or cost-utility of IVIG used to treat polyneuropathies is limited. Economic evaluations need to be incorporated into clinical trials and more use should be made of data that have already been generated, in order to produce cost-effectiveness models.

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Intravenous immunoglobulin in immunodeficiencies and severe infection—molecular aspects and clinical use

8
B-cell maturation to produce high-affinity antibodies of differents isotopes and its abnormalities

A.Durandy

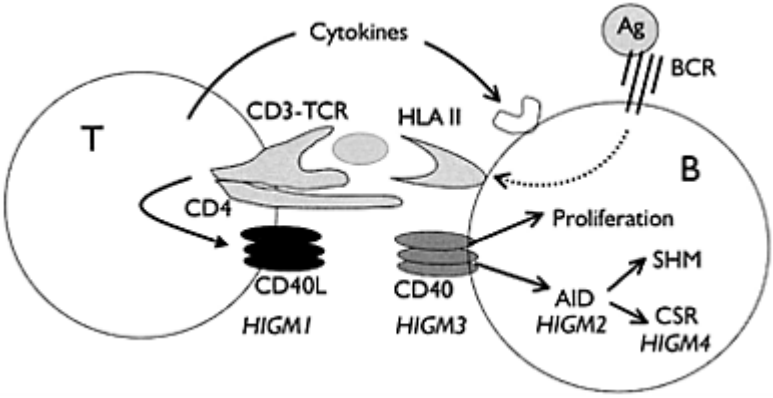
Maturation of the antibody repertoire consists of two successive steps. The first leads to the generation of the primary repertoire composed of immunoglobulin M (IgM) with a low affinity for antigen. It occurs in primary lymphoid organs (fetal liver and bone marrow) in an antigen- and T cell-independent manner. It is acquired by stochastic rearrangements of the different genetic regions of the immunoglobulin gene (rearrangement between the variable (V) region and the constant (C) region encoding the IgM isotype). The V region recognizes specifically the antigen, and the C region determines the isotype of the immunoglobulin (IgM). Mature B cells, therefore, express on their membranes IgM as the B-cell receptor (BCR), and can produce only IgM.

Mature B cells emigrate to the secondary lymphoid organs (lymph nodes, spleen, tonsils) where generation of the secondary repertoire occurs, in an antigen- and T cell-dependent manner. When a B cell specifically recognizes an antigen by its BCR (IgM), it strongly proliferates and gives birth to a peculiar lymphoid formation, the germinal center. There, in close contact with antigen-activated T cells, the two major events of antibody maturation occur: class switch recombination and the generation of somatic hypermutation (Figure 1).

Class switch recombination (CSR) leads to replacement of the genetic region encoding the IgM by another region encoding for other isotypes (IgG, IgA and IgE) with the same V region, and thus the same specificity and antigen affinity The nature of the isotype produced determines its activity (half-life, ability to bind Fc receptors or to activate complement) and the location at which it is delivered (in mucosa, for example, for IgA)[1].

Somatic hypermutation (SHM) generation introduces mutations with a high frequency in the V region of the immunoglobulin. SHM is eventually followed by the positive selection and expansion of B cells bearing a BCR with a high affinity for antigen, and the negative selection and deletion of B cells expressing a BCR with a low affinity for antigen or a BCR

Figure 1 The different molecules involved in antibody maturation are herein represented. The B cell recognizes specifically an antigen by its receptor BCR (immunoglobulin M, IgM). The antigen is processed and the antigenic peptides are presented in association with human leukocyte antigen (HLA) class II molecules to a CD4 helper T cell. The activated helper T cells produce cytokines and express transiently the CD40 ligand (CD40L). Triggering of CD40 molecules constitutively expressed on B cells leads to proliferation, expression of the activation-induced cytidine deaminase (AID) and class switch recombination (CSR) and somatic hypermutation (SHM). The main hyper-IgM (HIGM) syndromes, corresponding to molecular defects, are shown



recognizing an autoantigen. The SHM thus allows the production of efficient antibodies[2].

The recent elucidation of the molecular basis of inherited immunodeficiencies, the hyper-IgM (HIGM) syndromes, has made it possible to delineate some of the molecular events involved in both processes of antibody maturation.

HIGM syndromes are characterized by normal or elevated serum concentrations of IgM, with dramatically decreased or an absence of other isotypes, strongly suggesting a CSR defect. Most of them are associated with defective SHM and some are associated with cellular immune defect (Table 1).

CD40 LIGAND AND CD40 DEFICIENCY (HIGM1/HIGM3)

The first described HIGM, transmitted as an X-linked form, is caused by mutations in the gene encoding CD40 ligand (CD40L)[3–6]. Its counterpart, described more recently and transmitted as an autosomal recessive form, is caused by mutations in the *CD40* gene[7]. CD40L is transiently expressed by activated T cells and interacts with CD40, which is constitutively expressed on B cells and monocytes. CD40L/CD40 interaction is required for B cell proliferation in germinal centers, as well as CSR and SHM. The phenotype of both

Table 1 Hyperimmune immunoglobulin M syndromes (HIGM)

HIGM	Gene	Transmission	CSR	SHM frequency
T and B cell defects				
HIGM1	CD40L	XL	↓	↓
HIGM3	CD40	AR	↓	↓
B cell defects				
HIGM2	AID	AR	↓	↓
UNG deficiency	UNG	AR	↓	N
HIGM4	?	AR	↓	N

UNG, uracil-N glycosylase; XL, X-linked; AR, autosomal recessive; N, normal

syndromes is therefore identical, characterized by a defective germinal center formation, and an impaired CSR and SHM. Defective CD40L/CD40 interaction also leads to defective T cell/monocyte co-operation and an abnormal cellular response, which cannot be controlled by intravenous immunoglobulin substitution and results in a poor prognosis.

These two conditions demonstrate the essential role for B cell-CD40 activation in full antibody maturation.

HIGM CAUSED BY ACTIVATION-INDUCED CYTIDINE DEAMINASE DEFICIENCY (HIGM2)

This HIGM, the second to be described (hence its designation: HIGM2), is transmitted in an autosomal recessive pattern. It is secondary to mutations in the gene encoding the activation-induced cytidine deaminase (AID)[8]. AID is a B cell-specific molecule, expressed only in B cells undergoing CSR or SHM *in vivo* (in germinal centers) and *in vitro* (after activation by CD40). AID is absolutely required for both CSR and SHM; thus, AID-deficient patients produce only IgM with a low affinity for antigen. This specific B cell defect is controlled by regular intravenous immunoglobulin substitution.

The description of HIGM2 pinpoints the crucial role of AID, induced by CD40 activation, in both CSR and SHM.

HIGM CAUSED BY URACIL-N GLYCOSYLASE DEFICIENCY

A very rare autosomal recessive HIGM, characterized by a defective CSR and normal frequency of SHM, has recently been reported as caused by mutations in the gene encoding uracil-N glycosylase (UNG)[9]. This observation gives the opportunity to define precisely the role of AID in CSR. Indeed, AID deaminates cytosine into uracil residues on DNA and these uracil residues are processed in normal conditions by UNG. A defect in UNG is therefore responsible for a CSR defect, the role of AID in SHM remaining controversial.

HIGM CHARACTERIZED BY A DEFECTIVE CLASS SWITCH RECOMBINATION AND NORMAL SOMATIC HYPERMUTATION (HIGM4)

The most frequent autosomal recessive HIGM has, as yet, no defined molecular basis, but this condition appears homogeneous and is thus referred to as HIGM4[10]. It is characterized by a defective CSR, whereas SHMs are normally generated. The defect probably affects the CSR-specific DNA repair machinery, which is known to be different from SHM-DNA repair.

The phenotype is identical to that of HIGM2 (albeit the major difference in SHM generation in these two syndromes), and is controlled by regular intravenous immunoglobulin substitution.

The molecular definition of HIGM4 will afford in the near future much information concerning the last step of CSR (the DNA repair mechanism).

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9
The molecular basis of primary immune deficiency diseases

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Primary immune deficiency diseases (PIDDs), recognized more than 50 years ago, have provided unique opportunities to study the cellular, biochemical and molecular events that are involved in the function of a normal immune system. The discovery in recent years of single genes responsible for specific immunodeficiency disorders, and the understanding of the function of the products of these genes, have changed our approach from the descriptive to the molecular level, and have provided new tools to redefine genetic diseases of the immune system in molecular terms. We are now in a position to confirm the suspected diagnosis of a molecularly defined PIDD at the gene level, to recognize different clinical phenotypes resulting from mutations of the same gene and, conversely, to understand why a particular clinical phenotype can be caused by mutations of a number of different genes. The understanding of the molecular basis of PIDDs will allow us to design new and more effective therapeutic strategies. Finally, genetically determined PIDD are ‘experiments of nature’ that provide insight into the molecular events that are crucial and non-redundant for an effective cognate immune system. In this review, we use ‘entities of function’ to catalog the most common prototypes of PIDD, addressing both the cognate and innate immune systems (Table 1).

PRIMARY IMMUNE DEFICIENCY DISEASES ARE MODELS FOR B- AND T-CELL DEVELOPMENT

The generation of functional, polyclonal T and B lymphocytes depends on the sequential expression of genes that ultimately result in the creation of a cognate immune system with a broad repertoire of antigen recognition. Equally important is the development of secondary immune organs with characteristic histological structures that allow trapping, processing and presentation of antigen, and the functional interplay of differentiated cells directly or indirectly involved in the immune

Table 1 Molecular basis of primary immune deficiency diseases (PIDD)

Name of gene	Name of protein, syndrome	Inheritance	Treatment	Reference
Defective B- and T-cell development				
RAG1/RAG2	recombination-activating genes 1 and 2; V(D) J rearrangement protein 1, 2	AR	PCP-P; SCT; (IVIG)	1,2
	T^- B^- NK^+ SCID or Omenn syndrome (T^+ B^- NK^+)			
Artemis	Artemis corrects RAG-induced DNA breaks	AR	SCT, (IVIG)	3
	$T^-B^-NK^+$SCID		PCP-P	
IL-2Rγ	common γ-chain (IL-2R, 4R, 7R, 9R, 15R, 21R)	XL	SCT, (IVIG)	4
	T^- B^+ NK^- SCID		PCP-P	
Jak3	$T^-B^+NK^-$SCID	AR	SCT, (IVIG) PCP-P	5
Btk	Bruton's tyrosine kinase X-linked agammaglobulinemia	XL	IVIG	6
μHC	μ heavy chain mutated	AR	IVIG	6
λ/14.1	surrogate light-chain deficiency	AR	IVIG	
	BCR-signalling protein Igα	AR	IVIG	
BLNK	B-cell linker	AR	IVIG	
Fas, FasL Caspase 8, 10	autosomal recessive agammaglobulinemia defective lymphocyte apoptosis autoimmune lymphoproliferative syndrome (ALPS)	AR	CSA, FK506, prednisone	7
Defective ligand—receptor interaction, cytoplasmic signalling				
CD40L/CD40	CD40 signalling defect (HIGM)	XL/AR	IVIG, SCT	8
ICOS/ICOSL (B7RP) CD28/CTLA-	subgroup of CVID due to ICOS mutations	AR		19

4/B7

Name of gene	Name of protein, syndrome	Inheritance	Treatment	Reference
CD3λ; CD3ε	CD3 deficiency, T-cell receptor defect, CID, variable phenotype	AR		10
CD3δ	CD3 deficiency, SCID ($T^-B^+NK^+$)	AR	SCT	11
ZAP70	T-cell receptor ζ-chain-associated protein required for T-cell signalling, SCID (absent CD8+, normal number of non-functional CD4 +)	AR	SCT	12
WASP	WAS protein, cytoplasmic signalling, interacts via Arp2/3 with actin, WAS/XLT	XL	IVIG PCP-P SCT	13,14
Defective class-switch recombination (CSR) and somatic hypermutation (SHM) (requires CD40L/CD40/NEMO/NF- κB signal transduction)				
AID	activation-induced cytidine deaminase, HIGM	AR	IVIG	15,16
UNG	(nuclear) uracil glycosylase HIGM	AR	IVIG	17

Defective transcription factors/gene expression				
CIITA	MHC-II specific transcription factors	AR	IVIG	18
RFX-5	lack of MHC-II expression; bare lymphocyte		SCT	
RFXAP	syndrome (BLS)			
NEMO	NF-κB essential modulator or IKK-γ allows NF-κB signalling	XL	IVIG	19
AIRE	autoimmune regulator; APECED	AR	immune suppressive	20,21
FOXP3	forkhead protein 3 IPEX	XL	prednisone, FK506, CSA, (IVIG), SCT	22,23
DNA repair defects				
ATM	AT protein mutated, ataxia telengiectasia (AT)	AR	(IVIG)	24
NBS1 (Nibrin)	NBS protein; Nijmegen breakage syndrome	AR		25
BLM	BLM protein; Bloom syndrome	AR		26
Ligase IV	DNA ligase; developmental delay	AR		27
Deficiency of purine metabolism				
ADA	adenosine deaminase	AR	PEG-ADA	28
	↑ dATP		SCT	

Name of gene	Name of protein, syndrome	Inheritance	Treatment	Reference

	(_toxic product in ADA deficiency) ↑ apoptosis of T>B cells			
PNP	purine nucleoside phosphorylase ↑ dGTP (toxic), ↑ apoptosis	AR	(SCT)	29
Innate immune deficiencies				
Elastase	neutrophil elastase Kostmann (congenital neutropenia) cyclic neutropenia	AR	G-CSF	30
gp91 phox (CyBB)	cytochrome b-245β, 91-kDa protein CGD	XL	prophylactic antibiotics, antifungal, IFN-γ	31
p22 phox (CyBA)	22-kDa protein CGD	AR	SCT	
p47 phox	47-kDa protein, CGD	AR		
p67 phox	67-kDa protein, CGD CGD due to NADPH oxidase defect	AR		
C-component deficiences	classic or alternative pathway components (mostly AR, except: Properdin def (XL)	AR/XL	(antibiotics)	32

Name of gene	Name of protein, syndrome	Inheritance	Treatment	Reference
IRAK-4	IL-1R (Toll-like receptor) kinase-4	AR	(IVIG)	33
IFN-γR	atypical	AR	(IFN-γ)	34

1/2 def	mycobacteria, BCG		anti-Tb	
IL-12R deficiency		AR	IFN-γ anti-Tb	
CHS1	Chediak-Higashi syndrome	AR	SCT	35
MYO 5A	Griscelli syndrome	AR	SCT	36

RAG, recombination activating gene; SCT, stem cell transplantation; IVIG, intravenous immunoglobulin; AR, autosomal recessive; XL, X-linked; PCP-P, Pneumocystis carinii prophylaxis; SCID, severe combined immunodeficiency; CSA, cyclosporin A; HIGM, hyper-immunoglobulin M (IgM) syndrome; NEMO, NF-κB essential modulator; AID, activation-induced cytidine deaminase; UNG, uracil-DNA glycosylase; C-component, complement component; APECED, autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked; IL, interleukin; FasL, Fas ligand; CD40L, CD40 ligand; B7RP, B7 related protein; ICOS, inducible co-stimulatory molecule; ZAP10, zeta chain associated protein; WASP, Wiskott-Aldrich syndrome protein; XLT, X-linked thrombocytopenia; ATM, ataxia-telangiectasia protein mutated; ATP, adenosine triphosphate; GTP, guanosine triphosphate; CGD, chronic granulomatous disease; IFN, interferon; PEG, polyethylene glycol; G-CSF, granulocyte colony-stimulating factor; Tb, tuberculin; AIRE, autoimmune regulator

response. In the thymus, developing T lymphocytes undergo VDJ rearrangement and are exposed to self-antigens, a process requiring expression of the DNA binding protein, autoimmune regulator (AIRE) (mutated in patients with automimmune polyendocrinopathy, candidiasis, ectodermal dystrophy (APECED)), which is crucial for the elimination of anti-self lymphocyte clones[20,21]. In the cortex and medulla of the thymus, populations of single positive (CD4+ or CD8 +) T cells are being generated, released into the periphery and depleted of self-aggressive clones by apoptosis-inducing genes (e.g. Fas, Fas ligand, caspase 8, caspase 10). These gene products, if mutated, pave the way for autoimmune lymphoproliferative syndrome (ALPS)[7], caused by antigen-specific CD4+ T helper cells and CD8+ cytotoxic T cells.

In the bone marrow, pre-B lymphocytes undergo V(D)J rearrangement and express heavy and light chains, a process that leads to the recognition of a large array of antigens. Following exposure to antigen, the rearranged antigen-specific B cells, with the help of two crucial enzymes, activation-induced cytidine deaminase (AID) and nuclear uracil glycosylase (UNG), undergo class-switch recombination and DNA editing (somatic hypermutation)[8,37], a process that ultimately results in high-affinity antibodies of various immunoglobulin isotypes. Each of these steps may be disrupted by a single gene defect leading to characteristic deficiencies of the cognate immune system. Thus, humoral and

cellular immune responses are dependent on antigen exposure and a series of complex events that include antigen recognition and cognate interaction of T and B lymphocytes, which is amplified by cytokinesecreting modulatory cells, including macrophages, follicular dendritic cells and Langerhans cells. If T-cell maturation is interrupted, for instance by mutations of *RAG1* or *RAG2*, two closely related proteins required for VDJ rearrangement of the T-cell receptor (as well as the B-cell receptor), severe combined immune deficiency (T^-, B^- SCID) occurs[2]. If T-cell development is specifically disrupted at a later stage, as in deficiency of the common γ-chain, which participates in the formation of several lymphokine receptors, a clinical phenotype of T^- B^+ NK^- SCID develops[4], where NK is natural killer cell. On the other hand, if genes required solely for B-cell development are defective, patients will present with congenital agammaglobulinemia. The absence of B cells can be due to lack of the cytoplasmic tyrosine kinase Btk, as in X-linked agammaglobulinemia, or can be the consequence of mutations affecting the *μ heavy chain* gene, the surrogate light chain *λ-5/14.5* gene, the *Igα* gene or the *BLNK* gene[6]. Mutations of a specific gene may result in different clinical phenotypes, as has been exemplified by mutations of *RAG1/RAG2:* non-sense mutations cause T^- B^- SCID; missense mutations that allow some function result in limited V(D)J rearrangements, the generation of an oligoclonal set of T lymphocytes and the unique clinical entity of Omenn syndrome[1].

DEFECTIVE LIGAND-RECEPTOR INTERACTION AND ABNORMAL CYTOPLASMIC SIGNALLING

Signalling via the T- and B-cell receptors is a crucial step in the cognate immune response. The antigen-driven activation mechanism initiates signal transduction events that lead to cell cycle progression and differentiation of lymphocytes into antigen-specific cytotoxic or helper T cells and antigen-specific antibody-producing B cells.

Following the presentation by B cells of antigen-derived peptides (bound within the groove of major histocompatibility complex (MHC) class II molecules) to antigen-specific CD4+T helper cells via the T-cell receptor (TCR), cognate-bidirectional B-T interaction is initiated. However, for T cells to become fully activated and to express both membranebound and soluble factors required for the proper activation of T cell-dependent effector cells, e.g. B cells and monocytes, co-stimulatory signals are required. During this process, activated CD4+ T cells produce a series of lymphokines necessary for proliferation of T and B lymphocytes (e.g. interleukin-2 (IL-2), IL-4) and differentiation of B lymphocytes (e.g. IL-4, IL-10), and express receptors that recognize these lymphokines. In addition, activated T lymphocytes rapidly express ‘activation’ molecules, e.g. CD40 ligand (CD40L), a membrane protein that interacts via CD40 expressed constitutively by B cells, macrophages and other cells; and members of the CD28/CTLA-4 family, which includes ICOS (inducible co-stimulatory molecule) that interacts with its ligand, ICOSL/B7RP-1, expressed by B cells. The CD40-dependent pathway induces B cells to increase expression of B7 and B7RP-1 which interact with T cells via CD28 and ICOS, respectively, enhancing further T-cell activation and lymphokine production. As a result, B cells differentiate and undergo class-switch recombination (CSR) and somatic hypermutation (SHM)[8,37].

This complex cognate T-B-cell interaction fails if any of the major components are defective. For instance, mutation of CD40L, the gene responsible for X-linked hyper immunoglobulin M (IgM) syndrome (XHIGM), or of CD40 (responsible for an autosomal recessive form of HIGM syndrome), results in markedly abnormal responses to T-cell-dependent antigens, characterized by low antibody titers, lack of amplification, failure of CSR and lack of SHM[8].

Signalling via SLAM (signalling lymphocyte activation molecule), a cell surface protein expressed by T, B and NK cells, induces T-cell proliferation following interaction with SHP-2, a cytoplasmic phosphatase. SAP (SLAM-associated protein), a cytoplasmic protein expressed by T and NK cells, competes with SHP-2 for binding to SLAM, thus preventing excessive T-cell activation[38]. In NK cells, SAP binds to the surface molecule 2B4, which, if cross-linked, induces NK cell activation and killing of target cells[39]. Lack of SAP results in undesired (and detrimental) T-cell proliferation/activation, and defective NK cell function, mechanisms thought to be responsible for the XLP (X-linked lymphoproliferative) syndrome.

Wiskott—Aldrich syndrome protein (WASP) has several domains by which it participates in cytoplasmic signalling. Through its Pleckstrin homolog (PH) domain, WASP interacts with membrane-associated lipids (e.g. PIP2), and participates with other cell surface molecules in synapse formation. WASP interacts with WASP-interacting protein (WIP), forming a complex that associates with lipid rafts; if the two molecules dissociate, WASP can be activated by membrane-bound GTP-Cdc42 to initiate Arp2/3-dependent actin polymerization[14]. With its polyproline domain, WASP interacts with other SH3-containing cytoplasmic proteins, e.g. Btk, NcK, Grb2, Fyn, CrkL, thus participating in cytoplasmic signalling. Collagen activation of platelets induces rapid tyrosine phosphorylation of platelet-associated WASP13.

DEFECTIVE CLASS-SWITCH RECOMBINATION AND SOMATIC HYPERMUTATION

Immunoglobulin gene assembly by V(D)J recombination occurs prior to antigen encounter, and leads to a repertoire of B cells expressing IgM antibodies with typically low-affinity binding sites for a broad spectrum of antigens. After antigen encounter, those B cells expressing IgM of the highest affinity for the specific antigen undergo a period of rapid proliferation, and form germinal centers in the secondary lymphoid organs. As the immune response progresses, these rearranged antibody genes undergo localized SHM, with nucleotide substitutions being introduced in and around the rearranged V-gene segments. The purpose of SHM is to create new protein sequences in the variable regions that can bind a given antigen more strongly and specifically than their precursors. B cells that express somatically mutated variable genes that confer high affinity for the antigen of interest are then selectively enriched, generating high-affinity antibody in response to pathogens that were encountered by the host previously. In addition to hypermutation, the isotype of the expressed antibody can also be altered in a phenomenon called class-switch recombination (CSR). Both SH and CSR depend on the activation-induced cytidine deaminase (AID)[15]. Indeed, mutations of AID result in an autosomal recessive form of HIGM, characterized by defective CSR and SHM[16]. Using the chicken DT40 B-cell

model and UNG$^{-/-}$ mice, Neuberger and colleagues have demonstrated that a DNA-associated (nuclear) uracil glycosylase (UNG) is crucial for the process of SHM[40]. The proposed mechanism of SHM postulates that, in DNA, AID converts cytosine to uracil, thus producing large numbers of uracils within the V-genes. DNA polymerase enzymes that copy DNA by reading uracil as thymine during replication are the cause for the transition of cytosine to thymidine, an UNG-independent SHM mechanism. The role of UNG is to remove uracil, leaving a gap in the DNA sequence, which may provide the DNA break for CSR or, if filled in more or less at random by low-fidelity polymerases, result in both transitions and transversions at the cytosine positions. This latter mechanism ensures a high rate of SHM. Naturally occurring mutations of AID or UNG result in similar clinical phenotypes (e.g. HIGM with lack of SHM and CSR)[16,17].

DEFECTIVE REGULATION OF GENE EXPRESSION AS THE CAUSE OF PRIMARY IMMUNE DEFICIENCY DISEASES

Transcription factors play a major role in the control of gene activation and, if mutated, may result in a broad spectrum of immunodeficiencies. The bare lymphocyte syndrome (BLS) is caused by the failure of MHC class II expression by lymphocytes. Four DNA binding proteins required for MHC-II expression have been identified, each of which, if mutated, can cause BLS, a form of severe combined immune deficiency[18]. Mutations of the nuclear factor-κB (NF-κB) essential modulator (NEMO, also known as IKK-γ), impair signalling via NF-κB (a DNA-binding protein involved in activation of multiple genes in the immune response), and has been shown to cause hypohydrotic ectodermal dysplasia (HED) in affected males, as well as cellular and humoral immune deficiency, including defective CSR and SHM[19]. The *autoimmune regulator* (AIRE) is a DNA-binding protein most strikingly expressed by thymic epithelial and dendritic cell populations, also in lymph nodes and spleen. Mutations of this gene cause an autosomal recessive human syndrome of APECED[21]. AIRE-deficient knock-out mice fail to express selected gene products of endocrine organs that are expressed by the thymic epithelium of normal mice; interestingly, these self-antigens are precisely the proteins to which aire-/- mice and APECED patients develop autoimmune responses[20].

Immune dysfunction, polyendocrinopathy, enteropathy, an X-linked syndrome (IPEX), is a condition of overwhelming autoimmunity and immune dysregulation caused by mutations of the *FOXP3* gene[22]. A naturally occurring mouse disorder, scurfy, is due to a spontaneous mutation of the same gene and causes a similar phenotype in mice[23]. FOXP3 is a DNAbinding protein that has been shown to be absolutely required for the generation of regulatory T cells, a subgroup of CD4+, CD25+ cells that by direct contact inhibit the development of autoreactive lymphocyte clones[41]. The regulation of FOXP3 expression itself is unknown.

These regulatory DNA-binding proteins are not only involved in PIDDs, but appear to play a major role in up- and down-regulation of immune responses, and thus may have unique therapeutic potentials for the treatment of both immunodeficiency syndromes and autoimmune disorders.

DEFECTIVE DNA REPAIR

To achieve DNA recombination, a mechanism to induce DNA breaks and subsequently to repair DNA breaks is essential. Mutations of genes that assure repair of DNA breaks can be disastrous to T and B lymphocytes, which, by design, constantly undergo V(D)J recombination, CSR and SH. Mutations of ATM (ataxia—telangiectasia protein mutated) are responsible for the failure of ATM to repair DNA breaks, causing the clinical phenotype of ataxia-telangiectasia (AT)[24]. Similarly, in the Nijmegen breakage syndrome (NBS), mutations of Nibrin or NBS-1 result in a clinical phenotype that resembles AT and is often associated with malignancies[25]. Mutations of DNA ligase IV are responsible for a clinical phenotype characterized by immunodeficiency and developmental delay[27]. In Bloom syndrome, the molecular defect involves the BLM gene. Mutations of BLM result in chromosomal breaks, immune deficiency, skin rash and a high incidence of malignancies, similar to AT, NBS and DNA ligase IV deficiency[26]. Artemis is a gene that is required to correct DNA breaks that are caused by *RAG1/RAG2* deficiency, a pair of genes required for V(D)J recombination. Mutations of Artemis cause T^- B−SCID, described in Europe and Japan and in Athabascan-speaking North—American Indians[3].

DEFECTS IN PURINE METABOLISM

Adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) are enzymes involved in purine metabolism. If mutated, toxic purine metabolites accumulate (e.g. *d*-adenosine triphosphate and *d*-guanosine triphosphate), which interfere with DNA synthesis and cause apoptosis of T cells and, to a lesser extent, B cells. ADA deficiency is the cause of a T^- B^- form of autosomal recessive SCID; PNP deficiency presents with a less severe combined immune deficiency. The consequences of ADA deficiency can be prevented by treating ADA-deficient patients with regular injections of commercially available polyethylene glycol (PEG)-ADA[28].

INNATE IMMUNE DEFICIENCIES

Innate immunity represents a ‘shotgun’ approach to protecting higher organisms against microbial invaders. In contrast to the cognate immune system, which provides for ‘on the job’ education of its major players, T and B lymphocytes, the innate immune system is fully active without previous exposure to specific microbial antigens. Examples include the phagocytic and complement systems, Toll-like receptors and their signalling pathways, lymphokine receptors and adhesion molecules. The importance of the innate immune system is illustrated by the discovery of single gene defects that interfere with its function. For instance, mutations of neutrophil elastase result in Kostmann syndrome of congenital neutropenia or in cyclic neutropenia[30]. Mutations of genes involved in nicotinamide—adenine dinucleotide phosphate (NADPH) oxidase activity result in chronic granulomatous disease (CGD)[31]. Mutations of components of both the classic and the alternative complement pathways may be associated with recurrent infections and/or autoimmune disorders. IRAK-4 is an important cytoplasmic protein expressed by

lymphocytes through which Toll-like receptors and IL-1 receptors initiate signalling in monocytes. Mutations of IRAK-4 result in increased susceptibility of children to pyogenic and encapsulated organisms[33]. Lymphokine receptor deficiencies (e.g. interferon-γ receptor (R) 1/2 deficiency, IL-12R deficiency) are associated with susceptibility to atypical mycobacterial and salmonella infections[34]. Mutations of adhesion molecules result in failure of neutrophil adhesion to vascular endothelium and emigration of neutrophils into the extravascular space. Patients with adhesion molecule deficiencies (e.g. mutations of CD18 causing LAD1, and failure to express SLeX causing LAD2) present with recurrent infections without pus formation[42].

THERAPY

The molecular approach to understanding PIDD has lead to a rational review of treatment options. Primary B-cell defects respond well to intravenous immunoglobulin (IVIG) therapy If the antibody deficiency is caused by a lack of T help, as in X-linked HIGM, the response to IVIG is less dramatic. SCID patients are candidates for stem cell transplantation (SCT) or, in the future, gene therapy. Enzyme replacement in ADA deficiency is an alternative treatment to SCT. Granulocyte colony-stimulating factor (GCSF) is the treatment of choice for cyclic neutropenia and Kostmann syndrome. Prophylactic therapy with antibiotics, antifungals and interferon-γ markedly reduces the incidence of infections in CGD, and continuous treatment with FK506/cyclosporin A keeps patients with IPEX alive. One can only speculate what impact the exploitation of these newly discovered factors/proteins will have on Biotechnology's drug discovery programs. To design and carry out clinical trials safely and effectively will be a challenge to both scientists and clinical investigations, as well as industry and regulatory agencies.

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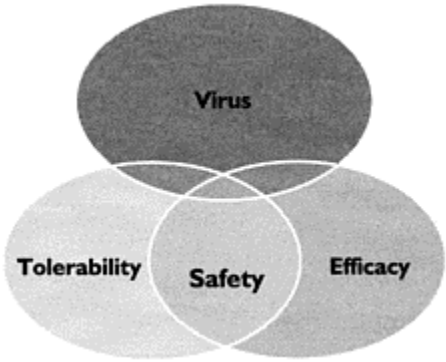
10
Safety in intravenous immunoglobulin therapy

E.W.Gelfand

Since their introduction in the 1950s, gammaglobulin injections have changed remarkably, both in their quality and in disease indications. The initial uses were as replacement for patients with compromised immunity by intramuscular injection; the general recommendations were for approximately 100mg/kg body weight to be given every 4 weeks. The injections were generally safe, albeit painful, and provided protection against recurrent infections. During the past two or more decades, the clinical indications for gammaglobulin therapy have expanded dramatically, and preparations of gammaglobulin suitable for intravenous administration have been developed. These preparations of intravenous immunoglobulin (IVIG) initially were chemically modified and enzyme-treated. Now they are manufactured in ways to avoid these harsh measures, preserving the integrity of immunoglobulin G (IgG) and increasing the circulating half-life of administered material.

With the introduction of IVIG, it became possible to administer much larger quantities of IgG, and with the broader disease base now targeted and larger numbers of adult patients, including elderly patients, being treated, new concerns emerged. Here, a broad approach to the concept of safety in IVIG therapy is discussed. As illustrated in Figure 1, safety is considered in a comprehensive way, based upon the potential for viral contamination and virus elimination, tolerability and efficacy. All three areas contribute to the overall safety of IVIG.

Figure 1 Comprehensive approach to intravenous immunoglobulin (IVIG) safety: virus inactivation/virus removal, tolerability (adverse events, infusion speed), efficacy



Safety is a prime concern for patients and health-care providers. In a recent survey of patients with primary immunodeficiency disease (PIDD), carried out by the Immune Deficiency Foundation (IDF), safety was a concern for more than 90% of the patients; in about half, the concern about safety was the most important. For most of these patients, safety reflected concern about disease transmission, hepatitis being the most prominent, but concerns about human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) were expressed by 25%.

VIRAL SAFETY

IVIGs are plasma-derived, and thus carry the risk of virus transmission. Except for an outbreak of hepatitis C (HCV) in 1992–93, the overall viral safety record has been good. The outbreak of HCV in recipients of IVIG was associated with two products, Gammagard® and Polygam®[1,2]. More than 100 cases were identified worldwide. This outbreak triggered the adoption of more vigorous standards by the industry, and no new cases have been identified since.

Viral safety begins with comprehensive donor screening, plasma testing and quarantining of batches of plasma until cleared. Remunerated donors form a large percentage of the source plasma for the production of IVIG. Repeat donors are believed to be a good source because of their lower incidence of exclusion disease markers[3]. Plasma donors are extensively screened. This involves a questionnaire, physical examination, cross-check with the National Donor Deferral Database and screening for antibodies against HIV-1, HIV-2, HCV, hepatitis B surface antigen, HIV p24 antigen, syphilis and liver transaminases. Plasma is quarantined for 60 days, until the tests prove negative. Polymerase chain reaction-based assays are also being introduced to detect, at a more sensitive level, viral genomic material[4].

To ensure further that IVIG carries a minimum risk for virus transmission owing to inadvertent contamination of the source plasma, different elimination methods during production have been utilized. The two distinct approaches include virus inactivation and virus removal. As listed in Table 1, viral inactivation methods include both physical and chemical/enzymatic methods. Among these, organic solvent/detergent (S/D) is highly effective against HIV and other lipid-enveloped viruses, but is not effective against non-lipidenveloped viruses (e.g. hepatitis A, parvovirus)[5]. Low pH can lower the risk of transmission of non-lipid-enveloped viruses[5]. Pasteurization has broad antivirus activity against lipid-enveloped and non-lipidenveloped viruses[5,6]. Caprylate, a natural plant-derived fatty acid, has been introduced in the preparation of Gamunex® the latest IVIG. Compared with S/D, the kinetics of viral inactivation by caprylate is much more rapid and probably gentler in terms of treatment of IgG[7].

The removal of viruses includes partitioning, entrapment, precipitation, chromatography and filtration. Nanofiltration removes viruses based on their size, and includes lipidenveloped and non-lipid-enveloped viruses. Nanofiltration and partitioning have been shown to clear prions effectively when added to the input solution.

To ensure the most effective virus reduction, increasing the safety margin by reducing the risks from contamination by known and unknown pathogens, the best approach is a combination of viral inactivation and viral

Table 1 Viral inactivation methods

Physical methods
Dry heat
Heat treatment of freeze-dried products
Pasteurization
Chemical/enzymatic methods
Solvent/detergent (e.g. Tween in tri-n-butyl phosphatase (TNBP))
Incubation at low pH
Incubation at low pH plus enzymatic treatment (pepsin digestion)
Chemical alteration of viral RNA to impede replication
Methylene blue (a photosensitizer requiring activation by white light)
Psoralens (photosensitizers requiring activation by long-wave ultraviolet light (UVA))
Riboflavin (a photosensitizer requiring activation by white light)
Caprylate (a plant-derived fatty acid)

removal steps, which act through independent mechanisms and are complementary to each other.

Although safety cannot be compromised, it is important to recognize that some of the approaches negatively impact upon the production process by increasing the time for production (e.g. S/D) or reducing yield (e.g. S/D), or can affect the integrity of the IgG

molecule (e.g. pasteurization, dry heat, S/D, β-propriolactone)[8]. Some procedures, such as low-pH, maintain IgG in monomeric form, while others, such as the integration of caprylate, shorten the production process[7].

TOLERABILITY

A second aspect of comprehensive safety is tolerability, which may be separated into two aspects, incidence of adverse events and rate of infusion. The two are closely linked as many of the reported adverse events may be infusion rate-related. The reported rates of adverse events vary widely among the IVIG products, and it is often difficult to compare products because of how the data are reported. When questioned in the IDF survey, about 55% of PIDD patients stated that they tolerated different products equally. On the other hand, more than 50% of patients surveyed stated that they avoided specific products because of adverse events. In general, the rate of adverse events is lower in patients with PIDD receiving lower doses than those receiving 1–2g/kg body weight for immune/inflammatory-mediated diseases.

The most common adverse event symptoms reported are headache, fever, nausea, cough, shortness of breath/chest tightness, chills and backache. Severe reactions are rare, and include aseptic meningitis and severe headaches, often associated with a previous history of migraine headaches. Anaphylactic reactions are very rare, and have been reported in patients with IgA deficiency associated with IgE anti-IgA antibodies: this is extremely rare[9].

A number of product features can affect clinical tolerability (Table 2). Products formulated at higher concentrations have the advantage of being administered in lower volumes. For example, a 70-kg individual given 1 g/kg body weight of a 5% solution would receive 1400 ml, and with a 10% solution only 700 ml. Osmolality of the final product can also be a concern: the more physiological the osmolality, the lower the potential incidence of adverse events. Similarly, sodium content, a major contributor to osmolality, has been implicated in adverse events[10]. Some but not all products contain sugars as stabilizers. Acute renal failure/dysfunction has been rarely associated with IVIG administration; epidemiological data have implicated sucrose as the causative factor in approximately 90% of reported cases[11]. An increasing incidence of thromboembolic phenomena has been reported. The etiology is unclear, but may relate to sodium/osmolality and total amount of immunoglobulin administered. Caution is also important when lyophilized preparations are reconstituted to a lower volume to increase the concentration of the infusion. This also results in increasing the concentration of sodium and sugars, contributing to sodium levels approaching 2% and a hyperosmolar

Table 2 Product features affecting clinical tolerability

Volume load (rate of infusion)
Osmolality
Sodium content
Sugar content
PH

Immunoglobulin A (IgA) content

solution. pH is not a factor, as the solutions are not buffered and the acid load is negligible.

The features listed in Table 2 are of greater importance to patients who may be at risk. In particular, the very young (neonates), elderly patients or those with cardiac or renal disease may be very susceptible to issues such as volume load, sodium or sugar content and osmolality. With the spectrum of available products, matching the appropriate product to an individual patient is critical.

EFFICACY

Given the many differences in the manufacturing process, the various approaches to viral elimination discussed and the differences in composition, it is not unreasonable to assume that the sum of these differences could impact upon clinical efficacy and outcome. However, it is difficult if not impossible to make comparisons among products as there have not been any head-to-head trials. Only recently has a direct comparison been carried out, evaluating Gamiomune® and Gamunex for infection prophylaxis in PIDD[12] and in restoring and maintaining platelet counts in idiopathic thrombocytopenic purpura[13]. Surprisingly, Gamunex appeared to achieve a better outcome in virtually all of the parameters monitored.

What emerges is that not all IVIGs are the same. Product attributes emerging during manufacture, formulation and composition contribute to these differences. From the patient's perspective (IDF survey), the three most important factors considered in switching products are efficacy, safety and tolerability. A comprehensive approach to IVIG safety must consider the interplay between virus elimination, tolerability and efficacy if we are to improve clinical outcomes.

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11
Immunoglobulin replacement by the subcutaneous route using preparations licensed in for administrations by other routes

M.Berger, K.Duff, J.Poll and S.Roy

Although Bruton treated the first agammaglobulinemic patient with subcutaneous injections of immune serum globulin[1], the intramuscular regimen used by Janeway, Gitlin and their colleagues in Boston soon became the standard of care[2,3]. In the late 1970s, Berger and colleagues introduced the use of small, battery-powered syringe driver pumps to administer, slowly, larger amounts of immune serum globulin than would be tolerated by intramuscular injection[4,5]. Shortly after that, however, the first preparations of immunoglobulin G (IgG) for intravenous use were licensed in the USA and became the predominant form of IgG replacement used in this country. Interest in subcutaneous infusions continued in Europe, nevertheless, particularly in the Scandanavian countries, and a number of protocols for both slow and rapid subcutaneous infusion were developed. Several studies, including large series, have shown the safety, practicality and advantages of the subcutaneous route of IgG replacement[6–8]. Stiehm and associates reported that patients who had severe adverse reactions to multiple intravenous immunoglobulin (IVIG) preparations could tolerate IVIG when it was given by slow subcutaneous infusion[9]. Despite these reports, there is no preparation of IgG licensed for subcutaneous administration in the USA.

In our large, university-based referral practice, out of about 100 patients receiving IgG replacement therapy, 11 are being given their treatment by the subcutaneous route, using preparations licensed for intramuscular (IM) or intravenous (IV) administration (Table 1). Reasons for use of the subcutaneous route of administration include poor IV access (three patients), excessive adverse events, including severe migraine headaches, nausea and/or vomiting despite trying multiple different IVIG preparations (four patients) and the convenience of self/home administration (four patients).

Five patients are receiving a liquid 16% preparation intended for IM use (Baygam^{R}), and six patients are receiving lyophilized preparations containing sucrose, which are intended

Table 1 Patients receiving immunoglobulin G therapy by subcutaneous route

Patient	Age (years)	Weight (kg)	Monthly dose (g)	mg/kg/ month	g/dose	ml/dose	Sites per infusion	Frequency (per week)	Duration of infusion (h)	Doses/ month
Lyophilized preparation with sucrose: Carimune™ NF or Panglobulin™ NF*										
#1	5	16.8	9.6	571	1.2	10	1	twice	2–3	8
#2	14	45.4	24	528	6	40	2	once	9.5	4
#3	24	63	24	380	6	40	2	once	5–6	4
#4	41	60.8	30	493	6	40	2	once	2–3	5
#5	41	75.8	54	712	6	40	2	twice	3–4	9
#6	42	106	54	509	12	80	4	once	2–3	4.5
Liquid 16% IM ISG: Baygam®										
#7	9	37.7	19.2	339	1.6	10	1	three times	4–5	12
#8	11	30.6	19.2	633	4.8	30	2	once	1	4
#9	12	39.6	19.2	676	1.6	10	1	three times	3	12
#10	49	77.4	51.2	661	6.4	40	2	twice	2–4	8
#11	50	69.5	51.2	724	6.4	40	2	twice	4–5	8

*Used at 15% except patient 1:12%; IM, intramuscular; ISG, immune serum globulin

for IV use (Carimune™ NF or Panglobulin™ NF). The latter products are available in bottles containing 6g of IgG and 10g of sucrose. For subcutaneous use, 40 ml of sterile water for injection is used to reconstitute the lyophilized material. We calculate that this yields a solution of approximately 15% IgG, which would have an osmolality of 960 mosmol/kg. One patient is using a lyophilized preparation reconstituted in the standard 12% IV form. The choice between use of the 16% IM preparation and the lyophilized material is partly dependent on the number of grams of IVIG to be given per dose. If this is under 6g, the liquid IM preparation is generally used, at increments of 1.6g (10ml). For patients receiving 6g or more per dose, the choice of products has been determined by local availability, price or logistic factors. Doses range between 340 and 724mg/kg/month. Four patients are scheduled to receive subcutaneous infusions once a week, five patients twice a week and two patients three times per week. Some patients require one additional dose per month to complete the designated total monthly dose (i.e. five or nine doses per month). Graseby model MS-16 pumps (MarCal Medical, Millersville, MD) are used for 30 ml or less per infusion, and Freedom-60 pumps (Repro-Med, Chester, NY) are used for 40 ml or more. Self-adhesive 27-gauge 6–8 mm plastic catheters (Sof-set, Minimed, Northridge, CA, USA or Clear-View, Norfolk Medical, Skokie, IL, USA) intended for subcutaneous infusion of insulin or narcotics are used by all patients. A maximum of 20 ml of solution is given into a single site. When larger total volumes are used for individual doses, the infusions are divided into multiple sites by the use of y-connectors and multiple catheters to infuse into several sites simultaneously, or by removing and reinserting a single catheter after 20ml has been infused into one site. Most patients complete their infusions in under 4h. However, one child routinely takes 30ml of 16% immune serum globulin into two sites in 1h, and one teenager with a history

of severe migraines during IVIG infusions prefers to take her 40ml of 15% solution into two sites in 9 h while sleeping.

Over 1500 subcutaneous infusions have been completed by these patients. No patient has had any significant systemic reaction to any subcutaneous infusion. One patient uses an antihistamine for premedication, but no other patient has required any routine medication before, during or immediately after subcutaneous infusions. None of the patients require treatment for migraine symptoms associated with these infusions. Most infusions are given in the anterior or lateral abdominal wall or the anterior or inner thigh. Several patients have reported small areas of induration, or painless swelling at subcutaneous infusion sites. In almost all cases this has been transient, and the infusion sites are no longer identifiable 24h after an infusion has been completed. One patient developed cellulitis after an infusion in which 40 ml was administered into a single site. This patient subsequently returned to the IV route. One patient, who was taking an infusion in the leg, developed a red streak going down the leg and feelings of numbness and tingling distally in that leg. We speculate that the infusion may have been inadvertently given into a superficial nerve or blood vessel, although there was no bleeding at the site when the needle was withdrawn. That particular infusion was stopped. The patient had previously used different vials of the same lot of product with no problem and has subsequently continued using that product, but now takes 0.5mg/kg Benadryl® before her infusions as a premedication. She has not experienced any further adverse reactions and continues to prefer to receive her IgG replacement by the subcutaneous route. One patient who was scheduled to self-administer 6g of IgG nine times per month required hospitalization for cellulitis surrounding a foreign body in the sole of his foot. At the time of admission, his serum IgG was 631mg/dl (lower limit of normal 700mg/dl). He then confessed that he had become complacent and was only taking the subcutaneous infusions once a week. He had also shown poor compliance previously with scheduled IVIG infusions.

We conclude that preparations of IgG currently licensed in the USA for IM or IV use can be safely administered subcutaneously. Reconstitution of lyophilized products to higher concentrations than would be given intravenously facilitates their delivery by small portable pumps, and neither nursing nor pharmacist involvement is necessary. The hyperosmolality of sucrose containing lyophilized products does not preclude their administration by the subcutaneous route and does not increase the incidence of local or systemic adverse effects. Careful selection of patients and routine physician monitoring are necessary to ensure proper compliance and efficacy of IgG replacement.

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12
The use of intranenous immunoglobulin for allogeneic stem cell transplantations: the US experience

R.H.Buckley

Intravenous immunoglobulin (IVIG) has been used for over two decades as part of the treatment regimen for chemoablated recipients of allogeneic bone marrow stem cell transplants, and has been approved by the US Food and Drug Administration for this indication[1]. Part of the rationale for using it is that a passive antibody might prevent infections in these iatrogenically neutropenic and T cell-immunosuppressed patients, particularly the highly problematic infections caused by cytomegalovirus and the interstitial pneumonia usually attributed to it[2]. A study performed before the availability of ganciclovir reported that IVIG reduced the incidences of both cytomegalovirus (CMV) infection and interstitial pneumonia in allogeneic bone marrow transplant recipients[2]. Most subsequent studies have shown that a combination of high-dose IVIG and ganciclovir is superior to each alone[3,4]. The development of hyperimmune anti-CMV IVIG preparations has led to the preferential use of it over polyclonal IVIG preparations. However, hyperimmune anti CMV IVIG alone did not prevent CMV viremia or pneumonitis nor did it improve 1-year survival in CMV-seropositive lung transplant recipients[5]. This is unfortunate, considering the rising incidence of ganciclovirresistant CMV organisms[6], which is being accelerated by the widespread practice of using ganciclovir prophylactically in many bone marrow and organ transplant centers. Thus, CMV infections continue to be a major contributor to morbidity and mortality after all types of transplantation.

Immunoglobulin prophylaxis against respiratory viruses has also been advocated in these immunosuppressed patients. Respiratory syncytial virus (RSV) immunoglobulin has been advocated for this purpose, because it contains not only high titers of antibodies to RSV but also high titers of antibodies to other respiratory viruses, such as parainfluenza-3 and the influenza viruses. However, in a recent study of the efficacy of RSV immunoglobulin in preventing RSV infection, no efficacy could be shown because of the low incidence of these infections in the study patients[7]. It was also observed that the impact that these infusions had on antibody titers to RSV in the recipients was minimal except in those who had low titers at the outset.

Although the above-cited reports supported the usefulness of IVIG for infection control in bone marrow transplant recipients, there were divergent conclusions about its efficacy from two large meta-analyses, with one supporting its use[8] and the other not[9]. It was noted that none of the trials reviewed in these metaanalyses were placebo-controlled, and most were carried out before effective drugs for CMV infection and disease were

available. No benefit was seen for IVIG infusions in the prevention of late infections after bone marrow transplantation in a non-immunodeficient patient population[10].

The authors' experience with IVIG is primarily with bone marrow transplants into non-chemoablated infants with severe combined immunodeficiency (SCID), who are profoundly hypogammaglobulinemic and usually infected with viral agents at presentation[11,12]. In these patients there is clearly a role for IVIG replacement therapy pre- and post-transplantation. These infusions are continued on a weekly or biweekly basis until T-cell function develops at around 3–4 months post-transplantation, then monthly until there is some evidence of endogenous immunoglobulin production. Development of B-cell function in these infants is variable, and largely dependent upon the molecular type of SCID, with those who have interleukin-7 receptor α (IL-7Rα) chain deficient SCID having the best B-cell function post-transplantation. Studies done elsewhere have shown that this problem is not improved by the use of pre-transplant chemotherapy in these SCID infants[13]. Currently, 102 (77%) of the 132 SCID patients transplanted at the authors' institution have survived at periods from 3 months to 21 years post-transplantation. Approximately 60% of the survivors receive monthly IVIG replacement therapy.

In addition to possibly preventing infections during the post-transplant period, IVIG has also been reported to have an immunomodulatory effect in lessening the occurrence and severity of acute graft-versus-host disease (aGVHD)[14]. However, this was not found to be the case for chronic GVHD[10]. The mechanism of action of IVIG in preventing aGVHD was investigated in a rat model of aGVHD, and both intact immunoglobulin G (IgG) molecules as well as the $F(ab)_2$ fragments of IgG were able to protect against aGVHD[15]. Protection against aGVHD was associated with a decreased ability of lymphocytes to proliferate spontaneously and to produce nitric oxide (NO) and interferon-γ (IFN-γ) *in vitro* in the absence of increased production of interleukin-10 (IL-10). In addition, protection was associated with a decrease in CD4+ T cells that bore the activation marker CD134 *in vivo*, and with enhanced apoptosis of activated CD4+ T cells by IVIG *in vitro*. This suggested to the investigators that the mechanism of action of IVIG is by the induction of apoptosis of activated alloreactive CD4+CD134+ donor T cells[15]. IVIG has also been shown to inhibit the differentiation and maturation of dendritic cells *in vitro*, and to abrogate the capacity of mature dendritic cells to secrete IL-12 on activation, while enhancing IL-10 production[16]. In those studies, IVIG also induced down-regulation of co-stimulatory molecules associated with the modulation of cytokine secretion, and inhibited autoreactive and alloreactive T-cell activation and proliferation.

A recent US multicenter, randomized, double-blind comparison of three different doses of IVIG (100, 250 and 500mg/kg) showed no differences in the rates of acute or chronic GVHD or infection after allogeneic bone marrow transplantation[17]. There was less GVHD in patients with unrelated marrow donors who were treated with the higher dose, but the difference was not statistically significant ($p<0.07$). Finally, results of the first randomized, double-blind, dose-effect, placebo-controlled, multicenter trial of IVIG in allogeneic-related marrow transplantation were recently reported from the GREFIG Study Group in France[18]. The 200 patients studied were from 19 different centers; all received human leukocyte antigen (HLA)-identical sibling marrow. Surprisingly, IVIG had no benefit over placebo in terms of infections experienced. In addition, there was no

difference in the cumulative incidences of interstitial pneumonia, GVHD, transplantation-related mortality or overall survival when the IVIG-treated groups were compared with each other or with placebo. There was a statistically higher incidence of grade 3 (severe) veno-occlusive disease as the immunoglobulin dose increased (p=0.01). Patients given higher doses of IVIG had side-effects, such as fever and chills, more often than did other patients. The authors concluded that the data provided no basis to recommend IVIG for HLA-identical sibling bone marrow transplants[18].

In summary, IVIG appears to offer protection against some types of infection when given in conjunction with antibiotics. Clearly it is of benefit to SCID infants and to those with other primary immunodeficiency diseases who are undergoing bone marrow transplantation. Routine use of IVIG appears to offer no benefit to other types of patients, such as those with malignancies, who are undergoing HLA-identical sibling bone marrow transplants. Moreover, high doses of IVIG could increase the risk of severe venoocclusive disease in these patients.

More studies are needed to determine whether IVIG is beneficial in the case of matched unrelated donor (MUD) bone marrow or cord blood transplants.

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13
Intravenous immunoglobulin for bone marrow/stem cell transplantation: the European experience

K.Theunissen, J.A.Maertens and M.A.Boogaerts

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) was originally developed for the treatment of aplastic anemia and hematological malignancies. However, with an increasing insight into risk factors, supportive care and mechanism of action over the past decade, its use has spread to the treatment of solid tumors, immunodeficiency syndromes, congenital enzyme deficiencies and autoimmune disorders. Nevertheless, these procedures include severe temporary alterations in the host defense mechanisms, generally caused by a combination of the underlying disease, previous treatments, the preparative myeloablative treatment for the transplantation and—in the case of allogeneic transplantation—immune suppressive drugs and graft-versus-host disease (GVHD).

Since its availability in the early 1980s, high-dose intravenous immunoglobulin (IVIG) therapy has been advocated as standard supportive care in both allogeneic and autolo gous HSCT. In the setting of defective humoral and cellular immune systems generally observed in the HSCT patient, it is expected to help in preventing serious infective complications, and play a critical adjuvant role in their treatment. Moreover, in allogeneic HSCT, IVIG has been attributed the role of immune modulator in GVHD.

Immune system deficiency and recovery in the hematopoietic stem cell transplant patient

The conditioning regimen used just prior to a HSCT, which is classically made up by a combination of high-dose chemotherapy and/or irradiation, is thought to treat the last trace of malignancy remaining in the patient. Unfortunately, also, normal hematopoiesis, humoral and cellular host defense mechanisms and mucosal barriers are destroyed. Indwelling intravenous catheters are usually placed to deliver the necessary supportive care, but at the same time they serve as an additional port of entry for opportunistic pathogens from organisms colonizing the skin. Engraftment, characterized by a sustained absolute neutrophil count of >500/mm^3, restores an effective phagocytic function, and usually occurs 2–3 weeks after transplantation. However, at the same time, recipients may experience acute GVHD that manifests as skin, gastrointestinal and liver injury. Although autologous or syngeneic recipients might experience a mild and self-limiting GVHD-like syndrome, GVHD occurs primarily in allogeneic recipients, particularly those receiving mismatched or unrelated donor grafts. GVHD *per se* is a major risk factor

for delayed immunological recovery, and the immunosuppressive agents used to prevent and treat GVHD make the recipient even more vulnerable to opportunistic viral and fungal pathogens. In the late post-transplantation phase, which starts around 100 days after HSCT, autologous recipients have a rapid recovery of immune functions and are at a lower risk of infections. Among allogeneic patients, however, some will experience chronic GVHD, which appears similar to autoimmune connective tissue disorders, and is associated with cellular and humoral immunodeficiency, including macrophage deficiency, impaired neutrophil chemotaxis, poor response to vaccination and varying degrees of mucositis. After the resolution of chronic GVHD, cell-mediated and humoral immunity functions are gradually restored, but this may take up to a couple of years.

CLINICAL RESULTS IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

The major challenge in autologous HSCT recipients is to prevent infectious complications in the neutropenic phase immediately after the preparative regimen, and before engraftment occurs. The use of myeloid growth factors and of growth factor-primed peripheral blood stem cells has drastically diminished the duration of severe neutropenia after autologous transplantation[1]. The risk of occurrence of viral infections, and especially cytomegalovirus (CMV) infection, CMV disease and interstitial pneumonitis, is markedly reduced, compared with allogeneic HSCT recipients[2,3]. This difference seems to parallel and be solely related to the occurrence of acute GVHD in allogeneic recipients.

Studies that have looked at prolonged IVIG administration after autologous HSCT in unselected patients have failed to show a benefit[4,5]. However, neither of these studies took into account whether patients had immunoglobulin deficiency or not. Grill and colleagues[6] have reported the critical influence of previous conventional chemotherapy on the development of immunoglobulin deficiency during the early post-transplantation period in children. It remains to be determined whether IVIG can be beneficial when autografting patients intrinsically at high risk for secondary immunodeficiency due to the underlying disease, such as chronic lymphatic leukemia (CLL) or multiple myeloma[7]. Also, the influence on immune reconstitution of the increasing use of newer drugs such as fludarabine[8], and monoclonal antibodies (rituximab or alemtuzumab) prior to the stem cell harvest, will need to be investigated.

CLINICAL OUTCOME IN RELATED ALLOGENEIC HEMOTOPOIETIC STEM CELL TRANSPLANTATION

The use of IVIG post-HSCT should have become standard practice after the pivotal study of Sullivan and colleagues in 19909. A recent survey from the European Blood and Marrow Transplantation Group[10], however, reported that 55% of 45 European centers give prophylactic immunoglobulin to all transplant recipients, indicating there has been no consensus about the usefulness of IVIG. Most of the large series reported to date have used different products (hyperimmune or polyvalent immunoglobulins), schedules and

dosing regimens, and a mixture of patient populations. Since the original Sullivan report, the beneficial role of myeloid growth factors in decreasing the duration of severe neutropenia and the incidence of severe infections in the early post-HSCT phase has been established[11,12]. Furthermore, protocols have become available either to prevent CMV and fungal infections, or to treat them pre-emptively, and are widely in use. This has led to confusing results and has encouraged new trials to define better the optimal dose and duration of IVIG therapy, and its clinical value.

One of these trials was recently concluded and reported by Cordonnier and colleagues[13]. In a multicenter trial they randomly assigned related allogeneic HSCT patients to receive 16 weekly doses of placebo or one of three dose levels of IVIG. No differences were observed with regard to infection prevention, incidence of interstitial pneumonia, and acute or chronic GVHD. Although there might have been a statistically non-significant trend, due to an underpowered study to detect a reduction in GVHD, towards prevention of acute GVHD in the 500-mg/kg group, this was countered by the observation that severe veno-occlusive disease (VOD) occurred more frequently when the immunoglobulin dose was increased. Transplant-related mortality at 6 months and overall survival at 24 months after transplanta tion were similar among groups. The results are similar to those reported earlier by Klaesson and colleagues[14] based on a smaller cohort and a historical control group.

However, it is of note that this trial excluded patients with hypogammaglobulinemia, with a previous autologous transplant, and patients with a mismatched or matched unrelated donor.

CLINICAL OUTCOME IN MATCHED UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION

The chance of finding a matched related donor is only 1/4, offering a suitable donor to only 30% of patients requiring a HSCT. This has prompted the transplant community to establish registers of volunteer donors, offering the possibility to use a matched unrelated donor HSCT as an alternative approach. In spite of better insights into major human leukocyte antigen (HLA) compatibility requirements for a successful transplantation, there is a substantially increased risk for severe acute and chronic GVHD and transplant-related mortality. Several approaches have been used to decrease the risk of GVHD, most of them focusing on *in vivo* (antithymocyte globulins, Campath®) and/or *in vitro* T-cell depletion (Campath, CD34 selection, (un)selective T- and/or B-cell depletion). These approaches will cause further immunodeficiency and impair immune reconstitution even more. On the other hand, studies addressing the potential use of IVIG are scarce, and difficult to perform because of the large heterogeneity of the study population. A preliminary report by Sullivan and co-workers[15] showed no difference in transplantrelated mortality, and acute or chronic GVHD in a placebo-controlled randomized study of 497 patients. In another study, Winston and associates[16] reported a trend towards a lower incidence of acute GVHD in unrelated recipient-donor pairs, although not significant because of a lack of power. Again, subpopulations will need to be defined to observe clear benefits.

DEVELOPING HEMATOPOIETIC STEM CELL TRANSPLANTATION MODALITIES

In spite of an increasing use of matched unrelated donors, there is still a large patient group that cannot find a suitable donor within due time. Umbilical cord blood grafts are an attractive alternative for transplantation in children, and are increasingly used in adults, despite the limited cell-dose available. This leads to a prolonged neutropenic phase, although ultimately immune reconstitution seems to be at least as fast as in classical bone marrow transplants, and severe GVHD is less frequent[17–19].

Another attractive approach is the use of haploidentical HSCT. Because of the increased risk of fatal GVHD, very efficient T-cell depletion is needed in this setting, which consequently leads to a very slow and sometimes incomplete immune reconstitution[20].

The last modality that needs to be discussed in this overview is of course the growing interest in non-myeloablative conditioning regimens. These regimens rely on the graft versus tumor effect rather than a strong cytotoxic effect of the drugs administered ultimately to treat the malignancy, and consist merely of a cocktail of strong immunosuppressive drugs to allow for engraftment of the donor stem cells. Although the first phase of this type of conditioning is generally characterized by a strong reduction in toxicity and time of neutropenia, the drugs that are used still cause a state of severe immune suppression in the host, and, especially in matched unrelated transplants, a high incidence of acute and chronic GVHD is observed[21–23].

Whether IVIG will have a role in the above-mentioned modalities of HSCT is a matter of pure speculation at this point. Just as for the more established transplant procedures, populations at risk who will benefit from prophylactic administration of IVIG will have to be identified by randomized, placebocontrolled trials.

CONCLUSIONS

There is an increasing body of evidence that the routine use of IVIG as prophylaxis in HSCT recipients is not indicated. There is no evidence supporting the use of IVIG beyond 100 days post-transplantation. However, there is a continuous need to identify further those categories at risk that do benefit from IVIG administration, to preserve an optimal cost-benefit ratio.

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14
Secondary antibody deficiency following treatment of vasculitis: the use of intravenous immunoglobulin

V.J.Tormey and A.Burns

Systemic vasculitides are diseases associated with high morbidity and mortality in which cyclophosphamide and corticosteroids are highly effective treatment. However, current treatment is toxic, and although successful in inducing remission is frequently complicated by infection[1]. Treatment-related toxicity, particularly infection, is a common cause of death or severe morbidity and is associated with leukopenia[2], increasing age and concomitant steroid dose[3]. Most trials and meta-analyses[4,5] monitor leukopenia and neutropenia, but not hypogammaglobulinemia, as an adverse effect of cyclophosphamide or azathioprine.

There have been several isolated reports of secondary hypogammaglobulinemia caused by drugs, including cyclophosphamide[6,7] and sulfasalazine[8] in systemic lupus erythematosus (SLE) and inflammatory arthritis. Severity has varied from selective immunoglobulin A (IgA) deficiency to panhypogammaglobulinemia, which is frequently reversible, although full recovery of antibody function may take months or years. Although considered uncommon, the incidence of drug-induced hypogammaglobulinemia in vasculitis is unknown. Following the identification of hypogammaglobulinemia in one patient after treatment of systemic vasculitis with prednisolone and cyclophosphamide, we introduced a screening program for anti-body deficiency as part of the routine follow-up in vasculitis.

We retrospectively reviewed a cohort of 53 consecutive patients with primary vasculitis who were treated between 1995 and 2002 in the renal vasculitis unit at the Royal Free Hospital, London. All patients were initially treated with prednisolone and oral cyclophosphamide. In addition, 17 were treated with intravenous methylprednisolone, ten by plasma exchange and three with intravenous immunoglobulin (IVIG) to induce remission. Eight to twelve weeks following achievement of remission, the cyclophosphamide was discontinued and azathioprine commenced. Five (9%) developed hypogammaglobulinemia, of whom four had infections including two with septicemia (Table 1). In the two patients

Table 1 Clinical course of patients with secondary hypogammaglobulinemia requiring intravenous immunoglobulin (IVIG)

Patient	Treatment	Time from decreasing Ig to nadir level	Ig pre-treatment (g/l)	Ig post-treatment (g/l)	Infection	Time from nadir to Ig recovery
1	prednisolone, cyclophosphamide, azathioprine (after 3 months)	2–7 months	IgA, 5.6 IgG, 12.9 IgM, 0.9	IgA, <0.1 IgG, 1.9 IgM, <0.1	CMV viremia, HSV with bacterial superinfection	no recovery after 22 months
2	methylprednisolone, cyclophosphamide	2–5 months	IgA, 2.0 IgG, 6.6 IgM, 0.2	IgA, 0.2 IgG, 0.8 IgM, <0.1	campylobacter diarrhea, staphylococcal, septicemia (hemodialysis line)	no recovery after 12 months

Ig, immunoglobulin; CMV, cytomegalovirus; HSV, herpes simplex virus

with more severe hypogammaglobulinemia, this merited the use of regular IVIG replacement at a dose of 400mg/kg/month. Both have been receiving IVIG for 12 and 22 months, respectively, maintaining their preinfusion trough IgG level of approximately 8g/l. They have remained free of infection, despite continued prednisolone alone or prednisolone with azathioprine treatment.

Severe hypogammaglobulinemia may prevent antineutrophil cytoplasmic antibodies (ANCA), antimyeloperoxidase and antiproteinase-3 antibodies being used as markers of disease activity[9]. However, none of the patients had relapses of vasculitis during the period of hypogammaglobulinemia, when as expected ANCA was negative.

With an annual incidence of primary systemic vasculitis of up to 19/million per year, approximately 100 in the UK and 500 in the USA will develop hypogammaglobulinemia each year. This issue has not been addressed in the European Vasculitis trials[5,10]. Although it is not the sole contributing factor to the infection risk in vasculitis, hypogammaglobulinemia is important because morbidity and mortality may be reduced by immunoglobulin replacement (IVIG) in selected patients. Future vasculitis trials should include immunoglobulin measurement as part of study protocols to determine whether hypogammaglobulinemia contributes to infectious complications or mortality. Further assessment of antibody function by measuring IgG responses to vaccination with tetanus toxoid and pneumococcal polysaccharide is recommended in those patients being considered for IVIG.

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15
Intravenous immunoglobulin in the prevention and treatment of severe infections

K.Werden

Intravenous immunoglobulin (IVIG) is widely used as prophylaxis against severe infections in intensive-care unit patients and as an adjunctive measure of sepsis treatment, especially in patients with severe sepsis and septic shock. When doing this, we intensivists, however, must bear in mind that this approach does not belong to the currently approved medical indications for IVIG products[1]. (Note that this chapter focuses on IVIG application in adult intensive-care unit patients. Sources for IVIG prophylaxis and treatment in neonates can be found in reference 2.)

RATIONALE

Low immunoglobulin G (IgG) and IgM levels predispose to postoperative infections. In patients with sepsis, serum immunoglobulin levels are often found to be in the lower normal range. Intravenous immunoglobulin therapy aims at raising the serum levels for at least several days to the upper normal range or even above. In adult patients this has been achieved, for instance, in the case of an IVIG(G) preparation by a total of 0.9g/kg body weight (0.6g/kg on day 1 and 0.3g/kg on day 2), and in the case of an IVIG(GMA) preparation (5% solution with 3.8g IgG, 0.6g IgM and 0.6g IgA per 100ml) with a total of 0.75g/kg (0.25g/kg=5ml/kg each on 3 consecutive days)[3]. Many mechanisms have been discussed as the basis for a beneficial role of IVIG in sepsis[2], with complement inhibition being one of the recent findings[4].

Intravenous immunoglobulin treatment of ‘the’ patient with severe sepsis and septic shock

Impressive reduction in mortality by IVIG treatment of sepsis and septic shock was reported in the Cochrane database[5], established on the results of several, relatively small controlled trials.

In a meta-analysis of eight studies including 413 patients with severe sepsis and septic shock, mortality was significantly reduced by 40% (relative risk (RR) 0.60, 95% confidence interval (CI) 0.47–0.76). Mortality reduction was more pronounced when treating with an IVIG(GMA) preparation (*n*=150; RR 0.38, 95% CI 0.22–0.67) than with IVIG(G) preparations(*n*=219; RR0.68, 95% CI 0.51–0.89).

The authors of the meta-analysis stated that polyclonal IVIG has a very promising role as adjuvant therapy in sepsis. Of course, they were also aware of the pitfalls of a meta-analysis based on relatively small IVIG-sepsis trials. Consequently, they postulated that 'large multicenter studies are needed to confirm the effectiveness of polyclonal IVIGs in reducing mortality in patients with sepsis'.

On the other hand, contrary conclusions have been drawn by the experts from the International Sepsis Forum[6]. They agree that some small, positive, IVIG-sepsis trials exist (grade C). However, they state that this evidence is not convincing enough to recommend IVIG treatment in sepsis. Also, the German Sepsis Society supports this statement.

In the meantime, the postulated 'large multicenter [study]…to confirm the effectiveness of polyclonal IVIGs in reducing mortality in patients with sepsis' has been carried out. The prospective, randomized, placebocontrolled SBITS study (score-based-immunoglobulin-therapy in sepsis study) has included 653 patients with score-defined septic multiorgan dysfunction syndrome[7,8]. Half of the patients were given a total of 0.9g/kg body weight IVIG(G) (Polyglobin®; Bayer, Germany: 0.6g/kg on the day of sepsis diagnosis and 0.3g/kg on the following day). Disappointingly, 28-day mortality in the IVIG(G) and in the placebo group was not significantly different. The failure to reduce mortality by IVIG(G) in the SBITS study[8], with more patients included than evaluated in the Cochrane database, seriously questions whether IVIG—at least, IVIG(G)—can reduce mortality in sepsis and septic shock. The list of currently approved medical indications for various IVIG products invariably does not include sepsis and septic shock[1].

IVIG TREATMENT OF SEPSIS IN WELL-DEFINED SUBGROUPS OF PATIENTS

In several, well-defined sepsis subgroups, a reduction in mortality has been described with IVIG in small yet low-powered clinical trials (Table 1). These subgroups consist mainly of:

(1) Surgical patients with severe, score-defined postoperative sepsis (sepsis score ≥17)[9];
(2) Patients with septic shock and endotoxemia[10];
(3) Patients with streptococcal toxic shock syndrome[11,12];
(4) Young patients with meningococcal septic shock[13].

Patients with disastrous streptococcal toxic shock syndrome probably benefit from high doses of IVIG (total amount 2g/kg[12] or even more[11]). The multicenter, randomized, double-blind, placebo-controlled trial of Darenberg and colleagues[12], however, was prematurely terminated because of slow patient recruitment, and results were obtained from 21 enrolled instead of 120 planned patients.

It remains questionable whether a large controlled trial will ever be available for this rare form of septic shock[2,11,14]. Databases of these patients and consensus statements concerning the role of IVIG would be helpful. Trial evidence argues for the application of IVIG(G)[11,12], while the antibody pattern argues for the application of IVIG(GMA)[15].

Table 1 Intravenous immunoglobulin (IVIG) treatment of sepsis subgroups

			Mortality					
Patients	*n*	Immune globulin	Control	IVIG	RR	*p* Value	Study design	Reference
Postoperative sepsis score ≥17	113	IgG	36/56 (64%)	19/57 (33%)	−48%	<0.005	prosp.	Dominioni *et al.*, 1996[9]
Endotoxemia+ septic shock	55	IgGMA	9/28 (32%)	1/27 (4%)	−88%	0.0063	prosp.	Schedel *et al.*, 1991[10]
Streptococcal toxic shock syndrome	53	IgG	14/21 (67%)	11/32 (34%)	−49%	0.009	histor.	Kaul *et al.*, 1999[11]
	21	IgG	4/11 (36%)	1/10 (10%)	−72%	0.3	prosp.	Darenberg *et al.*, 2003[12]
Meningococcal sepsis	32	IgGMA	15/21 (71%)	3/11 (27%)	−62%	0.019	histor.	Thomson *et al.*, 1989[13]

IgG, intravenous immunoglobulin G; RR, relative risk; prosp., prospective.; histor., historical

Cardiac surgery patients operated on with the support of cardiopulmonary bypass are at increased risk for sepsis and escalating systemic inflammatory response syndrome (SIRS). The results of the ESSICS study[16] do not support a beneficial role of IVIG(G) in early postoperative escalating SIRS in cardiac surgery patients. The ongoing Adjuvant Treatment of Media-stinitis with Immunoglobulins (ATMI) trial[17] will give information regarding whether the cardiac surgery patient with mediastinitis will benefit from adjunctive IVIG(GMA) treatment.

IVIG PROPHYLAXIS OF SEVERE INFECTIONS IN INTENSIVE-CARE UNIT PATIENTS

In high-risk patients, IVIG prophylaxis can undoubtedly reduce the occurrence of infec tions, especially pneumonias (Table 2). Recommendations for IVIG prophylaxis in selected patient groups will be strongly influenced by the success of IVIG propyhlaxis not only to reduce the rate of infections, but also to reduce infection-related mortality, length of stay in the intensive-care unit, antibiotics days, duration of mechanical ventilation and other expensive measures. For specific operations with a high risk of infection this additional requirement seems to be fulfilled[18], but not for patients with severe trauma[1,19], and in the case of anergic cardiac surgery patients, it is still an open question[20].

Of interest is the observation—found in a retrospective manner—that early IVIG(GMA) application may reduce the occurrence of critical illness neuropathy in

patients with Gram-negative sepsis[21]. Confirmative data from a prospective trial are, however, still lacking.

Table 2 Prophylaxis of severe infections in intensive-care unit (ICU) patients

Study	Patients *(n)*	Immune globulin	Prophylaxis (P)/ Therapy (T)	Success
Duswald *et al.*, 1980[23]	150 surgical	IgG	P	↓local infections
Glinz *et al.*, 1986[24]	150 polytrauma	IgG	P	↓pneumonias
Just *et al.*, 1986[25]	150 ICU	IgGMA	T	↓duration mech. vent.
				↓duration ICU in a risk group
Kress *et al.*, 1999[20]	40 cardiac surgery anergy	IgGMA		↓infections
Cafiori *et al.*, 1992[22]	80 surgical sepsis risk	IgG		↓infections
Douzinas *et al.*, 1999[19]	39 polytrauma, ISS 16–50	IgG		↓pneumonias
IICSG 1992[18]	352 postoperations with high infectious risk	IgG		↓infections (pneumonias)
				↓duration ICU
				↓duration hospital

Mech. vent., mechanical ventilation; IgG, immunoglobulin G; IICSG, the Intravenous Immunoglobulin Collaborative Study Group

IVIG FOR PREVENTION AND TREATMENT OF SEVERE INFECTIONS: STATE OF THE ART 2003

IVIG is undoubtedly not a 'magic bullet' of adjunctive sepsis treatment, but there are enough encouraging experimental and clinical data to maintain continuing interest in the IVIG field. Evidence-based medicine data and expert opinions at present are controversial, and may not be very helpful to the intensivist for decision-making.

The author's present view is the following (see also Table 3).

For 'the' patient with severe sepsis and septic shock, the results from the SBITS study especially argue against a survival benefit of IVIG(G) treatment with about 1g/kg body weight. It should be kept in mind that in the streptococcal toxic shock syndrome trials, accumulative IVIG(G) doses twice as high, 2g/kg and even higher, have been applied.

In the case of IVIG(GMA), some small trials argue for a survival benefit (evidence grade C); a confirming large trial is still not available.

Consequently, the author treats his patients in the early state of severe sepsis and septic

Table 3 Evidence-grading (EG) of intravenous immunoglobulin (IVIG) treatment of severe sepsis and septic shock. Grading is based on the recommendations of the International Sepsis Forum (ISF), 2001[6]

	Cochrane metaanalysis		Additional studies		ISF expert opinion		Author's statement	
Patients	Yes/no	EG	Yes/no	EG	Yes/no	EG	Yes/no	EG
Severe sepsis and septic shock								
IVIG	yes (+)	C			no	C		
IVIG(G)	yes(+)	C	no	B			no	B
IVIG(GMA)	yes(++)	C					yes	C
Sepsis subgroups								
Postoperative sepsis score ≥17 (IVIG(G))							yes	C
Septic shock with endotoxemia (IVIG(GMA))							yes	C
Meningococcal sepsis (IVIG(MA))							yes	E
Streptococcal toxic shock syndrome (IVIG(G))							yes	E

Grade A, at least two large, randomized, controlled trials with unequivocal results; Grade B, one large, randomized, controlled trial with unequivocal results; Grade C, only small randomized trials with uncertain results; Grade D, at least one non-randomized trial with concurrent control group and expert opinions; Grade E, non-randomized trial with historical control group, case report and expert opinions

shock with adjunctive IVIG(GMA), but not with IVIG(G). Of course, he is aware of the low evidence level of this approach, but this situation is not unusual in the case of sepsis treatment[6].

In well-characterized sepsis subgroups as described in Table 1, study results support, at the low evidence level, the application of IVIG(G) in surgical patients with score-defined postoperative severe sepsis and in patients with streptococcal toxic shock syndrome. The same holds true for the application of IVIG(GMA) in patients with septic shock and endotoxemia, as well as in young patients with meningococcal sepsis. In the case of postoperative sepsis and of septic shock with endotoxemia, the low evidence level of grade C urgently demands further studies; within the next few years either the positive results will have been confirmed or will have been unreproducible. In any case, the evidence must increase, in one way or another!

The author sees a prominent, currently underused, potential for IVIG in sepsis prophylaxis in risk patients as well as in risk situations.

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Immunomodulatory and anti-inflammatory potential of intravenous immunoglobulin

16
Mechanisms of intravenous immunoglobulin in autoimmune and inflammatory diseases

M.D.Kazatchkine, J.Bayry, S.Lacroix-Desmazes and S.V.Kavery

Clinical and experimental evidence suggests that a wide spectrum of immune-mediated conditions could benefit from intravenous immunoglobulin (IVIG), including acute and chronic/relapsing diseases, autoimmune diseases mediated by pathogenic autoantibodies or by autoaggressive T cells and inflammatory disorders associated with, for example, an imbalance in cytokine networks[1]. IVIG consists of intact immunoglobulin G (IgG) molecules with a distribution of IgG subclasses that corresponds to that of normal human serum. Most preparations contain traces of IgA, and carry the risk of sensitization to IgA in long-term treatment of IgA-deficient individuals. The preparations contain intact Fc moieties which allow IVIG to interact with and signal through Fc receptors on Fc receptorexpressing cells, including phagocytes and B lymphocytes, and with a number of Fc-binding plasma proteins, e.g. components of the complement system[2].

The mode of action of IVIG is complex, involving modulation of expression and function of Fc receptors, interference with complement activation and the cytokine network, provision of anti-idiotypic antibodies, inhibition of maturation and function of dendritic cells and modulation of T- and B-cell activation, differentiation and effector functions[1,3]. Such a broad range of activities reflects the functions of circulating immunoglobulins in the maintenance of tolerance to self and immune homeostasis in healthy individuals. A brief overview of various mechanisms that may be underlying the beneficial effects of IVIG in autoimmune and inflammatory conditions is presented here.

Fc-MEDIATED BLOCKADE OF Fcγ RECEPTORS ON MACROPHAGES

IVIG is able to block transiently the function of Fcγ receptors on splenic macrophages. Evidence for this mechanism of action was obtained through the following observations:

(1) Administration of IVIG is followed by a decrease in the clearance of anti-Dcoated autologous erythrocytes *in vivo*[4]*;*
(2) Peripheral blood monocytes from IVIG-treated patients with idiopathic thrombcytopenic purpura (ITP) exhibit a decreased ability to form rosettes with IgG-coated erythrocytes[5];
(3) Administration of antibodies against Fcγ-RIII or of Fc fragments of IVIG to patients with ITP results in similar effects to those of IVIG[6];

(4) Anti-D IgG induces an increase in platelet counts in rhesus-D-positive ITP patients.

Recent studies have further indicated that IVIG could also be effective in mouse models of ITP and arthritis models by up-regulating the expression of Fcγ RIIB[7–9]. These findings support the hypothesis that the interaction of IVIG with Fc receptors on macrophages plays a critical role in the beneficial effect of IVIG in peripheral autoimmune cytopenias.

ANTI-INFLAMMATORY EFFECTS OF IVIG

Anti-inflammatory effects of IVIG include its ability to neutralize microbial toxins, interfere with complement-mediated damage, alter the inflammatory potential of soluble immune complexes, modulate the production of proinflammatory and anti-inflammatory cytokines and of chemokines, and the expression of adhesion molecules.

Neutralization of microbial toxins

IVIG can inhibit superantigen-induced T-cell activation in the case of staphylococcal toxin superantigens that are targets for specific anti-superantigen antibodies in IVIG. Neutralization of the toxin is considered a likely mechanism of action of IVIG in Kawasaki disease[10]. In addition to containing antibodies to staphylococcal and streptococcal superantigens, IVIG contains neutralizing anti-bodies to the Shiga toxin and the SLT-1 toxin of *Escherichia coli* that are pathogens in the primary hemolytic—uremic syndrome[11]. The toxin-neutralizing properties of IVIG may contribute to its effect in chronic relapsing colitis caused by *Clostridium difficile* in children[12].

Attenuation of complement-mediated damage

The interaction of IVIG with complement prevents generation of the C5b—9 membrane attack complex and subsequent complement-mediated tissue damage, by scavenging active complement components and diverting complement attack from cellular targets. IVIG binds the activated components C3b and C4b in a C1q-independent[13] and C1q-dependent[14] fashion, thus preventing the deposition of these fragments on target surfaces of complement activation. IVIG also binds anaphylatoxins C3a and C5a, thereby neutralizing their proinflammatory effects[15]. This mode of action of IVIG is of relevance in the treatment of patients with severe dermatomyositis and for hyperacute and xenotransplant rejection. Thus, the effect of IVIG in dermatomyositis is associated with decreased plasma levels of C5b—9 and a significant decrease in the amounts of C3b and C5b—9 antigens deposited in endomysial capillaries[16].

Attenuation of immune complex-mediated inflammation

IVIG prevents damage mediated by C3b-carrying immune complexes in the circulation[17], by accelerating the decay of bound C3b into an inactive form, iC3b. By binding to free antigen or antibody valencies in the complexes, IVIG modifies the molecular content and

proinflammatory activity of complexes, as illustrated by the decrease in the amount of deposited IgG in renal biopsies of patients with immune complex-mediated glomerulonephritis following the addition of IVIG *in vitro*[18].

Induction of anti-inflammatory cytokines

Modulation by IVIG of the production of cytokines and cytokine antagonists is a major mechanism by which immunoglobulin exerts its anti-inflammatory effects *in vivo*[19]. IVIG selectively triggers the production of interleukin-1 receptor antagonist (IL-1ra), the natural antagonist of interleukin-1 (IL-1), in cultures of purified monocytes, without a concomitant effect on the production of the proinflammatory cytokines IL-1α, IL-1β, IL-6 and tumor necrosis factor (TNF-α)20. Circulating levels of IL-1 β decrease after treatment with IVIG of patients with the Guillain—Barré syndrome[21]. The anti-inflammatory effects of IVIG relating to the modulation of cytokine production are not restricted to monocytic cytokines, but are also largely dependent on the ability of IVIG to modulate T helper 1 (Th1) and T helper 2 (Th2) cytokine production[22].

Interaction of IVIG with antigen-presenting cells

We have recently examined the effects of IVIG on the differentiation, maturation and function of dendritic cells (DCs) from healthy blood donors and from patients with systemic lupus erythematosus (SLE)[23,24]. We have shown that DCs are primary targets for the immunosuppressive effects of IVIG on T-cell activation. IVIG inhibits the differentiation and maturation of DCs *in vitro*, and abrogates the capacity of mature DCs to secrete IL-12 upon activation, while enhancing IL-10 production. IVIG-induced down-regulation of costimulatory molecules associated with the modulation of cytokine secretion resulted in the inhibition of auto- and alloreactive T cell activation and proliferation. Modulation of DC maturation and function by IVIG is of potential relevance to its immunomodulatory effects in controlling specific immune responses in autoimmune diseases, transplantation and other immune-mediated conditions[23,24].

Effect of IVIG on activation of endothelial cells

IVIG controls endothelial cell activation in inflammatory conditions *in vitro*. TNF-α- and IL-1 β-induced transcription of the genes encoding the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and the cytokines monocyte chemoattractant protein-1 (MCP-1), macrophage colony stimulating factor (M-CSF) and granulocyte—macrophage colony stimulating factor (GM-CSF) is blocked by IVIG in cultured endothelial cells[25]. It has recently been shown that IVIG preparations induce apoptosis in TNF-α-stimulated endothelial cells via a mitochondria-dependent pathway[26].

V region-mediated interactions of IVIGwith antibodies and B-cell receptors

Interactions between IVIG and variable regions of autoantibodies provide the basis for the ability of IVIG to regulate autoreactive B-cell clones *in vivo*. We have gathered several lines of evidence that antibodies in IVIG recognize idiotypes of disease-associated and of natural autoantibodies and antigen receptors on B lymphocytes. IVIG and F(ab‘)2 fragments of IVIG neutralize the functional activity of various autoantibodies and/or inhibit the binding of the autoantibodies to their respective autoantigens *in vitro*. The inhibition of autoantibody activity by IVIG has been observed in the case of autoantibodies to factor VIII, thyroglobulin, DNA, intrinsic factor, peripheral nerve, neutrophil cytoplasmic antigens, platelet gpIIb, IIIa, the acetylcholine receptor, endothelial cells, phospholipids, nephritic factor and retinal autoantigens[27]. The neutralizing capacity of IVIG towards autoantibodies probably explains the rapid fall in the plasma titer of antifactor VIII and autoantibodies antineutrophil cytoplasm (ANCA) that has been seen in patients with antifactor VIII autoimmune disease and with ANCA-positive vasculitis following treatment with IVIG. In patients with these diseases, a direct relationship has been found between the ability of IVIG to neutralize autoantibody activity *in vitro* and that of IVIG to decrease autoantibody titers in treated patients *in vivo*.

Interaction of IVIG with membrane molecules of B and T lymphocytes

In addition to binding to idiotypes of immunoglobulins, IVIG reacts with a number of membrane molecules of T cells, B cells and monocytes that are relevant for the control of autoreactivity and induction of tolerance to self. Thus, IVIG has been shown to contain antibodies to variable and constant regions of the human αβ T-cell receptor[28], cytokines and cytokine receptors[29,30], CD5[31], CD4[32], human leukocyte antigen (HLA) class I molecules[33], RGD adhesion motif[34], Fas[35,36] and CCR5[37]. We have suggested that antibodies directed to such functional molecules of lymphocytes are essential for the immunomodulatory effects of normal immunoglobulin.

CONCLUDING REMARKS

Since it was first used in the treatment of idiopathic thrombocytopenic purpura, considerable progress has been made in understanding the mechanisms by which IVIG exerts immunomodulatory functions. The mode of action of IVIG is complex, involving modulation of expression and function of Fc receptors, interference with activation of complement and the cytokine network, provision of anti-idiotypic antibodies, inhibition of maturation and function of dendritic cells, regulation of cell growth, and effects on the activation differentiation and effector functions of T and B cells. The ability of IVIG to interact through V regions with complementary V regions of antibodies and antigen

receptors as well as with relevant soluble and surface molecules provides the basis for inducing the selection of immune repertoires.

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17
Role of intravenous immunoglobulin in reversing glucocorticoid insensitivity or resistance

E.W.Gelfand

In many chronic diseases, activation of the immune system and tissue inflammation play a central role in the pathogenesis of these disorders[1]. The consequences of immune activation are widespread, including immune recognition by T lymphocytes, the release of numerous proinflammatory cytokines and the recruitment of various inflammatory cells, which further release toxic mediators enhancing tissue inflammation. In some diseases the predominant inflammatory cell type is the neutrophil, in allergic diseases eosinophils, basophils and mast cells are prominent, while in others mononuclear cells (lymphocytes, monocytes) dominate. In virtually all of the chronic inflammatory diseases, glucocorticoids (GCs) remain the most effective form of therapy[2].

STRUCTURE AND FUNCTION OF THE GLUCOCORTICOID RECEPTOR

The anti-inflammatory effects of GCs are mediated through intracellular receptors that modulate (enhance or inhibit) inflammatory gene expression. As a result, GCs can reduce tissue inflammation at many different levels, including modulation of cytokine and chemokine production, adherence molecule expression and inflammatory cell accumulation. The GC receptors (GRs) are themselves members of a large steroid-nuclear receptor family. The GR is localized primarily in the cytoplasm and enters the nucleus of the cell after activation by hormone. In the unliganded form in the cytoplasm, the GR is part of a large heterodimeric complex, including dimers of heat shock protein (HSP) 90, a p23 subunit and immunophilin-related proteins[3]. The HSPs maintain the GR in a conformation that is appropriate for ligand binding and for inhibition of GR translocation to the nucleus.

The GR functions primarily as a ligandactivated transcription factor. After ligand activation and nuclear localization, the GR binds as a homodimer at GC response elements (GREs) in GR target genes. The GCs exhibit anti-inflammatory effects by controlling the expression of these specific target genes. The inhibition of gene expression probably contributes to many of the anti-inflammatory effects of the GCs. GCs limit expression of many cytokines (interleukins IL-6, IL-11, IL-13, IL-16), granulocyte—macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-α

(TNF-α) and chemokines (IL-8, RANTES, eotaxin, macrophage inflammatory protein 1a, monocyte chemoattractant protein 1)[4], as well as adhesion molecule expression (intercellular, ICAM-1, vascular cell, VCAM-1)[5,6], inducible nitric oxide synthase and cyclo-oxygenase[7,8]. Although more limited, GCs can increase the expression of a number of anti-inflammatory genes (e.g. type II IL-1 receptor, a decoy molecule for IL-1)[9].

The major ligand-activated GR is termed GRα. Alternative splicing of exon 9 of the GR gene results in the synthesis of a GRβ isoform, which differs (from GRα) only in the carboxyl terminus, with replacement of the last 50 amino acids with a unique 15-amino acid sequence. This variation between GRα and GRβ is located in the hormone-binding domain and, in contrast to GRα, GRβ does not bind ligand[10–13]. GRβ may inhibit GRα activity, and it is the dominant negative activity of GRβ which makes it a potentially important mechanism for GC insensitivity or resistance.

GLUCOCORTICOID INSENSITIVITY OR RESISTANCE

Despite their widespread potency and efficacy, it is becoming increasingly apparent that patients vary widely in their response to GCs. As illustrated in Figure 1, the response to steroids may follow distinct pathways. GC resistance has perhaps been best studied in chronic asthma, where a failure to respond to GCs is more readily demonstrated than in other diseases, that is by lack of improvement in pulmonary function after GC therapy[14]. As indicated in Table 1, a failure to respond to GC therapy, suggesting GC resistance, has been reported in asthma, rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, chronic sinusitis and atopic dermatitis.

Figure 1 Pathways of response to steroids

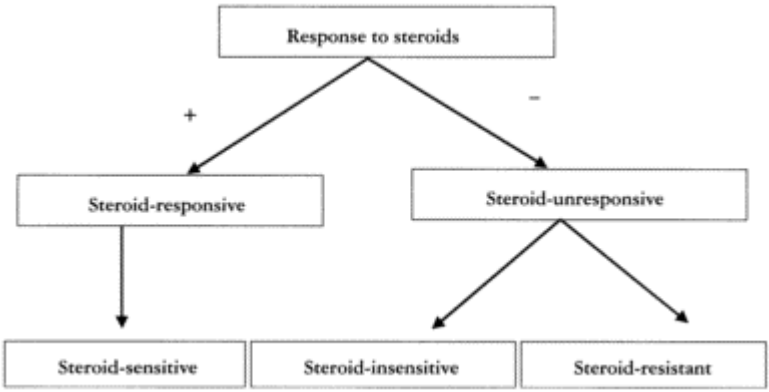

Table 1 Glucocorticoid insensitivity

Chronic inflammatory diseases
Asthma
steroid-dependent

fatal
nocturnal
Chronic sinusitis
Rheumatoid arthritis
Systemic lupus erythematosus
Inflammatory bowel disease
Transplant rejection

In patients with GC-resistant asthma, there is a defective response of peripheral blood mononuclear cells (PBMCs) to GC which has been defined in phytohemagglutinin (PHA)-stimulated T-cell proliferation assays[15–17]. In comparison with GC-sensitive patients, higher concentrations of steroids are required to inhibit T-cell proliferation in resistant patients. This assay has proved to be simple, sensitive and reliable. In a recent study in healthy volunteers, 30% of subjects had evidence of *in vitro* GC resistance[18], indicating that a significant proportion of the general population could fail to respond normally to steroids in chronic inflammatory conditions.

This type of GC resistance defined in patients with allergic and autoimmune diseases must be distinguished from the rarer, primary GC resistance syndrome which is the result of inactivation mutations of the GR gene[19]. GC resistance as defined here appears to be at the level of decreased GR ligand-binding affinity of mononuclear cells. The combination of IL-2 and IL-4 induces T-cell steroid resistance[20]. The precise mechanism by which cytokines such as IL-2 and IL-4 decrease responsiveness is not known.

A number of investigations have examined the potential role of GRβ in GC resistance. The dominant negative activity of GRβ makes it a potentially important mechanism for GC resistance. Combinations of IL-2 and IL-4, IL-13 or TNF-α induce the expression of GRβ in different cell types[21–23]. The increased expression of GRβ has been reported in a number of diseases associated with GC resistance, including chronic asthma[22,24], as well as fatal asthma[25] and nocturnal asthma[26], chronic sinusitis[27] and ulcerative colitis[28,29]. Up to 30% of patients with ulcerative colitis failed to respond to GC in one series, and were found to have *in vitro* T-cell resistance to steroids[28]. In another group of patients, GRβ mRNA and protein levels were much higher in GC-resist-ant patients with ulcerative colitis[29].

ROLE OF INTRAVENOUS IMMUNOGLOBULIN IN REVERSING GLUCOCORTICOID RESISTANCE

One of the major benefits of intravenous immunoglobulin (IVIG) therapy has been its potent anti-inflammatory effects in a number of inflammatory, allergic or autoimmune diseases. Although the underlying mechanism(s) for the beneficial effects of IVIG is not fully defined, one possibility is the inhibition of cytokine production. A number of investigators have shown the potency of IVIG to inhibit the release of a number of inflammatory cytokines including IL-2, IL-4 and TNF-α[30–32]. If these cytokines are important in inducing GC resistance through altering GR ligand-binding activity or the balance between active GRα and inactive GRβ expression, then inhibiting their

production with IVIG may explain the benefit, for example, as reported in severe asthma[31–36].

As shown in Figure 2, the addition of IVIG to PHA-stimulated cultures of T cells can markedly shift (increase) steroid sensitivity. We have also shown that IVIG can prevent the IL-2/IL-4 increased expression of GRβ in cultured T cells (Table 2). Together, these results suggest that IVIG can prevent or reverse a GC-resistant state. To test directly this concept *in vivo*, IVIG was administered to a group of severe steroid-dependent asthmatics with demonstrable decreased GR binding affinity[35]. Following 3 months and 6 months of therapy with IVIG (2g/kg body weight on a monthly basis), not only was steroid usage markedly decreased (as well as exacerbations and hospitalizations), but there was normalization of GR binding affinities. These preliminary results suggest that *in vivo*, IVIG treatment can restore GC sensitivity, an important factor when considering the widespread use of

Table 2 Intravenous immunoglobulin (IVIG) decreases interleukins IL-2/IL-4-induced glucocorticoid receptor β (GRβ) expression. Peripheral blood T lymphocytes were cultured overnight in the presence of medium (control), IVIG (10μg/ml), recombinant IL-2 and IL-4, or IL-2, IL-4 and IVIG. Mean fluorescence intensity following anti-GRβ antibody staining of the cells is indicated

	Mean fluorescence intensity
Control	490
IVIG	475
IL-2/IL-4	1210
IL-2/IL-4+IVIG	750

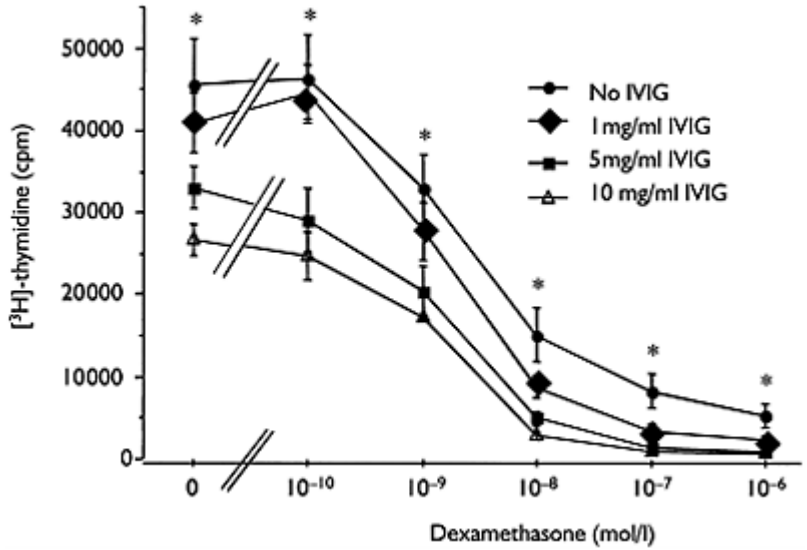

Figure 2 Intravenous immunoglobulin (IVIG) and dexamethasone act synergistically in suppressing lymphocyte proliferation. *$p<0.05$

GC in many chronic inflammatory diseases. Whether the increase in GC sensitivity and restoration of GC responsiveness is due to cytokine antagonism (e.g. of IL-2, IL-4 or TNF-α) remains to be defined.

CONCLUSIONS

GCs are the most effective drugs currently available for many chronic inflammatory disorders. The response to GC is not uniform, and varies from patient to patient and even in a given patient at various stages of their disease. In part, this heterogeneity in GC responsiveness may be due to inflammatory cytokines regulating GC responsiveness at the level of GR binding affinity, and relative expression of the GRα and GRβ isoforms. Therapies directed towards improving GR binding affinities and GRα expression (and inhibition of GRβ expression), perhaps through cytokine antagonism, should serve to maintain GC sensitivity and responsiveness. IVIG may be one such therapeutic option.

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18
Modulating the balance of activating and inhibiting Fcγ receptors: mechanism of intravenous immunoglobulin action

L.Pricop

In autoimmune diseases, prolonged cell activation, altered cytokine production, changes in cell surface receptors and unbalanced effector functions are components of the pathological process linked to the persistence of inflammation. For immune complex-mediated diseases, the inflammatory cascade is thought to be mediated at least in part by the interaction of autoantibodies with Fcγ receptors (FcγR)[1,2].

Three classes of activating FcγR, FcγRI, FcγRIIA and FcγRIIIA, are expressed on human monocytes and macrophages[3]. Interaction of immunoglobulin G (IgG) immune complexes with activating FcγR results in phagocytosis and release of inflammatory mediators. A distinct class of Fc receptors, FcγRIIB, exerts inhibitory functions upon coaggregation with activating FcγR[4]. Inhibiting FcγRIIB receptors set a threshold that must be overcome by activating signals in order to trigger effector functions[5,6].

Intravenous gammaglobulin (IVIG) has well-recognized anti-inflammatory properties leading to its broad therapeutic use in immune mediated inflammatory disorders. Various mechanisms account for the therapeutic activity of IVIG, including regulation of B- and Tcell responses, modulation of dendritic cell (DC) maturation and function, inhibition of complement-mediated tissue damage, blockade of FcγR and modulation of cytokine production (for review see reference 7). Recent experimental evidence in animal models of antibody-mediated autoimmune diseases suggests that the anti-inflammatory activity of IVIG is associated with the induction of the inhibitory receptor FcγRIIB[8,9].

In a murine model of immune thrombocytopenic purpura (ITP), the macrophage-mediated clearance of platelets opsonized with pathogenic antibodies could be prevented by IVIG[8]. Similarly, IVIG conferred protection against joint inflammation and cartilage damage induced by anti-glucose-6-phosphate isomerase antibodies present in K/BxN serum in a mouse model of rheumatoid arthritis[9]. In both cases, initiation of the inflammatory cascade was dependent on the interaction of pathogenic immune complexes with activating receptors FcγRIII, and IVIG-mediated protection was associated with increased expression of inhibitory FcγRIIB on macrophages. Complementing the concept of FcγR blockade, these studies revealed that IVIG could mediate its immunomodulatory effect by changing the balance of activating and inhibiting FcγR on macrophages.

The cytokine milieu constitutes a key component in the regulation of FcγR-mediated effector functions. Functionally distinct subsets of T lymphocytes produce different types

of cytokines. Whereas T helper 1 (Th1) cells secrete interferon-γ (IFN-γ) and tumor necrosis factor-β (TNF-β), Th2 cells secrete interleukin4 (IL-4), IL-5, IL-9 and IL-13.

An important consequence of the Th1/Th2 polarization is the differential modulation of activating and inhibiting FcγR[6]. IFN-γ, a prototypic Th1 cytokine, induced expression of activating FcγR, while it down-regulated inhibiting FcγRIIB isoforms, changing the balance of FcγR to an activating phenotype. In contrast, Th2 cytokines such as IL-4 and IL-13 increased the expression of inhibiting FcγRIIB on monocytes, while down-regulating all classes of activating FcγR[6,10,11].

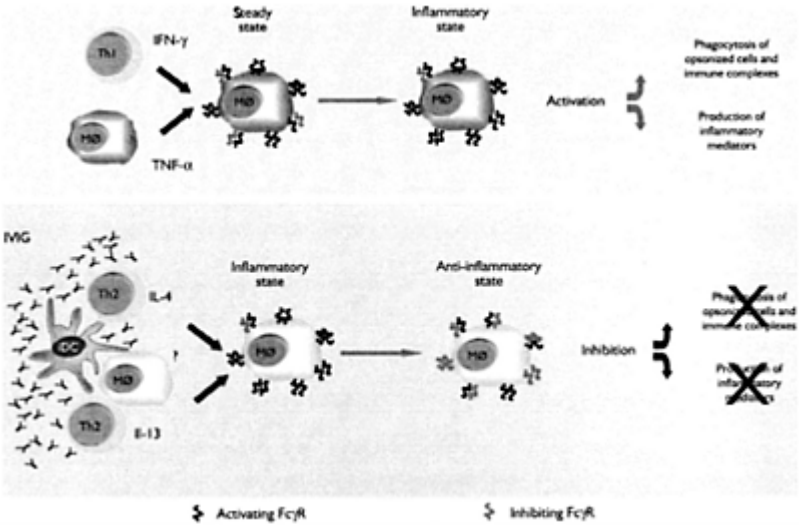
Recent experimental evidence obtained in our laboratory suggests that non-T cell-derived cytokines also have the ability to modulate the expression of the two opposing FcγR systems. TNF-α, a pivotal proinflammatory cytokine produced by myeloid cells, decreased the expression of inhibiting FcγRIIB on human monocytes. These molecular changes resulted in increased effector functions mediated by activating FcγRs, which could cause the amplification of immune complex-mediated inflammation in conditions associated with increased TNF-α production.

The activating phenotype induced by cytokines with described inflammatory properties, such as IFN-γ and TNF-α, resulted in a higher ratio of FcγR-mediating activation versus inhibition. In contrast, IL-4 and IL-13 led to a decrease in this ratio (Figure 1). Effective regulation of the two opposing FcγR systems may result from a decrease in proinflammatory cytokine production or an increase in anti-inflammatory cytokine production in the microenvironment where macrophages become primed.

Alterations in cytokine production following IVIG infusion are well documented[12–14]. Induction of cytokine release following IVIG infusion and *in vivo* modulation of cytokine activity due to high-avidity anticytokine antibodies present in IVIG preparations have been reported[15,16]. Interestingly, induction of Th2 cytokine expression patterns after IVIG treatment in children with ITP was associated with clinical remission[17]. Persistence of Th1 cytokine (IFN-γ) patterns after IVIG infusion in some patients was associated with refractoriness to IVIG treatment and poor prognosis.

The hypothesis that IVIG could result in the elaboration of cytokines that regulate the expression of inhibitory FcγRIIB represents an attractive avenue of investigation. The sources for potential mediators involved in the up-regulation of FcγRIIB expression following IVIG treatment remain elusive. The protective effect of IVIG was lost in mice deficient in colony-stimulating factor-1 (CSF-1) that lacked a subset of ‘sensor’ macrophages[9]. The up-regulation of FcγRIIB on effector macrophages infiltrating the arthritic joins of K/BxN mice was induced by a subset of CSF-1-dependent macrophages bearing distinct surface markers, and producing soluble mediators in response to IVIG treatment. In humans,

Figure 1 Model for cytokine-mediated modulation of activating and inhibiting Fcγ receptors by intravenous immunoglobulin (IVIG). Up-regulation of activating FcγR and down-regulation of inhibiting FcγR by interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) during inflammation (upper panel). Proposed model for the anti-inflammatory effect of IVIG via immature dendritic cells (iDCs)/macrophages mediating T helper 2 cell (Th2) polarization (interleukin-4 (IL-4) and IL-13)) resulting in down-regulation of activating FcγR and up-regulation of inhibiting FcγR (lower panel). MØ, monocyte/macrophage



the existence of distinct subsets of macrophages sensing IVIG, and endowed with the capacity to regulate FcγRIIB expression on effector macrophages, has yet to be determined.

The mechanisms by which IVIG might reorchestrate the production of T helperderived cytokines are under investigation in several laboratories. While a direct effect of IVIG on T-cell function is possible, an indirect effect mediated through the inhibition of DC

maturation was recently described[18]. The IVIG-mediated inhibition of differentiation of DCs was associated with decreased IL-12 production and enhanced IL-10 production. Polarization towards Th2 differentiation by immature, IL-10-producing DCs is a described phenomenon. In contrast, IL-12-producing DCs induce activation of IFN-γ-producing Th1 cells[19]. In this model, IVIG would interact with receptors on immature DCs or macrophages and skew the Th1/Th2 balance (Figure 1).

As in the case of 'sensor' CSF-1-dependent macrophages, the nature of the IVIG receptor on the surface of DCs remains undefined. A potential candidate is FcγRI, the high-affinity receptor for monomeric IgG. Cross-linking of FcγRI on the surface of macrophages led to down-regulation of IL-12 production and reciprocal up-regulation of IL-10 production, supporting this hypothesis[20].

Further insight into the molecular mechanisms that regulate activating and inhibiting FcγR expression and function by IVIG will be required to understand the basis of its therapeutic effect. The cytokine-mediated regulation of activating and inhibiting FcγRs provides a model in which lymphokines and monokines produced by several cell types could act as mediators of IVIG action. As the functional interplay between cytokines and FcγR determines the magnitude of effector functions, defining the IVIG mechanism of action on FcγR has important implications for its efficient therapeutic use for immune complex-mediated inflammatory conditions.

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19
Anti-inflammatory effects of intravenous immunoglobulin: Fcγ receptor inhibition/blockade and non-Fcγ receptor-mediated mechanism

J.W.Semple and A.H.Lazarus

INTRODUCTION

Immunoglobulin preparations extracted from human blood have been used since the early 1950s to treat immunodeficiency diseases[1]. Intravenous immunoglobulin (IVIG) therapy has also been shown to be effective in treating immune deficiency states[2,3], bacterial/viral infections[4] and immune regulatory disorders, particularly immunohematological disorders such as autoimmune neutropenia, autoimmune hemolytic anemia and autoimmune thrombocytopenia[5–7]. While the mechanisms of action of the anti-inflammatory nature of IVIG are complex and not fully elucidated, several theories have been postulated. In autoimmune thrombocytopenic purpura (AITP), four non-mutually exclusive major theories have evolved to explain IVIG's mechanism(s) of action. First, IVIG may cause competitive reticuloendothelial system (RES) blockade of activating Fc receptors on macrophages[8]; second, IVIG may act through interactions with the inhibitory FcγRIIB which result in paralysis of phagocytes[9]; third, IVIG may initiate FcγR-mediated cytokine alterations which shut off RES function[10]; and/or fourth, anti-idiotypic antibodies present in IVIG may neutralize/regulate antiplatelet autoantibodies[11]. This chapter focuses primarily on these four theories, and discusses how different IVIG products can utilize both FcγR- and non-FcγR-mediated events to affect platelet counts.

FcγR COMPETITIVE BLOCKADE

Early evidence demonstrated that IVIG may act by blocking the FcγR of the RES. Fehr and colleagues[8] showed that in patients with AITP who were not splenectomized, IVIG infusions prolonged the clearance of radiolabelled opsonized red blood cells *in vivo*. These results were subsequently confirmed using both erythrocytes and platelets as target cells[6,7]. Studies comparing intact IVIG with F(ab')2 fragments have also demonstrated that intact immunoglobulin G (IgG) was more efficacious in reversing thrombocytopenia in AITP, suggesting that the Fc portion of IVIG is a critical component of IVIG's mechanism of action[12]. Of interest, a case report suggested that blocking of the FcγRI had

no effect in AITP[13], whereas Clarkson and associates[14] showed that blocking both FcγRII and FcγRIII significantly increased platelet counts in AITP patients. Similarly, studies in animals have shown that the administration of a monoclonal antibody that blocks the FcγRII and FcγRIII can prevent the clearance of opsonized erythrocytes[15–17]. Taken together, these studies suggest that one of IVIG's major mechanisms of action may be to block physically the FcγR within the RES and release opsonized platelets into the circulation.

FcγRIIB-MEDIATED INHIBITION

The distinction between IVIG-mediated FcγR competitive blockade and inhibition was discovered in 2001 when three groups independently reported findings from a murine model of passively induced thrombocytopenia[9,18,19]. For example, Samuelsson and colleagues[9] showed that IVIG could rescue the platelet counts of mice injected with a monoclonal antibody specific for murine platelets. What was more intriguing is that they found that the inhibitory FcγRIIB receptor was necessary for the therapeutic effects of IVIG[9]. These results demonstrated for the first time that IVIG may not work by simple FcγR competitive blockade of the activating FcγRs, but rather by enhancing the expression of FcγRIIB inhibitory receptors on splenic macrophages[9]. Alternatively, in a similar mouse model, Song and associates[20] have recently shown that some monoclonal IgG antibodies with specificity for an erythrocyte antigen (CD24) may provide an alternative to IVIG in treating immune thrombocytopenia. These results suggest that in addition to FcγR-mediated inhibition, the specificity of the antibodies used in ameliorating thrombocytopenia may also be important.

Engagement of FcγRIIB in both B lymphocytes and mast cells normally induces an inhibitory transmembrane signalling cascade mediated by the recruitment of the SH2-containing inositol phosphatase (SHIP)[9]. However, a recent report by Crow and associates[21] has suggested that IVIG therapeutic effects in the murine passive AITP model are not mediated by SHIP but by another unknown mechanism. These results suggest that the mechanism of action of IVIG via FcγRIIB may be somewhat unique, and indeed complex. Furthermore, Bruhns and co-workers[22] have recently shown that IVIG-mediated FcγRIIB-dependent inhibition initially occurs on colony-stimulating factor (CSF)-1-dependent macrophages and not neutrophils. These results further suggest a two-step model for IVIG protection, in which CSF-1-dependent macrophages act as innate 'sensors' for the Fc fragment of IVIG, leading to the induction of FcγRIIB on CSF-1-independent 'effector' macrophages, thereby raising the threshold required for FcγRIII activation and preventing autoantibody-triggered inflammation[22].

Although competitive RES blockade and inhibition are currently the most accepted mechanisms to explain the effects of IVIG, several lines of evidence suggest that additional FcγR-dependent mechanisms may contribute to inhibiting the thrombocytopenia of AITP For example, anti-D has also been shown to raise the platelet counts of patients with AITP[23]. However, little is known about the mechanisms of action of anti-D, although the above theories of FcγR blockade/inhibition may apply. It has been suggested that anti-D opsonizes D+ red blood cells, and blocks platelet destruction by adhering to the FcγR of leukocytes[23]. Alternatively, it has been suggested that anti-D may

cause immunosuppression by interacting with FcγR and stimulating the production of immunosuppressive cytokines[24]. We recently demonstrated that alterations in *in vivo* levels of pro- and anti-inflammatory cytokines after anti-D treatment may play an important role in anti-D's mechanism of action. Within 3 h of anti-D administration, significant, but transient, changes in plasma levels of cytokines, such as interleukin (IL)-1 receptor antagonist (IL-1ra), IL-6 and tumor necrosis factor (TNF)-(α, occurred[25]. Subsequently, it was found that anti-D-opsonized red blood cells stimulated FcγR-dependent leukocyte activation and phagocytosis that was subsequently inhibited by the production of the inhibitory cytokine, IL-1ra[26]. These data suggest that anti-D, like IVIG, utilizes FcγR for its mechanism of action, but modulates the RES via the production of inhibitory cytokines that rescue platelet counts by inhibiting phagocytosis.

FcγR-INDEPENDENT MECHANISMS

Another major mechanism proposed for IVIG function involves the regulatory properties of a subset of antibodies called anti-idiotypic antibodies[11]. An idiotype is defined as the collection of antigenic determinants (termed idiotopes) contained within the variable (V) regions of an antibody molecule[27]. The diversity of variable regions generated during VDJ heavy-chain and VJ light-chain immunoglobulin gene rearrangements and antigen-driven somatic mutation permits the expression of large numbers of potential idiotypes[27].

One particular example of how anti-idiotypes may be involved in the mechanism of action of IVIG is that of transfusion-induced refractoriness. In contrast to the recognized efficacy of IVIG therapy in autoimmune and human platelet antigen-la (HPA-1a)-specific alloimmune disorders[5–7], most investigations have failed to document an effectiveness of IVIG in platelet transfusion-induced refractoriness due to human leukocyte antigen (HLA) alloimmunization[28,29]. These observations are intriguing because of the generally similar immunopathogenesis of platelet destruction in both auto- and alloimmune platelet disorders, i.e. antibody-opsonized platelet destruction via FcγR-mediated phagocytosis in the reticuloendothelial system (RES). The contrasting clinical results suggest that commercially available preparations of IVIG may lack a component necessary to affect anti-HLA-mediated destruction of platelets. This may relate to the particular type or quantity of anti-idiotypic antibodies contained in commercial IVIG preparations, resulting from the specific donor populations employed for the source material. For example, it has been shown that, compared with commercial IVIG or IgG prepared from the sera of male individuals, IgG prepared from the sera of multiparous women has higher anti-idiotypic binding capacity for anti-HLA, and can inhibit a secondary human anti-HLA response in humanized severe combined immune deficiency (SCID) mice[30]. Furthermore, the F(ab')2 fragments of IgG prepared from the pooled sera of multiparous women can also inhibit *in vivo* alloimmunization to HLA[30]. The latter result suggests that the anti-HLA inhibition mediated by IgG from multiparous women occurs primarily via an Fc-independent event, e.g. anti-idiotypic regulation. Thus, it appears that by simply pooling sera from selected multiparous donors, a more efficacious gammaglobulin product, useful in the prevention and management of HLA alloimmune platelet disorders, can be produced.

SUMMARY

It is clear that multiple mechanisms of action are related to the anti-inflammatory actions of various IVIG preparations. Standard IVIG preparations and hyperimmunes such as anti-D appear to mediate many of their effects via Fc-dependent actions, although other, Fc-inde-pendent mechanisms, such as idiotype/anti-idiotypes may also be utilized. The anti-idiotypic actions of IVIG can be observed clearly when donor selection is implemented, such as in the case of sera from mutiparous women. Nonetheless, although much work has been performed in this area, none of the current theories of the mechanism of action of IVIG can be eliminated to date. More research will be necessary to exploit the major mechanisms of any particular IVIG preparation.

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20
Immune modulation by intravenous immunoglobulin: some remarks on the role of Fc receptors

E.van Mirre and C.E.Hack

Polyspecific immunoglobulin G (IgG) for intravenous use (IVIG) was originally developed as a substitution therapy for hypo- and agammaglobulinemic patients. However, Imbach and colleagues observed that IVIG supplementation was in fact effective as a treatment in idiopathic thrombocytopenia purpura (ITP)[1]. This has led to the widespread use of IVIG in various immune diseases. Yet, the biological effects of IVIG explaining its efficacy in these diseases are still poorly understood, although they are frequently referred to as 'immunemodulatory' effects. One could postulate various mechanisms for these immunemodulatory effects of IVIG, some of which are dependent on a productive interaction of the Fcγ portion of infused immunoglobulin with the Fcγ receptors on effector cells[1–4] or with proteins of the complement system[5,6]. Here we discuss briefly some of our studies of the interaction of IVIG with Fc receptors.

As a main mechanism, IVIG has been proposed to enhance endogenous IgG catabolism, resulting in a reduction of (auto-)antibody titers. Saturation of the neonatal Fc receptor (FcRn), which was originally discovered to be involved in transplacental transport of IgG to the fetus, and later on shown to be involved in recycling of pinocytosed IgG into the circulation, by high-dose IVIG would result in increased IgG catabolism. Modelling studies, however, indicate that the decrease of endogenous IgG with high-dose IVIG via this mechanism is, at most, 40%[7]. As much more pronounced decreases of endogenous IgG have been described to occur upon IVIG infusion, saturation of FcRn cannot be the sole mechanism of action of IVIG in autoantibodymediated disease.

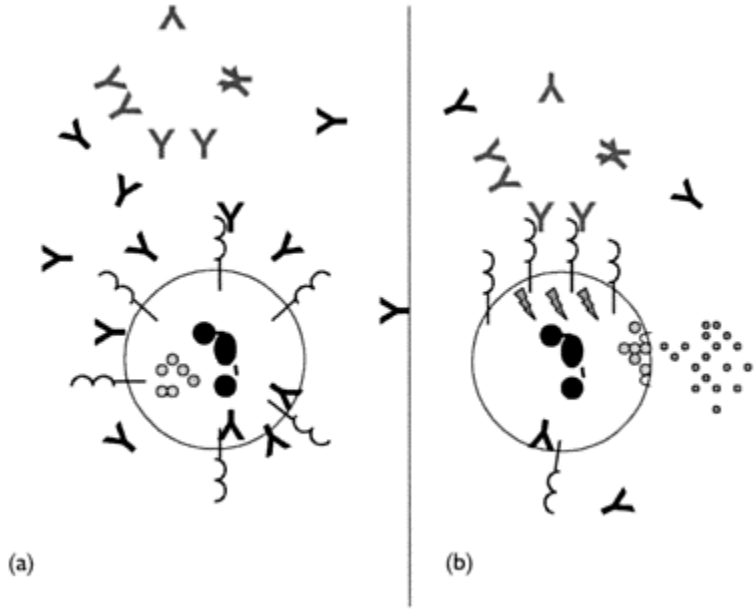
Blockade of the Fcγ receptors (FcγRs) on phagocytic cells, preventing the removal of sensitized platelets by macrophages in the spleen and liver, is believed to be the mechanism of action by which IVIG is effective as a treatment of ITP[1]. Indeed, blockade of FcγRs by IVIG on macrophages has been shown to inhibit macrophage-mediated phagocytosis of antigen-bearing target cells[2,8]. Depending on their expression on effector cells, FcγRs exert different effects. For example, on phagocytes they mediate phagocytosis, endocytosis, antibody-dependent cellular cytotoxicity and induction of respiratory burst. Three types of FcγR, types I, II and III, are distinguished, based on their affinity for IgG. Type I (affinity constant $K_a=10^8-10^9$/M) is considered a high-affinity receptor, whereas type II ($K_a=10^6$/M) and III ($K_a=5.5\times10^5$/M)[9,10] are considered to be low-affinity receptors. Hence, FcγRI binds monomeric IgG *in vivo*, whereas FcγRII and FcγRIII preferentially interact with immune complexes. Thus, regarding IVIG, FcγRII and FcγRIII will interact predominantly with di- or polymeric IgG, whereas FcγRI

probably reacts with monomeric IgG as well. IVIG preparations contain monomeric IgG as well as a variable amount of dimeric and polymeric IgG. In a mouse model for ITP, we have shown that dimeric IgG in IVIG potently inhibits the removal of antibody-sensitized platelets, whereas preparations with low dimeric content were hardly active[2]. Notably, the removal of sensitized platelets in this model is dependent on the low-affinity FcγRIII. Hence, one could postulate that dimeric IgG is the active component in IVIG explaining the efficacy of this drug in ITP. It could also be suggested that the dimeric content of IVIG co-crosslinks FcγRIIb with FcγRIII, thereby leading to attenuation of the signalling cascade. In fact, Samuelsson and colleagues have reported that the beneficial effect of IVIG in this model is mediated through FcγRIIb[11]. The signalling molecules recruited by FcRIIb in this effect of IVIG are not yet identified[12].

To what extent monomeric IgG in IVIG may contribute to the blockade of FcγRII and FcgRIII is not known. Although dimeric IgG is more potent in reducing immune complex mediated anaphylaxis in rats, we also observed that dimeric IgG induces anaphylaxis itself. In contrast, monomeric IgG did not itself induce anaphylaxis, but still had a protective effect at least at high dose (Teeling and colleagues, unpublished results). In fact, we now have evidence that monomeric IgG is indeed capable of blocking FcγRIIa and FcγRIII in the absence of signal transduction (Mirre and associates, manuscript in preparation) (Figure 1). We observed that aggregated IgG (aIgG)-mediated signal transduction, such as Ca^{2+} transients and elastase release from neutrophils, could be reduced by the addition of monomeric IgG in IVIG as well as in plasma. Furthermore, the attenuation of signalling was not due to the induction of a refractory state of the cells, as the cells remained fully responsive to FcγR-independent stimulation, such as formyl-Met-Leu-Phe (fMLP). Also, not only at physiological concentrations of monomeric IgG were aIgG-mediated effects attenuated, but also at therapeutic doses ≥20mg/ml) monomeric IgG could still exert its blocking effect.

This is unexpected because, given the affinity constants of the low-affinity FcγR, calculations show us that at 10 mg/ml, monomeric IgG occupies 98.5% and 97.4% of FcγRIIa and FcγRIII, respectively. Whereas at 21mg/ml, monomeric IgG occupies 99% and 98.7% of FcγRIIa and FcγRIII, respectively. Thus, even in the upper limit of FcγR saturation, therapeutic effects can still be reached by the addition of monomeric IgG. We therefore hypothesize that functional blockade of low-affinity FcγR might be an important early mechanism of action of IVIG.

Figure 1 Mechanisms of Fcγ receptor (FcγR) blockade by monomeric immunoglobulin (IgG). (a) Monomeric IgG at physiological and therapeutic concentrations occupies and saturates low-affinity FcγRs on the neutrophil without inducing signalling events. Immune complexes have less opportunity to bind and mediate signalling events. (b) Low concentrations or absence of monomeric IgG leaves the neutrophils with more free FcγRs and less competition on individual FcγRs for the immune complex. Immune complexes are able to bind and mediate signalling events, such as Ca^{2+} transients and elastase release from the granules



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21
Intravenous immunoglobulin in reactive hemophagocytic syndrome: a of Fcγ receptor and cytokine modulation

L.Mouthon

INTRODUCTION

Hemophagocytic syndrome (HPS) is a clinicopathological entity characterized by high-grade fever, hepatosplenomegaly, cytopenias, high ferritin level and increased proliferation and activation of benign macrophages, with hemophagocytosis throughout the reticuloendothelial system.

HPS may be primary, as observed in familial hemophagocytic lymphohistiocytosis (FHL), X-linked lymphoproliferative syndrome (XLP), Chediak—Higashi syndrome and Griscelli syndrome, or secondary to malignancy (hematological malignancies, solid tumors), infections (mainly viral and bacterial infections), autoimmune diseases (systemic lupus erythematosus (SLE), adult-onset Still's disease and juvenile chronic arthritis) or drugs (hypersensitivity syndrome)[1].

PATHOPHYSIOLOGY OF HEMOPHAGOCYTIC SYNDROME

Clinical and biological manifestations result from the secretion of huge amounts of cytokines and chemokines by activated T cells and macrophages.

Macrophages

Although HPS is characterized by the proliferation and activation of macrophages with hemophagocytosis, very few studies have been aimed at investigating the macrophage itself. Macrophages from primary HPS patients express complement receptors **(**CD35, CD21 and CD11b), CD36 and 'activation' markers such as CD25 and CD30, while those from patients with secondary HPS or healthy controls do not[2]. When assessed, macrophage proliferation is usually not clonal, and not correlated with the patient's outcome[3].

T lymphocytes and natural killer cells

Genetic defects specific for cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells have been identified in patients with primary HPS that are responsible for altered cell

death and apoptosis-induction or targetkilling. Perforin deficiency accounts for about one-third of FHL cases[4]. The establishment of perforin deficiency as a cause of the rapidly fatal FHL disease has demonstrated the essential role of perforin in the maintenance of human immune homeostasis.

In Griscelli syndrome, two closely linked genes localized on the human 15q21–22 region have recently been identified: *MYO-VA* and *RAB27A*. These genes encode for proteins involved in intracellular vesicular transport. In RAB27A-deficient T cells, the granule content is normal but release is impaired, leading to defective CTL activity and uncontrolled lymphocyte expansion[5]. Chediak—Higashi syndrome patients express deficient CTL and NK cell activities owing to an inability to secrete giant granules containing lytic proteins[5]. The XLP gene has been localized at Xq25, and named SAP[6]. The SAP protein, a small adapter molecule, interacts with SLAM (CD150), 2B4 (CD244) and NTB-A, members of an immune cell-specific receptor family called the CD2/SLAM family. Severe defects in 2B4- and NTB-A-mediated cytotoxicity are observed in XLP, responsible for NK cell defective function[7].

Cytokines, cytokine receptors and chemokines

HPS results from uncontrolled T-lymphocyte activation that promotes macrophage activation and the formation of a cytokine network. In patients with active HPS, serum levels of the T-helper cell Th1 cytokines interferon-γ (IFN-γ), interleukin-12 (IL-12) and IL-18 are significantly higher than in the remission phase of the disease or in healthy controls[8]. IL-18 seems to play a central role in inducing IFN-γ and IL-12 secretion, and serum levels of IFN-γ and IL-18 correlate positively with disease activity[8]. Serum levels of the proinflammatory cytokines tumor necrosis factor-α (TNF-α), IL-1β and IL-6 are also elevated in patients with active HPS, compared with controls[9], whereas this is not the case for the Th2 cytokine IL-4. In adult-onset Still's disease, juvenile chronic arthritis and probably SLE, IL-18 might play a role in initiating macrophage activation. Serum levels of IL-1ra and IL-10 are increased in the active phase and return to normal in the remission phase. The amounts of IL-10 produced, however, are not sufficient to influence Th1 cytokine production.

INTRAVENOUS IMMUNOGLOBULIN TREATMENT OF SECONDARY HPS

In primary HPS, bone-marrow allograft is usually proposed. In adult secondary HPS, various therapeutic strategies, including high-dose corticosteroids, immune suppressants such as cyclosporin A and etoposide have been proposed, with varying success.

IVIG has been tested in case reports and small series of patients[10,11]. Most series include children and adults, and the data are difficult to interpret, since HPS is usually primary in children whereas secondary HPS is dominant in adults. In order to analyze the results obtained with IVIG treatment in secondary HPS, we decided to group patients according to etiology: infection, systemic/autoimmune disease, malignancy.

IVIG in HPS secondary to infection

Twenty-six children, reported in six open studies and isolated case reports, were treated with IVIG for HPS secondary to proven or suspected infection. Identified infectious agents were viruses in most cases: Epstein—Barr virus (EBV) (*n*=13), human herpes virus-6 (HHV-6) (*n*=4), cytomegalovirus (*n*=1) and *Escherichia coli* (*n*=1), whereas in other patients an infectious cause was only suspected. All patients received IVIG alone, but in the case of failure, etoposide was added. Ten of them died, three survived despite IVIG failure and the others responded to IVIG treatment. In HPS secondary to EBV infection, IVIG fails to cure HPS, and etoposide represents the reference treatment[12].

Fifteen adult patients with HPS secondary to infection have been reported in four studies/case reports. Nine of them are reported by Larroche and colleagues[10] in a retrospective study. IVIG was administered in association with antibiotics or antiviral therapy. Most of the patients were immunosuppressed. Infectious agents were bacteria (*n*=4), fungi (*n*=3), EBV (*n*=1) and CMV (*n*=1), whereas for the others an infectious cause was only suspected. The efficacy of IVIG alone was difficult to assess since most of the patients also received corticosteroids. The outcome was favorable for 12 of the 15 patients[10,11]. In the study by Larroche and colleagues, efficacy of IVIG was observed in 59% of patients, with 78% improvement in patients with HPS secondary to infection, and only 37.5% improvement in HPS secondary to other causes[10]. We propose that for therapeutic strategy in HPS secondary to documented infection unrelated to EBV, an association of antimicrobial agents and IVIG could be administered, with addition of corticosteroids only in the case of failure. In HPS secondary to EBV infection, etoposide might represent the reference treatment, in association with corticosteroids[12].

IVIG in HPS secondary to systemic/autoimmune diseases

Emmenegger and colleagues treated 20 secondary HPS adult patients with IVIG, including 18 with non-infectious secondary HPS: Still's disease (*n*=9), SLE (*n*=1) or unknown (*n*=8)[11]. Eighty per cent of Still's disease and SLE patients and 50% of other patients improved, as assessed by clinical parameters and lowering of ferritin[11].

HPS may represent a specific manifestation of SLE, or may occur in otherwise inactive SLE and be related to iatrogenic immunosuppression. On the basis of a few reports, IVIG might be indicated in both situations[10,13].

Adult-onset Still's disease is a febrile disorder of unknown etiology, characterized by high fever, transient cutaneous rash and leukocytosis, which correspond to features similar to those of the systemic form of juvenile rheumatoid arthritis.

In addition to the nine Still's disease adult patients with HPS reported by Emmenegger and colleagues[11], 17 cases of adult-onset Still's disease treated with IVIG were reported in two series[14,15] and three case reports. Clinical remission was observed in 15 of them and four of these 15 presented relapses. However, based on the evolutive course of adult-onset Still's disease, with 50% spontaneous improvement, we cannot recommend the use of IVIG in this indication.

IVIG in HPS secondary to malignancy

There is no documented efficacy of IVIG in HPS secondary to lymphoma[10].

MECHANISMS OF ACTION OF INTRAVENOUS IMMUNOGLOBULIN IN SECONDARY HPS

Before discussing how IVIG acts in HPS, it is crucial to mention that predisposing factors to secondary HPS have not yet been identified. Genetic mutations associated with FHL, Chediak—Higashi syndrome, Griscelli syndrome and XLP should be investigated in these patients. The analysis of genetic polymorphisms of Toll-like receptors, cytokines, cytokine receptors and Fcγ receptors might also be of interest.

Although the mechanisms of action of IVIG in infectious diseases and in autoimmune and/or systemic inflammatory diseases are now well documented, there is no study available investigating these mechanisms in HPS.

Since IVIG is obtained from a pool of plasma obtained from more than 1000 healthy blood donors, and thus contains a wide range of antibody specificities, it can specifically neutralize a wide range of pathogens in HPS secondary to infection.

The putative immunomodulatory effects of IVIG in HPS are listed in Table 1. It is now well documented that IVIG is able to target the macrophage and induce expression of the inhibitory Fcγ receptor FcγRIIB. IVIG also modulates cytokine production and release by the macrophage, particularly by increasing the levels of IL-1ra and modulating cytokine production and release by dendritic cells.

CONCLUSION

Intravenous immunoglobulin could provide beneficial effects in secondary HPS: in combination with antimicrobial therapy in HPS secondary to infection (with the exception of EBV), or alone or in combination with corticosteroids in HPS secondary to SLE/Still's disease. This must be confirmed, however, in a randomized trial. Genetic factors of susceptibil-

Table 1 Putative mechanisms of action of intravenous immunoglobulin (IVIG) in hemophagocytic syndrome

Neutralization of pathogens
Modulation of antibody-dependent cell cytotoxicity (ADCC)
Modulation of FcγR expression on macrophages and dendritic cells
Modulation of cytokine production
down-regulation of proinflammatory cytokine production by monocytes/macrophages

increased IL-10 production and decreased IL-12 production by dendritic cells
modulation of IL-18 production by macrophages
Modulation of apoptosis
Modulation of CD8+ T-cell and NK-cell cytotoxicity
Modulation of cytokine production and release by lymphocytes
Specific recognition of cytokines, soluble receptors and cell surface molecules by infused IgG

IL-10, interleukin-10; NK, natural killer; IgG, immunoglobulin G

ity to secondary HPS and mechanisms of action of IVIG in these patients remain to be identified.

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22
Anti-inflammatory effects of intravenous immunoglobulin through modulation of complement activities

H.U.Lutz and P.J.Späth

The ability of pooled normal human immunoglobulin (IgG) for intravenous use (IVIG) to inhibit complement[1] was neither known before its clinical application nor expected. In fact, the opposite appeared more likely, because IVIG contains a huge variety of immune antibodies and a myriad of different naturally occurring autoantibodies (NAbs), capable of activating complement, when complexed with antigen. Some of these lowtiter, low-affinity NAbs have tissue-homeostatic roles and do not exist in saturating conditions in human plasma[2]. Hence, an IVIG-induced increase in their concentration could enhance complement consumption in the course of additional clearance of altered and senescent self. Some evidence was presented for enhanced, isoagglutinin-independent clearance of red blood cells[3], but the extent of an extra complement activation must have been minute, since the overall effect of IVIG is complement-inhibiting. To understand how IVIG may modulate complement, the following summarizes the relevant steps of complement activation.

Immune complexes activate the classical complement pathway that culminates in the generation of activated C3 in a number proportional to that of immune complexes. Activated C3 then triggers complement amplification, a positive feedback loop which generates activated C3 beyond proportionality and eventually activated C5. The result of a well-controlled complement activation is two-fold: it deposits C3b to immune complexes and thereby facilitates their removal, and it provides proinflammatory substances, the anaphylatoxins C3a and C5a. Both roles of complement activation are beneficial, but excessive complement activation is destructive and inflammatory. High amounts of locally generated C5b will allow assembly of the membrane attack complex, and high concentrations of anaphylatoxins will induce secretion of proinflammatory cytokines from attracted and activated white cells.

COMPLEMENT ACTIVATION AND AMPLIFICATION IN THE PRESENCE OF IgG

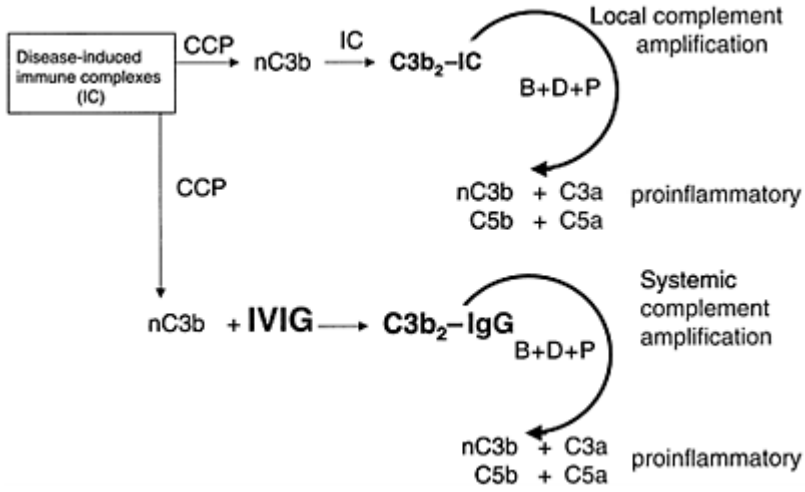
Complement activation generates C3b. Nascent C3b, a very short-lived intermediate generated during the activation of C3, forms covalent bonds with OH groups in its immediate vicinity[4]. The OH group-carrying acceptor can be a carbohydrate or a protein,

and even more probably a protein that was loosely associated with C3 before it was activated. IgG molecules have a low affinity within a constant region domain for C3[5]. This property, the short half-life of nascent C3b and the fact that classical complement activation occurs on antigencomplexed IgG, render IgG molecules predominant acceptors of nascent C3b. Nascent C3b links itself to a preferred site within IgG molecules[6,7] by forming $C3b_n$-IgG complexes. Plasma or serum IgG competes for nascent C3b with those IgG molecules that make up immune complexes. Hence, a 2–3-fold elevated IgG concentration, similar to that achieved during IVIG infusion, deflects nascent C3b away from immune complexes to fluid phase IgG with the formation of $C3b_n$-IgG complexes[8]. The generation of fluid phase $C3b_n$—IgG complexes is not a 'dead-end street' for complement amplification. It is known that such complexes activate alternative complement pathways[9]. In fact, these complexes contain never one, but two C3b molecules on one heavy chain (Jelezarova and colleagues, unpublished data) and are 7–10 times better activators of complement amplification than immobilized C3b[10]. Since such complexes provide dimeric C3b, C3/C5 convertase, assembled on such precursors, should have a decreased K_M for C5 and thus a substantial C5 convertase activity[11].

IS DISPLACEMENT OF C3b FROM LOCAL IMMUNE COMPLEXES SUFFICIENT TO ATTENUATE COMPLEMENT ACTIVATION?

Dermatomyositis is one of those autoimmune diseases, associated with complement activation beyond C3, where activated C5 allows assembly of the membrane attack complex, resulting in deposition of C5b-9 to endomysial capillaries. In this disease and two animal model systems[12], high-dose IgG (2g/kg body weight) displaced activated C3 from tissuebound immune complexes to fluid phase IgG, and thereby prevented complement-dependent tissue destruction[13,14]. At discovery, the phenomenon was poorly understood, despite a plausible explanation. A more detailed understanding is now emerging from the experimental work of several groups as outlined above, implying that the displacement of nascent C3b from local immune complexes to fluid phase IgG may stop tissue destruction, but not the generation of fluid phase $C3b_2$-IgG complexes. Thus, if displacement of nascent C3b to fluid phase IgG were the only complement-modulating effect of IVIG, complement amplification and with it generation of anaphylatoxins should continue in the plasma of patients with dermatomyositis (Figure 1). Data from such patients show the opposite, their serum C3 levels remaining virtually unchanged and levels of the inactivated terminal complex (SC5b9) almost as low as in controls after IVIG treatment[13] This implies that displacement of nascent C3b to fluid phase IgG is not the only effect of IVIG on complement. *In vitro* experiments revealed that IVIG preparations enriched in IgM not only displaced nascent C3b from a surrogate of an immune complex, but also inhibited C1q binding to it, and thereby prevented complement activation

Figure 1 Displacement of nascent C3b (nC3b) from local immune complexes (IC) does not reduce generation of proinflammatory substances. The scheme illustrates that classical complement pathway (CCP) activation by immune complexes in tissues (dermatomyositis) generates nascent C3b (nC3b) that either deposits to IC or in the presence of high-dose intravenous immunoglobulin (IVIG) primarily to fluid phase IgG. Either complex ($C3b_2$−IC on the tissue or $C3b_2$—IgG in the fluid phase) activates complement amplification and generates proinflammatory anaphylatoxins. Hence, displacement of nC3b from IC to immunoglobulin G (IgG) is not sufficient to attenuate complement activation



rather than redirected it[15]. Since 8mg IgG/ml was five times less efficient as IgM-enriched IVIG in C1q scavenging, it remains questionable whether IVIG acts by preventing classical pathway activation. Moreover, a dose of 0.5g/kg body weight of a pasteurized IVIG preparation activated classical and alternative complement pathways significantly[16], while as little as 0.1–0.2g/kg body weight of a pH4-treated preparation

elicited a moderately increased concentration of C3dg, without significant consumption of C4, C3 and B[17].

NEUTRALIZATION OF THE PROINFLAMMATORY EFFECT OF C3a AND C5a

With the awareness that the displacement of nascent C3b to fluid phase IgG as such cannot attenuate complement activation (Figure 1), the search for additional IVIG effects continued. Basta and colleagues[18] recently found that constant region domains of IgG or F(ab‘)2 have a low-affinity binding site for the anaphylatoxins C3a and C5a. In fact, a dose of 20mg IgG/ml almost completely inhibited the C3a-or C5a-induced histamine release from basophils. The data suggest that high-dose IVIG may indeed be capable of neutralizing the proinflammatory effect of anaphylatoxins. At present it remains unclear, however, why the neutralizing capacity of IVIG was significantly higher than that of pre-existing IgG in plasma and required 1 day or more to become effective *in vivo* as compared with 15min *in vitro*. Nevertheless, scavenging of anaphylatoxins by IgG is an interesting regulation of inflammation rather than of the complement system. It is unlikely to affect the rate of complement amplification.

INTRAVENOUS IMMUNOGLOBULIN ATTENUATES COMPLEMENT AMPLIFICATION

High-dose IgG stimulated inactivation of $C3b_n$—IgG complexes in serum in which complement was activated by immune aggregates[8]. Five mg/ml of IgG lowered the half-life of C3b2—IgG complexes from 3–4 to 1–2min in 20% serum. Hence, it appears that IVIG or components of it might have interacted with these complexes and either prevented C3 convertase assembly or directly stimulated their factor H- and I-dependent inactivation. High-dose IVIG had the same effect *in vivo* and induced a rapid decrease of plasma $C3b_2$-IgG complexes already during infusion, as we found in a recently completed study on patients with dermatomyositis[19]. While these relatively short-lived complexes may be of academic interest, the result of their enhanced inactivation is of clinical relevance. Infusion of 2g IgG/kg body weight resulted in a 30–40% consumption of C4 by day 2–3. This significant activation of the classical complement pathway, however, was not followed by a corresponding C3 consumption. The C3 concentration remained at initial levels in myopathic and even increased by 10–15% in amyopathic dermatomoysitis. Thus, IVIG stopped C3 activation in patients, and this attenuation even compensated for C3 that was activated via the classical pathway following application of IVIG. These findings suggest that IVIG attenuates complement amplification selectively, but does not prevent classical complement pathway activation, a fact important for host defense. We do not yet know how IVIG exerts this modulating effect. Experimental evidence suggests the involvement of certain NAbs in IVIG, and the attenuation of complement amplification may depend on specificities of IgG NAbs rather than on constant region domains.

CONCLUSIONS

IVIG exerts anti-inflammatory effects by interfering with complement components. Two of its modulating potentials, namely its abilities to displace nascent C3b from local immune complexes and to scavenge anaphylatoxins, are dependent on constant region domains of IgG. Its potential to attenuate complement amplification appears to be dependent on the specificity of certain physiological, naturally occurring antibodies. The complement-attenuating effect of IVIG in dermatomyositis may require both the displacement of nascent C3b from immune complexes and the selective inhibition of complement amplification.

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23

Changes in gene expression profile in the muscles of patients with dermatomyositis and inclusion body myositis after therapy with intravenous immunoglobulin: a microarray analysis correlating gene expression with clinical outcomes

R.Raju and M.C.Dalakas

INTRODUCTION

Dermatomyositis (DM) is an inflammatory muscle disease affecting muscle and skin. It is characterized by early deposition of complement on the intramuscular capillaries leading to capillary destruction, muscle ischemia, perifascicular atrophy, inflammation consisting of B cells, CD4+cells, and to a lesser degree CD8+ cells, intense up-regulation of cytokines and adhesion molecules, and endomysial fibrosis. In contrast, sporadic inclusion body myositis (sIBM) is a chronic inflammatory muscle disorder with intense endomysial inflammation consisting of CD8+ T cells that invade major histocompatibility complex (MHC) class-I expressing muscle fibers along with vacuolar degeneration of muscle fibers and amyloid deposits[1,2].

Intravenous immunoglobulin (IVIG) has been shown in a controlled study to be effective in DM patients, inducing not only clinical but also immunopathological improvement based on repeated muscle biopsy studies[3]. In contrast, IVIG in controlled studies did not statistically improve the strength of patients with IBM, even though T-cell activation, cytokines and chemokines are also up-regulated in these patients' muscles[4].

The immunoregulatory genes activated in these diseases, their changes after therapy with IVIG and the genes most relevant to the clinical response, are unknown. To understand the molecular basis that determines response to therapy with IVIG, we employed oligonucleotide microarray technique on muscle biopsy specimens of patients with DM and sIBM before and after therapy[3,5]. These specimens offer a unique opportunity to examine the relevant immunoregulatory genes manipulated by IVIG because we can compare the effects of IVIG in muscle specimens from patients who responded to the drug with those from patients who did not.

MATERIALS AND METHODS

Muscle biopsies from three DM patients before and after IVIG therapy were studied. All three patients had a major clinical improvement and underwent a repeated muscle biopsy at the end of three monthly IVIG infusions, when their muscle strength had normalized. Muscle biopsies were also studied from four patients with IBM, before and after IVIG infusions, treated the same way as the DM patients, but whose strength did not improve. Total RNA from muscle biopsies were reverse transcribed and biotinylated cRNA probes were generated by *in vitro* transcription. Fifteen micrograms of fragmented cRNA was hybridized to a human genome U133A array containing oligonucleotide probe sets representing more than 39 000 transcripts derived from approximately 23 000 well-substantiated human genes (Affymetrix, Santa Clara, CA, USA). The hybridized gene chips were scanned to quantitate the gene expression and the data analysis was performed using Microarray Suite and Data Mining Tool Softwares (Affymetrix, Santa Clara, CA, USA). We calculated the average 'fold' difference in gene expression between pre- and post-treatment in each disease group. The data were further filtered by selecting genes that produced only stronger signals as defined by an arbitrary cut-off point for the fluorescent signal intensity. All the specimens were blindly processed.

RESULTS

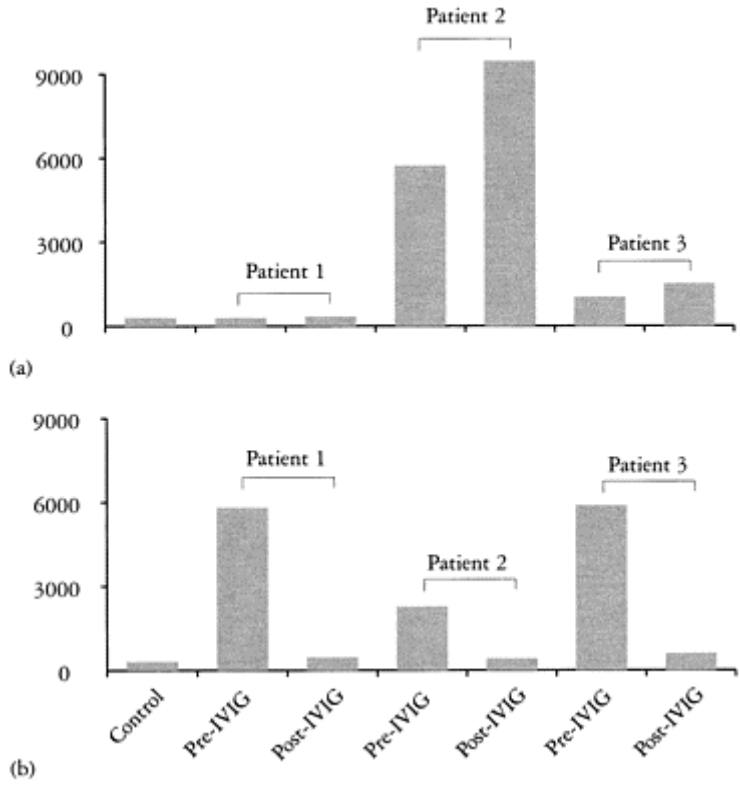
Among more than 22 000 genes probed, approximately 1800 genes were amplified with a minimum fluorescent signal of 100 in both DM and IBM muscles. Among those, over 1400 genes showed more than 1.5-fold expres sion differences after treatment. Over 700 genes showed consistent pattern of expression (either up-regulation or down-regulation). In DM, but not IBM, some of the genes that demonstrated strong down-regulation after IVIG included the FK506-binding protein, interleukin (IL)-22 and intercellular adhesion molecule (ICAM)-1. Most of the genes that showed down-regulation were either of immunological function or genes related to myocyte proliferation and differentiation. Among the genes that showed up-regulation in DM, but down-regulation in IBM, were those for the Mig chemokine (Figure 1). The effect on other genes and their confirmation with realtime polymerase chain reaction (PCR) is in process.

DISCUSSION

Microarray methodologies have been used recently to address gene expression profiles in neuroimmunological diseases as compared to healthy controls or to study the effect of various therapies[6,7]. For example, studies have addressed inflammatory myopathies like DM, polymyositis and IBM, as well as responder and non-responder phenotypes to interferon-β in multiple sclerosis[6,7]. Successful treatment of DM patients with IVIG results in changes in the expression levels of more than 700 genes involved in immune or regulatory pathways. One of the genes that is down-regulated in DM is that for IL-22. IL-

22 is produced by activated T cells. An important, cytokine-induced adhesion molecule gene that was affected by IVIG was the gene for ICAM-1 which was substantially down-regulated in DM patients who improved, but was modestly reduced in some IBM patients who did not. The down-regulation of ICAM-1 gene expression in DM

Figure 1 Expression of the chemokine Mig in controls, patients before intravenous immunoglobulin (IVIG) treatment and after IVIG treatment as demonstrated by microarray studies. Mig expression in three dermatomyositis patients was increased after IVIG treatment (a) whereas there is a significant down-regulation of Mig after IVIG treatment in three inclusion body myositis patients (b)



patients after IVIG as observed in the present study, was correlated with down-regulation of protein expression as we previously reported in the same DM biopsies after IVIG treatment[3].

Different (but clinically irrelevant) genes were also modified by IVIG in the muscles of patients with IBM who did not respond to therapy. The IVIG-induced expression levels of genes that were down-regulated by more than two-fold in DM patients who had improved, were unaffected in IBM patients who did not respond to IVIG. This clearly demonstrates a dichotomy in gene expression profiles induced by IVIG in two diseases, one responding to IVIG (DM) and the other not responding (IBM). Mig, an interferon-γ-induced chemokine, which was highly up-regulated in IBM and DM muscle was down-regulated several fold in IBM after IVIG. In contrast, Mig was up-regulated in patients with DM who improved after IVIG, suggesting that this chemokine has a different immunopathogenic role in DM compared to IBM.

Another gene down-regulated in DM subsequent to IVIG treatment was *N*-acetylgalactosamine-4-O-sulfotransferase (GALNAC-4-ST1). This enzyme adds sulfate to the non-terminal GalNAc residues in either chondroitin or dermatan[8,9]. Because this gene is involved with the glucoprotein expression on the surface of muscle fibers, it appears that IVIG has an effect on the reorganization of the structural proteins of the muscle membrane.

Our present preliminary results indicate that after IVIG administration, modulation of only a subset of immunoregulatory genes seems to be clinically relevant. Gene expression profiling is a new tool that provides information on the relevant genes responsible for the disease and for restoration of the muscle cytoarchitechture after successful therapy.

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24
In vitro studies of mechanisms of action of intravenous immunoglobulin in Guillain-Barré syndrome and Miller-Fisher syndrome: a review

R.Gold, A.Weishaupt, M.Stangel A.Chan and K.V.Toyka

INTRODUCTION

Intravenous immunoglobulin (IVIG) treatment now has an established role in the therapy of immunologically mediated demyelinating disorders including Guillain—Barré syndrome (GBS) and its subtypes, chronic inflammatory demyelinating polyneuritis (CIDP) and multi-focal motor neuropathy (for review see references 1 and 2). Both humoral and cell-mediated immune mechanisms are operative in these syndromes. Antibodies to different gangliosides were found in 30–40% of GBS patients. Immunoglobulin G (IgG) antibodies can access the peripheral nerve at its most proximal and distal parts, where the blood-nerve barrier is lacking. In addition, IgG antibodies may also interfere with neuromuscular transmission, as shown for sera from adult patients with GBS of the acute inflammatory demyelinating type[3]. There is overwhelming evidence that IVIG can manipulate the immune system at several levels, which may contribute to its therapeutic efficacy in GBS and its subtypes.

IVIG INHIBITS PATHOPHYSIOLOGICAL EFFECTS OF PATIENT SERA AT MOTOR NERVE TERMINALS

IVIG has been accepted as an effective alternative to plasmapheresis for the treatment of GBS (see review in reference 4). The clinical efficacy of IVIG in GBS was documented in adults by controlled trials, and by smaller open trials in children. IVIG may modulate the immune response by means of multiple mechanisms, including idiotypic—anti-idiotypic interactions, which have previously been demonstrated *in vitro*[5]. Because neuromuscular blocking antibodies are thought to represent one pathogenic factor for acute muscle weakness in GBS by exerting nerve conduction failure, studies were initiated to determine whether IVIG may directly influence functional properties of these blocking antibodies[6,7].

Experiments were conducted using the perfused macro-patch-clamp technique as described before[8]. Briefly, hemidiaphragms of at least 16-week-old male Balb/c mice were dissected with the adjacent phrenic nerve and kept at 20±0.5°C. End-plate currents were recorded with a perfused macro-patch-clamp electrode (Figure 1). The electrode,

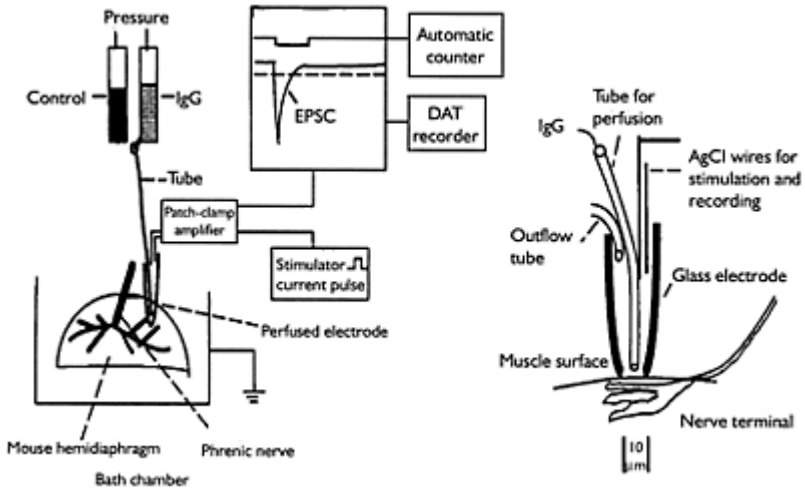
with a 10-µm-wide opening, covers a part of the presynaptic and postsynaptic membrane with its adjacent Schwann cell. The electrode contains a current clamp recording input, and a stimulation electrode through which current pulses depolarize the nerve terminal in a graded manner by shifting the extracellular field potential. Using this set-up, it is possible to record quantal excitatory postsynaptic currents corresponding to the release of one acetylcholine-containing presynaptic vesicle. In addition, the electrode is equipped with a fine tube that enables perfusion of the tip of the electrode with a pressurized solution of patient sera or immunoglobulins.

GBS immunoglobulins were purified by affinity chromatography as previously described[8]. Immunoglobulin fractions were free of complement and low-molecular-weight compounds. Commercially available IVIG preparations from two different manufacturers (Sandoglobulin® from Novartis, Basel, Switzerland, and Endobulin S/D® from Baxter, Heidelberg, Germany) were used for treatment and investigation.

Monovalent antigen-binding fragments (Fab) and Fc fragments were prepared from one of the two IVIG preparations (Sandoglobulin) as previously described[9]. IVIG, Fab and Fc fragments and sera from five patients with other non-neuropathic neurological diseases and healthy volunteers served as controls.

For coincubation experiments, a defined amount of serum obtained before IVIG therapy (subsequently called preinfusion serum) was

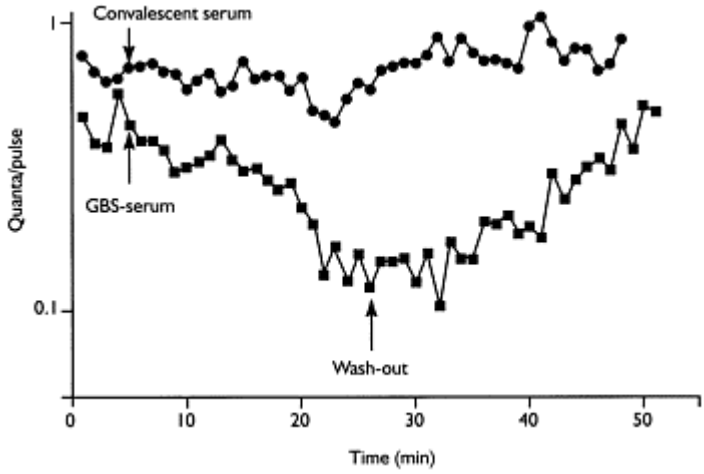
Figure 1 Macro-patch-clamp set-up (a) and detail of electrode (b). IgG, immunoglobulin G; EPSC, excitatory postsynaptic current; DAT, digital audio tape. Courtesy of Dr Buchwald, Munich



mixed with a defined amount of serum taken after IVIG treatment (postinfusion serum) or with a sample of therapeutic IVIG from the same batch that the patient had received.

GBS sera taken before IVIG treatment (preinfusion sera) induced a severe and virtually irreversible blockade of neuromuscular transmission. Quantal release was reduced by 80–90%, and in some cases did not return to normal after wash-out (Figure 2). In contrast, serum obtained 4–7 days later (i.e. after IVIG treatment; postinfusion sera) did not display blocking activity. To prove that the therapeutic IVIG circulating in the patients' sera might in itself exert this neutralizing effect, a defined amount of patient preinfusion (blocking) serum was mixed with a sample of the IVIG used for treatment. When this mixed sample was applied to motor nerve terminals, no blocking activity could be found, corroborating that the blocking activity of the pathogenic antibodies was directly inhibited by the therapeutic IVIG. Finally, monovalent and divalent Fab fragments and Fc fragments of one therapeutic IVIG preparation (Sandoglobulin) were coincubated with the blocking GBS-IgG fractions. Only Fab fragments neutralized the blocking activity of preimmune serum, whereas coincubation with the Fc fragments did not inhibit the blocking properties of the GBS-IgG.

Figure 2 Time course of reduction of quantal release (y-axis) by Guillain-Barré syndrome (GBS) serum in the acute phase, but not after recovery. Recording was done at the phrenic-diaphragm preparation during constant 5-Hz stimulation. Courtesy of Dr Buchwald, Munich



These results confirm earlier observations that sera of adults and children with acute GBS contain IgG antibodies capable of blocking neuromuscular transmission in the mouse hemidiaphragm[9]. They provide evidence that IVIG treatment may convey protection against these blocking antibodies in adult and childhood GBS. In a recent study from a

Dutch—Scottish co-operative group[7], similar findings were made for anti-GQ1b antibodies, which are important pathogenic factors in Miller—Fisher syndrome (MFS) and GBS, and act through effects at the neuromuscular junction. Using several electrophysiological and morphological read-out parameters, Jacobs and colleagues[7] showed that indeed IVIG inhibited the pathophysiological effects of these anti-GQ1b antibodies. Thus, further experimental background is provided to the clinical observation that IVIG is an effective treatment for GBS and MFS.

IVIG INHIBITS PHAGOCYTOSIS OF APOPTOTIC CELLS BY MICROGLIAL CELLS

Apoptosis is an established way to terminate an autoimmune T-cell response in the rodent or human brain and peripheral nervous system. In experimental autoimmune encephalomyelitis (EAE), an apoptosis rate of infiltrating T cells of up to 50% has been observed, and in experimental neuritis an apoptosis rate of 10–15%[10]. Ingestion of apoptotic leukocytes by microglia and macrophages results in an efficient clearance of the inflammatory infiltrate, followed by a profound down-regulation of proinflammatory phagocyte immune functions (reviewed in reference 11). This may further help to down-regulate inflammation in the nervous system, thus reducing autoimmune damage.

The effects of different immunomodulatory agents on Lewis rat microglial phagocytosis of apoptotic autologous thymocytes or myelin-basic protein (MBP)-specific, encephalitogenic T cells were investigated using a standardized, light microscopic *in vitro* phagocytosis assay[12]. Pretreatment of microglia with polyclonal 7S IVIG (Sandoglobulin) decreased the phagocytosis of apoptotic thymocytes by 38.2% (Figure 3). Interestingly, immunoglobulin F(ab)2 fragments (Gammavenin®) also microglial phagocytosis to a similar extent, suggesting an Fc receptor-independent mechanism. Similar results were obtained using pathogenic MBP-specific T cells. IVIG also partially reverted the phagocytosis-promoting effect of interferon-γ (IFN-γ).

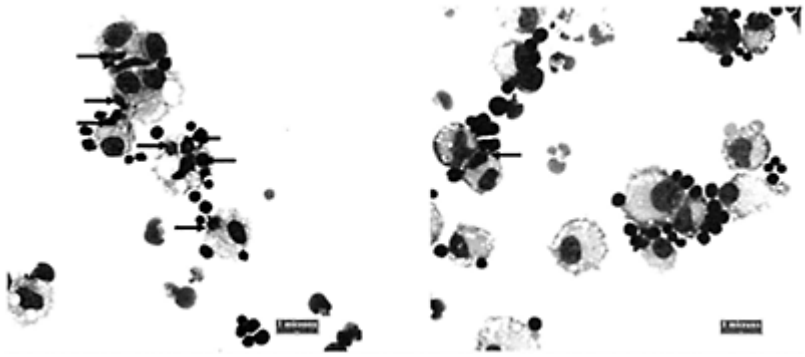
These results indicate that IVIG has a high potential to inhibit microglial phagocytosis of apoptotic inflammatory T cells even under proinflammatory conditions, and extend our view of the complex immunomodulatory effects of IVIG. Thus, in autoimmune central nervous system (CNS) inflammation, IVIG could interfere with the removal of the inflammatory infiltrate, at least during stages with a high prevalence of apoptotic inflammatory cells. Whether IVIG also inhibits the phagocytosis of apoptotic nerve cells is currently unknown. The binding site for IVIG has not been identified. As yet, a number of potential receptors for apoptotic cells have been characterized in murine and human phagocytosis models (see review in reference 14).

IVIG MODULATES NO PRODUCTION AND MICROGLIAL IMMUNE FUNCTION

The therapeutic benefit of IVIG in multiple sclerosis is probably mediated by modulation of the cytokine network and T-cell responses. Since there are no data on the influence of IVIG on the local immune reaction in the CNS, the effect of IVIG was studied on

cultured rat microglia[15], the main immune cell in the CNS. For these experiments, microglial cells were isolated from neonatal Sprague—Dawley rat cerebra and further processed to achieve a purity of >95% as judged by staining with isolectin B4.

Figure 3 Inhibition of microglial phagocytosis of apoptotic cells by intravenous immunoglobulin (IVIG). Photomicrographs of untreated (a) or IVIG (Sandoglobulin®)-pretreated (b) Lewis rat microglia interaction with autologous apoptotic thymocytes. Note that the number of ingested apoptotic fragments is greatly reduced after IVIG (b). May—Giemsa stain. Modified from reference 13



Sandoglobulin was used as the source of intact IVIG, and Gammavenin as the source of F(ab)2 fragments.

Treatment of unstimulated microglia with IVIG or albumin had no effect on NO production. Treatment of IFN-γ-stimulated microglia with IVIG for 24 h led to a dose-dependent increase in nitrite production, the stable end product of NO, while albumin had no effect. Nitrite production could be suppressed by specific inducible NO synthase (iNOS) inhibitors, but not by inhibitors of lipopolysaccharide. A preparation consisting of F(ab)2 fragments of immunoglobulins was used without a significant change in nitrite production. This suggested that the observed effect was mediated via the Fc part of immunoglobulins and the Fc receptor. This was supported by the finding that blockade of kinases down-stream of the Fc receptor could abolish the IVIG-induced NO production. Similar to NO production, secretion of tumor necrosis factor-α (TNF-α) was also augmented in microglial cells, even without cytokine pretreatment.

Thus, IVIG could participate in termination of inflammatory reactions in the CNS by enhancement of NO and TNF-α secretion, accelerating T-cell apoptosis. In addition to

such an immunomodulatory effect, the inhibition of microglial phagocytosis by IVIG could ameliorate oligodendrocyte damage.

CONCLUSIONS

There is overwhelming evidence that IVIG can manipulate the immune system at several levels. The history of IVIG treatments is a telling example of how much empirical knowledge can contribute to medical progress, even in the period of molecular medicine. Elucidation of the mode of action of IVIG may eventually lead to the devising of more specific immunoglobulin preparations.

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Intravenous immunoglobulin for autoimmune dieseases of the peripheral nervous system

25
The immunological rationale for intravenous immunoglobulin in Guillain-Barré syndrome and chronic inflammatory demylinating polyradiculoneuropathy

J.D.Pollard

INTRODUCTION

Intravenous immunoglobulin (IVIG) therapy has become first-line therapy for Guillain—Barré syndrome (GBS) based upon a demonstrated equal efficacy with plasma exchange (PE) but with greater convenience and fewer side-effects[1]. In chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), IVIG has also been shown to have equal efficacy to PE[2] and steroid therapy[1] and to be more effective than placebo[3–5]. Although it needs to be given repeatedly, the side-effects of such treatment are less severe than those resulting from chronic steroid or immunosuppressive therapy, and it is more convenient than PE; hence, in CIDP it has also become first-line therapy in most centers.

To review the immunological rationale for IVIG in GBS and CIDP, it is necessary to review the immunopathological basis to these disorders. GBS and CIDP are the acute and chronic varieties, respectively, of the inflammatory demyelinating neuropathies (IDNs). Both are characterized pathologically by multifocal areas throughout the peripheral nervous system of inflammatory infiltrates associated with demyelination and varying degrees of axonal loss. The mechanism of demyelination remains uncertain, but it is generally accepted that similar processes are involved to those which have been demonstrated in the animal model of IDN, experimental autoimmune neuritis (EAN).

THE IMMUNOPATHOGENESIS OF EXPERIMENTAL AUTOIMMUNE NEURITIS

This subject has been reviewed by Gold and colleagues[6]. EAN is induced in experimental animals by the injection of heterologous peripheral nerve myelin, myelin proteins or peptides derived from these, together with Freund's complete adjuvant. It may also be passively transferred by myelin-specific CD4-positive T-cell lines derived from EAN animals. It is generally accepted that T cells are activated in the periphery and that, by virtue of cell adhesion molecules which they express, attach to counter-receptors expressed on endothelial cells (Figure 1a). Other molecules produced by activated T cells

such as metalloproteinases and tumor necrosis factor-α (TNF-α) are able to degrade endothelial barriers, including the basal lamina, allowing these cells access to the endoneurium and, in the process, causing at least a transitory blood-nerve barrier (BNB) leakiness. This BNB leakiness, associated with T cell migration, in turn allows any circulating antinerve antibody access to the endoneurium.

Within the nerve, myelin-specific T cells may encounter their antigen on the surface of an antigen-presenting cell, possibly a perivascular dendritic cell or macrophage, inducing clonal expansion along a T-helper cell Th1 pathway with the production of proinflammatory cytokines such as interferon-γ (IFN-γ) and TNF-α, which may in turn activate macrophages to produce molecules injurious to Schwann cells or their myelin such as reactive oxygen species or proteinases. On the other hand, T cells may be activated to produce a Th2 suite of cytokines such as interleukin-4 (IL-4) or IL-10, which may have anti-inflammatory effects or help B cells to produce antibody. Anti-Schwann cell—myelin antibody, produced either locally or from the circulation, may target activated macrophages to the Schwann cell—myelin complex, resulting in demyelination (Figure 1b). Complement plays a significant role in this process, and EAN is markedly ameliorated in complement-deficient animals. Antibodies which can also be directed to axonal epitopes would be expected, by similar mechanisms, to induce axonal degener ation, but although these have not been well studied in EAN, they play a major role in some forms of GBS (see below). It is of interest also that in EAN induced by passive transfer of T cells, increased T cell numbers are accompanied by increasing degrees of axonal degeneration. It is only in actively induced disease characterized by both Schwann cell—myelin-specific antibody and activated T cells that widespread demyelination is seen[7]. A chronic form of EAN, highly analogous to CIDP, has been described in rabbits[8].

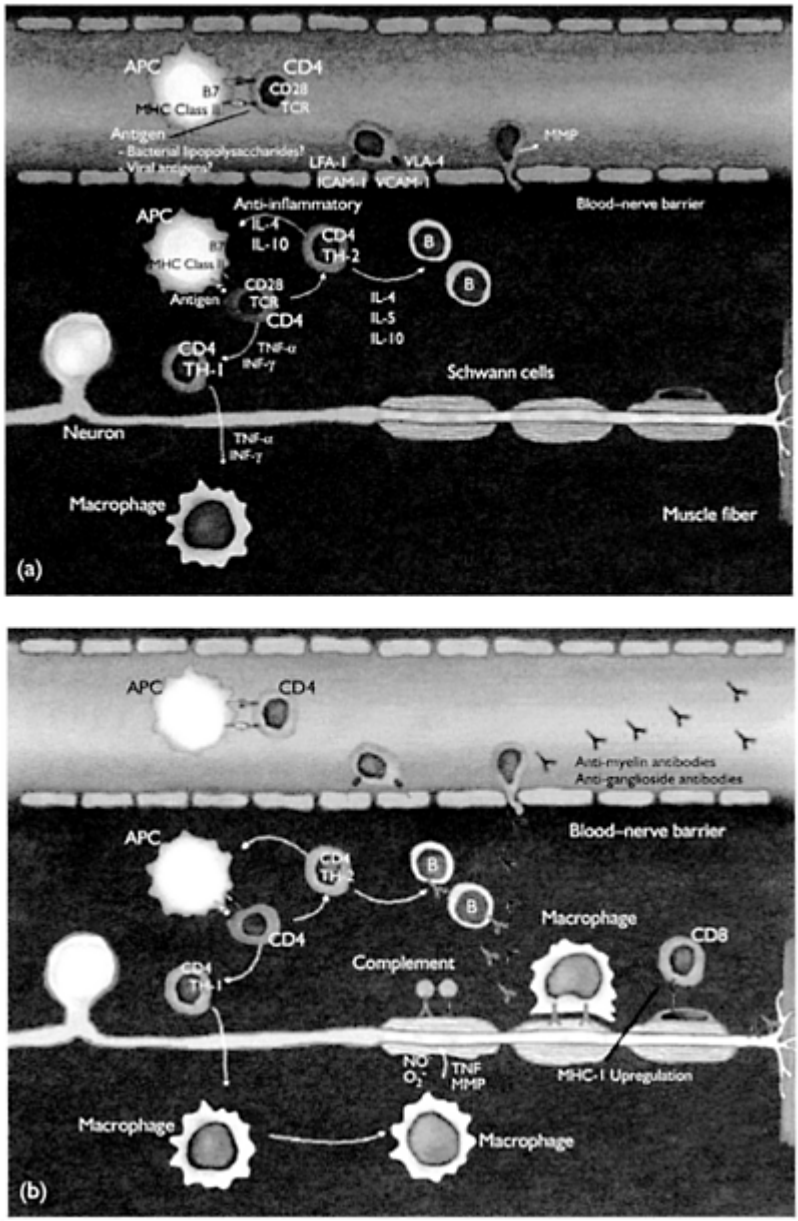
THE IMMUNOPATHOLOGY OF INFLAMMATORY DEMYELINATING NEUROPATHIES

Guillain-Barré syndrome

Although the immunopathology of human inflammatory demyelinating neuropathy and EAN appear similar, it is clearly the initiating events which remain unknown in the human disorders. However, a majority of cases of GBS follow a known precipitating bacterial or viral infection, and there is now convincing evidence that molecular mimicry plays an important role in disease induction.

A clearer understanding of disease pathogenesis in GBS has followed the definition of homogeneous subtypes of the disease. It is now generally recognized that GBS consists of at least four subtypes. Acute inflammatory demyelinating polyneuropathy (AIDP), which is the common form of GBS seen in the Western world, is characterized electrophysiologically and pathologically by demyelination. Acute motor axonal neuropathy (AMAN) is a mostly motor axonal neuropathy and has been well studied in northern China. The Miller-Fisher syndrome (MFS) is characterized

Figure 1 Diagrammatic representation of immune-mediated events within the peripheral nerve as described in the text



by ophthalmoplegia, ataxia and areflexia. Acute motor and sensory axonal neuropathy (AMSAN) is a rare condition with both severe motor and sensory loss and very poor prognosis. It is now clearly established that both AMAN and MFS are strongly associated with certain non-protein, antiganglioside antibodies.

Chiba and colleagues[9] first demonstrated that MFS was associated with antibodies to the GQ1b ganglioside, and that this ganglioside was enriched within the third, fourth and sixth cranial nerves. It is now clear that anti-GQ1b antibodies are present in at least 90% of MFS cases or GBS patients with ophthalmoplegia[10]. Yuki and colleagues[11,12] showed that antibodies generated against ganglioside epitopes on *Campylobacter jejuni*, a common antecedent infection in GBS, cross-reacted with similar epitopes on human nerves.

Thus, molecular mimicry involving *C. jejuni* or other initiating infective illnesses plays a central role in disease genesis. Since the BNB is deficient in the proximal and distal ends of nerves, i.e. the region of the nerve roots and the neuromuscular junction, these regions are relatively vulnerable to antibody-mediated disease (e.g. myasthenia gravis). MFS serum and purified immunoglobulin G (IgG) from MFS patients have been shown to exhibit an α-laterotoxin-like effect at the neuromuscular junction[13], causing massive release of acetyl-choline and block of neuromuscular transmission. The purified IgG has been shown to contain anti-GQ1b antibodies and the target of these antibodies to be GQ1b antigen at the nerve terminal[14]. This effect is complement-dependent. Thus, the weakness which occurs in MFS appears to be mediated by complement-dependent anti-GQ1b antibodies resulting in neuromuscular blockade.

AMAN is also associated with the presence of anti-ganglioside antibodies; in Chinese patients the association is with antibodies to GD1a[15], and in European patients with antibodies to GM1. Sheikh and colleagues have produced monoclonal antibodies to the major gangliosides including GD1a, and have shown by immunofluorescence that anti-GD1a antibodies bind preferentially to motor axons[16]. This interesting finding explains how a circulating antibody gaining access to mixed motor and sensory nerves may induce an entirely motor neuropathy such as AMAN. However, the pathogenicity of these antibodies has yet to be proven.

At this time, there is no clear association between AIDP and any ganglioside or antibody. However, studies of neuromuscular transmission in AIDP analogous to those described above by Plomp and colleagues[13] using MFS serum and IgG have shown the presence of antibodies which also interfere with neuromuscular transmission[17]. These antibodies have both a pre- and post-synaptic effect, mediated by purified IgG as well as whole serum, and are complement-independent. They are not associated with anti-ganglioside activity Nevertheless, they have been proposed as one cause of weakness in GBS[18,19].

Chronic inflammatory demyelinating polyneuropathy

It is probable that CIDP, like GBS, comprises several disease subtypes, although at this time no clearly defined groups can be differentiated on the basis of pathogenic mechanisms. However, differing responses to therapy suggest that humoral factors play a dominant role in some patients and cellular mechanisms in others. Certain patients with CIDP respond predictably and repeatedly to PE or IVIG, while others respond only to

powerful immunosuppression. Yan and associates[20,21] have shown pathogenic antibodies in a subgroup of CIDP patients who were PE-responsive. These antibodies were directed to the P0 protein of myelin, and caused conduction block and demyelination following intraneural injections or passive transfer in rats. Antibodies to PMP22 protein have been reported in 35% of 176 patients[22] and to P0 protein in 16–29%[20,23]. Antibodies to gangliosides have been reported in fewer than 10% of CIDP patients[24]. Since no common antigenic target has been defined in CIDP, it may vary from patient to patient.

MECHANISMS OF ACTION OF INTRAVENOUS IMMUNOGLOBULIN

Many different mechanisms of action for IVIG have been described, and it may well be that the principal action varies from disease to disease and that multiple actions may contribute to its efficacy in a particular disease. This would appear to apply to GBS and CIDP

Anti-idiotypic antibodies

Since therapeutic IVIG is sourced from a large number (>10 000) of donors, it contains many naturally occurring antibodies, including IgG dimers, which are evident by electron microscopy. These naturally occurring anti-idiotypic antibodies recognize the variable regions of autoantibodies, preventing them from binding to their target antigens[25]. Anti-idiotypic mechanisms have been shown to inhibit the action of autoantibodies against several systemic autoantigens, including anti-GM1 antibodies in multifocal motor neuropathy[11,26]. Anti-idiotypic binding of IgG has been shown to be the likely mechanism of action for the efficacy of IVIG in reducing the neuromuscular blockade induced by GBS serum and IgG[17]. In that study, the F(ab‘)2 portion of IVIG was as effective as whole IVIG, but the Fc portion was not. Moreover, the effect of IVIG in reducing blockade was dose-dependent when IVIG was added to GBS serum or IgG. Jacobs and colleagues have recently shown that IVIG prevents the binding of anti-GQ1b antibodies from Miller—Fisher syndrome patients[27], and can also displace antibodies already bound to GQ1b in an enzyme-linked immunosorbent assay (ELISA). In an *ex vivo* system of mouse phrenic nerve hemidiaphragm preparation, they showed that IVIG directly interfered with the action of anti-GQ1b at the neuromuscular junction. IVIG may in addition have more long-term effects by reducing the level of autoantibodies. Fc receptors on the B cell surface may be engaged by IVIG and the B cells thus down-regulated[28]. Increased catabolism of serum IgG including autoantibodies may result from saturation by IVIG of the endosomal receptor FcRn in endothelial cells, which functions to protect IgG[25,29].

Prevention of complement activation

IVIG has been shown to bind to complement factors C3, C4, C3a and C5a[30,31], and to inhibit the incorporation of C3b molecules into the C5 convertase assembly[32]. In dermatomyositis, IVIG inhibited the uptake of C3b and C4b and the deposition of

membrane attack complex on endomysial capillaries[32,33]. These findings are pertinent to GBS and CIDP since, in both disorders, complement deposition has been shown on the Schwann cell or myelin surface[34,35]. Jacobs and colleagues[27] have shown that IVIG reduces complement binding by anti-GQ1b antibodies from Miller—Fisher syndrome *in vitro*, and complement activation in the mouse diaphragm model *ex vivo*.

Fc receptor blockades

Blockade of Fc receptors on macrophages is accepted as the mechanism of action of IVIG in idiopathic thrombocytopenic purpura (ITP)[36]. The Fc fraction of IVIG or antibodies to the Fcγ receptor III are effective. Stimulation of the inhibitory FcγII receptor by IVIG was recently shown in an animal model of ITP to be an important mechanism of action. Modulation of FcγII/FcγIII receptors on the surface of circulating monocytes by IVIG, leading to a preponderance of inhibitory (FcγII) receptors, was demonstrated by Créange and colleagues[37]. Macrophages clearly play a central role in demyelination in both GBS and CIDP, and inhibition of their action by Fc mechanisms would provide a likely explanation for IVIG efficacy in these disorders.

Modulation of T-cell function

IVIG may regulate T-cell function by several mechanisms, including autoantibodies against the T-cell receptor[38] and down-regulation of surface adhesion molecules such as intercellular adhesion molecules (ICAM), which are essential for the process of transmigration of T cells from the circulation into the endoneurium[36,37]. IVIG also contains autoantibodies to superantigens, which may directly stimulate T cells.

T cells, both CD4 and CD8, found within the inflammatory lesions of both GBS and CIDP play a major role in inducing BNB leakiness, which allows antibody to access the endoneurium. Hence, regulation of T-cell function by IVIG may be another relevant mechanism of action in these disorders.

Effects on cytokines and chemokines

IVIG causes both suppression of cytokine and chemokine release and neutralization of these molecules in the circulation[39–41]. Levels of circulating TNF-α have been shown to fall in GBS following the administration of IVIG[42,43].

CONCLUSION

Although disease mechanisms for the inflammatory neuropathies GBS and CIDP remain to be clarified, it is apparent that similar processes are involved to those carefully studied in the animal model EAN. Antibody-mediated mechanisms are prominent in some disease subtypes, but T cell and B cell mechanisms with macrophage-mediated demyelination are evident pathologically. The efficacy of IVIG in these disorders may well involve several different mechanisms of action, but direct evidence is available for

anti-idiotypic antibodies, inhibition of complement activation, down-regulation of macrophage activity, regulation of T cell function and suppression of cytokine activity.

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26
Intravenous immunoglobulin for Guillain-Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy in adult patients

P.A.van Doorn

INTRODUCTION

Immune-mediated polyneuropathies are disorders of the peripheral nervous system, leading to muscle weakness and sensory disturbances. Most studies have been performed in patients with Guillain—Barré syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). GBS and CIDP have a wide range of variation in symptoms, severity and duration of progression[1,2]. GBS and CIDP seem to be variants of one disorder, with the very acute GBS patients on the one hand and slowly progressive CIDP patients on the other side of the clinical spectrum. Treatment trials have investigated whether prednisone, plasma exchange (PE) or intravenous immunoglobulin (IVIG) are effective. Recent trials have evaluated different dosage schedules of one type of treatment or have studied combinations of treatments. All major randomized controlled trials have been performed in adults. IVIG currently remains a cornerstone in the treatment of GBS, and in many centers also in CIDP. Studies of the mechanism of action of these various treatments in GBS and CIDP remain scarce. GBS is a disorder in which infections, antiganglioside antibodies and molecular mimicry may cause or at least contribute to the development of the disorder[3]. It is expected that PE at least removes possible pathogenic antibodies or other circulating agents, and IVIG interferes with either antibody production or function. Clinical indications and the effect of IVIG treatment are discussed for GBS and CIDP.

ASSESSMENT OF THE EFFECT OF TREATMENT

Improvement following treatment can be determined at various levels: impairment, disability, handicap and quality of life. In order to assess a relevant effect of treatment in immune-mediated neuropathies, appropriate scales should be applied. An outcome measure needs to be relatively simple, valid and reliable. Additionally, a scale needs to be responsive, which makes it suitable to study the effect of treatment during the course of disease. In GBS, a seven-point ‘GBS disability scale’ is most widely used. In CIDP the

'modified Rankin disability scale' has been used. Merkies and the Immune-mediated Neuropathy Cause and Treatment (INCAT) group have clinimetrically evaluated various assessment scales in patients with GBS and CIDP[4,5]. A selection of these scales can be used to evaluate the effect of IVIG treatment in patients with immune-mediated polyneuropathies. A trial comparing the effect of steroids and IVIG in patients with CIDP was the first study using these outcome measures[6].

Guillain—Barré syndrome

About 70% of GBS patients have had an infection prior to its onset, most frequently a *Campylobacter* infection. The patients then develop weakness and generally also sensory disturbances (Table 1). About 25% of patients need artificial ventilation owing to their muscle weakness. Despite treatment, about 20% of patients are still unable to walk unaided half a year after the onset of disease. Additionally, many patients remain fatigued for years. GBS can be subdivided into the most common form, named acute immune-mediated demyelinating polyneuropathy (AIDP), and the generally

Table 1 Diagnostic criteria for Guillain-Barré syndrome

General factors that need to be present
Progressive weakness with maximal deficit reached within 4 weeks
Weakness already from onset on both sides of the body, tends to be symmetrical
Hyporeflexia or areflexia
Other causes unlikely by history and neurological investigation
Features that argue for the diagnosis
Clinical
sensory disturbances, cranial nerve deficit, autonomic disturbances, absence of fever at onset of disease, antecedent infection (often diarrhea or urinary tract infection)
Cerebrospinal fluid (CSF)
increased total protein after first week, fewer than 30/3 (mononuclear) cells/mm^3
Electromyography
signs of a polyneuropathy, frequently (but not always) a demyelinating polyneuropathy
Doubt on the diagnosis
Persistent asymmetrical weakness, urinary or bowel disturbances, sensory level, more than 30/3 mononuclear cells/mm^3 in CSF, polynuclear cells in CSF, predominant respiratory weakness at onset of disease

more rarely occurring varieties, acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy (AMSAN). Since treatment studies are generally conducted in the whole group of severely affected GBS patients, further subclassification is not made here. The cranial nerve variant Miller-Fisher syndrome (MFS) is discussed separately since it occurs relatively more frequently and is related to the presence of anti-GQ1b immunoglobulin G (IgG) antibodies, and because recently developed research models (using MFS serum or anti-GQ1b antibodies) can be used to study the mechanism of action of IVIG and guide the search for possibly even more effective treatments in GBS (see 'Miller—Fisher syndrome and other variants of GBS' below). Most important in the treatment of GBS remains excellent general care and intensive-care facilities, including respiratory care, monitoring and early treatment of autonomic disturbances, anticoagulation and good nursing care. Treatment with IVIG or plasma exchange (PE) is discussed separately.

Intravenous immunoglobulin and plasma exchange

Plasma exchange is the first and only treatment in GBS that has been proven to be superior to supportive treatment alone[7]. PE reduces the time to independent locomotion by about 1 month (from 83 to 45 days) when applied within the first 2 weeks of the disease[7]. IVIG is an effective treatment for patients with GBS who are unable to walk unaided and are still within the first 2 weeks from onset of disease. IVIG and PE have a similar ability to hasten recovery from GBS[7–10]. The largest GBS trial ever conducted did not show significant differ ences between IVIG, PE, or PE followed by IVIG[11]. The referral pattern and outcome of GBS patients has been studied in The Netherlands. It was found that after the intro duction of IVIG as an effective treatment, fewer GBS patients were transferred from small to large hospitals, and this did not result in a worse outcome in those patients treated in small centers. It was concluded that the introduction of IVIG led to better use of different levels of health-care facilities[12]. For reasons of convenience and safety, particularly also in children and in patients with autonomic instability, IVIG is used as standard treatment in most centers[13].

IVIG dosage

The best dosage of IVIG in GBS is not known. IVIG is mostly administered in a dosage of 0.4g/kg body weight for 5 consecutive days. A total dosage of 2g IVIG/kg applied in 2 days, however, may act more rapidly, but might result in a higher rate of adverse effects. A study comparing different total dosages of IVIG was performed in a selected group of GBS patients. This randomized, double-blind, phase II trial was conducted in 39 GBS patients needing artificial respiration and having contraindications for PE[14]. It showed that patients treated for 6 days with IVIG 0.4g/kg/day improved faster compared with patients who received only 3 days of IVIG at 0.4g/kg/day (p=0.04). In the whole group of GBS patients unable to walk, there was a tendency for faster recovery when treated with the high dosage (p=0.08). This small study indicated that 6 days of IVIG treatment in a standard dosage of 0.4g/kg/day might be more beneficial rather than 3 days[14].

Corticosteroids alone and in combination with IVIG

Intravenous methylprednisolone alone did not show a significant difference in disabilityrelated outcome between the steroid and the placebo group[15]. It was concluded that corticosteroids alone should not be used in GBS[16]. A pilot study reported a positive effect of combined treatment with IVIG and methyl-prednisolone in 25 GBS patients[17]. The effect of the combination of IVIG and methylprednisolone has now been evaluated in a randomized controlled study conducted by the Dutch GBS study group. All 225 GBS patients unable to walk unaided and within the first 2 weeks of disease were treated with IVIG 0.4g/kg/day for 5 days. They were randomized for intravenous methylprednisolone 500mg/day or placebo for 5 consecutive days. The results of this trial are in press.

GBS patients with mild disease

Most treatment trials in GBS study only patients who are unable to walk unaided. Recently, more attention has been paid to GBS patients who remain able to walk during the course of disease, the so-called ‘mild patients’. One question is whether these patients need to be treated with IVIG or PE at all. The proportion of GBS patients who remained able to walk was 4.7% in a retrospective, single-hospital-based study in 254 patients[18]. Van Koningsveld and colleagues reported that 14% of GBS patients remained mildly affected, based on a prospective nationwide survey in 139 patients[19]. The results of the French Cooperative Group on PE in GBS argue for early treatment (with two PE sessions) in patients who are still able to walk[20]. Despite this study, the question whether one should treat GBS patients with minimal disease is still not fully answered, and more information about the course of the disease would be of help. The ultimate severity of weakness in patients remaining ‘mild’ and in those who became ‘severely’ affected appeared to be different already on the day of admission[19]. Thereafter, both groups showed a remarkably similar rate of progression. The patients with mild GBS reached a maximal degree of weakness after about 8 days, leading to the conclusion that treatment may be unnecessary in patients who remain able to walk during the second week of illness[18]. On the other hand, Van Koningsveld and colleagues reported that 38% of mildly affected patients had problems in hand function and an inability to run at 3 and 6 months[19]. Therefore, it seems appropriate that new trials should also consider treatment of mildly affected patients. In general it is important not only to study the effect of IVIG or PE after 4 weeks, but also to look for functional disability after a longer period of followup. Not only pure functional disability, but also residual fatigue and severe endurance intolerance may persist for years after the onset of GBS, even in patients who seem to have recovered well[21].

GBS patients with secondary deterioration

About 10% of GBS patients deteriorate after initial improvement or stabilization following IVIG treatment or PE[22]. In these patients, it is recommended to repeat the same treatment, and it can be anticipated that these patients improve again. Some patients who initially have a disease course compatible with GBS later on develop relapses and

remissions, and finally turn out to have chronic inflammatory demyelinating polyneuropathy. When it is clear that these patients indeed have CIDP, treatment according to the diagnosis of CIDP will have to be applied. In these patients, corticosteroids or intermittent IVIG infusions need to be given to prevent repeated deterioration.

Miller—Fisher syndrome and other variants of GBS

MFS can be considered as a variant of GBS. Almost all patients with MFS have IgG anti-GQ1b antibodies. There is now evidence that both pre- and postsynaptic mechanisms play a role in *in vitro/ex vivo* models[23,24]. There is experimental evidence that a complement-dependent mechanism plays a role. Recent experiments clearly demonstrate that IVIG, but moreover also $F(ab')_2$ and post-treatment GBS serum, inhibits impulse blocking[23]. Jacobs and colleagues recently showed that complement-mediated impulse blocking of the neuromuscular transmission by GQ1b antibodies could be blocked by IVIG[25]. Dalakas reviewed the mechanism of action of IVIG in GBS[26]. Currently it seems that most understanding of the pathogenesis of GBS originates from studies in MFS and the acute motor axonal (AMAN) variety, and not from the most frequent form, the acute immune-mediated demyelinating polyneuropathy (AIDP)[26].

CHRONIC INFLAMMATORY DEMYELINATING POLYRADICULONEUROPATHY

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) can be considered as a chronic variety of GBS, although there certainly are obvious clinical and immunological differences[27–29]. The course of CIDP may be one with gradual progression, with steps of progression or with spontaneous relapses and remissions. According to generally accepted criteria, the duration of progression in CIDP exceeds the duration of progression in GBS. Recently, new criteria for CIDP have been published[30]. Sometimes it is difficult to distinguish between relapsing GBS and CIDP Difficulties arise especially when patients initially have a course resembling GBS.

Corticosteroids

Dyck and associates have conducted the only randomized controlled open study of prednisone, and concluded that steroids are effective in CIDP[31]. Several large non-randomized studies suggest that steroids are beneficial in CIDP. A recent Cochrane review concluded that a single randomized controlled trial provided weak evidence to support the common opinion from non-randomized studies that oral corticosteroids reduce impairment in CIDP[32]. An advantage of steroids is their availability and low initial cost, but side-effects can be very serious (Table 2). Patients with CIDP may have a pure motor form. It has been reported that these patients do not respond, or even may deteriorate shortly after treatment with steroids.

Plasma exchange

PE is shown to be effective in CIDP[33,34]. A clear disadvantage of PE is its lack of availability, its high cost and it is a relatively invasive procedure. Patients treated with PE may improve rapidly, but need regular treatment to avoid clinical deterioration (Table 2).

IVIG

In placebo-controlled studies it was found that IVIG is an effective treatment for CIDP[35–37] (Table 2). The proportion of patients improving in strength was 76%. Improvement on a disability scale, however, was only found in one-third of the patients in the study by Mendell and colleagues[37]. One study did not show a positive effect of IVIG in CIDP[38]. A recent Cochrane review confirmed the favorable effect of IVIG[39]. If patients improve after IVIG, this starts within 2 weeks. The majority of patients need intermittent treatment during many months or several years to maintain the improved condition, which is a problem because IVIG is very expensive. According to our clinical experience, we have developed a guideline that we use in daily practice (Figure 1).

Comparison between steroids, PE and IVIG

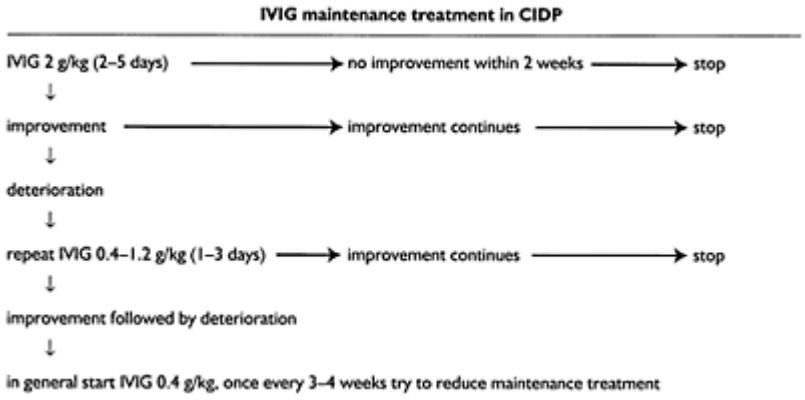
One study did not show a difference in treatment effect between PE and IVIG[40]. A recent

Table 2 Proven effective treatment for chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)

Treatment	Cochrane review	Effect	Side-effects (potential)	Availability	Direct costs
Prednisone[6,31]	ref. 32	+	severe	very good	low
PE[33,34]	—	+	minor	rather good	high
IVIG[6,35–38,40]	ref. 39	+	minor/none	good	high

PE, plasma exchange; IVIG, intravenous immunoglobulin; +, positive

Figure 1 Flowchart for treatment of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) with intravenous immunoglobulin (IVIG)



randomized double-blind, cross-over trial showed that IVIG (2g/kg given over 1 or 2 days) had a tendency to be superior during a treatment period of 6 weeks compared with oral prednisolone (tapered from 60 to 10mg daily during that period)[6]. This is the first trial comparing IVIG with steroids, but the treatment duration was relatively short for a chronic disorder generally needing treatment for many months or years. An editorial by Van den Bergh and a review by Van Schaik and associates provide a good overview[41,42].

Prognostic factors related to improvement

A better outcome after IVIG is reported to be related to younger age at onset, relapsing—remitting course and absence of axonal damage[43,44]. We recently reviewed the IVIG treatment results in our group of 90 CIDP patients who were followed for a period ranging up to 19 years. About 80% of patients initially improved. Of these patients about 50% needed intermittent IVIG for over 3.5 years and about 10% needed IVIG for over 11 years. Especially patients with pure motor CIDP needed IVIG for a long period[45].

CONCLUSION

Recent studies have added new information on the effect of treatment in patients with GBS and CIDP IVIG can clearly be of benefit in these disorders. Whether patients with MFS or those with mild GBS also need PE or IVIG warrants additional studies. Treatment with IVIG or PE is certainly not the final answer for GBS, since a significant proportion of patients have slow recovery or remain disabled or fatigued, even after many months or years. Combinations of treatment may reduce deteri oration and improve recovery. The final results of a trial evaluating the additional effect of methylprednisolone on standard treatment with IVIG in patients with GBS are expected to be published soon. The mechanisms of action of IVIG are complex, but recent experiments further disclose some of these mechanisms, at least in a subgroup of GBS.

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27
Clinical experience with intravenous immunoglobulin for treatment of pediatric Guillain-Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy

A.J.Kornberg

INTRODUCTION

Guillain—Barré syndrome (GBS) is the most common acute neuropathy in clinical practice. Evidence suggests that it is immune-mediated, although the exact pathogenic mechanisms have not been fully elucidated[1].

Great strides in treatment options have occurred over the past 10 years with considerable benefit to patients. Despite these therapies, GBS continues to have significant morbidity and mortality and places enormous burdens on the community. With the better understanding of mechanisms involved in the pathogenesis of GBS, more specific therapies and/or protective mechanisms for the axon will hopefully be developed.

GBS is a monophasic illness with symmetrical, ascending weakness involving proximal and distal muscles. Paresthesias and muscle pain may be presenting complaints, but sensory impairment is usually minimal[2]. Severe back pain and stiffness may occur especially in young children, and be a presenting symptom[3–5]. Tendon reflexes are lost early in the course of the illness. Cranial nerve involvement, particularly the facial nerve, is relatively common. Respiratory failure occurs in about 30% of patients and in addition may be associated with pharyngeal dysfunction. Autonomic involvement can cause wide fluctuations of the blood pressure as well as cardiac arrhythmias and bladder dysfunction. The bladder disturbance is usually transient and is rarely a presenting complaint. GBS typically progresses over a period of less than 4 weeks, with most patients reaching their maximal deficit within 2 weeks of onset.

Controlled trials have concluded that plasma exchange (PE) is an effective therapy in GBS[6–8]. Human intravenous immunoglobulin (IVIG) is at least as effective as PE and has fewer complications[7,9]. Clinical fluctuations occur after both IVIG and PE treatment, but anecdotal reports have suggested that relapse rates may be higher in patients treated with IVIG[10,11]. However, based on two large controlled trials[7,9], it is clear that relapse rates are similar in both IVIG- and PE-treated groups. The use of combined PE and IVIG therapy in an individual patient has not been shown in a recent trial to be more effective than PE or IVIG alone[7].

IVIG is more easily used in children for technical reasons, and appears to be as efficacious as in adults, although no controlled trials have been performed[12–16]. In a large,

cumulative multicenter study involving children, IVIG was shown to be effective, but interestingly a faster rate of recovery was seen in the patients treated over 2 days rather than 5 days[16]. PE in childhood GBS is also beneficial but technically difficult[17,18]. Recent studies have shed some light on the mode of action of IVIG in the therapy of GBS[19,20].

CLINICAL EXPERIENCE WITH IMMUNOMODULATING THERAPIES IN THE TREATMENT OF SEVERE PEDIATRIC GBS AT ROYAL CHILDREN'S HOSPITAL, MELBOURNE

Although the benefits of plasma exchange (PE) and intravenous immunoglobulin (IVIG) have been fairly well documented in the adult population, studies regarding the role of these immunomodulating therapies in the outcome of pediatric GBS are limited.

We have recently retrospectively reviewed the medical records of all children admitted to the Royal Children's Hospital, Melbourne, Australia, with the diagnosis of severe GBS over a 27-year period. We are in the process of analyzing the data of the entire cohort. A subset of this cohort is described here with a comparison between those treated with PE and/or IVIG, and a historic control group before the use of these therapies at our institution (1987 for PE and 1990 for IVIG).

Patients included in this subset had a Motor Disability Grading Score (MDGS) of 3 or greater during their clinical course. Thirty patients were identified in the treated group and 67 patients served as historical controls. Both groups were similar with regard to antecedent illness, clinical presentation (including time to reach nadir) and physical examination at the time of diagnosis. The untreated group had a statistically significant younger age at presentation. The outcome measures used included length of hospitalization, total days of assisted ventilation, days from nadir to subjective improvement, days from nadir to improvement by 1 MDGS and days from nadir until recovery of walking (this measure was used only in those patients with a maximal MDGS > 4).

The mean time of hospital stay was slightly shorter in the untreated group, although this was not statistically significant. This is probably related to a less severe illness in this group. The mean time to subjective improvement, improvement by one grade and improvement to walking were shorter in the treated group (improvement by one grade was statistically significant).

When analyzing the data from the different types of therapies (PE, IVIG or PE and IVIG) (Table 1), the children who received IVIG showed a shorter mean time of hospitalization, fewer days on assisted ventilation, and fewer days to subjective improvement and to improvement by one grade. The small numbers in these groups makes statistical analysis difficult.

Although further trials are required, our findings did not indicate a difference in the long-term outcome of severe pediatric GBS patients. This may reflect the relatively small number of treated patients and the bias

Table 1 Comparison between different treatments

	Days in hospital		Days ventilated		Days to subjective improvement		Days to improvement by grade 1	
	n	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
Plasma exchange	8	53.25 (46.55)	6	16.67 (12.76)	8	19.13 (21.54)	8	20 (19.5)
IVIG	14	15.75 (9.64)	2	22 (11.31)	14	3.86 (2.98)	14	5.07 (3.08)
PE and IVIG	8	33.5 (18.17)	7	12.14 (6.01)	8	7.63 (6.61)	8	9.75 (6.81)

IVIG, intravenous immunoglobulin; plasma exchange

associated with retrospective analysis. However, our results suggest a potential benefit from the use of immunomodulating therapies, particularly by speeding the recovery time and probably reducing the morbidity of these patients (by prolonged hospitalization) and reducing health-care costs.

CLINICAL EXPERIENCE WITH IMMUNOMODULATING THERAPIES CHRONIC INFLAMMATORY IN THE TREATMENT OF PEDIATRIC DEMYELINATING POLYRADICULONEUROPATHY

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a relatively rare disease in childhood. We have reviewed the clinical characteristics, response to therapy and long-term prognosis in 13 children (aged 1.5–16 years of age) diagnosed with CIDP at Washington University Medical Center, St Louis and the Royal Children's Hospital, Melbourne, Australia between 1979 and 1994[21] The most common presenting symptom (in 11/13 (85%)) was lower-extremity weakness associated with difficulty in walking. Preceding events within 1 month of onset, mostly intercurrent infections or vaccinations, occurred in seven children (54%). The disease was monophasic in three (23%) children. One relapse occurred in four (30%) and multiple relapses in six (46%). All patients had at least short-term response to steroids, and other therapies such as IVIG and more potent immunosuppressive agents were used. In the children treated with IVIG, sustained improvement over a 6-month period was noted. However, there are some issues with long-term use of IVIG in CIDP in childhood including the cost and availability of the product.

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Intravenous immunoglobulin for multifocal motor neuropathy

L.H.van den Berg

Lower motor neuron disease is a poorly defined disease entity characterized by (slowly) progressive asymmetrical weakness of limbs without sensory loss[1,2]. Only since the last decade has a subset of patients been recognized with an immune-mediated disorder which is designated as multifocal motor neuropathy (MMN)[3,4]. MMN is more common in men than in women (8:1). The age at onset of weakness ranges from 18 to 65 years; in contrast with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), MMN does not occur in childhood or old (>65 years) age. As MMN is a potentially treatable disorder, its differentiation from lower motor neuron disease is important. The presence of motor conduction block outside entrapment sites, which also occurs in CIDP, and elevated serum antibodies to GM1 ganglioside, a potential autoantigen on the nodes of Ranvier and the surface of motor neurons, support an immunemediated pathogenesis of MMN. Anti-GM1 antibodies are not specific for MMN, as these antibodies are also found in the Guillain—Barré syndrome and motor neuron disease. Whether the presence of conduction block is necessary to identify an immunological (treatable) disorder is still a matter of debate, and may depend highly on the criteria for conduction block and the electrodiagnostic protocol[5]. We recently found that 3 5% of the nerves with conduction block innervate non-weakened muscles[6]. This implies that an extensive electrodiagnostic protocol including nerves that innervate non-weakened muscles improves the diagnostic yield of patients with MMN. Another diagnostic feature may be an abnormal (swollen nerves, increased signal intensity) magnetic resonance imaging scan of the brachial plexus, which is found in approximately half of patients with MMN (and CIDP). Cerebrospinal fluid (CSF) protein levels are below 1g/l in most (>95%) patients with MMN, which is in contrast to patients with CIDP[7].

The hypothesis that MMN is an immune-mediated neuropathy has led to the trial of several immunological treatments. Pred-nisolone and plasma exchange are ineffective in most patients with MMN. Of the immunosuppressants, only cyclophosphamide seems to be consistently effective. Unfortunately, the considerable side-effects of cyclophosphamide, especially the increased risk of neoplasia, limits its utility in patients with MMN, who are of relatively young age[8]. Several open studies have shown a beneficial effect of intravenous immunoglobulin (IVIG) treatment[2,9]. The effect of IVIG in MMN was confirmed in four double-blind placebo-controlled trials[10–13]. However, as the effect of IVIG treatment only lasts a few weeks, IVIG maintenance is necessary to maintain the effect on muscle strength in most patients. Maintenance IVIG treatment is

expensive, and the frequent infusions may be burdensome to patients, but at present there is no therapeutic alternative to IVIG therapy Therefore, long-term studies of the effect of IVIG treatment are important. We recently evaluated the effect of long-term (4–8 years) IVIG treatment in 11 patients with MMN[14–16]. Muscle strength improved significantly within 3 weeks of the start of IVIG treatment, and was still significantly better at the last follow-up examination than before treatment, even though it decreased slightly and significantly during the follow-up period. The electrophysiological findings of this followup study imply that IVIG treatment favorably influences the mechanisms of remyelination or reinnervation, but that axon loss cannot be prevented. IVIG treatment did not induce remission in any of our patients; once IVIG treatment was stopped, substantial progression of weakness occurred. Side-effects were minor, the most disabling being skin changes (eczema) in the hands and trunk. Natural-history studies in MMN cannot be performed for ethical reasons. Because of this, we estimated disease progression by comparing the severity and duration of disease in 38 patients before IVIG treatment was started[17]. With increasing disease duration, weakness and disability became significantly more severe, and the distal and proximal CMAP amplitude decreased significantly. The number of conduction blocks was significantly higher in patients with a disease duration longer than 10 years. The patients who responded to IVIG treatment had a disease duration of up to 24 years and could have severe weakness, which provides indirect evidence that the progression of weakness in MMN is caused by an ongoing immunological process. These results indicate a slowly progressive disease course of MMN, and imply that early treatment may prevent future progression of weakness and disability in patients with MMN.

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Intravenous immunoglobulin in paraproteinemic demyelinating neuropathies

J.-M.Léger

INTRODUCTION

The association between peripheral neuropathy and paraproteinemia has been increasingly recognized in the past 25 years. The first descriptions concerned peripheral neuropathy associated with multiple myeloma, then Waldenström's macroglobulinemia[1]. In 1978, Kyle[2] proposed the term of monoclonal gammopathy of undetermined significance (MGUS) to regroup cases with 'benign' paraproteinemia, and Kelly and colleagues[3] drew attention to the frequency of the association between MGUS and polyneuropathy. Finally, an activity of the M-protein directed to peripheral nerve antigens (myelin-associated glycoprotein: MAG; glycolipids: sulfate glucuronyl paragloboside, sulfate glucuronyl lactosaminyl paragloboside, sulfatides) has been demonstrated in a high proportion of paraproteinemic demyelinating neuropathies (PDNs) associated with immunoglobulin M (IgM) MGUS[4–7], but has not been found in PDN associated with IgG and IgA MGUS. In this review the main characteristics of PDN, associated with either lymphoproliferative disorders or MGUS, are summarized, and the current place of therapies developed is discussed, with special attention to intravenous immunoglobulin (IVIG), which has been increasingly used in the treatment of immune-mediated neuromuscular diseases over the past decade (Table 1)[8,9].

PARAPROTEINEMIC DEMYELINATING NEUROPATHY ASSOCIATED WITH LYMPHOPROLIFERATIVE DISORDERS

Waldenström's macroglobulinemia

Waldenström's macroglobulinemia (WM) is a lymphoproliferative disorder of low malignancy. The usual clinical features are fatigue, weight loss, hepatosplenomegaly, lymphadenopathy and bleeding. A large study of 75 patients with IgM monoclonal gammopathy and neuropathy[10] showed a high frequency of predominantly sensory motor neuropathy, with a marked reduction of motor nerve conduction

Table 1 Summary of evidence for treatment in paraproteinemic demyelinating neuropathy (PDN)

Condition	Steroids	PE	IVIG	Immunosuppressive drugs
WM	0	+	+/0	+
Osteosclerotic myeloma	0	0	?	+
Solitary plasmocytoma	0	?	?	radiotherapy/surgery
IgM MGUS	0	+/0	+/0	+
IgG/IgA MGUS	+	+	+	?

WM, Waldenström's macroglobulinemia; IgM, immunoglobulin M; MGUS, monoclonal gammopathy of undetermined significance; PE, plasma exchange; IVIG, intravenous immunoglobulin; 0, no effect; +, beneficial effect; +/0, controversial; ?, not known

velocity (MNCV) and segmental demyelination and IgM deposits on peripheral nerves, consistent with PDN, but the majority of these cases had an IgM MGUS with anti-MAG immunoreactivity (see below). Other types of neuropathy include multiple mononeuropathy and distal axonopathy[11]. However, the treatment of WM by immunosuppressive agents (chlorambucil, fludarabine) may improve the PDN[12], which may also benefit from plasma exchange (PE)[13] or IVIG, unless no controlled study has been conducted in such cases.

Osteolytic-osteosclerotic myelomas

Neuropathy occurs in 1.4–13% of all patients with multiple myeloma, but is less frequent in osteolytic than in osteosclerotic myeloma[14,15]. In osteolytic myeloma, the usual presentation of the neuropathy is a painful sensorimotor axonal polyneuropathy with a rapidly disabling course and a poor response to treatment. In a retrospective study of 66 patients with neuropathy and monoclonal dysglobulinemia, Vallat and colleagues[16] reported eight cases of osteolytic myeloma, of whom four had an axonopathy, only one had PDN and the three others had a vincristine-induced neuropathy without direct link to the paraproteinemia. Amyloid deposition is a complication in 30–40% of cases but is by no means universal.

Osteosclerotic myelomas constitute only 3% of all myelomas, but a sensorimotor neuropathy is associated in 20–50% of cases. Electrophysiological studies disclose a 'mixed' pattern with slowing of MNCV consistent with PDN, associated with distal fibrillations and decreased recruitment on needle electro-myography (EMG), indicating secondary axonal degeneration. The M-protein, which is usually IgG or IgA present at low concentration, is found in 90% of cases, and the light chain is virtually always of the A, subtype. A constellation of systemic features may surface, referred to as the POEMS (polyneuropathy, organomegaly, endocrinopathy, M-spike and skin changes), or Crow—

Fukase syndrome[17]. As in neuropathy associated with osteolytic myeloma, PE and IVIG are generally ineffective.

Solitary plasmocytoma

Polyneuropathy associated with solitary plasmocytoma is a rare but well-documented combination[18]. The neuropathy, i.e. PDN, and the monoclonal gammopathy are similar to those observed in osteosclerotic myeloma, and may therefore be associated with a POEMS syndrome. Plasmocytoma is usually found in the ribs, spinal column, pelvis and skull. Treatment of the plasmocytoma by either surgery or radiotherapy may dramatically improve the neuropathy.

Cryoglobulinemic neuropathy

Cryoglobulins are proteins (usually IgG or IgM) that precipitate when cooled, redissolve after warming and are deposited as immune complexes in blood vessels. The immunoglobulin may be monoclonal (type I), both monoclonal and polyclonal (mixed essential cryoglobulinemia, type II) or polyclonal (type III). The observation that a majority of so-called 'mixed essential cryoglobulinemias' are secondary to hepatitis C virus (HCV) infection has led to a review of the spectrum of neuropathy-associated cryoglobulinemia[19]. Approximatively one-third are extensive multifocal mononeuropathies associated with vasculitis in nerve biopsy, and other systemic manifestations resulting from a disseminated vasculitis: purpura, liver involvement, arthralgia, glomerulonephritis. They have been treated successfully by antiviral agents, frequently given in conjunction with prednisone[20]. The remaining two-thirds are chronic distal sensorimotor neuropathies without evidence of vasculitis, and which may benefit from PE. IVIG has not been tried in these neuropathies.

PARAPROTEINEMIC DEMYELINATING NEUROPATHY ASSOCIATED WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

PDN associated with IgM MGUS

PDNs associated with anti-MAG IgM monoclonal gammopathy are mainly chronic sensory neuropathies occuring in the sixth and seventh decades[21,22]. On clinical examination, vibration and joint position sensation are impaired in the distal lower limbs and later in the distal upper limbs. Generalized areflexia is a common feature. Motor deficit occurs later in the course of the disease and affects mainly the distal lower limbs. Ataxia is present in two-thirds of patients, and a tremor of the upper limbs in 30% of cases. The course of the neuropathy is usually slowly progressive. Electrophysiological studies allow polyneuropathies associated with IgM monoclonal gammopathy to be classified as length-dependent PDN, with disproportionate prolongation of distal motor latencies[23]. This neuropathy is probably due to an indolent demyelination process caused by antibodies to carbohydrate epitopes on MAG and sulfated glucuronyl

paragloboside[15,21]. It may be so mild that patients are best observed and not given treatment, all of which are fraught with the danger of side-effects[24].

Several immunosuppressors have been proposed in open studies. A French controlled study in 40 patients[25] showed that chlorambu-cil given orally during 1 year, at 0.1mg/kg/day, significantly improved the neurological disability score, and that 15 PEs given in one-half of patients did not bring any additional beneficial effect. Cyclophosphamide has been proposed in intravenous combination with PE[26], or orally in association with prednisone[27]. Corticosteroids are considered to be ineffective[28]. PE did not show significant beneficial effect in a randomized trial[29] in 17 patients with PDN and IgM paraprotein, compared with 20 patients with PDN and IgG or IgA paraproteins (see below).

IVIG was first proposed by Cook and colleagues in 1990[30], then in open studies[31,32]. There are only three randomized trials of IVIG in PDN associated with IgM MGUS. All used a cross-over design. In one, only three of 11 patients showed benefit, and the overall result was not significant[33]. Another IVIG trial included 22 patients, and there was significantly more improvement in disability 4 weeks after treatment with IVIG than after placebo[34]. In the third trial[35], interferon-α produced a significant improvement in 80% of patients with PDN and IgM MGUS compared with 20% of those treated with IVIG, but the beneficial effect of interferon-α was not confirmed in a recent double-blind trial versus placebo[36]. Finally, fludarabine[12] and mainly rituximab[37,38] seem to be the more promising treatments.

PDN associated with IgG/IgA MGUS

Polyneuropathies associated with IgG/IgA MGUS are heterogeneous, but PDNs are found in more than 50% of cases[39–41]. Few therapeutic studies have been conducted. A randomized trial[29] showed that PE significantly reduced weakness in 20 patients with polyneuropathy associated with IgG or IgA paraproteins in true compared with sham exchanges. Recently, IVIG therapy was shown to increase the mean strength score by 1.1 points (p=0.22) and the sensory score by 1.7 points (p=0.11) in 20 consecutive patients with IgG MGUS and PDN treated over an 8-year period[42]. In conclusion, PDN associated with IgG/IgA MGUS may benefit from IVIG, similar to chronic inflammatory demyelinating polyradiculoneuropathy.

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Immunoglobulins in IgM antimyelin-associated glycoprotein and other paraproteinemic neuropathies

G.Comi

Intravenous immunoglobulin (IVIG), initially used as replacement therapy in primary and secondary antibody deficiency syndromes, has recently acquired an important role in the treatment of some dysimmune pathologies, including diseases of the central and peripheral nervous systems[1]. Compared with other immunoacting treatments, IVIG is characterized by a good safety profile. The main risk consists of the transmission of infectious agents that can only be excluded if the manufacturing process is optimal.

IVIG displays a wide range of effects on the immune system, including: anti-idiotypic suppression, down-regulation of B- and T-cell activation, blockade of Fc receptors on phagocytic cells, neutralization of superantigen (a complement-mediated effect), down-regulation of cytokine production and neutralization of cytokines. Which of these mechanisms, alone or in variable combination, plays a major role in the treatment of chronic inflammatory demyelinating neuropathies is still a matter of debate.

NEUROPATHIES ASSOCIATED WITH MONOCLONAL GAMMOPATHY

The association between neuropathies and monoclonal gammopathies was demonstrated in the early 1970s, but the frequency of such an association has not yet been determined. Monoclonal gammopathy, predominantly immunoglobulin G (IgG), has a high prevalence in the elderly population, and it has been estimated that about one-third of these patients have a related neuropathy[2]. In a low percentage of cases gammopathy is associated with malignancy; in these cases treatment is principally directed to the underlying disease. Prevalence studies of consecutive patients with benign monoclonal gammopathy show figures ranging from 5 to 70%. In the majority of patients with neuropathy and associated monoclonal protein, no underlying disease is found (monoclonal gammopathy of undetermined significance, MGUS). Neuropathies associated with monoclonal gammopathies represent a very heterogeneous group, and many syndromes have been described[3–5]. In about half of the patients with neuropathy associated with monoclonal gammopathy, neurophysiological and pathological studies reveal a predominantly myelin involvement that is indistinguishable from classical chronic inflammatory demyelinating polyradiculoneuropathy (CIDP); most of these patients have an IgM gammopathy, and about two-thirds have an IgM M-protein reactivity against myelin-associated glycoprotein (MAG). In these patients the

neuropathy is predominantly sensory, with insidious onset and slow progression, with prominent gait ataxia. In the other half of patients axonal damage is predominant, and the M-protein can be IgG or IgM, less frequently IgA. Evidence is emerging to suggest that anti-MAG antibodies detected in patients with IgM gammopathy and demyelinating neuropathy may have a pathogenic role. The hypothesis that neuropathy associated with anti-MAG IgM is an autoimmune disease has supported the use of treatments targeted to the immune system. Many open trials with steroids, immunosuppressive agents and plasma exchange (PE), alone or in variable combination, have been conducted in recent years with widely variable results, so that no conclusion can be derived. The only exception is a double-blind study which demonstrated the efficacy of PE versus sham exchange[6]. There are very few data on the efficacy of intravenous immunoglobulin (IVIG) in MGUS neuropathy, and they have been derived from small, open, uncontrolled studies. We treated five patients with IgG or IgA gammopathy; of these, three patients showed clinical and neurophysiological improvement. Cook and colleagues[7] treated two patients with IgM gammopathy, both of whom had a steadily progressive course over 3 years despite steroids and immunosuppressive treatment. Both patients showed rapid clinical improvement 5–10 days after the first immunoglobulin infusion, lasting 3–6 weeks. Retreatment determined improvement after each consecutive infusion. A positive response to IVIG has also been reported by Simmons and colleagues[8] in patients with IgA gammopathy. Léger and associates[9] treated four patients with IgG and 13 patients with IgM gammopathy with monthly infusions for 6–24 months. Two of the patients with IgG and six of the patients with IgM gammopathy had a persistent clinical improvement. Interestingly, some of the responders had not previously responded to other immunosuppressive agents. Ellie and co- workers[10] treated 17 patients with IgM gammopathy and anti-MAG reactivity; a clearcut improvement was observed in 24% of the cases and a transient, mostly subjective, improvement in another 35%. In a placebo, cross-over controlled trial[11] only a minority of patients with IgM gammopathy responded to IVIG.

More recently[12] in a double-blind, crossover, controlled trial, 22 patients with IgM monoclonal gammopathy and demyelinating polyneuropathy were randomized to receive IVIG or placebo. After 2 weeks, the overall disability grade decreased during both IVIG treatment and placebo, but neither change was significant nor was the mean difference between the treatment effects (Table 1). After 4 weeks the overall disability decreased significantly during the IVIG period, while it was substantially unmodified during the placebo period (Table 2). The mean difference between the treatment effects was significant (p=0.05). Overall, during the IVIG period, ten patients improved, 11 were stable and one became worse. During the placebo period, four

Table 1 Secondary end-points at week 2. Values are expressed as mean (SD)

	IVIG			Placebo			
Assessment	Baseline	Week 2	*p* Value*	Baseline	Week 2	*p* Value*	*p* Value†
Rankin scale	2.5 (0.9)	2.1 (0.8)	0.007	2.2 (0.9)	2.4 (0.8)	NS	0.007
Rotterdam	28.7 (5.6)	29.4 (5.9)	NS	30.0	29.6	NS	NS

scale				(5.2)	(5.5)		
10m Walking time (s)	10.5 (4.2)	9.5 (4.0)	0.047	10.8 (4.8)	10.7 (4.7)	NS	NS
Nine-hole peg board (s)	37.9(15.2)	36.7(16.0)	NS	38.7 (14.7)	37.1 (15.1)	NS	NS
Hand grip	68.6 (26.5)	75.2 (25.2)	0.014	71.8 (25.1)	73.2 (26.9)	NS	NS
MRC	55.9 (4.5)	56.6 (4.3)	NS	56.1 (3.4)	56.6 (3.8)	NS	NS
Sensory symptoms score	6.9 (3.6)	5.9 (3.4)	0.055	6.2 (3.1)	5.8 (3.4)	NS	NS

**p* Values for intragroup changes; †p values for differences between treatments; NS, not significant; MRC, Medical Research Council; IVIG, intravenous immunoglobulin

Table 2 Secondary end-points at week 4. Values are expressed as mean (SD)

	IVIG			Placebo			
Assessment	Baseline	Week 2	*p* Value*	Baseline	Week 2	*p* Value*	*p* Value†
Rankin scale	2.5 (0.9)	2.1 (1.0)	0.049	2.2 (0.9)	2.4 (0.9)	NS	NS
Rotterdam scale	28.7 (5.6)	29.8 (5.5)	0.07	30.0 (5.2)	29.4 (5.3)	NS	NS
10m Walking time (s)	10.5 (4.2)	9.5 (4.3)	NS	10.8 (4.8)	9.7 (4.0)	0.03	NS
Nine-hole peg board (s)	37.9 (15.2)	36.7 (16.0)	NS	38.7 (14.7)	38.1 (15.4)	NS	NS
SF36	43.3 (26.5)	48.6 (25.0)	NS	46.4 (26.3)	42.8 (25.0)	NS	NS
Hand grip	68.6 (26.5)	77.2 (28.6)	0.014	71.8 (25.1)	71.0 (24.8)	NS	0.049
MRC	55.9 (4.5)	56.7 (3.9)	NS	56.1 (3.4)	56.4 (3.9)	NS	NS
Distal CMAP amplitude	9.3 (5.9)	10 (6.3)	NS	10.6 (7.0)	10.2 (6.3)	NS	NS
Proximal CMAP amplitude	7.3 (6.1)	7.7 (5.8)	NS	7.8 (5.9)	7.5 (6.1)	NS	NS
Motor conduction velocity	30.6 (9–9)	29–9 (10.7)	NS	30.6 (10.9)	30.5 (10.3)	NS	NS

Sensory symptoms score	6.9 (3.6)	6.1 (3.4)	NS	6.2 (3.1)	6.0 (3.5)	NS	NS
Sensory sum score	10.1 (6.3)	7.2 (5.6)	0.002	10.2 (6.3)	8.7 (5.5)	NS	NS

*p Values for intragroup changes; †p values for differences between treatments; NS, not significant; SF36, short-form 36; MRC, Medical Research Council; CMAP, compound muscle action potential amplitude; IVIG, intravenous immunoglobulin

patients improved, four deteriorated and 14 were stable. Many secondary outcome measures, including Rankin scale, time to walk 10m, grip strength and sensory symptoms score, were significantly better during IVIG treatment. These results suggest that some patients with IgM paraproteinemic demyelinating neuropathy may benefit from IVIG treatment; however, owing to the short duration of the trial, the impact of treatment on the natural course of the disease remains undetermined.

CONCLUSIONS

Some positive effects have been observed in patients with IgM gammopathy associated with demyelinating neuropathy. An improvement of strength and/or sensory deficits commences very soon after IVIG infusion, suggesting that changes cannot be explained by remyelination or nerve fiber regeneration. Removing factors that affect the propagation of nerve impulses is probably the best explanation for this early improvement. Factors such as NO and tumor necrosis factor-α are products of the inflammatory process which may induce conduction block by interfering with the sodium channels at the Ranvier node[13]. The mechanism by which IVIG exerts its immunomodulatory effect is not known. Considerable emerging evidence suggests multiple sites of action of IVIG in immune disorders, at the levels of both cellular and humoral responses. Future studies will define whether short-term positive effects may lead to a reduction of long-term disability.

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Intravenous immunoglobulin for myasthenia gravis

P.Gaidos

Myasthenia gravis is an autoimmune disease. In up to 90% of generalized cases, auto-antibodies to the nicotinic acetylcholine receptor which mediate the neuromuscular transmission disorders are detectable. The disease is characterized by weakness and fatigability of voluntary muscle changing over shorter or longer periods. Acute exacerbations are life-threatening because of swallowing difficulties or respiratory failure. Intravenous immunoglobulin (IVIG) was used as early as 1984 for the treatment of myasthenia gravis (MG). The improvement rate achieved by IVIG in MG calculated in two reviews of previously published uncontrolled studies was 73 and 76%, respectively[1,2]. However, the methodological qualities of these studies leave many unanswered questions:

(1) Is IVIG as efficient for the treatment of MG exacerbation as other treatments (i.e. plasma exchange, steroids, etc.)?
(2) Is IVIG efficient for the treatment of severe but stable MG?
(3) What is the optimal dose regimen of IVIG?
(4) What is the mechanism(s) of action of IVIG?

The clinical efficacy of IVIG for MG has been reviewed and published in The Cochrane Library[3].

INTRAVENOUS IMMUNOGLOBULIN FOR MYASTHENIA GRAVIS EXACERBATION

Intravenous immunoglobulin versus plasma exchange

A randomized controlled trial[4] which included 87 patients compared three plasma exchanges (PEs) (*n*=41) and IVIG 0.4g/kg/day for 3 days (*n*=23) or 5 days (*n*=23). The main change in the myasthenic muscular score (MMS) between day 0 and day 15 was not different between the two treatment groups: 15.6 (95% confidence interval (CI) 10.9–20.3) in the IVIG group versus 16.6 (95% CI 11.6–21.6) in the PE group (*p*=0.65). There was no difference in the mean change in MMS between the 3-day IVIG and the 5-day IVIG group. However, given the number of patients allocated to receive a 3-day or a 5-day IVIG treatment, this trial could not detect a difference in efficacy between the two dose groups.

A second randomized controlled trial (Gajdos, unpublished data) compared the efficacy of IVIG 1g/kg for 1 day versus 1g/kg/day for 2 days. One hundred and seventy-three patients were randomized. At day 15 after randomization, the mean MMS increased by 15.5 (95% CI 11.9–18.8) in the 1-g/kg IVIG group compared with 19–3 (95% CI 15.82–22.85) in the 2-g/kg IVIG group (p=0.11).

IVIG versus methylprednisolone

A randomized controlled trial compared IVIG 30g daily for 5 days and methylprednisolone (MP) 1–1.5mg/kg daily (V Schuchardt and colleagues, personal communication). Thirty-three patients were included. The mean (± SD) change between day 0 and day 14 in the two most affected criteria of the quantified myasthenia gravis score (QMGS) was 0.93±1.1 in the IVIG group and 1.35±1.17 in the MP group (p=0.30). However, this trial was underpowered in relation to the study objectives.

INTRAVENOUS IMMUNOGLOBULIN FOR SEVERE BUT STABLE MYASTHENIA GRAVIS

IVIG versus placebo

A randomized controlled trial[5] compared IVIG 1g/kg on days 1, 2 and 22 with placebo. Fifteen patients were included. The mean change in the QMGS from day 0 to day 42 was 0.0±3.8 in the IVIG group and −1.6±2.7 in the placebo group (p=0.53). However, this study was underpowered.

IVIG versus plasma exchange

A cross-over study[6] compared IVIG 0.4g/kg/day for 5 days with five PEs. There was no difference in the change in QMGS from baseline to 1 and 4 weeks after treatment.

IVIG as maintenance therapy

In one open study[7], ten patients unresponsive to steroids and immunosuppressive drugs were treated with IVIG 0.4g/kg/day for 5 days with maintenance IVIG treatment at 0.4g/kg once every 6 weeks. The mean severity of the disease decreased by 2.5±0.8 grades on the Osserman scale after 1 year (p<0.001). At the same time, prednisone dosage was reduced from 60 mg/day to 9.4/alternate day. However, it is difficult to deduce from this uncontrolled study whether there is indeed a sparing effect of IVIG for steroids.

CONCLUSIONS

(1) IVIG may be used for the treatment of myasthenia gravis (MG) exacerbation;
(2) A dose of IVIG of 1g/kg in 1 day could be proposed for the treatment of MG exacerbation;
(3) There is no evidence to determine whether IVIG improves functional outcome of severe but stable MG;
(4) A sparing effect of IVIG on steroid dosage is suggested, but a controlled study should be undertaken.

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Intravenous immunoglobulin in polymyositis and dermatomyositis

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INTRODUCTION

Polymyositis (PM), dermatomyositis (DM) and sporadic inclusion body myositis (s-IBM) are three distinct idiopathic inflammatory myopathies, characterized by a common histological endomysial inflammation and distinct immune-mediated mechanisms[1]. In PM and s-IBM, sensitized CD8+ cytotoxic T cells recognize as yet unidentified muscle antigens. The cytotoxic cells surround, invade and destroy non-necrotic muscle fibers that express class I major histocompatibility complex (MHC) molecules[1,2]. DM differs from the other two diseases clinically, because of the characteristic rash that accompanies or often precedes the muscle weakness, and immunopathologically, with the presence of an intramuscular microangiopathy, mediated by the complement C5b—C9 membranolytic attack complex, which leads to the destruction of endothelial cells, loss of capillaries, muscle ischemia, muscle fiber necrosis and perifascicular atrophy[1,3].

Despite their distinct characteristics, treatment of PM and DM is similar[4]. The practical goals of therapy in PM and DM are to improve function in the activities of daily living and improve muscle strength. Treatment must induce remission of the dysimmune state and minimize muscle fiber loss. Although when muscle strength improves, the serum creatine kinase concentration tends to fall concurrently, the reverse is not always true, because most immunosuppressive therapies can result in a decrease in serum muscle enzymes without necessarily improving muscle strength.

The treatment of myositis is still largely empirical, as there are only limited data from controlled clinical trials to allow an evidence-based approach. Corticosteroids remain the mainstay of treatment, eventually associated with an immunosuppressive agent in patients refractory or intolerant to corticosteroids. Azathioprine, methotrexate, cyclosporin and, for some authors, cyclophosphamide, fludarabine, mycophenolate mofetil and FK506 are used when immunosuppressants are needed.

However, in some patients, the myositis remains active despite optimal immunosuppressive therapy, and other therapeutic options need to be considered. Plasmapheresis is rarely effective. High-dose intravenous immunoglobulin (IVIG) is an immunomodulating agent that has multiple activities, including modulation of complement activation products, suppression of idiotypic antibodies, saturation of Fc receptors on macrophages and suppression of various inflammatory mediators including cytokines, chemokines and metalloproteinases. Because all these factors are implicated to

various degrees in the pathogenesis of immune-mediated myopathies, administration of IVIG has been used with benefits in some uncontrolled and in one controlled trial[5–23] in myositis. This chapter summarizes the place of IVIG in dermatomyositis and polymyositis.

IVIG IN DERMATOMYOSITIS

Dermatomyositis is a distinct disease that affects muscle and skin. Involvement of the muscle results in mild to severe myopathy; involvement of the skin causes a heliotropic rash on the face and knuckles and a flat red rash on the trunk, knees, neck and chest. Muscle biopsy can be definitive in establishing the diagnosis of DM, showing characteristic abnormalities. The earliest lesion is deposition of the complement C5b—C9 membranolytic attack complex (MAC) on the intramuscular capillaries. The inflammation is predominantly perivascular and in the interfascicular septae. Endothelial hyperplasia of the endomysial vessels is associated, with obliteration of and a marked reduction in the number of capillaries that leads to ischemia and muscle fiber necrosis in a wedge-like shape or at the periphery of the fascicle. Dilatation of the lumen of the remaining capillaries compensates for the ischemia. The resulting perifascicular atrophy is diagnostic for DM. Cytokines, especially tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, interferon-γ and transforming growth factor-β (TGF-β), and adhesion molecules participate in the trafficking of sensitized lymphocytes and macrophages, up-regulated by MHC class I antigen expression on the muscle fibers.

High-dose IVIG has been shown to be beneficial in a few open prospective studies of adult and/or juvenile DM, with short follow-up periods[8–17] and in one controlled cross-over study[20]. These studies have demonstrated not only clinical benefit, but also improvement in the muscle cytoarchitecture, down-regulation of cytokines or adhesion molecules, an effect on complement activation and MAC deposition, and improvement of the muscle microvasculature.

Dalakas and colleagues conducted a double-blind, placebo-controlled study of 15 adult patients with biopsy-proven, treatment-resistant DM[20]. The patients were randomly assigned to receive one infusion of immunoglobulin (2g/kg of body weight) or placebo per month for 3 months. Significant improvement in scores of muscle strength, neuromuscular symptoms and heliotropic rash was observed only in the IVIG group. The clinical benefit was correlated with an improvement in the muscle cytoarchitecture.

In this study, repeated biopsies in five patients of muscles whose strength improved to almost normal showed a significant increase in muscle fiber diameter, a significant increase in the number and a decrease in the diameter of capillaries, resolution of complement deposits on capillaries, reduction of serum levels of the SC5b—9 complex, depletion of C3b NEO (a neoantigen that is expressed on the surface of activated C3 component upon incorporation into immune complexes) in muscles, down-regulation of the *in situ* expression of TGF-β and TGF-β mRNA and a reduction in the expression of intercellular adhesion molecule-1 and MHC class I antigens[20,24,25]. IVIG reduced serum levels of soluble interleukin-2 receptor (sIL-2R)[18].

IVIG may be effective for intractable DM skin lesions and severe amyopathic DM[15,17].

IVIG IN POLYMYOSITIS

Polymyositis seems to be an antigen-directed cytotoxicity mediated by cytotoxic T cells. Close interaction between T cells and non-necrotic fibers has been demonstrated. CD8 + T cells surround non-necrotic muscle fibers, then eventually invade and destroy. Muscle fiber necrosis occurs by the exocytosis granule model, releasing toxic proteins, especially perforin and granzyme B[26]. These muscle fibers strongly express the MHC class I antigen. Restricted T-cell receptor-V gene usage by invading T cells suggests an antigen-specific reaction[27]. Like DM, a variety of chemotactic, inflammatory chemokines and adhesion molecules are involved in the pathogenic process.

In PM, no controlled studies have been performed. Doubt about diagnosis (especially with s-IBM) may cause difficulties of interpretation. However, IVIG has been shown to be effective as add-on therapy in several open studies in patients with PM or overlap syndromes[6,21,23]. We recently performed an open prospective study in 35 cases of severe active PM refractory to traditional treatments, to evaluate the long-term efficacy and safety of IVIG used as a third line. The patients received 1g/kg/day twice per month during 4–6 months[28].

Significant clinical improvement was noted in 25 of the 35 patients (71.5%) after four IVIG infusions. The 25 patients who responded favorably to IVIG treatment were followed for more than 3 years. After discontinuation of the IVIG therapy, the efficacy remains stable in 50% of patients with a follow-up of over 3 years. Twelve of these 25 patients remained in full remission following their initial course of IVIG: complete stoppage of medication (five patients) or low doses of steroids (seven patients). The condition of six patients remained improved and no other drugs were prescribed, but the patients remained dependent on IVIG infusions. Seven of the 25 patients who responded well to IVIG treatment relapsed, an average of 17.1 (standard deviation 7.2) months after discontinuation of IVIG[28].

IVIG seems to be highly interesting in esophageal disorders of chronic refractory myositis[29].

IVIG is not effective in all cases. It is relevant that IVIG alone was not found to be effective when used as initial treatment in an open study of 11 patients with PM30. IVIG may therefore be more effective in patients who have already had or are on concurrent corticosteroids or other immunosuppressive therapy.

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Controlled studies with high-dose intravenous immunoglobulin in the treatment of inclusion body myositis

M.C.Dalakas

INTRODUCTION AND JUSTIFICATION FOR USING INTRAVENOUS IMMUNOGLOBULIN IN INCLUSION BODY MYOSITIS

The inflammatory myopathies are divided into three major and distinct subsets: polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM). This distinction is based on rather unique clinical, laboratory, prognostic, therapeutic, demographic, histological and immunopathological criteria that have been previously addressed[1–7]. Among these disorders, IBM is the most common acquired inflammatory myopathy, especially in patients above the age of 50 years[1–6]. The condition presents with selective atrophy of forearm flexor muscles, frequent falls, atrophy of the quadricep muscles and dysphagia.

The histological hallmarks of IBM are:

(1) Basophilic granular inclusions distributed around the edge of slit-like vacuoles (rimmed vacuoles);
(2) Angulated or round fibers, often in small groups;
(3) Eosinophilic cytoplasmic inclusions;
(4) Prominent endomysial inflammation in a pattern identical to that seen in PM;
(5) Congo red- or crystal violet-positive amyloid deposits next to the vacuoles[1–7].

In spite of the degenerative features consisting of the vacuoles and amyloid deposits, the fundamental process in IBM is the presence of endomysial inflammation, which is specific and independent of the degenerative features because the fibers invaded by lymphocytes are never vacuolated and the vacuolated fibers are never surrounded by inflammatory cells. Furthermore, the inflammation is not secondary, but primary, based on the following:

(1) Rearrangement of the T cell-receptor profile of the autoinvasive T cells[8];
(2) Association with specific human leukocyte antigen (HLA) alleles and autoantibodies[9,10];
(3) Specifically up-regulated cytokines and chemokines[11,12];

(4) The up-regulation of costimulatory molecules BB1 and inducible co-stimulatory molecule (ICOS) as well as their counter-receptors CTLA-4/CD28 and ICOS-Iigand[13,14].

In spite of the above immunopathological findings, and in contrast to PM and DM, patients with IBM respond poorly to steroids, methotrexate, cyclosporin and azathioprine. The need for an effective therapy prompted us to use intravenous immunoglobulin (IVIG) based on our previous experience with this drug, and the impressive results we observed in a controlled study conducted in patients with DM15.

METHODS AND RESULTS

In a first, open, pilot trial that we conducted, IVIG seemed to be helpful in some patients with IBM because the strength improved in some muscle groups after treatment[16]. Although the improvement was not dramatic, it made a difference to the patients' life-styles. However, another uncontrolled series of patients collected from 2–3 institutions[17], showed no benefit, prompting us to perform a controlled, double-blind study[18].

In the first controlled study, we enrolled 19 patients with IBM. Efficacy was assessed by quantitative muscle strength testing and quantification of swallowing function, which is so commonly affected in IBM patients. The study, a 3-month, randomized, cross-over trial, was designed similarly to the one we reported for DM[15]. No statistically significant differences were noted in the strength of the limb muscles between placebo and IVIG. Whether the small sample size might have been a factor is unknown. However, significant regional differences were observed in the IVIG-randomized patients, especially in the muscles of swallowing measured objectively by an ultrasound technique[18] (Table 1). The non-significant effect in the limb muscles, compared with the swallowing muscles, may be due to the more precise, objective and reproducible measurement of the swallowing function. Because the test—test variability with the ultrasound technique that measures the duration of swallowing in seconds is minimal, this technique allows us to capture even minor improvements.

Although in the limb muscles the study did not overall establish efficacy of IVIG, six of 19 IBM patients (31%) showed a mild improvement in muscle strength (Figure 1a), which was functionally important for their daily activities. Because, in our experience, IBM patients do not respond to therapies and the disease is incapacitating, even a minor improvement may have a positive impact on some of the patients' daily activities. On this basis, we conducted a larger study combining, this time, IVIG with prednisone. This was a double-blind, placebocontrolled, study involving 33 patients, 17 of whom were randomized to IVIG plus high-dose steroids and 16 to placebo plus high-dose steroids[19]. Changes in muscle strength were accessed using quantitative muscle strength testing and the MRC scale. After 3 months of treatment, there was no clear benefit in any of the two groups. Minor gains in strength were noted in the IVIG-randomized patients, but were not significant (Figure 2). Of interest, subjective improvements were noted in ten of the 17 patients randomized to IVIG compared with one of 16 randomized to placebo. The repeated muscle biopsies showed a reduction in T-cell count in both groups, probably related to

Table 1 Mean duration (s) of ultrasound swallows at baseline, 3 months and end of cross-over in 19 patients with inclusion body myositis

Patients	Type of swallow	Baseline	3 months	Cross-over
			Placebo	*IVIG*
Randomized to placebo (*n*=10)*	D1	2.33	1.83	1.91
	D2	2.15	1.75	1.88
	D3	1.84**	2.84**	1.83**
	W1	1.59**	2.25**	1.86**
	W2	2.49	1.96	2.00
	W3	1.59**	2.07**	2.44**
			Placebo	*IVIG*
Randomized to IVIG (*n*=9)*	D1	3.00**	1.62**	1.47
	D2	3.37**	2.02**	2.31
	D3	2.35	2.74**	1.85**
	W1	1.82	1.86	1.47
	W2	1.73**	1.24**	1.54
	W3	1.98**	1.49**	1.48

*p=0.05; **p=0.05

steroids. No changes in the muscle cytoarchitecture, vacuoles, amyloid deposits or adhesion molecules (neural cell adhesion molecule, NCAM) were noted. The transforming growth factor-β (TGF-β) in the muscle remained unchanged at both the protein and the mRNA level[20]. This is in contrast to the TGF-β in DM patients, which was down-regulated, and correlated with clinical improvement[20]. This second study also failed to show efficacy of IVIG. A third study, conducted by Walter and colleagues, demonstrated similar results[21]. Other observations based on open-label trials have recently shown that IVIG has a dramatic effect on swallowing function, a very disabling and life-threatening symptom in IBM patients[22].

DISCUSSION

In spite of these negative trials, it is our clinical impression that a few patients may show transient signs of improvement which are minor and difficult to capture with the methods used, but can be clinically significant for the patients' activities and life-styles, at least for a period of time. Whether such a mild improvement in a small number of IBM patients justifies a 2–3-month trial with IVIG remains a matter of clinical judgement and should be viewed on a case-by-case basis. Safety, age, economics and

Figure 1 Placebo-controlled study with intravenous immunoglobulin (IVIG) in inclusion body myositis. Difference in scores at baseline and 3 months after treatment with placebo or IVIG

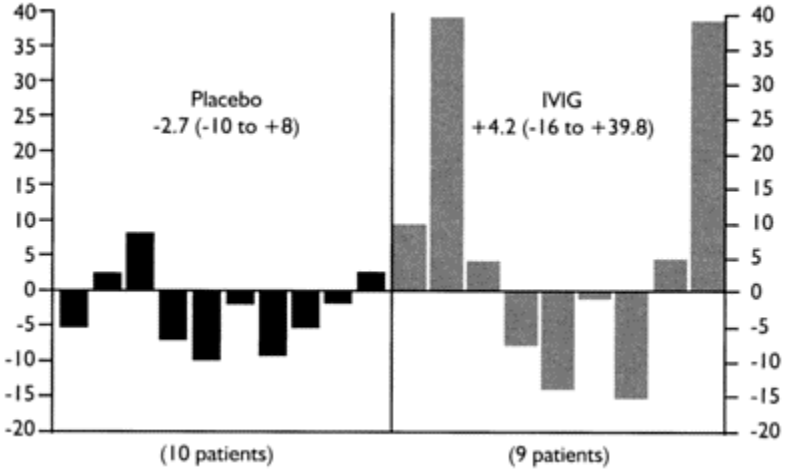
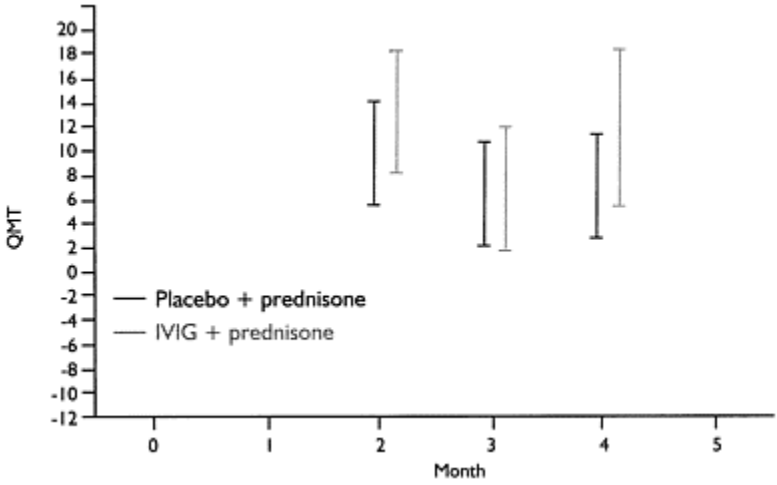


Figure 2 Placebo-controlled study with intravenous immunoglobulin (IVIG) and prednisone in inclusion body myositis. Mean per cent change from 1 month in quantitative muscle testing (QMT) of the lower extremity



the reminder that nothing else (including steroids) offers even minor clinical improvement to IBM patients should be taken into account. The impressive results of the swallowing function in open trials, supported by our double-blinded study, justifies a trial of IVIG in patients whose dysphagia appears to be life-threatening.

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Intravenous immunoglobulin for autoimmune diseases of the central nervous system

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Immune mechanism and treatment perspectives in multiple sclerosis

R.Hohlfeld

IMMUNE MECHANISMS IN RELATION TO THERAPY

In multiple sclerosis (MS) as in other diseases, rational treatment depends on a thorough understanding of the etiology and pathogenesis. Research into the pathogenesis of MS and especially the rapidly growing number of different animal models are beginning to reveal a remarkable heterogeneity and complexity of the pathogenic mechanisms of inflammatory demyelinating central nervous system (CNS) disease[1–3]. In view of these developments, it seems likely that much more refined classifications can be developed in the future for the disease we today call MS. Clearly, this will improve the chances for a more differentiated therapeutic approach.

Although transfer experiments demonstrate that autoreactive T cells are critically important in the immunopathogenesis of experimental autoimmune encephalomyelitis (EAE) (and by analogy, probably also MS), it is becoming increasingly clear that B cells and their prod ucts, antibodies, are equally important, especially for demyelination. Assays using the recombinant extracellular domain of the myelin oligodendrocyte glycoprotein (MOG) allow the detection of anti-MOG autoantibodies in serum by Western blotting and enzyme-linked immunosorbent assay (ELISA). Although these antibodies are clearly not specific for MS[4], they seem to be important predictors of the clinical course[5].

Common sense and clinical judgement would suggest that the earlier immunotherapy is initiated, the better the chances are for preventing deficit. Arguments against very early treatment include the high cost of long-term therapy and adverse reactions, which could diminish or abolish therapeutic effectiveness at a later stage when therapy is urgently needed (e.g. development of neutralizing antibodies to interferon-β). At the other end of the clinical spectrum, patients with severe impairment have an increased risk for various adverse reactions to immunotherapy. Furthermore, immunotherapy cannot be expected to reverse a pre-existing, stable chronic deficit. Therefore, patients with advanced disease are bad candidates for immunotherapy, and aggressive immunosuppressive treatments are usually contraindicated.

It seems logical to consider combining different immunotherapies that work via different mechanisms. Obviously, the difficulty is to select the right agents to combine, as well as the right type of MS. An important potential problem is that the different immune mechanisms targeted by different agents may be interdependent, so that one agent

depends on the intactness of mechanisms inhibited by another agent. Clinical trials need to look carefully for such adverse interactions, which are difficult to predict.

TREATMENT PERSPECTIVES

Interferons

Interferons were initially tried in MS for their antiviral effect. A pilot trial of systemic recombinant interferon-γ resulted in a sharp increase of exacerbations. In contrast, interferon-β turned out to have a beneficial effect, which has meanwhile been corroborated in several large controlled trials.

Numerous immunomodulatory effects of type I interferons have been described[6]. For example, interferon-β has been shown to up-regulate interleukin-10 (IL-10) expression and secretion by T cells and monocytes, indicating that some of the clinical effects of interferon-β are in fact mediated by IL-10. Both interferon-β1a and interferon-β1b induce the production of IL-10 in myelin basic protein (MBP)-specific CD4+ T-cell lines, but inhibit proliferation and production of lymphotoxin. Furthermore, interferon-β inhibits T-cell migration across basement membrane *in vitro*, presumably by decreasing the secretion of matrix-degrading enzymes. This interferon (IFN)-mediated inhibitory effect on the secretion of matrix metalloproteinases, as well as other consequences of IFN cellular responses, may be pertinent for suppressing inflammation (i.e. reduced numbers of enhancing magnetic resonance imaging (MRI) lesions and lessened cerebrospinal fluid (CSF) pleocytosis) observed in IFN-treated MS patients. Potential mechanisms include an increase in soluble vascular cell adhesion molecule-1 (VCAM-1) and concomitant down-regulation of the corresponding partner adhesion molecule very late antigen (VLA)-4 on peripheral blood lymphocytes. Increased soluble VCAM-1 and decreased cellular VLA-4 would both tend to block leukocyte—endothelial adhesion at the blood—brain barrier. Leukocytes also require stimulation by chemoattractants, called chemokines, during extravasation, and several chemokines are increased in the CSF of patients with relapses of MS. Interestingly, IFNs up-regulate the expression of chemokines by numerous cell types, including hematopoietic cells. Elevated levels of chemokines in the circulation would tend to reduce transvascular chemokine gradients favoring entry of cells into the CNS.

Myriad other effects of interferon-β have been described[6,7]. The relative importance and mutual interdependence of this bewildering variety of actions is not well understood, so that currently the mechanisms of interferon-β treatment of MS remain unknown.

Copaxone

Copaxone (copolymer-1, Cop-1, glatiramer acetate (GA)) is a synthetic basic random copolymer of L-alanine, L-glutamic acid, L-lysine and L-tyrosine in a molar residue ratio of 6.1:1.9:4.7:1.0. It was originally studied along with other basic copolymers in an attempt to simulate the activity of MBP in inducing EAE, but was then found to suppress EAE in various species including guinea pig, rabbit, mouse, rhesus monkey and baboon (reviewed in reference 8). Different encephalitogenic determinants of MBP are involved

in the different species. Furthermore, there are some indications that the suppressive effect of copolymer-1 is not restricted to MBP, but extends to EAE induced in mice with encephalitogenic proteolipid protein (PLP) or MOG peptides. On the other hand, copolymer-1 had no effect on experimental myasthenia gravis and experimental thyroiditis, indicating some specificity for myelin-induced autoimmunity[8].

It seems unlikely that a single mechanism can explain all these observations. It has been proposed that copolymer-1 competes with MBP and, perhaps, other myelin autoantigens for binding to major histocompatibility complex (MHC) class II molecules expressed on antigen-presenting cells (APCs)[8]. However, it is difficult to understand how Cop-1 can compete in MS patients with myelin antigens for MHC binding at the subcutaneous injection site. The following effects of Cop-1 on human T cells have been described (reviewed in reference 9):

(1) Cop-1 binds 'promiscuously' to MHC class II and perhaps MHC class I molecules, thereby competing with the MHC binding of other antigens. This effect, which by its nature is antigen-non-specific, is unlikely to play a role *in vivo*, since after subcutaneous administration, Cop-1 is quickly degraded to free amino acids and small oligopeptides, and thus it is not likely to reach the CNS where it could compete with the relevant autoantigens for MHC binding.

(2) Cop/MHC competes with MBP for binding to the antigen-specific surface receptor of MBP-specific T cells ('T-cell receptor antagonism'). The experimental evidence supporting this effect is controversial. If it occurs, it is unlikely to be relevant *in vivo*, since GA is unlikely to reach sites where it could compete with MBP.

(3) Cop/MHC binds to the T-cell receptor of T cells specific for MBP and, perhaps, other myelin antigens. In this view, Cop-1 acts like an 'altered peptide ligand' (APL) relative to MBP As a consequence, some of the myelin-specific, pathogenic T cells might become 'anergic' or be otherwise changed in their properties, e.g. in their migratory potential. This effect would be relatively antigen-specific and presumably occur in the periphery at the injection sites or in their draining lymph nodes where the MBP-specific T cells might be confronted with Cop-1. Although some *in vitro* findings support this mechanism, it is not yet known whether the functional properties of MBP-specific T cells are altered in Cop-treated patients. It may be of relevance in this connection that we were unable to isolate MBP-specific T-cell receptor from Cop-treated patients[9].

(4) Cop-1 treatment induces a T helper cell Th1-to-Th2 shift in Cop-1-reactive T cells *in vivo*. The Cop-reactive T cells act as regulatory cells and have beneficial effects on the pathogenic autoimmune reaction[10]. Compared with the other putative mechanisms, this currently has the strongest experimental support. We would like to propose the following scenario. Cop-reactive Th2-like T cells are able to cross the blood-brain barrier, since they are activated by daily immunization. During treatment, the properties of the Cop-reactive T cells are changed in such a way that they become increasingly Th2-like[10]. Inside the CNS, the Cop-reactive T cells are confronted with products of myelin turnover presented by local APCs. Some of the GA-reactive cells cross-react with MBP or MOG, and are therefore stimulated to release anti-inflammatory cytokines such as IL-4, IL-6 and IL-10 and even neurotrophic factors[11–13]. Subsequently, the production of proinflammatory cytokines such as IL-2 and IFN-γ by other inflammatory cells is reduced via a suppressive bystander effect.

Hypothetically, a similar process might occur in the periphery, if the Cop-reactive T cells are stimulated by a foreign (viral or bacterial) antigen cross-reacting with GA or MBP by molecular mimicry.

Other imunosuppressive and immunomodulatory agents

Apart from interferon-β and Cop-1, many other agents for the immunosuppressive or immunomodulatory treatment of MS have previously been tested or are currently being tried. Three double-blind, randomized, placebo-controlled trials of intravenous immunoglobulin have been performed in MS, all in patients with a relapsing-remitting course[14–16]. Collectively, results from these trials have provided the basis for a consensus recommendation to consider intravenous immunoglobulin as a second- or third-line immunomodulatory treatment in MS[17]. The mechanism of action of intravenous immunogloblin in MS seems to be mediated via

Table 1 Conventional immunomodulatory and immunosuppressive treatments. For most of the listed treatments, there are currently insufficient data to judge their usefulness for multiple sclerosis (MS) therapy definitely

Azathioprine
Bone marrow and stem cell transplantation
Cladribine
Corticosteroids
Cyclophosphamide
Cyclosporin-A
15-Deoxyspergualin
Intravenous immunoglobulin
Irradiation; total lymphoid, low-dose total-body
Linomide*
Metalloprotease inhibitors
Methotrexate
Mitoxantrone
Mycophenolate mofetil
Pentoxyfilline
Phosphodiesterase (PDE) IV inhibitors
Plasma exchange
Psoralen UV-A irradiation (PUVA)
Sirolimus (rapamycin)
Sulfasalazine
Tacrolimus (FK506)

*Treatment shown to be ineffective or unfavorable in MS, or had unacceptable toxic effects

Table 2 Biotechnological agents and experimental approaches for the immunomodulatory therapy of multiple sclerosis (MS)

Immunosuppressive agents
Anti-CD3 mAbs
Anti-CD4 mAbs
Anti-CD52 mAb (Campath-1H)
Anti-interleukin-2 receptor α-subunit mAbs (e.g. daclizumab, basiliximab)
Cytokines and cytokine inhibitors
Interferons
interferon-β1a
interferon- β1b
interferon-α
other interferons
Tumor necrosis factor (TNF) inhibitors
TNF-receptor-IgG soluble dimeric p-55 (Lenercept®)*
anti-TNF human/murine chimeric mAb cA2*
metalloprotease inhibitors (e.g. BB-3644)
Down-regulatory cytokines
interleukin-1 inhibitors
interleukin-4
interleukin-10
interleukin-13
TGF-β2 (BetaKine®)*
Chemokine antagonists and receptor blockers
neurotactin antagonist
MCP-1 receptor antagonist
CXCR3 receptor antagonist
CCR1 receptor antagonist
CCR5 receptor antagonist

Table 2

Therapies directed at cell interaction molecules
Adhesion molecules
humanized anti-CD11/CD18 mAb (Hu23F2G)
antagonistic peptide inhibitors of integrins
anti-VLA-4 and anti-α4 integrin mAbs or peptide inhibitors
anti-ICAM-1 (CD54) mAb
Co-stimulatory molecules
anti-CD2 mAb
anti-LFA-3 (CD58) mAb

anti-CD154 mAb
CTLA4-Ig
anti-CD45 mAb
Immunotherapies targeting the 'trimolecular complex'
Copolymer-1
MHC blockers
Alterered peptide ligands (so far mostly MBP analogs)
Oral tolerance (oral bovine myelin)*
Other strategies of tolerance induction by modification of antigen presentation
Vaccination with T cells or TCR peptides
Anti-TCR mAbs
Agents affecting both the immune and nervous systems
e.g. Neurotrophic factors

Food and Drug Administration (FDA)-approved agents are printed in bold face; *treatments shown to be ineffective or unfavorable, or had unacceptable toxic effects: most of the remaining treatments are considered experimental; mAbs, monoclonal antibodies; IgG, immunoglobulin G; TGF, transforming growth factor; VLA, very late antigen; ICAM, intercellular adhesion molecule; MHC, major histocompatibility complex; MBP, myelin basic protein; TCR, T-cell receptor

its immunomodulatory rather than remyelination-inducing effects[18]. Overviews of additional therapies may be found in references 1 and 19 (Tables 1 and 2).

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A review of of intravenous immunoglobulin in multiple sclerosis

P.S.Sorensen

INTRODUCTION

Intravenous immunoglobulin (IVIG) has a number of potential effects that may be beneficial in multiple sclerosis (MS): neutralization of circulating autoantibodies against myelin proteins, down-regulation of proinflammatory cytokines, functional blockade of Fc receptors on macrophages, suppression of inducer T cells and B cells, inhibition of complement activation and, in animal models, promotion of remyelination[1,2].

Hence, IVIG therapy has been applied in controlled trials in patients in different phases of MS.

CLINICAL TRIALS

Relapsing-remitting MS

Four randomized double-blind studies have been performed in relapsing—remitting MS, and all showed a beneficial effect[3–6]. The studies used different designs, different doses of IVIG and different trial durations, and had different primary efficacy end-points (Table 1).

The largest trial was performed by Fazekas and colleagues[3], who randomized 150 patients to treatment with either IVIG 0.15–0.20g/kg/month or placebo for a period of 2 years. Patients treated with IVIG had a significant reduction in the primary efficacy end-point, change in Expanded Disability Status Scale (EDSS), but the most remarkable result was a reduction in the mean annual relapse rate from a pretreatment rate of 1.30 to 0.52 during IVIG, while the mean annual relapse rate in the placebo group only fell from a pretreatment rate of 1.41 to 1.26 during placebo treatment. The difference between IVIG and placebo was a 59% reduction in the relapse rate (p=0.004). Also, the proportion of relapse-free patients was significantly higher in the IVIG group (p=0.03). With this low dose of IVIG, adverse effects were infrequent and mild. Magnetic resonance imaging (MRI) scans were not obtained in this study.

Table 1 Study point, patient characteristis, intranenous immunoglobulin (IVIG) doses, trial duration and primary endpoint of randomized trials of IVIG in relapsing-remitting multiple sclerosis

Study	Design	*n*	Age (year)	Duration of MS (yreas)	EDSS	IVIG monthly (g/kg)	Trial Duration (months)	Primary end-point
Fazekas *et al.*[3]	PG	150	150	37	3.3	0.2	24	EDSS changes
Achiron *et al.*[4]	PG	40	35	7	2.9	0.2	24	relapse rate
Sorensen *et al.*[5]	DG	26	35	5	3.5	2.0	6×2	MRI lesion
Lewanska *et al.*[6]	PG	49	38	8.5	3.0	0.2 0.4	12	relapse rate

PG, parellal groups; DC, double cross over; EDSS, Expanded Disability Status Scale; MRI, magnetic imaging

In a study of 40 patients, Achiron and associates[4] randomly assigned 40 patients with relapsing—remitting MS to treatment with either IVIG at a loading dose of 0.4g/kg/day for 5 days followed by 0.4g/kg every 2 months, or placebo, for 2 years. They observed a significant reduction of relapse rate in the IVIG group from 1.50 in the pretreatment period to 0.75 in the first year and 0.52 in the second year ($p<0.05$). In the placebo group, the annual relapse was 1.55 in the pretreatment period, 1.80 in the first year and 1.42 in the second year. The reduction of relapse rate in the IVIG group for the 2-year study period was 63%, compared with placebo ($p<0.01$). No significant changes were found in EDSS or MRI measured semiquantitatively on T2-weighted images.

Sorensen and colleagues[5] performed a double-blind cross-over study in 26 patients. Half of the patients were treated with IVIG 2g/kg/month for 6 months and, after a 3-month wash-out period, they were treated with placebo for 6 months. The other half of the patients were treated in the reverse order. MRI was performed at monthly intervals. The total number of gadolinium-enhancing lesions per patient per month was 1.3 in the IVIG group compared with 2.9 in the placebo group ($p=0.03$), equivalent to a reduction of 60% in IVIG-treated patients compared with placebotreated patients ($p<0.05$). The median percentage change in T2 lesion load was −2.6 during IVIG treatment and −0.4 during placebo ($p<0.27$). A trend towards a reduction in the number of relapses was observed during IVIG treatment (11 relapses) compared with placebo treatment (19 relapses) ($p=0.13$), and significantly more IVIG-treated patients were exacerbation-free compared with placebo-treated patients ($p=0.02$). Unfortunately, this high dose of IVIG was associated with a high number of adverse events, including one lethal pulmonary embolus, one case of hepatitis C and severe eczema primarily on the hands and feet in 11 patients.

Lewanska and co-workers[6] conducted a small-dose comparison study of IVIG 0.2g/kg ($n=17$), IVIG 0.4g/kg ($n=15$) and placebo ($n=17$), given at monthly intervals for 12 months in 49 patients. The annual relapse rate was significantly lower in both IVIG groups, 0.88 for 0.2g/kg and 0.6 for 0.4g/kg, compared with 1.24 in the placebo group ($p<0.05$). The mean EDSS score decreased by 0.03 in the 0.2-g/kg IVIG group and 0.07 in the 0.4-g/kg IVIG group, compared with an increase of 0.29 in the placebo group ($p=0.01$). Also, the mean number of gadolinium-enhancing lesions and the change in T2 lesion volume were reduced in both IVIG groups compared with placebo. There were no

significant differences between the effects of the two IVIG doses in any of the chosen end-points.

All four studies showed beneficial effects on various outcome measures, but the studies were relatively small, and, hence, a meta-analysis was performed to provide an overall assessment of the benefits of IVIG[7]. The meta-analysis showed significant positive effects of IVIG on the annual relapse rate (effect size −0.5; p=0.00003) (Table 2) and on the proportion of relapse-free patients (0.29 difference; $p=2.1\times10^{-8}$). The meta-analysis also showed a favorable effect of IVIG on disease progression, expressed as the change in EDSS score (effect size −0.25; p=0.04), and a reduction in the proportion of patients who deteriorated (p=0.03) (Figure 1).

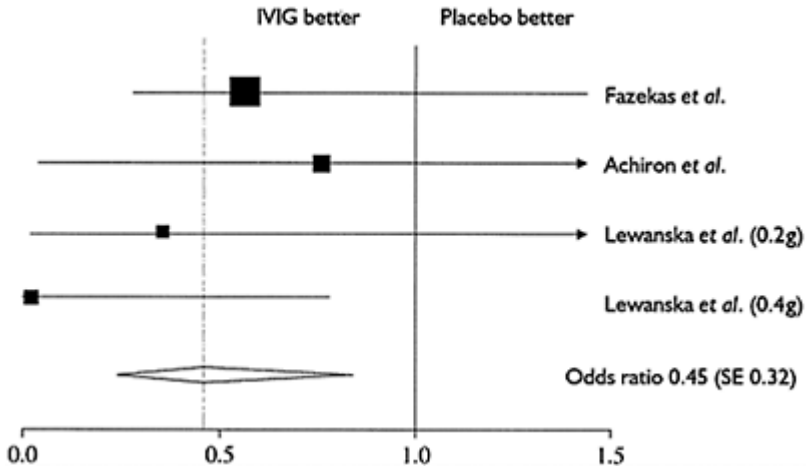
Very recently, Achiron and colleagues concluded a placebo-controlled trial of IVIG in 91 patients with clinical isolated syndromes. Significantly fewer patients in the IVIG group

Table 2 Annual relapse rate (mean±SD) in randomized trials of intravenous immunoglobulin (IVIG) in relapsing-remitting multiple sclerosis[7]

Study	IVIG	Placebo	Effect size*	Weight†
Fazekas *et al.*[3]	0.52±0.87	1.26±2.2	−0.44	0.51
Achiron *et al.*[4]	0.59±0.67	1.61±0.98	−1.22	0.12
Sorensen *et al.*[5‡]	1.04±1.74	1.80±3.14	−0.30	0.15
Lewanska *et al.*[6]				
0.2g/kg	0.88±1.26	1.24±0.75	−0.35	0.12
0.4g/kg	0.87±0.99	1.24±0.75	−0.43	0.11
Overall effect size* (95% confidence interval):−0.5 (−0.73 to −0.27), p=0.00003				

*Effect size: IVIG—placebo/SD; †proportion of reciprocal of total variation attributable to given study; ‡extrapolated from 6-month data

Figure 1 Odds ratios (intravenous immunoglobulin (IVIG)/placebo) for deterioration in Expanded Disability Status Scale (EDSS). Trial results and 95% confidence intervals are shown. Area of square symbol is proportional to amount of information contributed. Diamond shape indicates overview of results and 95% confidence limits. From reference 7



converted to clinical definite MS (Achiron, personal communication).

Acute relapses

Sorensen and associates[8] treated 76 patients, who had suffered an acute relapse with onset of symptoms between 24h and 14 days before, with either IVIG 1g/kg or placebo, 24h prior to treatment with intravenous methylprednisolone 1g on three consecutive days. Both groups improved and no significant difference between IVIG and placebo was found, but a trend towards more improvement in the IVIG group was observed.

Chronic deficits in MS

Noseworthy and co-workers[9] studied the effect of IVIG on persistent loss of visual acuity after optic neuritis. Fifty-five patients were treated with either IVIG 0.4g/kg daily for 5 days followed by 0.4g/kg every 4 weeks for 3 months, or placebo. No difference in improvement in visual acuity was found at 6 months, but a positive trend favoring IVIG was found at 12 months (p=0.132). IVIG did not improve visual fields or visual evoked potentials.

Two trials have been performed in MS patients with stable motor deficits. Noseworthy and co-workers[10] studied 67 patients with persistent muscle weakness that had been stable for 4–18 months. The patients received either IVIG 0.4g/kg daily for 5 days followed by 0.4g/kg every 2 weeks for 3 months, or placebo. No difference in the degree of change in strength in the targeted weak muscles was found between the two treatment groups.

In a small placebo-controlled cross-over study, Stangel and associates[11] treated ten patients with stable motor deficits with placebo, and 6 weeks later with IVIG 0.4g/kg daily on five consecutive days. No change in the primary outcome measure, the central motor conduction time, was found 6 weeks after each treatment, but a trend towards an improvement in the neurological rating scale was seen after IVIG compared with placebo.

Secondary progressive MS

In secondary progressive MS, IVIG was assessed in a large placebo-controlled trial (European Study of Intravenous Immunoglobulin in Multiple Sclerosis (ESIMS)) of 318 patients who were treated with either IVIG 1g/kg or placebo every 4 weeks for 26 months[12]. No significant difference between IVIG and placebo was found in the primary outcome measure, time to sustained deterioration of one point in EDSS, or in other clinical outcomes. The only secondary outcome measure that showed a beneficial effect of IVIG was less reduction in brain atrophy in the IVIG-treated patients, compared with placebo.

Ongoing trials

A dose-finding study in 120 relapsing-remitting MS patients is currently being conducted comparing monthly infusion of IVIG 0.2g/kg or 0.4g/kg with placebo in a 1-year trial, with relapse-free patient as the primary outcome measure.

The first combination study of IVIG and interferon-β (a double-blind, randomized, placebo-controlled trial of intravenous immunoglobulin as add-on therapy to interferon-β for the treatment of relapsing—remitting multiple sclerosis (IVIMS)) has recently been launched. IVIG 0.2g/kg or placebo as add-on therapy to interferon-β (Avonex®) will be given monthly for 2 years to patients with breakthrough disease on interferon-β. The primary outcome measure is the annual relapse rate, and secondary end-points include other clinical and MRI variables.

CONCLUSIONS

Randomized studies in relapsing—remitting MS and clinical isolated syndromes have shown concordant evidence of a beneficial effect of IVIG on relapses, disability and MRI changes. It is difficult to compare the effect of IVIG with the effect of the approved therapies, interferon-β and glatiramer acetate, but the results of a meta-analysis indicate that the effect of IVIG on the relapse rate is equivalent to that of the approved therapies[7]. The results of the ESIMS trial show that IVIG has no clinical effect in the secondary progressive phase of MS. IVIG can be considered a valuable second-line alternative to the established therapies in relapsing—remitting MS, and ongoing studies will hopefully determine the optimal dose of IVIG.

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Intravenous immunoglobulin treatment for patients with primary or secondary progressive multiple sclerosis

D.Pöhlau and U.Kallweit

INTRODUCTION

In the treatment of patients with secondary progressive multiple sclerosis (MS) interferon-β1b had a significant effect on progression of the expanded disability status scale (EDSS)[1]. This result could neither be confirmed in another study with the same drug, conducted in slightly older and more disabled American MS patients, nor in a study with interferon-β1a. Some authors conclude, that prescribing interferon-β should be limited to those patients who have had disabling relapses in the past 2 years (reviewed in reference 2). Mitoxantrone was effective in secondary progressive MS, but the use may be limited by cardiotoxicity[3].

In the case of the treatment of primary progressive MS no treatment to date has proved efficacious. Therefore, further treatment options for the group of patients with chronic progressive MS need to be tested.

OBJECTIVE AND DESIGN

The objective of this prospective, randomized, double-blind, placebo-controlled multicenter trial was to demonstrate both the safety and efficacy of IVIG treatment in patients with progressive MS. Two groups of patients were stratified and included, one with primary progressive MS and the other with secondary progressive MS. Within each group, patients were randomized and treated with IVIG or with placebo. The study was approved by the ethics committee of the Ruhr-University Bochum and carried out according to the 'good clinical practice' regulations. The study was initially sponsored by Novartis and later by ZLB Bioplasma AG.

TREATMENT

Patients were treated with an IVIG dose of 0.4g/kg body weight or placebo every 4 weeks. Human albumin preparation visually indistinguishable from IVIG was used as placebo. Patients have been treated for 24 months and followed up for an additional 12 months.

Inclusion criteria

(1) Clinically definite or laboratory-supported definite MS for more than 2 years;
(2) Chronic progressive course of disease, no acute relapse in the last 12 months (i.e. appearance of new neurological symptoms or worsening of pre-existing symptoms lasting at least 48h in a patient who had been neurologically stable or improving for the previous 30 days; worsening must reach or exceed 0.5 points on EDSS or 1.0 point on the pyramidal, cerebellar, brainstem or visual functional system scores);
(3) Clinical disease activity: disease progression in the last 12 months must reach at least 0.5 points on EDSS;
(4) Age 18–65 years, male and female patients;
(5) EDSS $\geq$3 and $\leq$7.0;
(6) No corticosteroids for at least 1 month prior to the study;
(7) No other immunotherapy for at least 3 months prior to the study;
(8) Normal X-ray of the chest taken no longer than 1 year before entering the study;
(9) Normal electrocardiogram no older than 6 months.

Exclusion criteria

(1) Concomitant diseases which may cause a worsening on the EDSS;
(2) Psychiatric, medical or other conditions that compromise the ability to give informed consent and will probably cause non-compliance;
(3) History of allergic diathesis with multiple allergic reactions;
(4) History of anaphylactic reaction;
(5) Renal insufficiency;
(6) Heart insufficiency;
(7) History of stroke or ischemic heart attack;
(8) History of thrombosis;
(9) Pregnancy or lactation;
(10) Second- or third-degree atrioventricular block, ventricular extrasystoles or repolarization abnormalities.

OUTCOME MEASURES

Primary outcome measure

The time to sustained progression ('treatment failure') as measured by the EDSS[4] was used as the primary end-point. Depending on the patients' EDSS at the beginning of the study, changes in EDSS are regarded as significant, when they reach one point on the EDSS if the EDSS at entry was $\leq$5.0 points and 0.5 points when the EDSS score at entry was >5.0. Treatment failure was be only accepted if it was sustained after 3 months.

Secondary outcome measures

(1) Nine-hole-peg test;

(2) Box-and-block test;
(3) Visual acuity;
(4) Visual contrast sensitivity;
(5) Visual evoked potentials;
(6) Depression;
(7) Neuropsychological examination assessing memory, attention and conceptual abilities (months 0, 6, 12, 24);
(8) Quality of life;
(9) Number and duration of infections as documented in the patient's diary.

With the exception of neuropsychological tests, which were carried out at 3, 6, 12 and 24 months, all outcome measures were taken every 3 months. EDSS was also scored at every relapse.

Safety analysis

Besides the analysis of the daily diary for adverse events and the clinical visits, blood samples were assessed for white and red blood count, cell differentiation, liver and renal function. After about 1200 infusions in 99 patients an interim analysis on drug safety was carried out by the sponsor.

PRELIMINARY RESULTS

Drug safety

After about 1200 infusions in 99 patients (about half of them IVIG and half placebo) 25 serious adverse effects (SAEs) in 25 patients have been reported.

SAEs were defined according to German drug law ('Arzneimittelgesetz') and the guidelines of the sponsor as follows: 'Any event which is fatal, considered life-threatening, requires hospitalization, prolongs in-patient hospitalization, causes permanent disability, constitutes cancer, congenital anomaly or over-dose', due to former reports on adverse effects of IVIG[5].

None of these SAEs were judged to be drug-related, but they were instead attributable to the formal criterion of 'hospitalization' (Table 1).

Two patients were admitted to hospital after grand mal seizures. One female patient had two complex focal epileptic seizures with secondary generalization to grand mal seizure prior to inclusion in the study. Despite anti-epileptic treatment this patient suffered one more grand mal seizure. Another female had suffered generalized epilepsy of the grand mal type for some years, but was stable with anti-epileptic treatment for more than a year prior to inclusion.

No kidney problems, either clinical or relating to laboratory parameters, and similarly no cases of aseptic meningitis, rheological problems, hepatitis, severe skin problems and other adverse events were reported. Biographical data of the hospitalized patients are given in Table 2.

Efficacy

The study has been completed and the database was closed according to good clinical practice. A first analysis of the primary end-point indicates a reduction of progression in the whole group (primary and secondary chronic progressive MS pooled together (*n*=231));

Table 1 Reasons for hospitalizations

Serious adverse effect	Number of patients
Worsening of disability	9 (6 admissions to hospital, 3 to in-house rehabilitation)
Relapse	5
Urinary tract infections	4
Epileptic seizure	2 (both with epilepsy prior to inclusion)
Pneumonia	1
Appendectomy	1
Dyspnea for some hours some days after infusion	1 (no evidence for embolism)
Painful dysesthesia in the legs	1
Tremor upper limbs	1

Table 2 Biographical data of the hospitalized patients

Age (years)	mean 46.9 (25–65)
Duration of disease (years)	mean 12.72 (2–42)
EDSS at point of inclusion	mean 5.6 (3.0–7.0); median 6.0
EDSS 12 months previously	4.88
Sex	male 44; female 54
Type of multiple sclerosis	secondary progressive 67; primary progressive 31
Time in study	1—17 infusions

EDSS, expanded disability status scale

this effect is more pronounced in the small subgroup of primary progressive MS (*n*=34) than in the group of secondary progressive MS (*n*=197). The analysis is ongoing; final data will be prepared for publication in peer-reviewed journals.

DISCUSSION

This study used different clinical and paraclinical end-points.

With the mathematical model of factor analyses the clinical data from different MS trials have identified several areas of disability in multiple sclerosis. These areas are considered to be independent in this mathematical model:

(1) Leg dysfunction;
(2) Arm dysfunction;
(3) Sensory dysfunction (superficial touch, position sense and vibration threshold);
(4) Visual dysfunction;
(5) Mental or cognitive dysfunction;
(6) Bowel, bladder and sexual dysfunction.

These finding have been found to be consistent across different datasets[6,7]. From a clinical perspective, these six areas are all of clinical importance and represent the major clinical symptoms of MS disability[8].

Patients may worsen in one factor while being stable in remaining symptoms. The value of a clinical approach by the multi-area analysis is underlined by the proposition of an international task force appointed by the US National Multiple Sclerosis Society. This task force recommended a multidimensional assessment measure comprising these relatively independent clinical dimensions of MS disability[8].

Despite the promising use of surrogate markers in multiple sclerosis such as magnetic resonance imaging (MRI) for assessment of the disease activity or the estimation of the cerebral pathology, there is unanimous agreement that proof of efficacy of a new treatment for multiple sclerosis should be based on clinical outcome, i.e. relapse rate or disease progression.

The Kurtzke expanded disability status scale (EDSS) was established back in 1983 and is the most widely used instrument in clinical trials of MS. The advantages of this scoring system are that it is familiar to clinicians interested in clinical trials, it is easy to apply and its intra- and inter-rate reliability is well estab lished[4]. Disadvantages of the EDSS are that it is a non-linear ordinal scale. It relies heavily on capability to walk, namely at elevated EDSS values where a poor assessment of upper limb functions occurs. Finally, it is insensitive to cognitive decline. However, there is currently no other widely accepted scoring system which has proven to be superior to EDSS[9]. A standardized scoring protocol and rating training program for all centers should minimize problems related to the use of the EDSS and increase the intra- and inter-rating reliability.

Secondary end-points covered disability areas which are poorly represented in the EDSS: upper limb functions (box-and-block test; nine-hole-peg test), visual function and cognitive dysfunction (neuropsychological test battery, depression questionnaire). The visual system was intensively examined in this study using visual evoked potentials, contrast sensitivity and visual acuity.

The potential shortcomings of this study with regard to MRI examinations may be compensated by these broad examinations of clinically relevant end-points. The IVIG used in this study was demonstrated to be a safe treatment even for severely disabled chronic progressive MS patients.

In general, IVIG has been reported to be an effective treatment for different neurological autoimmune diseases. In experimental allergic encephalomyelitis (EAE), an animal model representing distinct aspects of MS, IVIG was shown to exert a beneficial effect[10]. Furthermore, it has been suggested that different mechanisms of action for IVIG[11] could also be of benefit in the treatment of MS.

IVIG is effective in relapsing—remitting MS and is recommended as a second-line therapy[12], and has been reported to be effective in other forms of MS, including chronic progressive MS (reviewed in reference 13).

An open study on 18 MS patients with primary or secondary progessive MS recently reported that 11 of 18 patients treated with IVIG improved and seven remained stable for 12 months[14].

In contrast, one double-blind, placebo-controlled study on the treatment of secondary progressive MS with IVIG (the so-called European Study of Intravenous Immunoglobulin in Multiple Sclerosis (ESIMS) study) could not show a difference between IVIG- and placebo-treated patients in the primary endpoint (sustained progression measured with EDSS) or burden of disease (T2-weighted MRI images). Interestingly, there was a significant reduction of progression of brain atrophy (T1-weighted MRI images)[15]. A valid comparison of both studies will only be possible after final analysis and publication of the clinical data.

The primary progressive MS subtype clearly differs from the relapsing—remitting MS and secondary progressive MS, by showing more pronounced neurodegeneration and more rapid progression[16]. Our small stratified population of primary progressive MS will not allow a final conclusion, but the preliminary data give hope that IVIG might also be effective in this condition. If this is confirmed by the final analysis and also by the secondary end-points, a larger double-blind, placebo-controlled study should be initiated to confirm data from the present study.

Interestingly, the only difference between the IVIG- and the placebo-treated patients which was found in the ESIMS study was seen in MRI-measured brain atrophy which reflects the neurodegenerative aspect of MS.

IVIG is effective in autoimmune diseases where autoantibodies and/or complement mechanisms may play a pathogenic role. Many studies on the pathogenesis of MS show that MS is not a single entity but that there are different subtypes with different pathological mechanisms. In some of the subtypes, autoantibodies and complement essentially contribute to pathogenesis[17]. It is expected that in such subtypes the immunomodulatory and anti-inflammatory potential of IVIG should provide most benefit for patients.

Future studies should help in identifying the predominantly autoantibody and complement-mediated subtypes of MS by using blood and/or MRI markers.

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37
What is the effect of intravenous immunoglobulin for treatment of ralapsing-remitting multiple sclerosis?

M.Lewanska, M.siger-Zajdel and K.Selmaj

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Although the pathogenesis of MS remains unknown, the majority of data have favored an autoimmune hypothesis of MS[1–3]. Therefore, the search for therapeutic strategies in MS has been focused on agents influencing immune system function. Intravenous immunoglobulin (IVIG) represents a non-specific immune therapy which has been successfully applied to a number of autoimmune conditions during the past decade[4,5].

The efficacy of high-dose IVIG in auto-immune disease was first shown in idiopathic thrombocytopenic purpura at the daily dose of 0.4g/kg body weight for 5 days[6]. Thereafter, IVIG has been proposed as a therapy of several autoimmune diseases, including neurological disorders such as acute and chronic inflammatory demyelinating radiculoneuropathies[7–10], myasthenia gravis[11–14] and dermatomyositis[15]. During the two past decades, a number of small open trials have been published using low doses of IVIG for MS treatment. Most of these studies claimed a beneficial effect of IVIG on this disease[16,17]. Higher doses of IVIG were effective in the prevention of acute exacerbations of MS[18,19]. The first larger-scale, placebocontrolled trial on the efficacy of long-term IVIG therapy in relapsing—remitting MS demonstrated a reduction of relapse frequency and a beneficial influence on the course of clinical disability[20]. Subsequent trials have shown additionally the reduction of gadoliniumenhancing lesions on magnetic resonance imaging (MRI) after IVIG treatment[21,22]. However, the optimal dose of IVIG in MS treatment still has not been established. Studies in MS with IVIG used a wide range of IVIG doses from 0.15 to 2.0g/kg with various administration regimens. In the present double-blind, placebo-controlled study, we have compared the efficacy of two IVIG doses, 0.2g/kg and 0.4g/kg. The dose of 0.4g/kg has been most often used in the treatment of autoimmune conditions, but lower doses have been suggested to show a beneficial effect on clinical measures of MS in earlier non-controlled studies[17,23].

METHODS

Patients

Forty-nine patients, 20 males and 29 females, with clinically definite relapsing—remitting MS (RR MS)[24] and MRI confirming the diagnosis[25] entered the study. Baseline clinical and demographic characteristics in the three study groups are presented in Table 1. The groups were statistically comparable.

The inclusion criteria were: duration of MS more than 2 years, a baseline Kurtzke expanded disability status scale (EDSS) score of between 0 and 6.5, a history of at least two clearly documented relapses during the previous 2 years and age range 18–55 years (Table 1).

The exclusion criteria were: pregnancy, lactation, lack of acceptable form of contracep tion, any other diseases of the CNS or peripheral nervous system, any diseases of the kidney, liver or heart, patients with immune deficiencies or psychiatric diseases, patients with claustrophobia and metal implants. Patients were excluded if they were in clinical relapse, or under treatment with corticosteroids during the 3 months prior to the screening visit, or had had immunosuppressive therapy within the 12 months before screening. Patients signed a written informed consent, approved by the University Human Studies Committee.

Study design

Patients were randomly allocated to three groups: two groups were treated with two doses of IVIG, 0.4g/kg/day (15 patients), 0.2g/kg/day (17 patients) at monthly intervals, and the placebo group (17 patients) was treated in the same manner. Generation of the allocation sequence was based on a random-number table (see Figure 1 for trial profile). Placebo consisted of saline, to avoid a non-specific protein effect. The treatment duration was 12 months, with 3 months' follow-up.

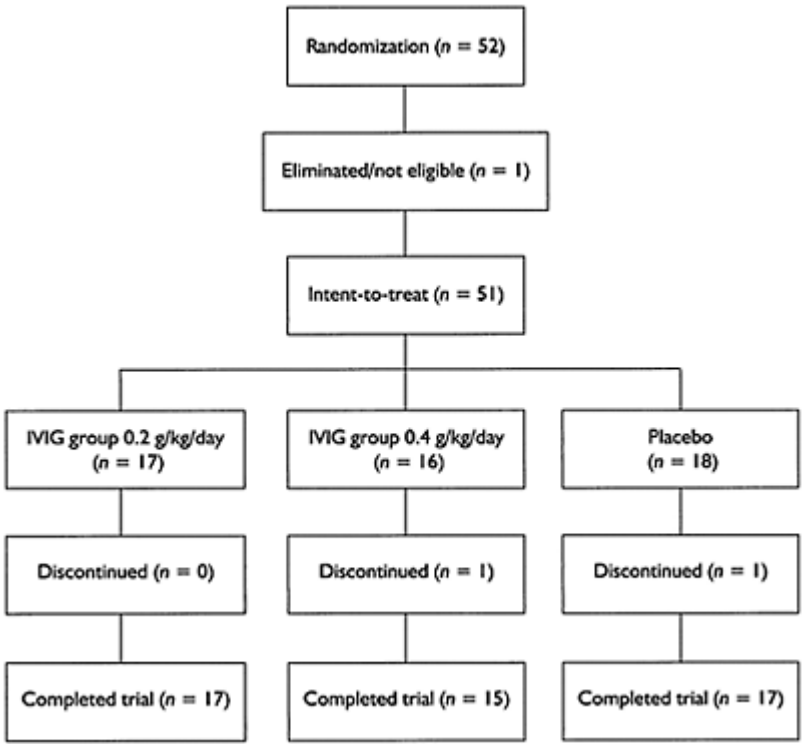
Table 1 Characteristics of study population. Values are expressed as mean (SD) where appropriate

Group	IVIG 0.2g/kg/day (*n*=17)	IVIG 0.2g/kg/day (*n*=15)	Placebo (*n*=17)
Male/female	6/11	4/11	10/7
Age (years)	38 (6.96)	31.1 (6.08)	41.8 (6.98)
Duration of MS (years)	10.7 (7.57)	7.2 (5.48)	7.5 (4.70)
Baseline EDSS	3.0 (1.35)	3.0 (2.08)	2.97 (1.58)
Baseline NRSS	81.6 (9.13)	83.07 (12.96)	81.35 (9.34)

ARR prior to study	1.65 (1.11)	1.73 (0.79)	1.29 (0.58)

MS, multiple sclerosis; EDSS, expanded disability status scale; NRSS, neurological rating scale score; ARR, annual relapse rate; IVIG, intravenous immunoglobulin

Figure 1 Study design flow chart. IVIG, intravenous immunoglobulin



Infusions of IVIG and placebo were stored in identical opaque plastic bags for concealment during administration. The treatment physician was unaware of the actual treatment allocation. Before entry to the study, and monthly thereafter during the study and at 3 months after the end of the study, each patient was examinated blindly by the same neurologist who was unaware of the treatment allocation. Monitoring and recording of relapses, concomitant treatment, side-effects or other medical events were documented throughout the study. The trial-stopping rules were defined as significant worsening of clinical end-points and the safety profile. A relapse was defined as the appearance of a new symptom or worsening of an old symptom attributable to MS, persisting for more than 24h and accompanied by stability or improvement for at least 30 days before-hand[26]. In the case of acute relapse, patients were administered 1g methylprednisolone intravenously daily, for 5 consecutive days. Mild relapses were not treated with steroids.

During an exacerbation study medication was stopped, and continued beginning 4 weeks from the last dose of steroids as in other trials[20]. Laboratory safety examinations were carried out at the beginning and end of the study period and included hemoglobin, complete blood cell count, hepatitis B and C serologies, serum creatinine, blood urea, nitrogen, electrolytes, blood glucose, liver enzymes and urine analysis.

Brain MRI examination

MRI was performed before the start of the study and thereafter every 3 months. In the case of relapse and corticosteroid administration, the MRI scan was postponed until 4 weeks after the last dose of steroids. Patients were positioned using a standard protocol to ensure a reproducible slice position[27]. Brain imaging was performed using a 1.5-T Siemens machine (Magnetom Vision Plus; Erlangen, Germany). MRI scanner images in the transverse plane were obtained with a double spinecho sequence (TR 2500 ms, TE 20 and 80 ms, one acquisition, 224×256 matrix, 4mm slice thickness, disphactor 0.1, number of slices 21), thus providing a proton and T2-weighted image (PD/T2WI); furthermore, a T1WI (TR 650ms, TE 14ms, two acquisitions) was applied with 21 slices as the double spin-echo sequence. Ten minutes after injection of gadolinium—diethylenetriamine penta-acetic acid (DTPA) (Schering, Berlin, Germany) at 0.1mmol/kg body weight, the T1WI scan was repeated. All scans were evaluated blindly, and the number of enhancing lesions and new lesions on T2WI were registered. The areas of the lesions were summed to the total lesion area, then multiplied by the slice thickness for a straightforward estimate of volume. The area of the lesion was measured using a semi-automatic program based on segmentation techniques.

Evaluation of efficacy

The primary clinical end-point was the annual relapse rate (ARR) during the study period and a comparison between the ARR pre-study and during the study period. All patients were followed in the MS clinic for at least 2 years before entering the study, according to the clinical protocol used during the study. The secondary clinical end-points were the proportion of relapse-free patients, mean changes in EDSS and neurological rating scale score (NRSS) from baseline to study conclusion and the proportion of patients with worse clinical disability by the end of the study (as defined by an increase or decrease of at least 0.5 grade in the EDSS score sustained for at least 3 months and by an increase or decrease by 7 points in the NRS score).

The MRI end-point measures were the change of mean lesion volume on T2WI, the mean number of new lesions on T2WI and the mean number of Gd-enhancing lesions on T1WI every 3 months.

Statistical methods

As descriptive statistics, mean values and standard deviations are given.

A repeated-measures analysis of variance was used to compare the variability in time of the examined clinical and MRI parameters

Table 2 Annual relapse rate (ARR) analysis. Values are expressed as mean (SD) where appropriate

				p Value		
	IVIG 0.2g/kg	IVIG 0.4g/kg	Placebo	0.2g/kg vs. placebo*	0.4g/kg vs. placebo*	0.2g/kg vs. 0.4g/kg*
Pre-study	1.65 (1.11)	1.71 (0.82)	1.29 (0.59)	0.464 648	0.353 648	0.959 75
Study ARR	0.88 (1.26)	0.87 (0.99)	1.24 (0.75)	0.579738	0.591 541	0.999 913
Change ARR	−0.76** (1.15)	−0.87** (0.92)	−0.06 (0.75)	0.089 055	0.062 673	0.953 870

*Tukey *B* test significance level 0.05; ***p*<0.05 for within-group evaluation, Wilcoxon matched pairs signed-ranks test; IVIG, intravenous immunoglobulin

(ARR, EDSS, NRSS, total lesion volume, new lesions on T2WI and Gd-enhanced lesions on T1WI) during the study between the placebo group and the two treatment IVIG groups[28].

One-way analysis of variance was used to test the hypothesis that the three populations, placebo group and two treatment groups, at separate times of the study, were equal[29]. For determining which population means were different from each other, the Tukey *B* multiple comparison test[30] was employed. Statistical significance was defined as $p<0.05$

RESULTS

IVIG effect on the annual relapse rate

The annual relapse rate (ARR) during the study period was lower in both IVIG groups than in the placebo group. The mean ARR was 0.88 in the 0.2-g/kg IVIG, 0.87 in the 0.4-g/kg IVIG group vs. 1.24 in the placebo group (Table 2). The reduction of ARR during the treatment period compared with ARR prior to the study was significant for both IVIG groups; for the 0.2-g/kg dose the reduction was −0.76 (p=0.0208) and for the 0.4-g/kg dose was −0.87 (p=0.0099), whereas the ARR reduction in the placebo group was only −0.06 (p=0.7532) (Table 2). The proportion of relapse-free patients during the study was higher in both IVIG groups: 47.07% in the 0.2-g/kg group, 50% in the 0.4-g/kg group vs. 11.76% in the placebo group.

IVIG effect on disease progression

The mean changes in EDSS from baseline to study conclusion were −0.029 in the 0.2-g/kg IVIG group (p=0.7794), −0.066 in the 0.4-g/kg IVIG group (p=0.4227) and +0.294

in the placebo group (p=0.0117). Neurological impairment measured by the NRS showed a similar positive trend towards reduced neurological disability in both IVIG groups; the mean change in the 0.2-g/kg group was +1.117 (p=0.2891), in the 0.4-g/kg group was +0.066 (p=0.2367) and in the placebo group was −5.176 (p=0.0022) (Table 3).

Serial EDSS and NRS scores at the monthly clinical evaluation in the three study groups showed that disability in the placebo group worsened during 6 months of observation, whereas the IVIG groups remained stable

Table 3 Disease progression analysis. Values are expressed as mean (SD) where appropriate

				p Value		
	IVIG 0.2g/kg	IVIG 0.4g/kg	Placebo	0.2g/kg vs. placebo*	0.4g/kg vs. placebo*	0.2g/kg vs. 0.4g/kg*
Baseline EDSS	3.03 (1.35)	3 (2.08)	2.97 (1.58)	0.994 383	0.998 824	0.998 824
Final EDSS	3 (1.43)	2.93 (2.18)	3.26 (1.65)	0.900 406	0.865 082	0.994 204
Change in EDSS	−0.029 (0.41)	−0.0667 (0.32)	0.29** (0.37)	0.035 432*	0.026 352*	0.958 43
Baseline NRSS	81.647 (9.13)	83.06 (12.96)	81.35 (9.34)	0.996 424	0.896 262	0.927 563
Final NRSS	82.764 (10.94)	83 (14.68)	76.18 (12.61)	0.297 484	0.316852	0.998 664
Change in NRSS	1.117 (5.09)	−0.0667 (4.94)	−5. 176** (5. 16)	0.002 184*	0.022 273*	0.799 25

*Tukey *B* test with significance level 0.05; **p<0.05 for within-group evaluation, Wilcoxon matched pairs signed-ranks test; EDSS, expanded disability status scale; NRSS, neurological rating scale score; IVIG, intravenous immunoglobulin

throughout 12 months of treatment in both EDSS and NRS scores, without serious fluctuation.

The proportion of patients who worsened in EDDS in the 0.2-g/kg group was 23.5%, and in the group treated with 0.4g/kg of IVIG 6.7% of patients worsened compared with 47.1% patients with progression of disability in the placebo group. The proportion of patients with progression of disability measured by the NRSS was 5.9% in the 0.2-g/kg group and 6.7% in the 0.4-g/kg group, compared with 35.3% in the placebo group.

Efficacy of IVIG on MRI end-points

The total T2WI lesion volume in the placebo group increased by 13.56% (p=0.0026) during 12 months of study. This increase, although relatively high, was still within the range of T2WI increase during natural progression of the MS process[31–34]. In the IVIG-

treated groups the change in the total T2WI lesion volume was significantly lower than in the placebo group, and in the 0.4-g/kg group this decreased by −3.95% (p=0.0712) and in the 0.2-g/kg group increased by 3.6% (p=0.6233).

The mean number of Gd-enhancing lesions in the IVIG groups was reduced, compared with the placebo group. In the 0.4-g/kg group the mean number of Gd-enhancing lesions dropped from 1.67 at the baseline level to 1.0 (p=0.2076) at study completion, and in the 0.2-g/kg group from 1.0 to 0.4 (p=0.1551), whereas in the placebo group the mean Gd-enhancing lesions rose from 1.12 to 1.29 (p=0.6356) (Table 4).

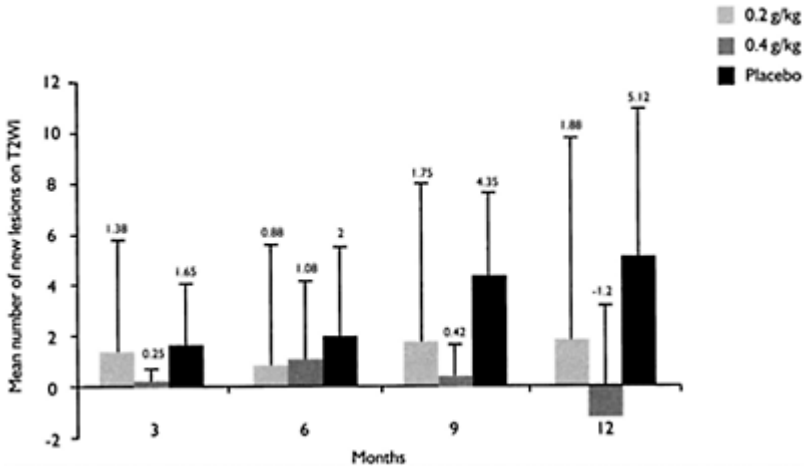
The mean number of new lesions on T2WI during the study increased in the placebo group by 5.12 (p=0.0003), compared with only a slight increase in the 0.2-g/kg IVIG group by 1.88 (p=0.0006). In the 0.4-g/kg IVIG-treated group the mean number of new lesions on T2WI decreased by 1.2 (p=0.0022) (Figure 2). The number of new lesions was

Table 4 Mean number of Gd-enhancing lesions on T1-weighted image. Values are expressed as mean (SD) where appropriate

				p Value		
	IVIG 0.2g/kg	IVIG 0.4g/kg	Placebo	0.2g/kg vs. placebo*	0.4g/kg vs. placebo*	0.2g/kg vs. 0.4g/kg*
Baseline	1 (1.46)	1.67 (2.26)	1.12 (1.31)	0.978312	0.700451	0.592 996
3rd month	0.81 (1.37)	0(0)	2 (2.47)	0.141 379	0.019 501	0.491 123
6th month	0.81 (2.25)	1.83 (2.12)	1.59 (1.8)	0.540 775	0.954 399	0.451 808
9th month	1.44 (2.94)	0.83 (1.4)	1.24 (1.88)	0.964 469	0.898 359	0.785 604
12th month	0.44 (0.89)	1 (1.65)	1.29 (1.72)	0.232 526	0.8748	0.615911
Change of mean number of Gd-enhancing lesions	−0.5625 (1.6721)	−0.6667 (1.7233)	0.1765** (1.5506)	0.417692	0.426202	0.986848

*Tukey *B* test with significance level 0.05; **p<0.05 Wilcoxon matched pairs signed-ranks test; IVIG, intravenous immunoglobulin

Figure 2 Mean number (±SD) of new lesions on T2-weighted image (T2WI) every 3 months of the study



lower in the 0.4-g/kg group vs. placebo (p=0.052335), and was not significantly different between IVIG groups (p=0.479512).

The frequent MRI analysis of total lesion volume on T2WI showed that progression in the placebo group occurred after 6 months of treatment, whereas in both IVIG groups the volume remained stable throughout 12 months (Figure 3).

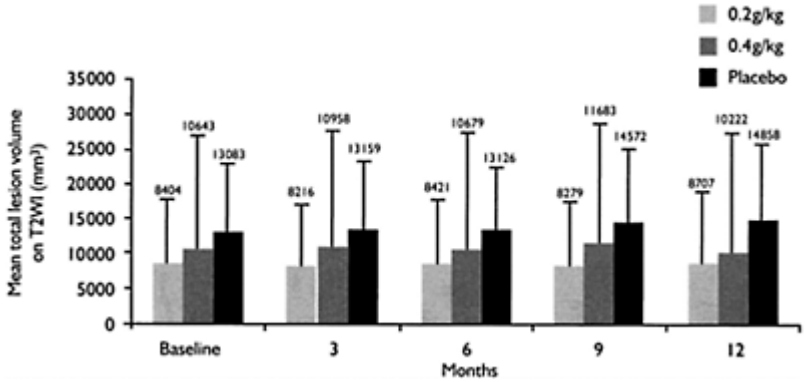
Frequent MRI analysis of Gd-enhancing lesions showed initially, at the 3-month time point, a reduction of mean Gd-enhancing lesions in both IVIG groups. Thereafter, a fluctuation of the number of Gd-enhancing lesions was observed, with a reduction at the end of the treatment period. The frequent MRI analysis of Gd-enhancing lesions in the placebo group showed an early increase in number of the lesions (Table 4).

Frequent MRI analysis of new lesions on T2WI showed insignificant fluctuations, with a trend towards increasing numbers in both IVIG groups but with a final reduction of new T2 lesions in the 0.4-g/kg group. A gradual increase of the number of new lesions on T2WI was observed in the placebo group (Figure 2).

Safety profile of IVIG treatment

Generally, patients tolerated immunoglobulin infusions very well. There were no serious side-effects in the treated patients. One patient discontinued treatment after 3 months because of dyspnea and tachycardia during the third infusion of IVIG. These symptoms spontaneously resolved after discontinuing the infusion. One patient had slightly elevated serum bilirubin, but the hepatitis serological studies were negative. This symptom was self-limited

Figure 3 Mean number (±SD) lesions volume on T2-weighted image (T2WI) measured longitudinally every 3 months of the study



and disappeared after a month, and the patient continued treatment. Another patient had elevated serum bilirubin and transaminases. The hepatitis serological studies were negative, and the enzymes and bilirubin level resolved after discontinuing IVIG treatment. One patient had short-lived nausea for about 2h after infusions of IVIG Tolerance to the two doses of IVIG was comparable.

DISCUSSION

In this study we aimed to compare two doses of IVIG, 0.2g/kg and 0.4g/kg, in the treatment of MS. Clinical and MRI parameters have been applied for analysis of the effectiveness of IVIG treatment. The results of our study have confirmed a beneficial effect of IVIG on the clinical course of RR MS[20–22]. In both IVIG-treated groups, 0.2g/kg and 0.4g/kg, reduction of the ARR was significant versus placebo, although not different from each other. A decrease of the ARR and increase in the proportion of relapse-free patients in both IVIG groups suggests that IVIG diminishes the activity of the disease process in MS. Additionally, fewer patients treated with IVIG worsened in EDSS and NRSS. Also, the annual change in EDSS and NRS score from baseline to study conclusion was significantly different between IVIG and placebo groups. The mean in EDSS and NRS changes in both IVIG groups improved slightly, but again they were not different from each other.

The clinical findings were confirmed with MRI data. Frequent MRI scans were performed to obtain a continued insight into the disease activity. This type of study also increases the statistical power to evaluate changes within a shorter time of observation and using a limited number of patients[35]. The mean annual change in total T2 burden of the disease in both IVIG groups was significantly lower, compared with placebo. The 0.4-g/kg IVIG group showed a slight reduction of the total T2 burden whereas the 0.2-

g/kg IVIG group showed only a small but insignificant increase. The mean number of Gd-enhancing lesions decreased significantly in both IVIG groups, indicating that IVIG might specifically influence the blood—brain barrier function. The third MRI parameter, number of new lesions on T2WI, also showed a beneficial effect of IVIG treatment over placebo. Patients receiving 0.4g/kg IVIG showed a slight reduction in new T2 lesions, whereas patients receiving 0.2g/kg IVIG showed an insignificant increased number of new T2 lesions, but lower than in the placebo group.

Previous studies of IVIG in MS used various doses of IVIG ranging from 0.15 to 0.2g/kg, and with differing regimens of IVIG administration[16,17,19,36]. More recently, Fazekas and colleagues[20] reported a study of IVIG in MS using a dose of 0.15–0.2g/kg once a month for 2 years. In that study, a 50% reduction in ARR in the IVIG-treated patients, compared with the placebo group, as well as a modest beneficial effect on disease progression measured by mean EDSS analysis, was observed. No MRI analysis was done. Sorensen and associates[22] reported MRI findings in 26 MS patients treated with a high dose of 0.2g/kg IVIG per 2 days monthly for 6 months. A significant reduction in Gd-enhancing lesions was observed in patients treated with IVIG. In that study, clinical analysis did not meet statistical significance possibly because of the short, 6-month, treatment period. Achiron and colleagues[21] reported an IVIG trial in MS patients using 0.4g/kg every 2 months after initial loading treatment for 5 consecutive days. Treated patients showed a significant reduction in ARR compared with the placebo group. There was also a difference in the mean EDSS between the IVIG-treated group (decreased by 0.3) and the placebo group (increased by 0.25). There was no difference in MRI lesion score between the treated group and placebo, but the complex MRI measure used in this study was not directly comparable to the three most often used MRI parameters of: total T2 disease burden, new T2 lesions number and Gd-enhancing lesions. Instead, the MRI analysis involved only pre- and posttrial scanning for T2 lesion load. This insensitive measure, together with only a 0.5-T magnet, might have been insufficient to detect a difference before and after treatment.

In our study, we combined clinical analysis with frequent MRI scans in MS patients treated with IVIG. These results confirmed and extended previous studies of the effectiveness of IVIG in the treatment of MS. Our study demonstrated that IVIG treatment has a beneficial effect on MS, using both clinical and MRI end-points. There was good correlation between reduction of the ARR and disease stability measured by EDSS and NRS, and lack of progression or improvement of MRI parameters. Since higher doses of IVIG are linked with higher risks of side-effects[37,38], we attempted to assess the effectiveness of a relatively low dose of 0.2g/kg, once a month, and compare it with the dose of IVIG 0.4g/kg, which has been most often used in treatment in many other autoimmune conditions. Our results from both clinical and MRI analysis indicate that there is no significant difference in effectiveness of the 0.2-g/kg IVIG dose from the 0.4-g/kg dose in RR MS. In MRI analysis there was a non-statistically significant trend that enlargement of T2 lesion load and increase in new T2 lesions were better controlled with the dose of 0.4g/kg IVIG. All clinical parameters indicate, however, that differences between the 0.2-g/kg and 0.4-g/kg doses are insignificant.

The mechanism of action of IVIG in autoimmune conditions, including MS, is poorly defined. According to a recent report, manipulation of the inhibitory Fc receptor (FcR) pathway is a practical therapeutic means for controlling autoantibody-mediated

inflamation. IVIG mediates its protective effect by its ability to induce the expression of the inhibitory FcγRIIB receptor on effector cells[39]. It has been suggested that IVIG modulates the immune system through multiple mechanisms, not only by blocking of the Fc receptor on macrophages[40], but also by other mechanisms such as: inducing regulatory effects of anti-idiotypic antibodies[41], inhibiting complement binding to the oligodendrocyte surface and myelin proteins[42] and suppression of pathogenic cytokine production[43]. Rodriguez and Lennon[44] have also shown that IVIG may promote remyelination within demyelinative lesions induced by Theiler's virus. All of these activities might contribute to the mechanism responsible for IVIG efficacy in MS.

In summary, our data from a 12-month study suggest that MS patients would benefit from a lower dose of IVIG (0.2g/kg/day) to the same extent as from using the standard dose recommended for treatment of other autoimmune diseases (0.4g/kg/day).

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38
High-dose intravenous immunoglobulin In the treatment of patients with stiff person syndrome: a controlled study

M.C.Dalakas

INTRODUCTION

Stiff person syndrome (SPS) is a rare central nervous system disorder characterized by fluctuating muscle rigidity of truncal and proximal limb muscles with superimposed spasms[1–4]. Continuous contraction of agonist and antagonist muscles caused by involuntary motor unit firing at rest are the hallmark clinical and electrophysiological signs of the disease[2]. The cause of SPS is unknown, but an autoimmune pathogenesis is suspected based on:

(1) The presence of antibodies against glutamic acid decarboxylase (GAD_{65}), the rate-limiting enzyme for the synthesis of γ-aminobutyric acid (GABA)[2,5–7];
(2) The observation that these antibodies are produced intrathecally and may be pathogenic because they inhibit the activity of the inhibitory neurotransmitter GABA, resulting in low GABA levels in the brain and cerebrospinal fluid (CSF)[8–10];
(3) Association with other autoimmune disorders, autoantibodies and alleles in the DR and DQ phenotypes 1, 2.

The main therapy of SPS is with diazepam, which helps most patients for a period of time, but the required doses are often so high (up to 60mg daily) that they cannot be tolerated[2,11]. As a result, most patients are left with significant disability requiring a cane or a walker, owing to truncal stiffness and frequent falls[1]. Anecdotal reports had suggested that intravenous immunoglobulin (IVIG) could help patients with SPS[12,13], but the efficacy of the drug was not shown in a large number of patients in a randomized trial. Because IVIG is a potent immunomodulating agent whose efficacy has been demonstrated in other auto-immune disorders[14], there was a clear rationale for examining its effect in SPS. The results have been reported[15].

METHODS

We enrolled 16 SPS patients (nine women, seven men), mean age 46 years (range 28–59 years). Patients were incompletely responding to therapies, and fulfilled the following strictly defined clinical criteria[1]:

(1) Muscular rigidity in limbs and axial (trunk) muscles, prominent in the abdominal and thoracolumbar para-spinals;
(2) Continuous co-contraction of agonist and antagonist muscles confirmed clinically and electrophysiologically;
(3) Episodic spasms precipitated by unexpected noises, tactile stimuli or emotional upset;
(4) Absence of any other neurological disease that could explain stiffness and rigidity;
(5) Positive anti-GAD_{65} antibodies assessed by immunocytochemistry, Western blot and enzyme-linked immunosorbent assay (ELISA), as described[1,2,8].

At enrolment, 16 patients were receiving benzodiazepines, six baclofen, three gabapentin and one valproic acid. The doses of these drugs remained unchanged throughout the study. The study was supported by the National Institutes of Health (NIH) and the patients signed an Institutional Review Board-approved clinical protocol.

The protocol was a randomized, double-blind, placebo-controlled design that followed the dose and schedules we previously applied in other disorders[16]. We administered 2g/kg body weight of IVIG, divided into two daily doses, or placebo consisting of half-normal saline every month for 3 months. After a washout period of 1 month, the patients crossed over to the alternative therapy for another 3 months. All patients were followed for at least 3 months after the infusions. The randomization code was not broken until all patients completed the study and the results were analyzed.

Clinical assessments every month

Clinical evaluations were performed every month based on the following: first, change in stiffness using the distribution of stiffness index that we have previously validated, according to the number of stiff areas quantified as follows: no stiff areas=0, stiffness of lower trunk=1, stiffness of upper trunk=2, stiffness of both legs=3, stiffness of both arms=4, stiffness of face=5 and stiffness of abdomen=6; and second, change in the degree of spasms using the heightened sensitivity scores, which have also been validated as a reproducible means of assessing the number of factors triggering spasms, as follows: spasms induced by unexpected noises=1, by visual stimuli=2, by somatosensory stimuli=3, by voluntary activities=4, by emotional upset or stress=5, untriggered=6 and awakenings due to nocturnal spasms=7.

Statistical analysis

The primary end-point was a change in the scores of distribution of stiffness index (total =6) and heightened sensitivity (total=7) from baseline to the second and third month after each treatment. The net differences in the stiffness index and heightened sensitivity

scores from baseline to the end of 3 months of treat-ment and the first- or second-order carry-over effects were compared between patients randomized to the two treatment groups for each period, as described[15]. We obtained unbiased estimates of:

(1) Baseline sequence group difference;
(2) Direct treatment effect on the month following each infusion;
(3) First-order carry-over effect (the residual effect after three monthly infusions);
(4) Second-order carry-over effect (the residual effect after 'wash-out'), using least squares techniques.

Significance testing was performed by applying 'permutation' techniques.

Patient's own assessment of response to therapy

At the end of the study and prior to breaking the code, the patients were asked to:

(1) Speculate as to which phase they had received placebo or IVIG;
(2) Define the phase in which they experienced an improvement;
(3) Indicate if improvement was meaningful in their activities of daily living;
(4) Specify if their disease had remained stable, improved or worsened;
(5) Indicate if after the study they independently pursued IVIG on the assumption that the improvement attained during one arm of the study was IVIG-related;
(6) Specify how long the improvement lasted after each open-label infusion.

Anti-GAD_{65} antibody titers

Anti-GAD_{65} antibody titers were measured by ELISA[8] in coded specimens, before and after 3 months of treatment with IVIG or placebo, in 12 patients in whom post-infusion sera from both phases of the study were available. The Student *t* test was applied to determine significance.

RESULTS

Of the 16 enrolled patients, eight (four women, four men) were randomly assigned to placebo for the first 3 months (group A) and eight (five women, three men) to IVIG (group B). Balanced randomization was achieved for both groups with regard to age, disease duration, onset of symptoms, disease severity and other associated conditions (Table 1).

The complete results for two patients could not be obtained and were not entered for analysis. None of the other 14 patients had any side-effects to raise suspicion of receiving IVIG.

The mean number of stiff areas of the placebo-randomized patients (open circles) remained constant for the first 4 months but dropped significantly during the next 3 months when they crossed to IVIG (Figure 1a). In contrast, the scores of the IVIG-randomized patients (closed circles) dropped for the first 3 months, remained constant during the 'washout' period and rebounded from months 5–8, never reaching baseline.

The non-overlapping areas for a given visit between the standard error bars for the effect of IVIG and those for the effect of placebo indicate a significant difference at that visit (Figure 1a). The difference was most prominent in months 2–3, 3–4, 4–5, 6–7 and 7–8. The mean difference between groups at each visit is given in Table 2.

Table 1 Baseline characteristics of stiff person syndrome (SPS) patients randomized to receive placebo for the first 3 months (group A) or intravenous immunoglobulin (group B)

Patient characteristics	Group A (*n*=8)	Group B (*n*=8)
Age of onset (years)	35–47 (mean 41)	27–54 (mean 39)
Sex	4M, 4 W	3M, 5 W
Disease duration (years)	5–22 (mean 11)	3–23 (mean 12)
Disease severity		
mean heightened sensitivity scores	4.85±1.7 (SD)†	4.85±1.4 (SD)†
mean distribution of stiff areas	4.71±1.3 (SD)	4.57±1.6 (SD)
Coexisting autoimmune diseases *(n)*		
diabetes*	4	3
polymyositis	1	0
pernicious anemia	1	0
thyroiditis	1	3
vitiligo	0	1
Seizures	1	1
Medications on enrolment *(n)*		
benzodiazapines	10	6
baclofen	1	5
gabapentin	1	2
valproic acid	0	1

*Two additional patients in group A and one in group B developed diabetes during the 3-year follow-up period; †standard error of the mean: 0.6 and 0.5 for group A and 0.5 and 0.6 for group B, for heightened sensitivity scores and distribution of stiff areas, respectively; M, men; W, women; SD, standard deviation

When changes in the stiff areas were analyzed between the two groups, there was a significant group difference for the direct treatment effect (p=0.01) and the first-order carry-over effect (p<0.001). Subanalysis of each of the stiffness areas showed a

statistically significant reduction of stiffness in the trunk ($p<0.001$ for the direct effect and $p=0.04$ for the first-order carry-over effect), the abdomen ($p<0.001$) and the face ($p<0.001$). A similar, although less prominent, effect was noted for the scores of heightened sensitivity rating (Figure 1b). Although the difference at each visit did not reach significance (Table 3), when changes were analyzed between the two groups there was a significant group difference in the decline of heightened sensitivity scores between IVIG and placebo for the direct treatment effect ($p=0.03$).

The time to walk 30 ft also dropped significantly in the IVIG group for the direct and first-order effects ($p=0.02$), as well as the second-order carry-over effect ($p=0.03$), indicating a faster gait owing to reduced stiffness and spasms.

Clinical observations

Six of seven patients assigned first to IVIG had a noticeable improvement. They were able to

Figure 1 Mean number of stiff areas (a) and heightened sensitivity rating (b) among patients randomized to placebo (gray circles) or intravenous immunoglobulin (IVIG) (black circles). The interrupted line denotes the wash-out period. Bars represent standard error. For the stiff areas (a), the most significant difference between IVIG and placebo was noted in months 2–3, 3–4, 4–5 and between 6–7, 7–8. For the heightened sensitivity (b), the difference of individual visits was most prominent in months 5–6, 6–7

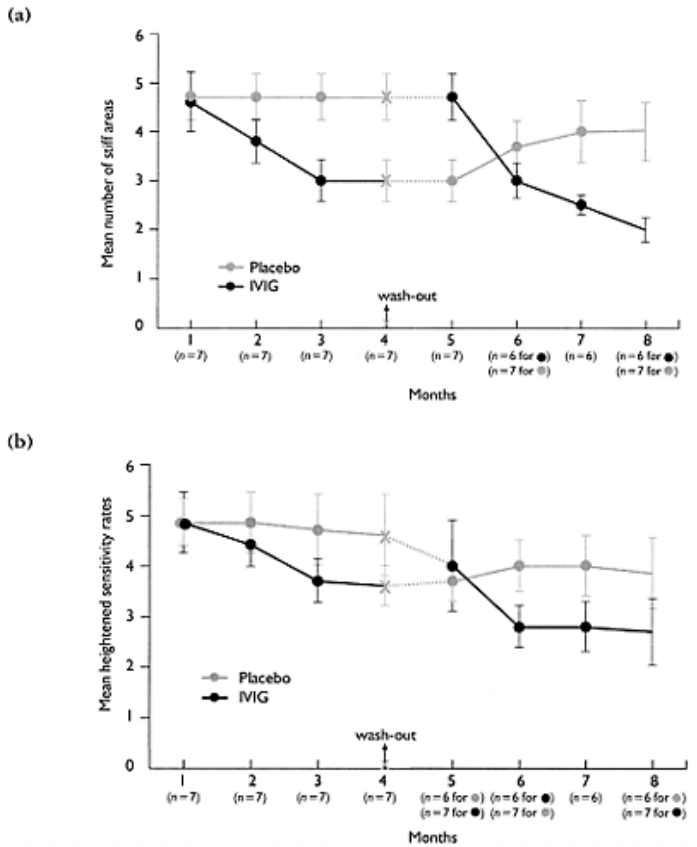


Table 2 Differences in scores of stiff areas at each visit between patients randomized to intravenous immunoglobulin (IVIG) or placebo and after crossing to the alternative treatment

Months	Score		*p* Value
	Mean (95% CI)	Mean (95% CI)	
	Baseline	*Baseline*	
	4.6 (3.4–5.7), *n*=7	4.7 (3.7–5.7), *n*=7	0.85
	IVIG	*Placebo*	
1–2	3.8 (2.9–4.7), *n*=7	4.7 (3.7–5.7), *n*=7	0.21
2–3	3.0 (2.2–3.8), *n*=7	4.7 (3.8–5.6), *n*=7	0.02
3–4	3.0 (2.2–3.8), *n*=7	4.7 (3.7–5.7), *n*=7	0.02
	Washout	*Washout*	
4–5	3.0 (2.2–3.8), *n*=7	4.7 (3.7–5.7), *n*=7	0.02
	Placebo	*IVIG*	
5–6	3.7 (2.7–4.7), *n*=7	3.0 (2.3–3.7), *n*=6	0.30
6–7	4.0 (2.8–5.2), *n*=6	2.5 (2.1–2.9), *n*=6	0.05
7–8	4.0 (2.8–5.2), *n*=7	2.0 (1.5–2.5), *n*=6	0.01

n, indicates number of patients with data collected at each visit; CI, confidence interval

Table 3 Differences in scores of heightened sensitivity at each visit between patients randomized to intravenous immunoglobulin (IVIG) or placebo and after crossing to the alternative treatment

Months	Score		*p* Value
	Mean (95% CI)	Mean (95% CI)	
	Baseline	*Baseline*	
	4.8 (3.9–5.7)*n*=7	4.8 (3.7–6.0) *n*=7	1.00
	IVIG	*Placebo*	
1–2	4.4 (3.6–5.2), *n*=7	4.9 (3.7–6.0), *n*=7	0.56

2–3	3.7 (2.9–4.5), *n*=7	4.7 (3.3–6.0), *n*=7	0.23
3–4	3.6 (2.9–4.3), *n*=7	4.6 (3.1–6.1), *n*=7	0.27
	Washout	*Washout*	
4–5	3.7 (2.9–4.5), *n*=7	4.0 (2.2–5.8), *n*=6	0.77
	Placebo	*IVIG*	
5–6	4.0 (3–5.0), *n*=7	2.8 (2.0–3.6), *n*=6	0.09
6–7	4.0 (2.8–5.2), *n*=6	2.8 (1.9–3.7), *n*=6	0.15
7–8	3.8 (1.4–5.2), *n*=7	2.6 (1.3–4.0), *n*=6	0.25

n, indicates number of patients with data collected at each visit; CI, confidence interval

walk unassisted for the first time in months or years, stopped falling, were able to appear in public, socialize, cross a street without help, shower without spasms and assume work or household chores. Their phobias for open spaces diminished. The face became animated and more expressive. In contrast, no objective changes were noted in the seven patients assigned first to placebo. Five of seven patients assigned first to placebo improved after IVIG, and four of seven assigned first to IVIG worsened after placebo. One placebo-randomized patient worsened substantially during the first 3 months, and was in a continuous state of spasms and stiffness (status spasticus)[1] when he entered the cross-over phase. The patient improved dramatically after crossing over to IVIG.

Among the 14 patients (two spouses) contacted after the results were analyzed, 13 readily identified the treatment phase and stated unequivocal improvement; one assigned first to IVIG experienced slight improvement throughout the study, presumably owing to a sustained carry-over effect. Two patients had died: one from gastrointestinal bleeding a year after the study and the other from cardiac arrest 2 years after the study. Of the patients who successfully pursued IVIG, six require treatment every 4–12 weeks to secure normal daily activities and two others every 4–6 months. Three patients did not need additional treatment for up to a year. Three patients were unable to obtain IVIG through their insurance companies.

Anti-GAD_{65} antibody titers

Antibody titers remained unchanged after placebo, but dropped by 33% after IVIG and rebounded significantly (p=0.03) after crossing to placebo (Figure 2). At 3 months, the reduction in antibody titers, however, was not significant between the two groups of six patients each (Figure 2). Weekly determinations of antibodies in two patients showed that titers started to fall by the 7th day and reached a nadir within 3 weeks.

DISCUSSION

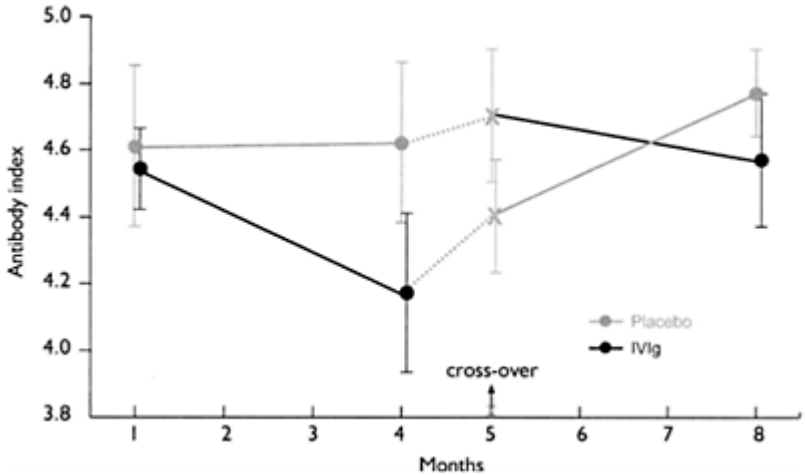
The study demonstrates that IVIG is a safe and effective therapy for patients with SPS. The conclusion is based on the objective reduction of stiffness parameters and factors of heightened sensitivity after administration of IVIG, compared with placebo, and is supported by the increased ability of the patients to perform activities of daily living.

Up to 65% of SPS patients cannot independently perform daily activities owing to total body stiffness, phobias, anxiety-triggered spasms and frequent falls[3]. Others use walkers or wheelchairs, and still others are bedridden because of severe stiffness. The results of this study show a clear improvement in all the patients' symptoms, including degree of stiffness, frequency of falls, need to walk with assistive devices, shower independently without spasms, ability to perform work or household chores and a reduced sensitivity to episodic spasms triggered by unexpected noises, tactile stimuli, fear or emotional upset.

Although the role of anti-GAD_{65} antibodies in SPS remains unclear because GAD is a cytoplasmic antigen, there is *in vitro* evidence that the patients' immunogloblin G (IgG) inhibits GAD activity and impairs GABA synthesis[9,10]. Even if the pathogenic antibody is different from GAD, as we suspect and have proposed, the end result caused by this putative antibody should be the reduction of GABA, the brain's predominant inhibitory neurotransmitter. As 25–35% of all synapses are GABAergic, a reduction of GABA due to anti-GAD65 or other related antibodies could easily explain the patients' muscle hyperactivity. The noted improvement after IVIG is consistent with the above, and supports the view that SPS is a functional rather than a structural disorder, with an ongoing immune response that impairs GABAergic transmission without effecting structural changes in the brain. Because GABA is involved in many brain circuits controlling muscle tone, autonomic responses, fear, arousal and behavior[17], an enhanced GABAergic transmission resulting from the immunoregulatory effects of IVIG on the underlying immunopathology can explain not only the reduction in muscle stiffness but also in the frequency of spasms triggered by phobias and emotional upsets.

In some of the patients, the efficacy of IVIG was short-lived (mean duration 6 weeks), as commonly seen in other autoimmune neuro-muscular disorders[14]. In five patients, however, the benefit was surprisingly long-lasting, even up to a year. The mechanism of such a long-lasting effect is unclear. Although the anti-GAD65 antibodies declined after IVIG, the titers did not correlate with disease severity and the reduction was unrelated to the magnitude of the clinical response.

Figure 2 Mean anti-glutamic acid decarboxylase (GAD65) antibody titers expressed as logarithmic antibody index in six patients after treatment with placebo (open circles) or IVIG (closed circles). The mean titers decreased after 3 months of intravenous immunoglobulin (IVIG) (but not after placebo) and rebounded significantly (p=0.03) after crossing over to placebo



This is the first proven effective therapy for SPS patients resulting in a significant improvement in their activities of daily living and quality of life. Considering that SPS, if untreated, can be serious or result in total disability, the results from the present study should be of major help to SPS patients who rarely respond adequately to diazepam or to other available agents.

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Intravenous immunoglobulin in acute disseminated encephalomyelitis

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INTRODUCTION

Acute disseminated encephalomyelitis (ADEM) is classified in the group of inflammatory demyelinating diseases of the central nervous system (CNS), and it is generally considered a complication of infections or vaccinations. The outcome can be favorable with spontaneous recovery, but pharmacological treatment is required in most cases. The best therapeutic option has not yet been established by controlled trials[1,2], but high-dose steroids are usually employed, resembling treatment of multiple sclerosis relapse. In a few cases the disease is particularly aggressive, leading to severe disability or death, in spite of early steroid administration. Although ADEM is classically considered a monophasic disease of the CNS, it is now accepted that both disseminated and site-restricted ADEM variants[3] could show a multiphasic or chronic course[4,5], and could be complicated by severe impairment of the peripheral nervous system. In these cases a chronic or 'pulsed' pharmacological treatment could be required, but treatment choice is difficult as chronic steroids are often contraindicated and other immunosuppressive drugs, such as azathioprine and cyclophosphamide, are not advisable owing to severe toxicity and poor effectiveness. Further options are, at present, not available. During the past decade some authors have reported positive results following intravenous immunoglobulin (IVIG) administration as a second-line treatment in both pediatric and adult series of steroid-resistant ADEM cases[6–12].

During the period from January 1998 to December 2002 we observed 49 adult patients affected by post-infectious encephalomyelitis (data partially published in reference 13). Twenty-nine of them (24 monophasic and five with the recurrent variant) had a good recovery after high-dose steroid treatment. One patient, affected by seizures and tetraplegia due to multifocal encephalomyelitic lesions, died 1 month after disease onset from systemic complications. We describe here the other 19 patients, 14 of them having a monophasic course and the other five the recurrent ADEM variant (ARDEM), who were treated with high-dose IVIG after inadequate response to steroids.

PATIENTS

The clinical and paraclinical findings, during the acute stage, of all 49 patients are given in Table 1. Seventeen patients (34.6%) underwent IVIG administration owing to steroid

failure. Twelve of them (70.5 %), showed a good recovery (Table 2). We describe here, in more detail, six patients showing a peculiar clinical course (Table 3).

Case A

A 71-year-old man developed headache, confusion, weakness of the four limbs and urinary retention 8 days after a brief febrile illness. On the first day of hospitalization the neurological examination revealed consciousness impairment, rigor and tetraparesis, more pronounced in the lower limbs, with depressed deep tendon reflexes, bilateral Babinski sign and urinary retention (Scripps Neurological Scale, SNS: 20/100). Routine laboratory testing and serum and cerebrospinal fluid (CSF) antibody titers of Epstein—Barr virus, human immunodeficiency-1 and -2 virus, cytomegalovirus, herpes virus types 1 and 2, and *Borrelia burgdoferi* were normal. CSF examination revealed mildly increased albumin (78mg/dl) and immunoglobulin G (IgG) content (10mg/dl), and lymphocytic pleocytosis (35cells/mm^3). Brain magnetic resonance imaging (MRI) was normal, while spinal cord MRI revealed a vast area of hyperintensity on T2-weighted images, extending C2—C6, with contrast enhancement (see Table 3). On the second and third days after admission, patient consciousness worsened and nystagmus occurred. A further brain MRI was still normal. The patient was treated with high-dose 6-methylprednisolone (6-MP),1g/day for 6 days, followed by oral prednisone (PN), 100mg/day for 10 days, tapered over 15 days. Despite an improvement in consciousness impairment and meningeal signs, no significant recovery of motor functions occurred. Two months later, after the end of steroid treatment, the patient was still tetraparetic and unable to walk (SNS: 45/100). At this time, a further CSF examination revealed persistence of raised inflammatory parameters. Owing to the ineffectiveness of the previous steroid cycle the patient was administered a 5-day course of IVIG (400mg/kg/day). Within 4 days of administration, strength improved. Two weeks later he could stand and walk without assistance, although bladder function did not significantly improve (SNS: 86/100). One year later, 10 days following an anti-flu vaccination, he had a relapse characterized by sudden strength worsening of the lower limbs. A new IVIG 5-day cycle was administered, with recovery of autonomous gait within 10 days.

Case B

A week after the occurrence of fever, diarrhea and vomiting, a 63-year-old woman was hospitalized for sudden-onset paraplegia and urinary retention. Neurological examination revealed flaccid areflexic paraplegia with bilateral Babinski sign, and D10 sensory deficit level for all modalities (SNS: 46/100). Routine laboratory and virological testing as listed above was normal. MRI of the spinal cord showed multifocal T2-weighted signal hyperintensities at D5—D9 level, without enhancement (see Table 3). The patient received high-dose 6-MP followed by oral PN tapered over 30 days, with

Table 1 Main features of whole acute disseminated encephalomyelitis (ADEM) group according to clinical syndromes

	Encephalitis	Encephalomyelitis	Myelitis	Encephalomyeloradiculitis	Myeloradiculitis	Total
n (M:F)	10 (4:6)	7 (2:5)	11 (6:5)	13 (5:8)	8 (4:4)	49 (21:28)
Mean age (SD) (years)	38.2 (16.71)	49.43 (19.86)	44.82 (19.2)	51.62 (16.85)	64.38 (12.76)	49.12 (18.53)
Relapses	0/10	3/7	2/11	4/13	5/8	14/49 (28%)
CSF (barrier damage)	5/10	7/7	6/11	12/13	6/8	36/49 (73%)
CSF (oligoclonal bands)	0/10	0/7	0/11	2/13 (both serum and CSF)	0/8	2/49 (4%)
MRI (brain or spinal cord T_2 hyperintensities)	8/10	5/7	10/11	10/13	7/8	40/49 (81%)
MRI (brain or spinal cord Gd enhancement)	7/8	4/5	4/10	8/10	5/7	28/40 (70%)
Steroid effectiveness*	8/10	4/7	9/11	6/13	2/7	29/49 (63%)
IVIG effectiveness*	—	3/3	2/2	4/8	5/6	14/19 (73.6%)

M, male; F, female; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; IVIG, intravenous immunoglobulin *at the first episode

Table 2 Main features of patients who underwent intravenous immunoglobulin (IVIG) treatment according to clinical syndromes

	Encephalitis	Encephalomyelitis	Myelitis	Encephalomyeloradiculitis	Myeloradiculitis	Total
n (M: F)	0	3 (2:1)	2 (1:1)	8 (4:4)	6 (2:4)	19 (9:10)
Mean age (SD) (years)	—	65	—	54.33 (12.35)	66.41 (11.52)	57.85 (19.32)
Relapses	—	2/3	—	2/8	1/6	5/19 (26.3%)
CSF (barrier damage)	—	3/3	1/2	8/8	6/6	18/19 (94.7%)
CSF (oligoclonal bands)	—	1/3 (both serum and CSF)	—	1/8 (both serum and CSF)	0/6	2/19 (10.5%)
MRI (brain or spinal cord T2 hyperintensities)	—	3/3	2/2	8/8	6/6	19/19 (100%)
MRI (brain or	—	0/3	1/3	7/8	6/6	14/19

spinal cord Gd enhancement)						(73.7%)
Steroid effectiveness	—	0/3	0/2	2/8 (incomplete)	0/6	2/19 (10.5%)
IVIG effectiveness	—	3/3	2/2	4/8	5/6	14/19 (73.7%)

M, male; F, female; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging

Table 3 Clinical, cerebrospinal fluid (CSF) and instrumental findings during acute stage (cases A—F)

					CSF findings			MRI: T2-weighted hyperintensities	
Case (age)	Clinical syndrome	Episode	VEP	SSEP: upper limbs/lower limbs	Cells (*n*/mm3)	Proteins (mg/dl)	Oligoclonal bands	Brain	Spinal cord
Patient A (71 years)	EM	I	normal	central delay/ central delay	35	78.3	4 (in both CSF and serum)	absent	C2–C6
	M	II	NE	NE	55	76.2	absent	absent	absent
Patient B (63 years)	M	I	normal	normal/ central delay	20	<30	absent	absent	D5–D9
Patient C (54 years)	EM	I	normal	central delay/ central delay	25	272	absent	bihemispheric, subcortical	absent
	M	II	normal	NE	23	105	absent	absent	D7
Patient (46 years)	DM	I	normal	central delay/ central delay	190	67.5	absent	absent	absent
Patient E (45 years)	EM	I	normal	central delay/ central delay	125	116	absent	bihemispheric, subcortical	D8–D10
Patient F (47 years)	EMRN	I	normal	central delay	3	34	2 (in both CSF and serum)	pons, basal ganglia, temporal lobe	T1–T4
	MRN	III	normal	central delay	3	70	absent	reduced	T1–T4

Glucose level was normal in all cases; M, myelitis; EM, encephalomyelitis; EMRN, encephalomyeloradiculoneuritis; MRN, myeloradiculoneuritis; VEP, pattern reversal visual evoked potential (30-min and 15-min checks); SSEP, somatosensory evoked potential by median and tibial nerve stimulation (central delay: increase in N13–N20 or N22–P40 times); CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; NE, not executed

no benefits. Then she received an IVIG 5-day cycle (400mg/kg). Within a few days she obtained rapid improvement of strength and sensory deficits (SNS: 76/100). One month later she was able to walk with a stick, while bladder dysfunction recovery was delayed and incomplete (SNS: 85/100 at 1 year).

Case C

Ten days after a bout of fever, a 54-year-old man developed confusion, myoclonic jerks, inco-ordination of the four limbs and ataxic gait. During hospitalization his condition worsened until he became comatose and tetraplegic (SNS: 20/100). Routine and virological testing results were normal. Brain MRI showed diffuse bihemispheric T2-weighted hyperintensities with mild contrast enhancement, while spinal cord MRI was normal. He was initially treated with intravenous 6-MP (1g/day for 6 days) followed by oral PN (1mg/kg) tapered over 30 days. Despite successful recovery of his mental status and co-ordination, limb strength only partially improved (SNS: 54/100). Two months after steroid discontinuation he developed fever, bilateral visual blurring, flaccid tetraparesis and urinary retention (SNS: 30/100). Lumbar puncture showed lymphocytic pleocytosis and mildly increased protein concentration. Brain MRI was substantially unchanged compared to the previous one, while spinal MRI showed a T2-weighted hyperintensity at the D7 level. A further high-dose 6-MP cycle was ineffective. Therefore, we administered IVIG, 400 mg/day, for 5 days. Within a few days, upper limb strength completely recovered and lower limb strength significantly improved, until he was able to walk with a stick (SNS: 70/100). Bladder dysfunction did not improve.

Case D

A 46-year-old man presented with subacute paraplegia and lower limb anesthesia at the L1 upper level. Neurological dysfunction occurred a few days after a febrile illness with headache, flu and cough, and neurological examination showed a spastic paraplegia, left Babinski sign, bilateral Oppenheim sign, L1 sensory level for all modalities and severe bladder dysfunction (SNS: 52/100). MRI of the brain and spinal cord was normal. He was started on a high-dose intravenous 6-MP cycle followed by a 1-month PN course (doses as above) with mild benefit. A few days after steroid discontinuation, lower limb strength rapidly worsened, leading to paraplegia; he failed to improve after further administration of a 6-MP course. After 1 month, while his neurological dysfunction appeared stabilized, he underwent a 5-day cycle of IVIG, 400mg/kg/day, thus obtaining, within a few days, a marked improvement of lower limb strength (SNS: 85/100). One month later he recovered autonomous walking.

Case E

A 45-year-old woman, 4 days after the occurrence of a mild fever, acutely developed lower limb strength deficit. Neurological examination revealed severe spastic paraparesis prominent in the left lower limb, bilateral Babinski sign, D9 sensory deficit level for all modalities, stypsis and urinary retention (SNS: 62/100). Routine laboratory and virological tests, as listed above, were normal. Brain MRI detected three T2-weighted

hyperintensity lesions in the white matter of both hemispheres. Spinal MRI showed a D8–D10 T2 signal hyperintensity with contrast enhancement. CSF examination showed lymphocytic pleocytosis and raised protein content (see Table 3). A 6-MP cycle did not significantly modify the clinical picture, which remained unchanged 20 days following the onset of neurological symptoms. The patient underwent a 5-day cycle of IVIG, 400mg/kg/day, following which we could observe a rapidly progressive improvement of her muscular strength, together with spasticity reduction and partial resolution of sensitive dysfunction. Seven days after treatment the patient was able to walk with a stick and she further improved during a 2-month follow-up. Bladder dysfunction persisted (SNS: 85/100). The disease did not recur over a 1-year follow-up.

Case F

A 47-year-old woman developed diplopia and lower limb paresthesias 10 days after an upper respiratory tract infection characterized by a productive cough, without fever. Neurological examination revealed right sixth cranial nerve palsy and moderate spastic paraparesis with D10 upper sensory level (SNS: 60/100). Within the first day of hospitalization, she developed additional higher CNS dysfunction characterized by recent memory impairment and disorientation, rapidly progressing to mild coma. Brain MRI revealed multiple areas of hyperintensity on T2-weighted images, involving the pons, bilateral basal ganglia and left temporal lobe, with focal areas of mild enhancement. Spinal cord MRI revealed an area of T2 hyperintensity within the spinal cord, T1–T4, enhanced following contrast administration. An identical pattern of oligoclonal bands was detected in both CSF and serum; CSF examination was otherwise normal, as were other laboratory parameters and virological screening. Visual evoked potentials were normal. She was administered a 6-MP cycle, thus obtaining a significant improvement (SNS: 90/100). One year later she developed a new episode of paraparesis with acute onset; spinal cord MRI findings were identical to those observed during the previous episode, while brain MRI revealed almost complete disappearance of the previously observed multifocal lesions. CSF findings showed mildly increased albumin, without oligoclonal bands. Visual evoked potentials were still normal. Again, she obtained satisfactory clinical improvement following a 6-MP cycle (SNS: 86/100). Sixteen months later, she developed a third episode retracing the second one in both clinical and instrumental findings (see Table 3). On this occasion, the usual steroid course was ineffective, and she was administered IVIG 0.4g/day for 5 days. Within the second day of administration, motor dysfunction began to improve until she could walk without assistance after about 2 months.

DISCUSSION

Despite the often dramatic presentation, ADEM usually shows a benign course with complete recovery. However, data in the literature indicate that, in a subset of patients, severe disability or death[14] may occur, owing to steroid failure. In our ADEM group, as many as 40.8% (20/49) of patients failed to improve after steroid treatment; of these, 14/19 (73.7%) showed a good recovery following IVIG administration. Steroid

ineffectiveness seems more likely to occur in post-infectious syndromes involving both central and peripheral nervous systems (60% of myeloradiculoneuritis and encephalomyeloradiculoneuritis). In our patients the diagnosis of ADEM, suggested by the clinical context, was supported by biochemical and instrumental evidence: cases A, C, E and F were affected by typical disseminated encephalomyelitis, while cases B and D presented with isolated post-infectious myelitis, which is now recognized to be a site-restricted ADEM variant[3]. In two patients, high-dose steroids lost their effectiveness, respectively, during the second (patient C) and the third episode (patient F). In the other patients steroid treatment was substantially ineffective, while IVIG administration was followed within a few days by marked functional improvement both in patients treated during the acute phase of the illness, and in patients treated in the context of an apparently stabilized disability. Timing and duration of functional recovery seem unlikely to be spontaneous. Case A is particularly notable, as he received IVIG after 2 months of severe motor impairment and, within 2 weeks, he recovered an autonomous gait. One year later, during a relapse, he obtained the same favorable effect following IVIG treatment, on this occasion without a previous course of steroids. This further supports the effectiveness of IVIG alone, although in some cases a synergistic action of steroids cannot be ruled out.

Patients A, C and F had one or two relapses. Recurrent variants of ADEM have been described in previous reports[4,5]. Khan and colleagues distinguished these cases as ‘multiphasic disseminated encephalomyelitis’ or ‘recurrent encephalomyelitis’, depending on the distribution of lesions during the relapse, compared with those observed at the first episode. The clinical and laboratory findings observed in our relapsing cases suggest the diagnosis of ‘recurrent ADEM' (ARDEM), rather than of multiple sclerosis (MS): the age of onset is unusual for MS; CSF examination showed blood—brain barrier damage without intrathecal oligoclonal bands; and the visual evoked potential was normal in all cases. Therefore, the criteria for MS diagnosis are not fulfilled. Moreover, all patients had a fever during the disease onset, and in patient A the relapse followed an anti-flu vaccination.

In all patients, IVIG therapeutic effects started during the 5 days of drug administration and reached a maximum within 3 weeks. Clinical results are also supported by the improvement of post-treatment MRI findings. Also, we emphasize IVIG treatment effectiveness in post-infectious myelitic syndromes, which frequently show poor prognosis[15]. The usefulness of IVIG in CNS demyelinating diseases has been shown in MS[16] and in ADEM[6,7]. Experimental data suggest that IVIG may modulate the local immune reaction in CNS inflammatory diseases via regulation of nitric oxide production and microglial functions[17]. Other IVIG modes of action have to be considered. They include supply of idiotypic antibodies with a wide spectrum of idiotypic—anti-idiotypic specificity, which have the potential of binding and neutralizing pathogenic antibodies and of suppressing autoantibody production[18,19].

Interpretation of our results should be made cautiously as the study was neither randomized nor controlled, but we believe that the timing and duration of functional recovery in these patients seem unlikely to be spontaneous. Unfortunately, the application of an adequate study design in this field raises serious difficulties owing to the intrinsic features of ADEM. However, our findings in adults, together with the recently reported

positive results in small pediatric series[6,7], suggest the usefulness of IVIG as second-line treatment in steroid-resistant ADEM.

CONCLUSIONS

(1) In our ADEM group 30.6% (15/49) of patients failed to improve after steroid treatment and 60% of these (9/15) showed a good recovery following IVIG administration.
(2) Steroid ineffectiveness seems to be more frequent in post-infectious syndromes involving both central and peripheral nervous systems (60% of myeloradiculoneuritis plus encephalomyeloradiculoneuritis).
(3) IVIG therapeutic effects started during the 5 days of drug administration and reached a maximum within 3 weeks. Functional improvement was observed both in patients treated during the acute phase of the illness and in patients with a steady disability.
(4) Our results need to be interpreted with caution as the study was neither randomized nor controlled, although we maintain that the timing and duration of functional recovery in these patients seem unlikely to be spontaneous.

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The use of intravenous immunoglobulin in dermatology

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Intravenous immunoglobulin in dermatology

S.Jolles

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Intravenous immunoglobulin (IVIG) has been used as an immunomodulator in a range of specialties including hematology, neurology, rheumatology and, more recently, dermatology[1]. Dermatological conditions treated with high-dose IVIG represent a small but expanding area encompassing autoantibody-mediated disorders (e.g. blistering diseases) as well as diseases characterized by cellular and complement-mediated pathogenesis (e.g. dermatomyositis) (Table 1). Dermatological disorders have contributed considerably to our understanding of the mechanism of action of IVIG[2], and in view of the ready access to skin can be easily documented photographically or by established scoring systems as well as by biopsy. There has been the perception that dermatological conditions are trivial and not life-threatening, and this has led to these conditions sometimes being given a lower priority than other diseases. It is clear, however, that indications such as toxic epidermal necrolysis (TEN) have a mortality of between 25 and 35%, and that other conditions have a major impact on the quality of life of the sufferers. The main indications for the use of high-dose IVIG (outside conditions such as Kawasaki syndrome

Table 1 The main current uses of high-dose intravenous immunoglobulin (hd IVIG) in dermatology

Condition	Number of reports (Medline)
Kawasaki syndrome	242
Dermatomyositis	80
Pemphigus vulgaris	28
Epidermolysis bullosa acquisita	13
Atopic dermatitis	18
Bullous pemphigoid	13
Toxic epidermal necrolysis	24
Chronic urticaria	7

Mucous membrane pemphigoid	9
Gestational pemphigoid	2
Linear IgA disease	2
Pyoderma gangrenosum	6
Scleromyxedema	3
Erythema multiforme	4
Pretibial myxedema	2
Psoriasis	1

IgA, immunoglobulin A

and TEN, where treatment is generally a single cycle) are treatment-resistant disease or unacceptable side-effects from conventional therapy[3].

Our knowledge regarding the mechanisms of action of high-dose IVIG has moved forward greatly in the past few years, and this has helped in understanding not only the complexity of IVIG, but also the pathogenesis of the conditions being treated. IVIG may for clarity be considered to have four separate mechanistic components:

(1) Actions mediated by the antibody variable regions F(ab‘)2;
(2) Actions of the Fc fragment on a range of Fc receptors (FcR);
(3) Actions mediated by complement binding within the Fc fragment;
(4) Immunomodulatory substances other than antibody in the IVIG preparations.

It is likely that these components act concurrently, and different mechanisms may be important in different settings. A summary of the mechanisms of action is given in Table 2[4]. Taking blistering diseases as an example, although the precise mechanisms of action are not understood it is likely that effects on the production, catabolism and effector functions of the autoantibodies involved play a major role, and it has been established in numerous studies that high-dose IVIG reduces the titer of autoantibodies.

The numbers of patients in most of the conditions listed are small, and there are few properly performed controlled studies outside Kawasaki syndrome and dermatomyositis. The data from these largely uncontrolled and heterogeneous studies must be interpreted with caution in view of the likely reporting bias for favorable outcomes, differences in IVIG preparations, dosing schedules, use of concurrent therapy, severity of disease and previous exposure to immunosuppressive agents.

Taking as a whole all patients treated for dermatological indications, the success rate for IVIG as monotherapy is approximately 40%, whereas when used adjunctively this increases to 80%. This raises the question of the choice of agent to combine with IVIG, and there is currently no clear answer. There are insufficient data to choose the other agents, although steroids, in view of their synergy with IVIG, and mycophenolate, because of its effects on B cells, are potential candidates. It will clearly be of importance to establish the role of the newer biologicals such as rituximab in some of the dermatological disorders.

Trial design is important for those wishing to treat a single patient, as well as in the context of controlled studies. Patients must be carefully selected, with clear documentation of disease and end-points, and with the prior understanding and agreement that should the end-points not be reached, therapy with IVIG will be discontinued.

Stratification of disease severity is vital as this allows the comparison of studies through established scoring systems. For most chronic conditions, the current evidence would support the use of 2g/kg/month of IVIG used adjunctively and given for 3–4 cycles, before a clear assessment of efficacy can be made. In terms of larger studies it is important to ensure that there is the statistical power to yield an unambiguous answer, and this may involve multicenter studies in view of the rarity of some of the conditions, in addition to reducing bias.

Any assessment of the pharmacoeconomics of the use of high-dose IVIG in dermatology must take into account that only those patients

Table 2 Immunomodulatory actions of intravenous immunoglobulin (IVIG)

Immunomodulatory category	Effects
Effects due to F(ab‘)2	antiproliferative effects
	modulation of apoptosis and cell cycle
	activation of specific cells
	effects on cell adhesion
	antibodies to pathogens and superantigens
	anti-idiotypes
	antibodies to immunoregulatory molecules (cytokines, TCR, CD4, CD5)
	effects on cytokine levels
	modulation of matrix metalloproteinases
Effects due to Fc receptors	inhibition of phagocytosis
	inhibition of ADCC
	effects on antibody production and recycling
	effects on glucocorticoid receptor binding affinity
	modulation of DC maturation
Effects due to complement—Fc binding	inhibition of deposition of activated complement
Effects due to substances other than	IVIG contains cytokines, cytokine

antibody within IVIG	receptors, CD4, MHC class II and stabilizing agents, mainly sugars

TCR, T-cell receptor; ADCC, antibody-dependent cell cytotoxicity; DC, dendritic cell; MHC, major histocompatibility complex

with severe treatment-resistant disease or unacceptable side-effects are likely to be selected for therapy, and the impact of the disease on quality of life for this small subset of patients will be very different from that of the disease as a whole.

What does the future hold for high-dose IVIG in dermatological disorders? It seems that, with the increased numbers of reported patients (especially in the blistering diseases) with successful outcomes, a 'critical mass' has been reached to justify double-blind, placebo controlled, randomized, multicenter studies to define clearly the role of IVIG, an increasingly expensive and scarce resource, which needs to be used appropriately. It will be critical to extend the evidence base in a range of dermatological conditions to guide prescribing of IVIG where appropriate. Second, further understanding of the optimization of current therapy in terms of dose, number of cycles required and route of administration (e.g. subcutaneous immunoglobulin to deliver high concentrations to localized lesions in conditions such as pyoderma gangrenosum) is needed. In the longer term, information learned about the pathogenesis of disease and the mechanism of action of high-dose IVIG may allow the development of cheaper and more specific treatments.

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The use of intravenous immunoglobulin therapy in of autoimmune mucocutaneous blistering diesases

A.R.Ahmed

INTRODUCTION

This chapter focuses on the use of intravenous immunoglobulin (IVIG) in the treatment of autoimmune mucocutaneous blistering diseases (AMBDs). In order to avoid speculation and controversy, only published data on these clinical entities are discussed. The principal contents of this chapter are the published experiences of treating 125 patients suffering from AMBDs with IVIG, at one center in the USA.

AMBDs are a group of uncommon diseases that have several features in common[1]. Three of the most important and relevant features are:

(1) Involvement of the skin and one or more mucous membranes derived from the stratified squamous epithelium; (2) An autoimmune basis for pathogenesis;
(3) Consequences and sequelae which frequently result in a poor quality of life and which can sometimes cause death.

During the past four decades, the advocated conventional therapy for treating these diseases has been the use of high-dose corticosteroids, in conjunction with an immunosuppressive agent[2]. Such therapies can result in several side-effects, some reversible, others not. Another concerning consequence of long-term immunosuppression is the subsequent development of malignancies.

INDICATIONS FOR USE

Indications for the use of IVIG in the treatment of the above patients were:

(1) Failure of conventional therapy;
(2) Significant adverse effects;
(3) Contraindications to the use of conventional therapy;
(4) Rapidly progressing and debilitating disease;
(5) The age of the patient, and pregnancy.

PROTOCOL

The same protocol was used to treat all patients. The published experience recommended that a dose of 2g/kg/cycle would be optimal and necessary to produce the expected clinical results. A cycle would consist of the total dose of IVIG, divided into three equal doses, given on three consecutive days. The infusion was given slowly over 4–4.5h.

FREQUENCY

The initial frequency was one cycle every 3–4 weeks. In patients with aggressive ocular cicatricial pemphigoid, the infusion was given every 2 weeks. Once clinical control of the disease was achieved, as defined by a lack of new lesions for 3 weeks and significant healing of existing lesions, the frequency was reduced. The interval between infusions was increased to 6 weeks, then 8, 10, 12, 14 and 16 weeks. If at a 16-week interval the disease continued to remain in clinical remission, it was considered the end-point of the therapy.

OUTCOME PARAMETERS

To evaluate objectively the clinical efficacy and benefit of using IVIG in the 125 patients from the published cases, the following parameters were used. In all patients, prior to giving IVIG, the duration of the disease, the dose and duration of prednisone used, the dose and duration of immunosuppressive agents used, the side-effects of these drugs, and the number of hospitalizations and duration of each hospitalization were recorded. The quality of life owing to the disease and the side-effects of the drugs, as assessed by each individual patient prior to IVIG therapy, was documented.

Once IVIG was initiated, tapering and eventual discontinuation of prednisone and immunosuppressive agents was done. In all 125 patients, IVIG was then used as monotherapy. After initiation of IVIG therapy, the doses and durations of prednisone and immunosuppressive agents were recorded. Regarding the IVIG therapy, the total number of cycles, duration of therapy and any side-effects were recorded. The length of follow-up after discontinuation of IVIG therapy and the quality of life resulting from its use were noted.

These objective outcome criteria clearly indicated that, in all of these patients, there were statistically significant differences between pre- and post-IVIG indices. IVIG was successful in bringing about clinical control of the disease. Prednisone and immunosuppressive agents could be gradually tapered and discontinued. The number of relapses and recurrences was significantly less; disease progression was arrested; in these patients, IVIG induced long-term sustained clinical remission.

RESULTS OF USE OF IVIG IN AUTOIMMUNE MUCOCUTANEOUS BLISTERING DISEASES

A summary of these results is presented in Table 1.

Pemphigus vulgaris

In a review of 21 patients with severe pemphigus, 81% showed clinical improvement and in their ability to undergo reduced systemic immunosuppressive therapy. A lack

Table 1 Data on 125 patients with autoimmune mucocutaneous blistering diseases treated with intravenous immunoglobulin (IVIG). Values are expressed as mean (range)

Study	Patients *(n)*	Duration of disease prior to IVIG (months)	Duration of prednisone therapy (months)	Duration of immuno-suppressive therapy (months)
Pemphigus vulgaris				
Ahmed[3], 2001	21	35.9(12–96)	15.8 (3−48)	24.9 (3–48)
Sami *et al.*[4] 2002	15	16.7 (8–23)	19.9 (4–60)	not applicable
Pemphigus foliaceus				
Ahmed and Sami[5], 2002	11	29.5 (16–45)	25.6(14–40)	36.5 (18–72)
Sami et al.[6], 2001		30.4 (2–72)	22.7 (12–34)	37.0 (14–39)
Sami *et al.*[7] 2002		34.3 (14–39)	10.6 (3–24)	not applicable
Bullous pemphigoid				
Ahmed[8], 2001	15	28.3 (10–90)	8.3 (1–24)	8.5 (4–20)
Mucous membrane pemphigoid				
Foster and Ahmed[9], 1999	10	81.6 (36–168)	36.7 (29–76)	70 (29–87)
Ahmed and	8	12.2 (4–	14.7 (10–	23.4 (16–37)

Colon[10], 2001		22)	42)	
Sami *et al.*[11], 2002	7	10.6 (7–15)	not applicable	not applicable
Sami *et al.*[12], 2002	15	48.5 (18–67)	32.5 (10–72)	40 (22–52)
Letko *et al.*[13], 2003	8	26.3 (16–35)	not applicable	not applicable
Overall	125	32.8 (2–168)	20.8 (1–76)	34.4 (3–87)

of response was attributed to an inadequate dose of IVIG, used for brief periods[14].

In a study of 21 patients with severe pemphigus who had not responded to high doses of systemic corticosteroids and immunosuppressive agents, IVIG produced prolonged clinical remission, which was sustained after the IVIG was discontinued[3]. In another study of 15 patients who failed to respond to high-dose systemic corticosteroids and in whom the use of immunosuppressive agents was contraindicated, IVIG produced sustained clinical remission[4]. Recently, Bystryn and colleagues treated six patients with IVIG[15]. Their patients were simultaneously treated with cyclophosphamide and prednisone. The follow-up was limited. A dramatic clinical response was observed, and the dose of systemic corticosteroids was reduced by 35%. In summary, 58 patients with severe pemphigus vulgaris, when treated with IVIG, demonstrated significant clinical improvement.

Pemphigus foliaceus

In the literature, 26 patients with pemphigus foliaceus, resistant to conventional therapy were successfully treated with IVIG. In one study 11 patients, and in a second study eight patients with widespread pemphigus foliaceus, non-responsive to high-dose prednisone and immunosuppressive agents, achieved sustained clinical remission when treated with IVIG[5,6,15]. In addition, seven patients demonstrated a significant steroid-sparing effect[7].

Bullous pemphigoid

In a review of 17 patients with bullous pemphigoid treated with different protocols of IVIG, 70% responded favorably. Those who did not respond had received lower dosages and a single dose[16].

In a series of 15 patients with recurrent bullous pemphigoid who were failures on conventional therapy, treatment with IVIG as monotherapy, using a prescribed protocol, resulted in long-term clinical remission[8].

Thus, in 27 of the 32 cases reported in the literature, a significant response with IVIG was noted.

Mucous membrane pemphigoid (cicatricial pemphigoid)

In the first published study, IVIG was used to treat ten patients with severe and progressive ocular pemphigoid[9]. These patients were blind in one eye, and vision in the second eye was rapidly deteriorating. Hence, the principal objective of using IVIG was to prevent any further deterioration. Significant stabilization of the disease process occurred, and the patients were able to retain their vision. In a series of 15 patients with severe mucous membrane pemphigoid involving several mucosal surfaces non-responsive to conventional therapy, IVIG resulted in prolonged and sustained clinical remission[12].

Twenty patients with severe oral pemphigoid, in whom dapsone could not be continued because of side-effects, were divided into two groups[10]. Eight patients received only IVIG. Twelve patients were treated with conventional therapy using immuno-suppressive agents and prednisone. When the two groups were compared, the patients who had received IVIG went into long-term clinical remission and did not have additional mucosal surfaces involved. In contrast, the patients treated with conventional therapy had involvement of additional mucosal surfaces and only 50% achieved clinical remission.

In a recently completed study of 16 patients who had disease involving other mucosal surfaces, the indication for the use of IVIG, was new involvement of the eye[13]. Eight of the 16 patients were treated with IVIG. Eight continued to be treated with changes in immuno-suppressive agents and prednisone. A 5-year follow-up demonstrated that patients who had received IVIG did not show any progression of eye disease, notably scarring or deterioration of vision. In contrast, patients on conventional therapy had marked scarring.

In a study of seven patients with severe oral pemphigoid who did not respond to dapsone and in whom systemic corticosteroids and immunosuppressives were contraindicated, IVIG produced long-term clinical remission which was sustained after the discontinuation of IVIG therapy[11].

Thus, in 48 patients with severe mucous membrane pemphigoid, IVIG has been of significant benefit. IVIG produced clinical remission, and arrested progression of the disease to involve new sites. It also prevented scarring.

Epidermolysis bullosa acquisita

In a recent review of the literature, nine patients with epidermolysis bullosa acquisita (EBA), resistant and non-responsive to other therapies, were treated with IVIG[17]. It produced significant improvement without any adverse side-effects. Since the available therapies for EBA are limited and produce significant side-effects, it is recommended that IVIG should be the drug of choice and first-line therapy in patients with severe EBA, especially those in whom multiple mucosal surfaces are involved and scarring has been initiated, or the potential threat of scarring exists.

MECHANISM OF ACTION

The exact mechanism of action of IVIG in the treatment of AMBDs is not completely understood[18]. Here, only the mechanism of action relevant to autoimmune mucocutaneous blistering diseases is discussed. In the experience of the author, the mechanism by which IVIG works could be in two phases. There is an early response and a delayed or late response. Studies have demonstrated that patients with pemphigus vulgaris and mucous membrane pemhigoid have high serum levels of interleukin (IL-1, α and β) and lower levels of IL-1 receptor antagonist (IL-1RA), before beginning IVIG treatment[19,20]. Once IVIG therapy has been initiated, this ratio is reversed, and it is observed that serum levels of IL-1 decrease, while those of IL-IRA increase. In these patients, peripheral blood leukocytes (PBLs) before IVIG therapy were found to produce high levels of IL-1 and low levels of IL-1RA. When IVIG was added to the PBL cultures, the levels of IL-1 decreased, while those of IL-1RA were increased. Titers of the pathogenic antibody were measured in patients from initiation of IVIG therapy for a period of 18–24 months. In patients with pemphigus vulgaris and pemphigus foliaceus, antibodies to desmoglein-1 and desmoglein-3 were measured[21,22]. In patients with bullous pemphigoid, antibodies to BPAg1 and BPAg2 were measured[23]. In patients with ocular cicatricial pemphigoid, antibodies to human β4 integrin were measured[24]. In all three disease entities, there was an initial high level of the antibody. Thereafter, there was a very slow decline in the antibody titer, until it became negative or non-detectable. For the remainder of the observation, these antibody titers continued to remain undetectable. The fall in autoantibody titers paralleled clinical improvement. The author is aware that these are very preliminary observations and that additional studies need to be done to understand the complete and exact mechanism of action of IVIG in AMBDs.

FUTURE GOALS

The existing data have been beneficial in convincing the US Department of Health and Human Services, Center for Medicare and Medicaid Services, to announce a policy that approves the use of IVIG in the treatment of AMBDs. Recently a Consensus Development Group consisting of physicians from several specialties, from the USA, Canada and Europe, met to produce a consensus statement on the use of IVIG in the treatment of AMBDs[25].

Thus, there is emerging acceptance of IVIG as an option to treat patients with AMBDs, especially those in whom conventional therapy has been a failure, has produced significant side-effects or is contraindicated. Future directions include defining those patient populations in whom the response to IVIG is less than optimal, and the use of additional agents to boost this response to IVIG. The available data and experience would indicate that a multicenter trial is warranted. Such a study would help further define several parameters of therapy, including the optimal protocol for, side-effects of and contraindications to the use of IVIG, in these potentially life-threatening diseases.

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Treatment of atopic dermatitis, urticaria and psoriasis with intravenous immunoglobulin

M.Rustin

ATOPIC DERMATITIS

Atopic dermatitis (AD) is the most common inflammatory skin disease, affecting 20% of children and up to 4% of adults. The pathogenesis is multifactorial with a dominant genetic component, but in addition having environmental, bacterial, dietary, epidermal barrier and immunological elements. Since 1952, topical corticosteroids, have been the mainstay of treating the inflammatory component of the disease. Attempts have been made to improve receptor targeting of corticosteroids, but there has been no escape from the link between increased potency and side-effects. The availability of tacrolimus ointment and pimecrolimus cream for moderate to severe and mild to moderate AD, respectively, has revolutionized the topical treatment of this disease[1,2]. Tacrolimus ointment is as efficacious as potent topical corticosteroids, whereas pimecrolimus cream is as efficacious as mild to moderately potent topical corticosteroids[3]. Neither treatment produces cutaneous atrophy, striae or pigmentary changes, and they can be used on the face without fear of inducing glaucoma. These treatments will probably reduce the need for systemic therapy, but for those patients with resistant disease, treatment with phototherapy (ultraviolet B (UVB), UVA1, psoralen and ultraviolet A (PUVA)), cyclosporin, mycophenolate or azathioprine would be appropriate. Nevertheless, there are still patients with recalcitrant disease, and intravenous immunoglobulin (IVIG) has been reported to be beneficial in case reports and clinical trials.

Kimata first described the benefits of IVIG when an incidental improvement in severe AD was noted in four children treated with IVIG (0.4g/kg/day for 5 days) as sole therapy of Kawasaki disease and idiopathic thrombocytopenic purpura (ITP)[4]. All patients experienced at least a 50% improvement in their AD by day 7 and near complete remission by day 14. Improvement was noted in skin scores and sleep patterns, and a decrease in immunoglobulin E (IgE) level, *in vitro* spontaneous IgE production and eosinophil count was recorded. At 6 months the two patients with Kawasaki disease had no further treatment requirements for their AD, and similarly at 1 year the two patients with ITP were no longer reliant on treatment (Table 1).

An open study of three patients with severe AD treated with IVIG 2g/kg/month for 6 months demonstrated an improvement in symptom scores and a reduction in pruritus but no change in serum IgE levels[5]. Cessation of the IVIG at 6 months was associated with a recurrence of symptoms which disappeared on reinstitution of the IVIG.

A case report of an 8-month-old having Wiskott—Aldrich syndrome and AD showed no benefit with one cycle of IVIG 1g/kg[6].

A further open study in which nine patients with AD and one patient with hyper-IgE syndrome were treated with IVIG 2g/kg monthly for 7 months revealed no statistically significant improvement in skin scores but skin scores did improve slightly in six patients, were unchanged in two and worsened in one[7]. One patient was withdrawn because of a serum sickness-type reaction. There were no changes in IgE levels, IgE synthesis or other measures of immunological function.

A larger, open, uncontrolled study in which 41 patients with AD received different amounts of IVIG depending upon their weight (<30kg received 500mg/kg, >30kg received 15g) showed a significant improvement at 7 and 21 days post-treatment[8]. There was an associated reduction in serum IgE levels but no change in eosinophil counts.

A small controlled study in children aged 7–12 months compared the effect of IVIG 2g/kg/month for 3 months with that of topical steroid therapy alone[9]. After IVIG therapy there was a significant reduction in clinical scores and serum levels of intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1) and eosinophil cationic protein (ECP). No significant difference was detected in CD4+T-cell intracellular interferon-γ (IFN-γ) or interleukin-4 levels.

A group of investigators initially reported benefit in three patients with severe AD treated with IVIG 2g/kg/month for 11 months, and these patients were able either to stop or to taper their dose of systemic corticosteroids[10]. Then in a subsequent open study by the same authors, six adult patients with AD were treated with IVIG 2g/kg/month for 6 months and were continued on their second-line systemic therapies. A significant improvement in clinical scores was noted in four out of the six patients[11]. Lymphocyte phenotypes showed a decrease in CD4 T cells following the infusions, which had recovered by the next cycle. CD69 expression in both CD4+ and CD8+ cells decreased during the 6 months to 60% of baseline values, but this did not reach statistical significance. No significant changes in intracellular IFN-γ and tumor necrosis factor-α (TNF-α) in CD4 and CD8 populations were observed.

Recently, in a randomized evaluator-blinded trial, ten patients with severe AD received one infusion of IVIG 2g/kg[12]. Five patients received the IVIG at day 0 and the other five patients received their IVIG at day 30. There was no significant difference in clinical scores between the two groups at day 30 but in the total cohort of ten there was a significant improvement in clinical severity scores at days 30 and 60.

In conclusion, there are undoubtedly benefits observed following IVIG therapy, but this is not a universally observed finding. Not surprisingly, in a chronic disease one infusion is unlikely to have any impact on the long-term control of the disease, and further double-blind, placebo-controlled trials are required to confirm efficacy and to determine whether

Table 1 Treatment of atopic dermatitis with intravenous immunoglobulin (IVIG)

Number of patients	Age	IVIG dose	Outcome	Response time	Duration of remission	Reference
2M, 2F	2–6 years	0.4g/kg for 5 days	all improved	4–7 days	1 year	Kimata[4]
3	31–40 years	2g/kg/ month	all improved	NA	short lived	*Gelfandet al.*[5]
1 (child)	8 months	1g/kg×1	no improvement	NA	NA	Weiss *et al.*[6]
10	7–64 years	2g/kg×7	no significant improvement	NA	NA	Wakim *et al.*[7]
19M, 22F	NA	<30kg 500mg/kg, >30kg 15g ×1	significant improvement	NA	NA	Noh and Lee[8]
5 (children)	7–12 months	2g/kg/month ×3	all improved	3 months	>6 months	Huang *et al.*[9]
3M	19–45 years	2g/kg/month ×11	all improved	2–4 months, maximal 11 months	1 year	Jolles *et al.*[10]
6M	18–53 years	2g/kg/month	4/6 improved	2–4 months	2/4>3 months	Jolles *et al.*[11]
10	18–50 years	2g/kg×1	? no significant improvement	NA	NA	Paul *et al.*[12]

M, male; F, female; NA, not available

continued adjunctive therapy provides a better and more sustained response.

URTICARIA

Urticaria describes an eruption of cutaneous swellings that are usually itchy, and the characteristic lesion is a weal which is an edematous, erythematous papule or plaque. Urticaria can be classified into ordinary, physical, angioedema, contact and urticarial vasculitis. The etiology of urticaria can be subdivided into idiopathic, non-immunological and immunological. The non-immunological causes include opiates, angiotensin-converting enzyme inhibitors, radiocontrast media, salicylates, non-steroidal anti-inflammatory drugs, azo dyes and food preservatives. Immunological causes include autoimmune triggers, type I hypersensitivity reactions, immune complexes and C1 esterase inhibitor deficiency[13]. Chronic urticaria is defined as urticaria lasting longer than 6 weeks, and many of these patients (up to 60%) have autoimmune urticaria. Such

patients were identified by the development of weals at sites of intradermal injection of autologous serum, and the finding that their sera released histamine from basophil leukocytes of healthy donors *in vitro*. This histaminereleasing activity has been identified as immunoglobulin G (IgG) autoantibodies directed against IgE, the IgE binding domain of the high-affinity IgE receptor FcεR1 or the non-IgE-binding domain of FcεR1. These effects may occur separately or together. Some patients with positive autologous intradermal tests fail to release histamine from basophil leukocytes but have a mast cell-specific histamine-releasing activity which can be differentiated from anti-FcεR1γ or anti-IgE antibodies. There are further patients (40%) who have consistently negative autologous intradermal serum tests.

The treatment of urticaria involves the exclusion of any underlying cause (e.g. occult infections, thyroid disease and drugs) and the administration of antihistamines. Non-sedat-ing H1 antagonists (e.g. fexofenadine, loratadine, mizolastine or acrivastine) and the minimally sedating H1 antagonist cetirizine can be given alone or in combination with a sedating antihistamine at night (e.g. chlorpheniramine, hydroxyzine or doxepin). The response to anti-histamines is variable, with up to 44% of patients with chronic urticaria gaining good control, and the combining of H1 with H2 antagonists may produce better control than H1 antagonists alone. Severe acute urticaria may be treated with corticosteroids, but their long-term use should be avoided. There is probably limited benefit with the mast cell-stabilizing drug ketotifen, the calcium channel blocker nifedipine, β-adrenergic stimulants and phototherapy; however, cyclosporin in a double-blind randomized study has been shown to be effective in patients with chronic urticaria, but only 25% of the responders remained clear or much improved 4–5 months later[14]. Plasmapheresis and IVIG have been shown to produce benefit in selected patients.

The first study recruited ten patients with severe autoimmune urticaria, poorly responsive to conventional treatment, and all patients had positive autologous intradermal serum tests and their sera produced histamine release from basophil leukocytes[15]. One cycle of IVIG 0.4g/kg per day for 5 days produced a reduction in mean urticaria activity scores from 24.6 at baseline to 12.6 and 7.8, 2 weeks and 6 weeks post-IVIG, respectively. Sustained remission of the urticaria was observed in two patients followed up for 3 years post-treatment, and another patient whose urticaria relapsed after 21 weeks and who was retreated with a further cycle of IVIG experienced a remission of 3 years. Three patients had a complete remission, but relapsed after 6, 8 and 21 weeks, respectively. There was a reduction in the autologous intradermal serum test value post-treatment in seven of ten patients.

A case report of a patient with recalcitrant chronic urticaria and a negative autologous intradermal serum test, treated with low-dose IVIG (0.2g/kg) repeated once monthly, documented suppression of the urticaria[16]. Another study of three patients having recalcitrant and treatment-unresponsive urticaria and positive autologous serum tests treated with IVIG (0.4mg/kg/day for 5 days) reported no benefit in one patient and total remission for 3 weeks in another patient, but disease activity returned to pre-IVIG levels 5 weeks after treatment and better control of the urticaria in the third patient[17].

In conclusion, IVIG appears to be efficacious in some patients with treatment-resistant chronic urticaria, but to date no placebo-controlled studies have been undertaken. Such future studies require the recruitment of rigorously defined subsets of patients with chronic immune and non-immune urticaria treated with multiple cycles of IVIG.

Furthermore, the requirement for continuing adjunctive treatment also needs to be addressed.

PSORIASIS

Psoriasis is a chronic, genetically determined and immunologically mediated inflammatory skin disease that affects 1–3% of the population. The underlying abnormalities in psoriasis are increased proliferation of keratinocytes, which accounts for the thickened scaly plaques, and an inflammatory cell infiltrate composed of neutrophils and lymphocytes. Both keratinocytes and T cells produce chemokines and cytokines that are actively involved in the pathogenesis of the disease. There is no cure for psoriasis, but standard topical treatments include vitamin D analogs, topical corticosteroids, retinoids, coal tar and dithranol preparations. Temporary remission may be induced with phototherapy (UVB, UVA, PUVA), and in more severe cases the systemic agents methotrexate, retinoids, hydroxyurea, fumaric acid and cyclosporin may be required. Biological therapies using etanercept and infliximab are employed in resistant cases.

So far only one report describing the use of IVIG in psoriasis has been published[18]. These patients had concomitant active psoriatic arthritis and were treated with monthly IVIG (2g/kg), two continuing to receive their usual systemic therapies. Improvement in joint symptoms and inflammatory markers was observed in all patients in 1 month (C-reactive protein falling from 39 to 9, 61 to 8, 44 to 12mg/l, respectively). The psoriasis had cleared in one patient after one cycle and in the second after three cycles, and the third patient's skin disease had become less active but the affected area was unchanged. Clearly, further trials are warranted to confirm efficacy.

CONCLUSION

There is difficulty in interpreting the results of many of the above studies, since some patients have continued to receive concomitant adjunctive therapy and the diseases have varied in the severity and type of conditions. There is now a need for appropriately designed, adequately powered, double-blind, placebo-controlled randomized trials in these three conditions to confirm efficacy Unfortunately, owing to the cost of IVIG these may have to be funded by pharmaceutical companies, especially if there is a desire to have IVIG licensed for the treatment of atopic dermatitis, urticaria and psoriasis.

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Intravenous immunoglobulin in toxic epidermal necrolysis and Stevens-Johnson syndrome

L.E.French

Toxic epidermal necrolysis (TEN, Lyell's syndrome) is a rare adverse cutaneous drug reaction characterized by an average mortality of 30%[1]. Stevens—Johnson syndrome (SJS) is a severe mucocutaneous disease that is frequently drug-induced and can progress to TEN[2]. SJS and TEN are in fact considered to be part of a spectrum of adverse cutaneous drug reactions with increasing severity and extent of skin detachment, ranging from SJS (less than 10% body surface area skin detachment) to SJS-TEN overlap syndrome (10–30% skin detachment) and TEN (greater than 30% skin detachment)[3]. Both SJS and TEN are characterized morphologically by ongoing apoptotic keratinocyte cell death that results in the separation of the epidermis from the dermis. Although mortality rates in SJS are not as high as in TEN (on average 30%), fatal outcomes have been reported in about 1–5% of patients[1,4]. Furthermore, visceral complications, and especially ocular complications which can occasionally lead to permanent impairment of eyesight, can be as severe in SJS as in TEN, since mucous membrane involvement is similar in both.

No specific treatment for SJS or TEN exists to date. TEN is caused by extensive Fas-mediated keratinocyte death, a process that can be inhibited by high-dose intravenous immunoglobulin (IVIG) *in vitro*[5]. Recently, we were able to elucidate the mechanisms involved in triggering keratinocyte apoptosis during TEN. We demonstrated that keratinocyte apoptosis occurs as a result of enhanced keratinocyte Fas ligand (FasL) (CD95L) expression and Fas-mediated keratinocyte cell death[5]. We also showed that commercially available IVIG preparations inhibited the progression of TEN in ten patients treated within a pilot study[5]. Recently, a few additional case reports have reported the treatment of TEN with IVIG[6–8]. In addition, we demonstrated that IVIG contains anti-Fas antibodies and is able to inhibit Fas-mediated keratinocyte apoptosis *in vitro*.

Over the past 3 years, we have evaluated in a larger number of patients the therapeutic potential of IVIG in TEN, and parameters that may affect response to treatment[9]. Forty-eight consecutive patients with TEN (>10% body surface area skin detachment, mean 44.8%, range 10–95%) were treated with standard care and IVIG (mean total dose 2.7g/kg body weight, range 0.8–5.8g/kg) in 14 dermatology centers. Objective response to treatment, final outcome at day 45 and parameters that may affect response to treatment were retrospectively analyzed. IVIG infusion was associated with a rapid (mean 2.3 days, range 1–6 days) interruption of skin and mucosal detachment in 89–6%

of patients, and 87.5% overall survival. Patients who responded to IVIG had received, on average, higher doses of IVIG and earlier treatment.

Two other reports have recently analyzed the clinical efficacy of IVIG in TEN. Trent and colleagues performed a retrospective monocenter analysis of IVIG at an average total dose of 4g/kg in 16 consecutive patients (mean age 43 years, mean skin detachment 43%)[10]. Using a validated severity of illness score (SCORTEN) as a predictor of outcome according to standard therapeutic procedures, 5.8 deaths (36%) were predicted, and only one (6%) occurred in IVIG-treated patients. The authors claim that IVIG treatment reduced the expected mortality by 83%. The second study reported by Bachot and associates was a prospective open monocenter trial assessing IVIG at an average total dose of 2g/kg in 34 consecutive patients (mean age 47 years, mean skin detachment 19%)[11]. SCORTEN predicted 8.2 (24%) deaths in their cohort, and 11 deaths were observed (32%). An unusually high mortality rate due to renal failure (six patients) was also reported in this study. This was not observed in the other two studies, even in patients with pre-existing abnormal renal function. With the results taken together, early infusion of high-dose intravenous immunoglobulin is safe, well tolerated and likely to be effective in improving the survival of patients with TEN (Table 1). From the currently available data we would recommend early treatment with IVIG at a total dose of 3g/kg over 3–4 consecutive days (1g/kg/day).

There is currently no proven effective treatment for SJS. Since clinical and histological characteristics suggest that the pathogenesis of epidermal detachment in SJS is similar to that of TEN, and since clinical data suggest that IVIG may be useful for the treatment of TEN, we have assessed the same therapeutic approach in patients suffering from SJS[12]. Data concerning 12 consecutive patients diagnosed with SJS according to a recent consensus definition, and treated with IVIG at seven European and North American university dermatology centers, were analyzed retrospectively. All patients had progressive ongoing epidermal and/or mucosal detachment at the time of treatment initiation. SJS-TEN overlap syndromes and TEN were excluded. Tolerance to IVIG, survival at 45 days after onset and total healing time were assessed. Twelve SJS patients (mean age, 44 years) were treated with IVIG at a mean dose of 0.6g/kg/day for an average of 4 days. An objective response to IVIG infusion was observed in all patients within a mean of 2 days, and no patients developed progressive disease or died. Total skin healing occurred in 8.3 days (mean). Time to total healing was found to be shorter in a group of patients who had, on average, less concomitant severe disease(s) of unrelated origin, and had received IVIG infusion earlier after the onset of skin lesions. High-dose IVIG seems therefore to be effective in blocking the progression of SJS and reducing the time to complete skin healing.

Table 1 Comparison of four studies available

	Viard *et al.*[5], 1998	Prins *et al*[9], 2003	Trente *et al.*[10], 2003	Bachot *et al.*[11], 2003
Study type	prospective, non-controlled	retrospective, non-controlled	retrospective, non-controlled	prospective, non-controlled
Number of patients	10	48	16	34
Average age (years)	39	43	43	47
Average detachment (%)	28.5	45	43	19
Dose IVIG (total) (g/kg)	3	3	4	2
Predicted deaths	—(30%)	—(30%)	5.8 (36%)	8.2 (24%)
Actual deaths	0/10 (0%)	6/48 (12%)	1/16 (6%)	11/34(32%)
Deaths from renal failure	0	0	0	6

IVIG, intravenous immunoglobulin

In conclusion, infusion of high-dose IVIG is safe, well tolerated and likely to be effective in improving the survival of patients with TEN (level of evidence IIb—III), and blocking the progression of SJS. From the currently available data, early treatment with 1 g/kg/day over 3–4 consecutive days is recommended in TEN and SJS.

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Intravenous immunoglobulin in the treatment of vasculitis

D.Jayne

INTRODUCTION

The primary systemic vasculitides have an annual incidence of 40/million and are classified according to their association with anti-neutrophil cytoplasm autoantibodies (ANCAs). Those predominantly involving microscopic vessels without immune deposits include Wegener's granulomatosis, microscopic polyangiitis and Churg—Strauss angiitis, and are usually ANCA-positive and represent 50–75% of the total. The ANCA-negative group include those with immune deposits, such as Henoch—Schönlein purpura and cryoglobulinemia or those restricted to muscular arteries, including polyarteritis nodosa and giant cell and Takayasu's arteritis[1,2]. The treatment of primary systemic vasculitis with high-dose corticosteroids and immune-suppressive drugs is generally effective in controlling clinical evidence of disease activity in new presentations[3,4]. This approach is complicated by frequent, severe toxicity and infections, a high relapse rate and chronic morbidity with increasing incapacity. Following demonstration of the efficacy of intravenous immunoglobulin (IVIG) in reducing the incidence of coronary artery aneurysms in the childhood vasculitis, Kawasaki disease, there has been interest in treating other vasculitides with IVIG.

THERAPEUTIC MECHANISM OF INTRAVENOUS IMMUNOGLOBULIN IN VASCULITIS

The pathogenesis of primary vasculitis includes widespread immune activation, endothelial cytotoxicity and infiltration of vessels with inflammatory cells causing necrosis of vessel walls and thrombosis of the vascular lumen. Limited evidence suggests a possible contribution of microbial infections, including *Staphylococcus aureus* in Wegener's granulomato sis, parvovirus in polyarteritis and *Streptococcus* sp. in Henoch—Schönlein purpura[5–7]. Neutralization of microbial superantigens by IVIG has been demonstrated in Kawasaki disease and may therefore be relevant to other forms of vasculitis[8,9].

Although they have proved difficult to isolate, autoreactive CD4+ and CD8+T cells are strongly implicated in vasculitis, and their depletion abrogates disease and lowers autoantibody levels[10–12]. IVIG has the potential to influence T-cell activities in vasculitis through binding to cell surface molecules or modulating cytokines: IVIG has suppressed

circulating interleukin-10 (IL-10) levels and has normalized the T-cell repertoire in Kawasaki disease.

There is evidence of idiotypic regulation of ANCA autoantibodies, with rises in the level of circulating ANCA anti-idiotype antibodies at disease remission when ANCA levels themselves fall or become undetectable[13]. The binding of the vasculitis-associated autoantibody, ANCA, is inhibited by IVIG F(ab‘)2 fragments, and ANCA autoantibodies are preferentially adsorbed onto columns of immobilized IVIG indicating the presence of idiotypic interactions between variable regions on antibodies in IVIG and ANCAs[14–16]. Common idiotypes have been identified on at least 50% of ANCA antibodies reacting with proteinase 3, 5/7 idiotype, and myeloperoxidase, 7F2C11 idiotype, but their direct interaction with IVIG has not been investigated[17,18]. Functional effects of ANCA inhibition have been confirmed with the reduction in ANCA-induced neutrophil interleukin-1 (IL-1) release *in vitro*[19]. The immunoglobulin M (IgM) fraction of normal plasma is enriched in natural antibody activity, and the increase in total IgM seen in patients with vasculitis after IVIG administration has been linked with concurrent falls in ANCA levels.

The reduction in IL-1 activity following IVIG administration in Kawasaki disease appears to be related to anti-IL-1 antibodies in IVIG rather than a fall in IL-1 production[20]. Other anticytokine effects would be important in vasculitis where cytokines, especially tumor necrosis factor-α (TNF-α), IL-1 and IL-6, play central roles, and therapeutic anti-TNF-α antibodies lead to disease remission[21–24]. Endothelial activation is a key stage in the pathogenesis of vasculitis and contributes to its late cardiovascular morbidity; IVIG partially inhibits TNF-α-induced endothelial activation *in vitro* and in animal models, and impairs endothelial release of thromboxane, endothelin and metalloproteinases, of direct relevance to human disease[25–27].

In common with its potential role in other autoimmune syndromes, including idiopathic thrombocytopenic purpura, many of the anti-inflammatory effects of IVIG in vasculitis can be explained by the interaction of IVIG with Fc receptors on macrophages and other immune cells. A more specific role for Fc receptors in vasculitis is seen in ANCA-induced neutrophil activation, which is inhibited by Fc receptor blockade[28–32]. Modulation of endothelial functions, including chemokine release, expression of adhesion molecules and thrombosis, by IVIG is also likely to contribute to its effects in vasculitis[33–35].

The solubilization of immune complexes by IVIG has been observed in systemic lupus erythematosus (SLE) and may contribute to its therapeutic mechanism in immune complex vasculitides, such as Henoch—Schönlein purpura and cryoglobulinemia[36]. The role of complement in most vasculitides is poorly understood, but complement activation is usually detectable, and the reduction of complement-mediated inflammation by IVIG is of potential importance[37–39]. Possible mechanisms of IVIG relevant to vasculitis are listed in Table 1.

Table 1 Immunomodulatory mechanisms of intravenous immunoglobulin (IVIG) of relevance to vasculitis

Target for effect	Nature of effect	Evidence in vasculitis
T cell	inhibition of cytokine release	
	release of cytokine antagonists	
	reversal of counter-regulatory subsets imbalance	Brown—Norway rat model
	inhibition of T-cell activity through	
	binding to cell surface antigens	
	increase in CD4+ and CD8+subsets	Kawasaki disease
B cell/autoantibody	reduction in autoantibody secretion	falls in ANCA after IVIG
	stimulation of natural antibody secretion	increase in IgM and natural antibody levels
	blockade of autoantibody binding	inhibition of ANCA-induced neutrophil IL-1 release
	idiotypic regulation	fall in ANCA?
Cytokines	inhibition of effect	fall in CRP and anti-inflammatory
	inhibition of monocyte TNF secretion	effect in vasculitis?
Complement	inhibition of complement-mediated injury	Henoch—Schönlein purpura?
Fc receptor	increased Ig catabolism blockade of autoantibody-	

	mediated cytotoxicity	
	resorption of immunoglobulin deposits	Henoch—Schönlein purpura and cryoglobulinemia?
Endothelial cell	inhibition of TNF- or IL-1-induced adhesion molecule expression inhibition of leukocyte adhesion reduction in thromboxane A2 and endothelin release	
Infectious agents	neutralization of superantigen-induced T-cell activation control of viral infection	Kawasaki disease, Wegener's granulomatosis? parvovirus B19?
Nerves	promotes myelination	vasculitic neuropathy?
Fas (CD95)	promotes apoptosis	Churg-Strauss angiitis?

TNF, tumor necrosis factor; Ig, immunoglobulin; IL-1, interleukin-1; ANCA, antineutrophil cytoplasm autoantibodies; CRP, C-reactive protein

CLINICAL EXPERIENCE WITH INTRAVENOUS IMMUNOGLOBULIN IN VASCULITIS

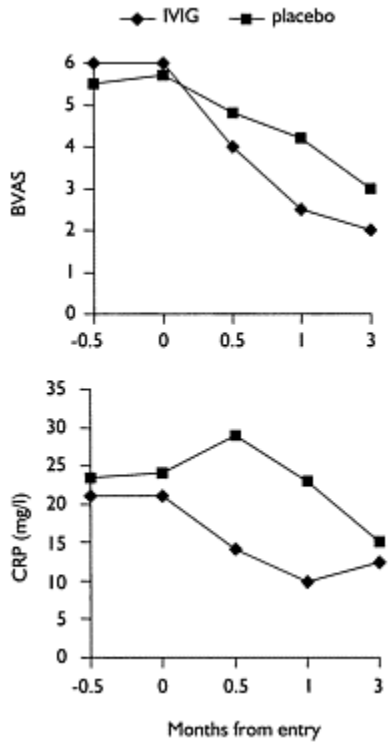
ANCA-associated vasculitis

Initial experience with IVIG in Wegener's granulomatosis and microscopic polyangiitis used IVIG as an additional agent to standard immunosuppressive therapies in patients with poor disease control. Individual cases have shown dramatic clinical improvement, while others have not; response rates following IVIG in published series have varied from 40 to 75% [14,40–42] Studies have varied in the number of courses of IVIG, and it is unclear whether multiple courses are effective where a single course has failed[14].

The difficulty of divorcing the effect of IVIG from that of continuing immunosuppressive therapy prompted a prospective study of six cases where IVIG was used as sole therapy for untreated disease without threatened vital organ failure[43]. Four

exhibited a sustained improvement in disease activity with falls in C-reactive protein and ANCAs, two of whom relapsed after 1 year, while two had only a transient response. More recently, a double-blind, placebo-controlled trial of IVIG for persistent ANCA-associated vasculitis involving 34 patients revealed a reduction in disease activity and C-reactive protein after a single course of IVIG (2g/kg)[44]. The benefit was not maintained beyond 3 months, indicating the need to evaluate a repeated dose regimen, at 1–3-month intervals (Figure 1). The fall of ANCAs after IVIG *in vivo* has been compared to the *in vitro* ANCA-inhibitory activity of IVIG and to clinical responses; a preliminary study showed a correlation but confirmatory data are lacking[42].

Figure 1 Birmingham Vasculitis Activity Score (BVAS) and C-reactive protein (CRP) levels after high-dose intravenous immunoglobulin (IVIG) or placebo in antineutrophil cytoplasm autoantibody (ANCA)-associated vasculitis[44]



Churg—Strauss angiitis

A small number of case reports have demonstrated a therapeutic response to IVIG in refractory Churg-Strauss angiitis[45–47]. This vasculitis is associated with eosinophilia, a feature of T helper cell Th2-dominant immune responses analogous to that seen in the Brown—Norway rat model of vasculitis, where disease expression is partially prevented by IVIG[48].

Henoch-Schönlein purpura

Two prospective studies involving 24 patients with IgA nephropathy or Henoch—Schönlein purpura have found improvements in nephritis accompanied by reductions in IgA and β1-microglobulin levels, and glomerular IgA and C3 deposition following prolonged immunoglobulin therapy[49,50]. These results suggest an influence of exogenous IgG on IgA dysregulation with consequent reductions in IgA-mediated inflammation and injury.

Polyarteritis nodosa

Recurrent, cutaneous polyarteritis nodosa refractory to immunosuppression has been controlled by IVIG in case reports[51,52]. A further treatment-resistant case exhibited improvement during monthly IVIG treatment but relapsed subsequently[52]. Polyarteritis associated with hepatitis B, parvovirus and streptococcal infection has been controlled by IVIG after the failure of other treatments[6,52].

Cryoglobulinemia

Improvements in cyroglobulinemic vasculitis have occurred following IVIG[54]. However, there is a risk of precipitating a cryoglobulinemic crisis with IVIG[55–57]. This can be avoided by the sequential combination of IVIG after plasma exchange to reduce cryoglobulin levels.

CONCLUSIONS AND FUTURE DIRECTIONS

There is now considerable evidence of the anti-vasculitic potential of IVIG in some, but not all, cases[58]. Routine clinical application is complicated by a poor understanding of the dominant therapeutic mechanisms in vasculitis, which allow rational integration of IVIG with other therapies. No dose-ranging studies have been performed, and the role of repeated doses, either to improve the primary therapeutic response or to maintain disease control, also needs further study. ANCA-inhibitory activity of IVIG varies between batches and between manufacturers; batch or plasma donor selection has the potential to improve its efficacy in ANCA-associated vasculitis[15]. Concurrent therapy in vasculitis patients complicates evaluation of the efficacy of IVIG, and important interactions may

exist such as promotion of the immunoregulatory effects of IVIG by plasma exchange[59,60].

Current immunosuppressive protocols have a high rate of adverse effects, of which infection is the most serious. IVIG offers the possibility of reducing immunosuppressive doses while maintaining or improving therapeutic efficacy and possibly reducing the incidence of infection. This is particularly relevant to elderly or pregnant patients, in whom the adverse consequences of immunosuppression are more serious.

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Emerging applications for intravenous immunoglobulin

45
Immunomodulation by intravenous immunoglobulin of alloantibodies to human leukocyte antigen: clinical aspect in solid organ transplantation

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INTRODUCTION

Up to 30% of patients waiting for a renal allograft are immunized, that is, possess in their sera antibodies directed against allogeneic human leukocyte antigen (HLA) molecules. The presence of antibodies of the immunoglobulin G (IgG) isotype, directed against HLA class I molecules borne by the graft, is very strongly correlated with episodes of severe acute rejection and graft loss[1]. The existence of these antibodies is demonstrated by a technique called 'cross-match', in which the serum of the recipient is incubated with lymphocytes from the donor and the potential binding revealed, either through cytotoxicity with rabbit complement or through flow cytometry using a secondary antibody. The positivity of a classic cytotoxic T-cell IgG cross-match is an absolute contraindication to transplantation[2]. Transplantation of such patients is thus hampered by the need to find a compatible organ which does not harbor any of the HLA molecules recognized by the patient's antibodies, so the waiting time of these patients extends frequently to more than 3 years[3]. Moreover, once grafted, these patients are at risk, with more rejections and a lower graft survival than in naive patients.

This chapter reports the experience of our center and others, using intravenous immunoglobulin (IVIG) alone or in combination with plasmapheresis, in the transplantation of such patients.

INTRAVENOUS IMMUNOGLOBULINS

IVIG administration has proven efficacy in a number of autoimmune diseases such as idiopathic thrombocytopenic purpura, hemolytic anemias, autoimmune neutropenias, etc. (reviewed in reference 4). The immunological mechanisms at play are diverse, and most probably vary from one disease to another. Among these various mechanisms of action, some may have particular relevance in transplantation, as a prophylactic (P) or curative (C) treatment of rejection:

(1) Neutralization of circulating antibodies, through idiotype—anti-idiotype inter-interactions, as seen in autoi in autoimmune hemophilia (P, C)[5];
(2) Inhibition of secretion of cytokines and other soluble mediators, as seen in Kawasaki's disease (C)[6];
(3) Inhibition of the binding of complement fractions to their target cells, as seen in neuromuscular disorders (C)[7];
(4) Inhibition of B- and T-cell proliferation, with down-regulation of antibody synthesis, which has been demonstrated *in vitro* (P, C)[8];
(5) Inhibition of endothelial cell activation (P, C)[9];
(6) Inhibition of CD8 T-cell cytotoxicity (P, C)[10];
(7) Increased apoptosis of B cells (P, C)[11];
(8) Inhibition of maturation and function of dendritic cells (P)[12].

ANTI-HUMAN LEUKOCYTE ANTIGEN IMMUNIZATION

There are three different causes of immunization, namely transfusion, pregnancy or transplantation. This immunization is much stronger (as determined by the antibody titer), polyreactive (number of HLA antigens recognized) and prolonged when, in the same patient, different causes of immunization act together. Thus, transfusion alone induces immunization in only 10% of the patients. After transplant failure and return to dialysis, less than 10% of patients develop anti-HLA antibodies, but if, at the time of transplantectomy, patients receive a blood transfusion, this percentage rises to 80%[13]. Likewise, only 5% of pregnant women become immunized, but if a blood transfusion occurs, this percentage rises to 50%[14]. Thus, strong and durable immunization is nearly always the consequence of a combination of transplantation and transfusion or pregnancy and transfusion.

As time goes by, this immunization may simply disappear, persist or disappear through an active phenomenon, the generation of anti-idiotypic antibodies[15]. These anti-idiotypic antibodies inhibit anti-HLA antibodies, blocking their cytotoxic effect[16], and are clearly associated with a better graft prognosis[17].

INTRAVENOUS IMMUNOGLOBULIN AND ANTI-HLA ANTIBODIES

Laboratory data

We first tested the hypothesis that commercial preparations of IVIG could block the cytotoxicity induced by anti-HLA antibodies. To this end, we slightly modified the classic panel reactive antibody assay (PRA) by diluting the sera to be tested in the ratio 1:2, either in phosphate-buffered saline or in IVIG (20mg/ml). Lymphocyte target cells were incubated with the sera, rabbit complement was added and lysis detected after the addition of a vital dye. Results can be expressed either in absolute numbers (10/20 meaning that cells of half the donors have been lysed) or as a percentage (50%).

Sera from 19 immunized patients were studied[18]. The addition of IVIG significantly reduced cytotoxicity ($p<0.0001$). Nearly half of the sera had a 50% or more inhibition of cytotoxicity, and only one serum showed no inhibition. It is of note that IVIG alone never caused cytotoxicity, and that adding IVIG never led to an increase of cytotoxicity.

The precise mechanism at play was investigated through analysis by flow cytometry. IVIG itself does not bind lymphocytes, but inhibits the binding of anti-HLA antibodies to the cells in a dose-dependent manner. Equivalent results were found using F(ab‘)2 fragments of IVIG, evoking the presence, among the IVIG, of anti-idiotypic antibodies directed against anti-HLA antibodies.

Clinical findings

Ten patients on the transplant waiting-list received 0.4g/kg of IVIG (Gamma-PEG; Pasteur-Mérieux, Lyon, France) during four consecutive dialysis sessions, and their anti-HLA titers were monitored by cytotoxicity during the following weeks and months[19]. Mean antibody titer pretreatment was 50% (range 30–90%). None of these patients had either immunoglobulin M (IgM) anti-HLA or autoantibodies. In more than half of the patients, an important and prolonged decrease was observed, with a stable titer 3 weeks after the last IVIG infusion. Similar studies performed on patients awaiting cardiac or renal transplants yielded equivalent results. The decrease observed in antibody titer was long-lasting and far exceeded the half-life of the infused IVIG, suggesting a modification of the immune repertoire of the patients, as seen with IVIG therapy after bone-marrow grafting[20].

INTRAVENOUS IMMUNOGLOBULIN AND TRANSPLANTATION

Allowing transplantation through desimmunization

Following this first trial and the subsequent transplantation of some patients[21], we initiated another trial with the goal of transplanting those patients with a decrease in antibody titers in the few weeks following desimmunization. To date, 15 patients have been included, and 13 have been transplanted[22,23]. These patients have the following characteristics: mean age 28 years, mean time on dialysis more than 9 years and 13 of them already with at least one previous renal transplant.

Eleven of these patients were transplanted with a cadaveric kidney after a mean decrease of 77% of their anti-HLA antibody titer, from 64% (86–42%) to 15% (0–30%). Two patients had a living related donor against whom the cross-match was positive before IVIG treatment.

The immunosuppressive regimen included IVIG (1g/kg at days 0 and 1, days 20 and 21, and days 42 and 43), Thymoglobulin® (1mg/kg/day) for 10 days, tacrolimus (from day 10 onwards), Cellcept® and steroids.

One graft was lost as a result of thrombosis in a few hours post-transplant, and one patient lost his graft owing to rejection. All other patients, including those with living related kidneys, had normal renal function and did not experience any episode of

rejection after a mean follow-up of more than 18 months. Similar results using 1–4 administrations of 2g/kg of IVIG have been reported by Jordan[24]. Very recently, the preliminary results of the first randomized, controlled study of the use of IVIG to allow transplantation were presented by Jordan and colleagues[25]: 101 immunized patients (PRA>50%) were allocated to receive either IVIG (49 patients) or albumin (51 patients) at a dose of 2g/kg body weight on four occasions. The level of anti-HLA antibodies was clearly more decreased in the IVIG group, and, more importantly, 37% of the patients in the IVIG group were transplanted within a year, compared with only 17% of the control group ($p<0.02$). Similar studies have been published in heart transplantation, with a decrease of 33% of anti-HLA titers and a reduction of the waiting time[26].

Another technique uses repeated plasmapheresis, coupled with low-dose (100mg/kg) IVIG, to prevent the rebound in antibody synthesis seen in earlier protocols. Montgomery and colleagues first reported the successful treatment of four patients[27], although all these patients experienced acute humoral rejection (AHR) post-transplant, reversed by the same association of plasmapheresis and IVIG. In follow-up reports of 33 patients by the same group, the rate of rejection fell to 64%[28]. Gloor and colleagues added one dose of rituximab as well as splenectomy to the plasmapheresis/IVIG approach, and could transplant 14 patients, with an overall rate of AHR (clinical or subclinical) of 43%[29]. Along the same lines, some heart transplantations have been performed against a positive cross-match pre-IVIG, with good results, using a treatment of both plasmapheresis and IVIG[30,31]. This is most important in the case of patients under circulatory assistance, where the necessary multiple transfusions induce, in the vast majority of patients, important immunization[32].

IVIG at the time of transplantation for prevention of acute rejection

IVIG has been used in the prophylaxis of acute rejection in patients deemed at high risk of rejection, such as hyperimmunized patients, those with second transplants and children, in renal as well as cardiac transplantation.

A retrospective study was performed in 21 immunized patients receiving their first transplant[33]. These patients received IVIG (0.4g/kg/day from day 0 to day 5) on top of a quadruple sequential immunosuppressive regimen consisting of antilymphocyte antibodies, azathioprine, steroids and delayed cyclosporin A. Graft survival at 2 years was 95%, well above published results for this type of patient (around 80%). A pediatric study of 52 children demonstrated better graft survival at 1, 2 and 3 years (95%, 95% and 88% versus 88%, 79% and 79%) in children at high risk for cytomegalovirus infection treated by IVIG, compared with a control, low-risk group without IVIG therapy[34]. Finally, a randomized study using IVIG as prophylaxis for acute rejection was performed on 41 patients receiving their second graft[35]. Twenty-one patients received 0.4g/kg/day of IVIG from day 0 to day 5, with a classic sequential quadruple immunosuppressive regimen. Five-year graft survival was significantly higher in the IVIG-treated group (68% versus 50% in the control group). A similar study in heart transplantation demonstrated that the survival of 16 immunized patients treated by IVIG and plasma-pheresis was identical to the survival of non-immunized patients[36].

IVIG and curative treatment of rejection

IVIG is sometimes used to treat established rejection, most notably in patients with poor health status, to avoid the consequences of antilymphocyte antibody therapy. In most cases, the rejection is steroid-resistant, and often with ominous vascular lesions. IVIG may act in these cases either by neutralization of circulating antiendothelium antibodies, as demonstrated in xenotransplantation[37–39], or by blocking endothelial cell activation, an essential step in the genesis of vascular lesions[9]. In a prospective randomized study of 30 steroid-resistant rejections, Casadei and colleagues showed that IVIG had the same efficacy as OKT3®, rescuing 73% of the grafts, without the well-known side-effects of OKT3[40]. The combination of IVIG and plasmapheresis has also been used in this setting, first by the Johns Hopkins group[27], with remarkable results, and confirmed by Rocha and associates in a retrospective study of 16 acute humoral rejections with an 81% graft survival 1 year post-rejection[41].

CONCLUSIONS

IVIG is now an integral part of the therapeutic tools commonly used in allotransplantation, with three major indications. Besides its application, alone or in combination with plasmapheresis, to allow transplantation of immunized patients, its place in the prophylaxis and treatment of rejection needs to be better defined. The use of IVIG, together with the more sophisticated techniques now employed for the detection of anti-HLA antibodies, clearly calls for a reappraisal of the contraindication of transplantation in the case of anti-donor antibodies.

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46
Role of intravenous immunoglobulin in the management of heart failure

H.Krum

PATHOPHYSIOLOGY OF HEART FAILURE

Over recent years there has been a dramatic improvement in our understanding of the pathophysiology that underlies heart failure. In response to myocardial injury or inflammation, a series of processes are activated in an attempt to restore cardiovascular homeostasis[1] (Figure 1). Whilst activation of these processes may be of hemodynamic benefit in the short term, chronic activation contributes to an ongoing and progressive decline in cardiac function. This is manifest as the process of ventricular 'remodelling'.

Among the best characterized of these activated processes are neurohormonal vasoconstrictor pathways such as the renin—angiotensin—aldosterone system, sympathetic nervous system, endothelin and vasopressin. Indeed, blockade of both the renin—angiotensin—aldosterone and sympathetic nervous systems represents the cornerstone of therapy for chronic heart failure[2]. More recently, additional systems activated following myocardial injury or inflammation have been characterized, including activation of proinflammatory cytokines.

IMMUNE ACTIVATION IN HEART FAILURE

A broad-based proinflammatory response is observed in patients with chronic heart failure, comprising activation of tumor necrosis factor-α, γ-interferon and interleukin-1β, −2 and −6 among a range of other proinflammatory factors[3,4].

There are a number of key issues that are still to be fully elucidated with regard to this proinflammatory cytokine response. The first issue is what induces this response, even remote from events such as myocarditis or myocardial infarction. One theory is that gut edema in the setting of heart failure leads to increased permeability of the gut wall to entry of Gram-negative bacteria, which in turn trigger the

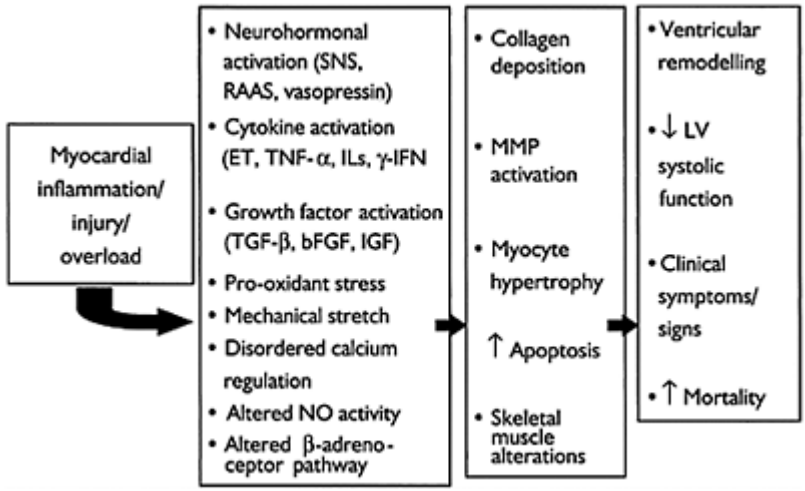


Figure 1 Mechanisms of heart failure disease progression following the initial myocardial insult. SNS, sympathetic nervous system; RAAS, renin-angiotensin-aldosterone system; ET, endothelin; TNF-α, tumor necrosis factor-α; IL, interleukin; γ-IFN, γ-interferon; TGF-β, transforming growth factor-β; bFGF, basic fibroblast growth factor; IGF, insulin-like growth factor; MMP, matrix metalloproteinase; LV, left ventricular

proinflammatory response[5]. However, the evidence to support this hypothesis is still somewhat lacking.

The other main issue relates to whether the proinflammatory cytokine activation observed in heart failure is of itself pathogenic in driving progression of disease, or merely represents an epiphenomenon. There is considerable evidence to support the former proposition. Tumor necrosis factor-α (TNF-α) is by far the best characterized of these cytokines. TNF-α overexpression or exogenous infusion results in a cardiac phenotype characteristic of dilated cardiomyopathy with impaired systolic function, fibrosis and reduced contractility of myocytes[6,7]. TNF-α plasma levels[8] and expression of this cytokine within the myocardium itself[9] are increased in heart failure. Furthermore, TNF-α mediates a series of further detrimental actions, including release of inducible nitric oxide, endothelin and other mediators that may have a progressive deleterious effect on cardiac function[10].

BLOCKADE OF IMMUNE ACTIVATION IN HEART FAILURE

A number of clinical strategies have been proposed to block proinflammatory cytokine activation in heart failure. The most widely studied approach has been to inhibit TNF-α activity. As mentioned, TNF-α has been the best characterized of the proinflammatory cytokines activated in heart failure. Furthermore, agents which block TNF-α have already been used for a number of years in the treatment of inflammatory disorders such as rheumatoid arthritis[11] and Crohn's disease.

The two main approaches to TNF-α blockade studied have been the humanized Fc receptor fusion protein, etanercept, and the monoclonal antibody, infliximab.

Preclinical studies in models of TNF-α overexpression have demonstrated inhibition of the progression of heart failure with etanercept[12], and this has been supported by short-term early-phase clinical data with the agent[13,14].

The Randomized Etanercept Worldwide Evaluation (RENEWAL) program with etanercept comprised two large-scale phase III studies of differing doses and geographical diversity, examining the utility of the agent on clinical events in patients with chronic heart failure. The study program was stopped early because of a lack of clear-cut evidence of clinical benefit, with a neutral result for the co-primary end-points of clinical composite score as well as mortality and heart failure hospitalization[15].

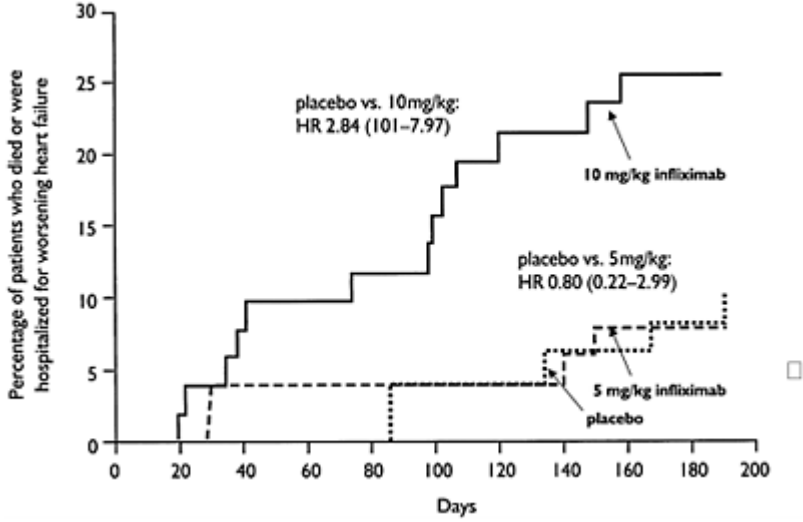
Similarly, the smaller phase II Anti-TNF Therapy Against Congestive Heart Failure (ATTACH) study with infliximab demonstrated no significant difference in clinical status with two doses of infliximab compared with placebo[16]. There was an almost three-fold increase in death and heart failure hospitalization at the higher dose of infliximab used, in comparison with placebo (Figure 2). This led to a 'black box' warning against the use of infliximab in patients treated with this agent for inflammatory disorders who also have concomitant heart failure.

Perhaps even more worrying have been recent reports of *new* heart failure in patients with rheumatoid arthritis and Crohn's disease receiving these drugs for these indications, without known pre-existing heart failure[17].

There are a number of potential explanations to be considered in assessing why the TNF-α blockade strategy was not successful in the treatment of heart failure[15]. First, it has proved extremely difficult for many agents to be demonstrated to be of significant benefit in addition to background standard heart failure therapies such as angiotensin-converting enzyme (ACE) inhibitors and β-blockers. The same may be true of TNF-blocking strategies; however, this does not explain the excess of cardiac events with infliximab in ATTACH. Next, TNF-α may be important in the regulation of cardiovascular homeostasis and not all of its effects detrimental in the setting of overt heart failure[18]. Indeed, it may be that there are short-term benefits of blockade (as observed in phase II studies with etanercept) that are offset by long-term adverse effects with this strategy.

Finally, and most likely, the TNF-α blockade strategies utilized, i.e. Fc receptor fusion protein and monoclonal antibody approaches, are highly specific against TNF-α alone. Given that heart failure is a condition associated with an extremely broad-based proinflammatory immune response (as described above), blockade of one specific cytokine may have been too selective an approach to produce sustained clinical benefit. Indeed, specific TNF-α blockade

Figure 2 Kaplan-Meier plot of death and hospitalization for heart failure in the Anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial[16]. HR, hazard ratio



may result in negative feedback activation of other proinflammatory cytokines to compensate for this blockade.

On the basis of these considerations, a number of broader-based anti-inflammatory strategies have been considered, and in some cases clinically evaluated, in the heart failure setting.

Immunoadsorption has been utilized as a means of removing circulating autoantibodies[19,20] that may be adversely contributing to patients' heart failure status. The most widely assessed of these are β-adrenoceptor autoantibodies. This approach has been commercialized and is currently undergoing further evaluation.

Another approach is to augment anti-inflammatory cytokines to counteract the predominance of proinflammatory cytokine activation in this setting. Interleukins such as −4 and −10 are potent anti-inflammatory cytokines and result in the suppression of proinflammatory status, at least *in vitro*[21]. This approach remains to be evaluated in the *in vivo* setting in both preclinical models and in patients themselves.

'Immune modulation therapy' has been studied in patients with heart failure. This approach involves removal of blood from the patient, the application of physicochemical stress to that blood (heat, light and oxidative stress) and subsequent reinjection into the patient, which is claimed to result in a broad-based anti-inflammatory response. The results of a phase II study have been presented in a 'Hotline' session at the Heart Failure Society of America Meeting, 2002[15]. Interestingly, there was no effect observed on patients' ventricular function or clinical status. In contrast, however, there was a highly

significant reduction in mortality and heart failure hospitalization, as well as mortality alone in these patients, in comparison with the control group.

Finally, and the subject of the remainder of this chapter, intravenous immunoglobulin therapy has been extensively studied as a broad-based approach to block proinflammatory cytokine activation in heart failure, both acute and chronic.

INTRAVENOUS IMMUNOGLOBULIN IN THE TREATMENT OF HEART FAILURE

Intravenous immunoglobulin (IVIG) appears to be a rational approach to the management of proinflammatory immune activation in heart failure. This concentration of pooled polyclonal human antibodies has significant immunomodulatory properties, extensively described elsewhere in this book.

IVIG has been explored in a number of areas related to myocardial dysfunction. These include recent-onset heart failure (presumed acute myocarditis), chronic heart failure and peripartum cardiomyopathy. Use of IVIG in each of these clinical scenarios is discussed in turn.

Recent-onset heart failure

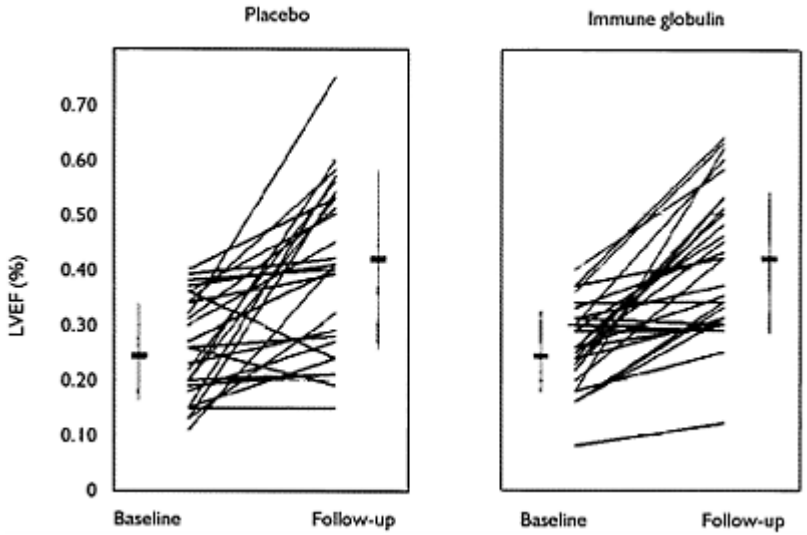
The background to clinical use of anti-inflammatory strategies in acute heart failure is that most of these episodes are thought to be viral or immune-mediated. Indeed, various anti-inflammatory strategies have been studied, most comprehensively the use of exogenous glucocorticoids together with standard immunosuppressive agents[22,23]. Long-term outcome studies with these strategies have been equivocal at best. Hence, there is an urgent need for effective therapy(ies) in this setting.

There is considerable preclinical evidence to support the concept that IVIG can suppress proinflammatory cytokine activation in the period immediately following myocardial injury and/or inflammation. In a murine encephalomyocarditis-related viral myocarditis, IVIG treatment prevented the development of clinical heart failure and improved survival[24]. IVIG in this model was associated with significant reductions in TNF-α, γ-interferon, macrophage inflammatory protein-2 and interleukin-6. At the level of the myocardium, myocardial necrosis was suppressed in association with the T-cell infiltration that is characteristic of this model of myocardial injury.

These and other preclinical data have supported the study of IVIG in the acute myocarditis/heart failure setting.

An uncontrolled series of adults with recent-onset dilated cardiomyopathy appeared to derive benefit from IVIG therapy, leading to a definitive placebo-controlled trial in 62 patients with recent-onset heart failure (less than 6 months of symptoms) and left ventricular ejection fraction (LVEF) less than 40%[25]. Patients were randomized to 2 g/kg of IVIG or placebo. The primary end-point was change in LVEF at 6 and 12 months post-randomization. Overall, LVEF improved from 0.25±0.08 to 0.41±0.17 at 6 months and 0.42±0.14 at 12 months. However, the increases were virtually identical in patients receiving IVIG and

Figure 3 Left ventricular ejection fraction (LVEF) at baseline and follow-up in patients with recent-onset dilated cardiomyopathy receiving placebo (left panel) or immune globulin (right panel). No differences by treatment group were evident



those given placebo (Figure 3). Indeed, 36% of the 56 completing patients normalized their ejection fraction to greater than 50%.

These data demonstrate the difficulty in assessing any intervention in the setting of a condition such as myocarditis/acute heart failure, where there is a high rate of spontaneous recovery.

Chronic heart failure

Based on the preceding considerations, chronic heart failure may be a more readily achievable target for clinical trials of IVIG therapy. Spontaneous resolution of ventricular function beyond the immediate period of injury is rela tively rare. Thus, changes in ejection fraction are usually minimal in the placebo group. Indeed, with progressive myocardial dysfunction, ventricular function often worsens with placebo over the period of evaluation.

The mechanistic rationale for such an approach in chronic heart failure is well described earlier in this chapter.

There have been a number of recent small-scale studies which have evaluated the potential of IVIG in chronic heart failure. Gullestad and colleagues[26] studied 40 patients with chronic heart failure (i.e. no previous heart failure and no evidence of myocardial

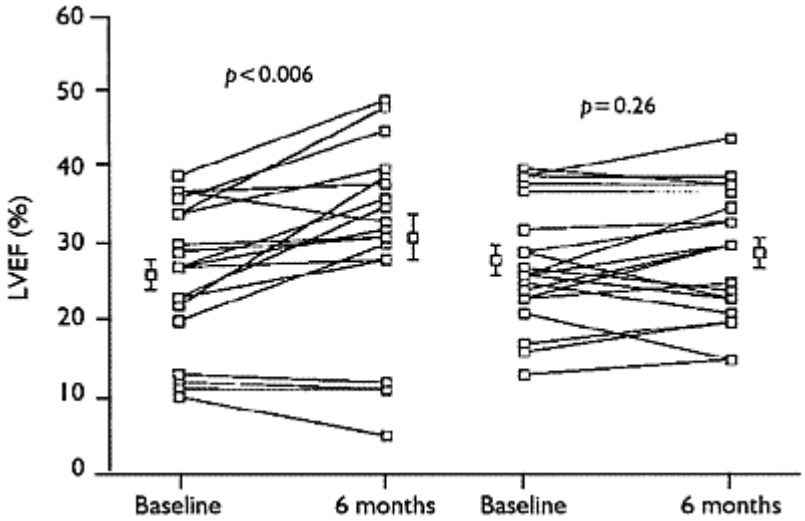
infarction or unstable angina during the past 6 months) and no change in medication during the past 3 months, together with an LVEF <40% and New York Heart Association (NYHA) class II–III symptoms. Patients received IVIG or placebo for a total period of 26 weeks. The chief finding of this proof of concept study was that, in contrast to placebo (where there was no change), there was a 5% increase in LVEF from 26±2% to 31±3% with IVIG therapy (Figure 4). However, between-group differences did not reach statistical significance. Of note, there was a significant increase in the anti-inflammatory interleukin-10 (IL-10), and a reduction in N-terminal proatrial natriuretic peptide (ANP), a powerful vasodilator and antinatriuretic factor which is a crude marker of cardiac dysfunction.

The authors concluded that IVIG was able to shift inflammatory balance in heart failure towards a more anti-inflammatory milieu with IVIG, and this was associated with an improvement in ejection fraction. In a follow-up paper[27], the authors demonstrated that there was an enhancement of systemic complement activation in heart failure with IVIG compared with placebo, involving both the classic and alternative pathways. The clinical relevance of this further complement activation is currently uncertain.

Peripartum cardiomyopathy

Peripartum cardiomyopathy is another potential target for IVIG, as its etiology is thought to

Figure 4 Left ventricular ejection fraction (LVEF) before and following 6 months' treatment in patients with congestive heart failure receiving intravenous immunoglobulin (IVIG) (left panel) or placebo (right panel). There was a statistically significant within-group difference in LVEF observed with IVIG-treated patients, but not with placebo. Between-group differences were not significant



be also on an immune basis, with a frequent finding of lymphocytic myocarditis on endomyocardial biopsy[28]. Significant numbers of patients recover in the first 6 months after presentation (30–50%). However, for the remainder, the prognosis is similar to those with idiopathic dilated cardiomyopathy.

A small (17-patient) retrospective analysis of treatment with IVIG (2g/kg) involved a comparison with 11 historical control subjects[29]. The authors found a greater improvement in LVEF with IVIG (increase of 26±8%), compared with 13±3% with placebo. Indeed, all patients treated with IVIG had an improvement of at least 10 ejection fracture units and only one patient was left with severe left ventricular dysfunction.

Thus, although lacking prospective randomized control clinical trial data, the use of IVIG therapy is deserving of further investigation for patients in this setting.

SUMMARY

Immune activation is almost certainly a major contributor to the progression of heart failure that follows the initial myocardial injury or inflammatory insult. On this basis, therapies targeting this activation would be expected to yield beneficial effects, complementary to and independent of blockade of key neurohormonal systems, such as the sympathetic nervous system and renin—angiotensin—aldosterone system.

Specific cytokine blocking strategies, such as with the TNF-α antagonists etanercept or infliximab, have not been successful in heart failure, raising the prospect that more broad-based therapies may be of greater potential utility. Although a number of approaches are currently being assessed, the known safety profile and powerful immune-modulating activity of intravenous immunoglobulin places this therapy at the forefront of potential add-on therapies for the treatment of this condition. Large-scale prospective trials are urgently needed to address this issue definitively and to influence patterns of prescribing by clinicians.

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Intravenous immunoglobulin: potential role in the management of severe acute respiratory syndrome

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INTRODUCTION

On 12 March 2003, the World Health Organization (WHO) issued a worldwide alert regarding cases of a rapidly progressive respiratory illness in Guangdong Province (China), Hong Kong, Vietnam, Singapore and Canada. WHO coined the term 'severe acute respiratory syndrome (SARS)' for this highly infectious disease. The causative agent was identified to be a novel coronavirus which has been named the SARS-CoV[1,2]. As of 26 September 2003, there were 8098 cases of SARS in 29 countries with 774 deaths (WHO website).

In Singapore, the SARS outbreak which began in early March 2003 was traced to a traveller returning from Hong Kong[3]. On 22 March 2003, Tan Tock Seng Hospital (TTSH), a 1200-bed acute general hospital where the national Communicable Disease Center is co-located, was designated the national center for managing SARS. During this outbreak, 199 SARS patients were admitted to TTSH, with 46 to our SARS intensive-care unit (ICU).

SARS-CoV INFECTION

SARS is spread mainly from person to person, and can be acquired from face-to-face contact suggesting droplet spread. The incubation period is reported to be 2–16 days (median 6 days). SARS-CoV causes a spectrum of manifestations from subclinical infection to severe respiratory distress. The first symptom is typically fever (>38°C). Other symptoms include rigors, malaise, myalgia, headache, diarrhea, dry cough and dyspnea. Some 19–32% of patients require ICU care, and the majority of patients need mechanical ventilation[4,5]. Mortality is greater than 10% in many countries. The majority of deaths occur in the second to fourth week of ICU stay, when patients with acute respiratory dis tress syndrome (ARDS) progress to multiorgan failure, sepsis and thromboembolic complications[4,5]. The infection is milder in younger children[6]. Older patients have a poorer prognosis[7].

Lymphopenia is present in the majority of patients, and low CD4 and CD8 cell counts in peripheral blood at presentation are reported to be associated with adverse outcomes[8]. More than half have thrombocytopenia. Elevated lactate dehydrogenase (LDH) is a

marker of disease severity. Chest X-ray findings vary from normal to diffuse interstitial infiltrates characteristic of ARDS. Diffuse alveolar damage with classical hyaline membranes, intravascular fibrin thrombi and focal necrosis of bronchial mucosa are found on pathological examination of lung tissues at post-mortem[9,10]. Fibroproliferation has been noted in some cases, and there is report of an increase in macrophages in the lung[9]. Cytokine studies of sera of 60 of our patients with SARS showed elevated transforming growth factor-β (TGF-β) in 55 patients (91.7%), interleukin-8 (IL-8) in 32 (53–3%) and IL-6 in 27 (45%). These peak in the second and third weeks of illness, corresponding to the development of respiratory distress (unpublished data). IL-10 was elevated in only 13 patients (21.7%) and interferon-γ (IFN-γ) in 20 (33.3%).

IMMUNOMODULATION IN THE TREATMENT OF CRITICALLY ILL SARS PATIENTS

No antiviral drug is proven effective, and treatment strategies for SARS remain diverse and experimental without clear evidence of efficacy[11,12]. Early in the outbreak, all patients in our hospital with radiographic abnormalities received either levofloxacin or a combination of a macrolide and intravenous ceftriaxone. Oseltamivir, ribavirin and intravenous corticos teroids were also prescribed to some patients in the initial stages. Severely ill SARS patients responded poorly to these drugs, despite maximal therapy in the ICU, and several patients succumbed[3]. A SARS Clinical Management Workgroup was formed in early April 2003 to oversee protocol development. Based on the clinical and initial pathological findings on post-mortem examination, we hypothesized that death in the group with a severe and protracted ARDS course was related to an overwhelming host inflammatory and immune response to the viral infection. An immunomodulatory protocol was thus formulated for use in SARS patients admitted to the ICU.

SARS patients were admitted to our ICU if they were clinically in respiratory distress and fulfilled acute lung injury (ALI) or ARDS criteria based on an arterial oxygen tension/fraction of inspired oxygen *(PaO2/FiO2)* ratio of ≤300mmHg or ≤200mmHg, respectively[13]. Patients who deteriorated were mechanically ventilated and best supportive therapy was prescribed[4]. The immunomodulatory regimen consisted of intravenous methylprednisolone 200mg daily for 3 days followed by intravenous hydrocortisone 100mg 8-hourly if body weight was 50kg or lower and 100mg 6-hourly if body weight exceeded 50 kg. Intravenous hydrocortisone was tapered every 3 days and converted to oral prednisolone where appropriate. The total duration of corticosteroids was 3–4 weeks. Corticosteroids have been used in the treatment of ARDS, as steroids reduce the production of many inflammatory and profibrotic mediators by several mechanisms. Studies have shown that late-phase ARDS patients may benefit from high-dose corticosteroids. However, the use of corticosteroids in ARDS is still controversial[14]. Low-dose intravenous methylprednisolone was chosen because of concern about opportunistic infections. In addition, electron microscopic studies showed viral particles in the lung of one of our earlier post-mortem cases (unpublished), and we did not want to oversuppress the patients, which could potentially result in over-whelming viremia. Corticosteroid therapy was prolonged with the aim of achieving effective containment of the overwhelming host response and resolution of the acute respiratory distress[15].

Since low-dose intravenous methylprednisolone alone may not be sufficient to modulate the excessive host response, moderate-dose intravenous immunoglobulin (IVIG) was added for synergistic effects[16], and because it is known to be associated with few adverse reactions. IVIG was administered simultaneously at 0.4g/kg body weight every day for 3 days. IVIG has a complex mode of action, including the modulation of expression and function of Fc receptors, inhibition of complement-mediated damage and modulation of cytokine and cytokine antagonist production, provision of anti-idiotypic antibodies, regulation of cell growth and effects on the activation, differentiation and the effector functions of T cells and B cells[17]. The use of IVIG in ARDS has not been reported in the literature.

Our hospital's experience in the management of SARS ICU patients is reported elsewhere[4]. The first patient received this treatment protocol on 5 April 2003. We evaluated the efficacy of this regimen using mortality as the outcome. Survival time was defined as the interval between date of admission to the ICU and date of death or date of last follow-up as of 14 May 2003 (when censoring occurred). The Cox proportional hazards model was used to analyze the survival data. Fisher's exact test was used to compare the occurrence of complications in the two groups. The patient characteristics are listed in Table 1. Fifteen of these patients were prescribed intravenous methylprednisolone (IVMP) and IVIG. The other 30 patients did not receive this therapy, because 25 were treated before this protocol was formalized and five were deemed to be too ill. As of 14 May 2003, there were four deaths in the group that received IVIG and IVMP and 18 deaths in the other group. The hazard ratio for mortality in the group treated with IVIG and IVMP was 0.35 (95% confidence interval (CI) 0.12–1.05; p=0.061) compared with those who were not given this form of treatment (Figure 1). The difference in mortality failed to reach statistical significance (p=0.061) probably because of the small number of patients. After adjusting for APACHE scores (acute physiology and chronic health evaluation), there was minimal change to the hazard ratio (adjusted hazard ratio 0.41, 95% CI 0.14–1.23, p=0.113), suggesting that the difference in survival was not due to differences in disease severity. There was no difference in the incidence of infective, thromboembolic and renal complications. We conclude that the above combination therapy is associated with improved survival compared with the control group, and does not lead to unacceptable adverse events.

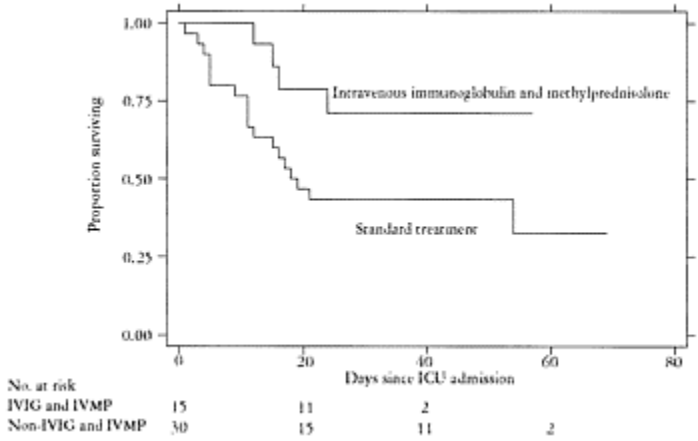
The correct timing of any immunomodulating protocol is crucial, because delayed therapy may not be efficacious. From the Hong Kong experience, early use of corticosteroids in SARS does not prevent the development of ARDS[18]. The authors of this study concluded that the worsening of SARS patients in week 2 of the illness is probably unrelated to uncontrolled viral replication, but may be related to immunopathological damage.

Table 1 Biodata of 45 severe acute respiratory syndrome (SARS) patients admitted to the intensive-care unit. Values are expressed as n (%) or mean ± SD

	All patients (*n*=45)	IVMP and IVIG (*n*=15)	Other forms of treatment (*n*=30)
Sex			
male	23(51%)	8(53%)	15(50%)
female	22(49%)	7(47%)	15(50%)
Age (years)	50.0±16.5	51.1±18.2	49.5±15.9
Race			
Chinese	33(73%)	12(80%)	21(70%)
Malay	6(13%)	1(7%)	5(17%)
Indian	4(8%)	1(7%)	3(10%)
others	2(4%)	1(7%)	1(3%)
PaO_2/FiO_2 ratio	142±96.5	165±118.5	132±84.6
APACHE II score	19.4±9.7	17.3±7.2	20.4±10.6
Required mechanical ventilation	38(84%)	13(87%)	25(83%)

PaO_2, arterial oxygen tension; FiO_2, fraction of inspired oxygen; APACHE, acute physiology and chronic health evaluation; IVMP, intravenous methylprednisolone ; IVIG, intravenous immunogloblin

Figure 1 Kaplan-Meier estimates of the survival of two groups of patients, the upper curve representing those who received intravenous immunoglobulin (IVIG) and methylprednisolone (IVMP) the lower those who did not. ICU, intensive-care unit



SARS AND HYPERIMMUNE GLOBULIN

Serum immunoglobulin M (IgM) and IgG antibodies to SARS-CoV could be demonstrated from the second week of infection in the majority of patients. Serum IgG to a recombinant SARS-CoV nucleocapsid antigen could be detected as early as the first week of symptoms, and remained detectable beyond 56 days of infection (unpublished). Convalescent plasma may be collected from recovered SARS patients and hyperimmune globulin produced. Its value in protecting patients at high risk, if given upon contact with SARS, needs to be studied.

CONCLUSION

SARS is an infection that is of worldwide concern because of its highly infectious nature and associated high mortality. It is increasingly clear that respiratory distress is the result of a dysregulated inflammatory and immune response. The result of our experience using IVMP and IVIG as immunomodulatory agents during the ARDS phase is encouraging, and needs to be confirmed. The potential role of a SARS hyperimmune globulin should also be addressed.

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Enhancement of the immune response to *Mycobacterium tuberculosis* by high-dose intravenous immunoglobulin in mice

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Tuberculosis (TB) kills approximately 2 million people each year. The global epidemic is growing and becoming more dangerous. The breakdown in health services, the spread of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) and the emergence of multidrug-resistant TB are contributing to the worsening impact of this disease. In 1993, the World Health Organization (WHO) took an unprecedented step and declared tuberculosis a global emergency, so great was the concern about the modern TB epidemic. It is estimated that between the years 2002 and 2020, approximately 1000 million people will be newly infected, over 150 million people will become sick and 36 million will die of TB, if control is not further strengthened.

TB is a contagious disease spread in droplets by coughing, sneezing, talking or spitting by those with pulmonary disease. A person needs only to inhale a small number of bacilli to be infected. Left untreated, each person with active TB will infect on average between 10 and 15 people every year. Someone in the world is newly infected with TB every second:

(1) Nearly 1% of the world's population is newly infected with TB each year;
(2) Overall, one-third of the world's population is currently infected with the TB bacillus;
(3) Of people who are infected with TB (but who are not infected with HIV) 5–10% become sick or infectious at some time during their life.

Each year, more people are dying of TB. In Eastern Europe and Africa, TB deaths are increasing after almost 40 years of decline. In terms of numbers of cases, the biggest burden of TB is in South-east Asia.

TB accounts for about 11% of AIDS deaths worldwide. In Africa, HIV is the single most important factor determining the increased incidence of TB in the past 10 years.

In addition, poorly managed TB programs are threatening to make TB incurable owing to the emergence of multidrug-resistant TB (MDR-TB), caused by inconsistent or partial treatment, because of poor compliance, incorrect prescribing or unreliable drug supply. Rates of MDR-TB are high in some countries, especially in the former Soviet Union, and threaten TB control efforts. From a public-health perspective, poorly supervised or incomplete treatment of TB is worse than no treatment at all. When people fail to complete standard treatment regimens, or are given the wrong treatment regimen, they may remain infectious. The bacilli in their lungs may develop resistance to anti-TB drugs. People they infect will have the same drug-resistant strain. While drug-resistant TB is treatable, it requires extensive chemotherapy (up to 2 years of treatment) that is often

prohibitively expensive (often more than 100 times more expensive than treatment of drug-susceptible TB), and is also more toxic[1].

The only currently licensed vaccine for TB is bacille Calmette-Guerin (BCG), which has variable efficacy of between 0 and 80%. It appears to protect from serious TB in children but is much less effective in adult pulmonary disease, which is the major health burden. There has been much recent work attempting to develop an improved vaccine using subunit, DNA, BCG-expressing immunodominant epitopes, attenuated *Mycobacterium tuberculosis* (Mtb) and prime boost strategies[2]. The first of these approaches using BCG prime and MVA (modified vaccinia virus Ankara) antigen 85 boost has commenced clinical trials (personal communication, Helen McShane, 2003), and appears to be promising. One of the problems with studies of this type is the lack of good immunological correlates of protection. Although the role of CD4 and CD8 T cells as well as interferon-γ is undisputed, measurement of interferon-γ-producing T cells does not always correlate with protection or even the generation of effective long-lasting memory.

The immune response to Mtb is suboptimal, and Mtb is known to evade an effective immune response resulting in chronicity. Several mechanisms may allow Mtb to escape, including its waxy coat, down-regulation of CD40 and CD80, and interference with phagosome lysosome fusion within the macrophage preventing access to the major histocompatibility complex (MHC) class I pathway of antigen presentation. Vaccine strategies are aimed at modification of the pathogen or parts thereof to induce protective immunity better than BCG; we have aimed to modify the immune response itself to improve control of TB using intravenous immunoglobulin (IVIG).

IVIG is a blood product prepared from the serum of between 1000 and 15000 donors per batch. It is the treatment of choice for patients with antibody deficiencies, where it is used at a 'replacement dose' of 200–400 mg/kg body weight, 3-weekly[3]. In contrast, 'high-dose' IVIG (hdIVIG), usually given at 2g/kg/month, is used as an 'immunomodulatory' agent in an increasing number of immune and inflammatory disorders[4].

Here, we report that mice treated with hdIVIG following infection with Mtb have 2 log lower colony counts in the lungs and spleen; this is much greater than the reduction in colony counts observed when mice are vaccinated with BCG 9–12 weeks prior to infection with Mtb. Lung histology in IVIG-treated mice shows a denser mononuclear cell infiltrate, and individual granulomas in treated mice appear to be more lymphocyte-predominated than in controls. IVIG *in vitro* does not inhibit the growth of Mtb in murine bone marrow-derived macrophages, suggesting that an enhanced immune response rather than an effect on macrophages is responsible for the observed reduction in colony counts.

The mechanism of action of hdIVIG remains incompletely understood; however, in this setting it is possible that enhancement of cross-priming[5,6] or alterations in regulatory T cells may play a role, as the pool of antibodies in IVIG is derived from Western donors, some of whom will have been vaccinated with BCG. Approaches with IVIG or components thereof could be used therapeutically and to enhance vaccine efficacy.

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Potential applications for intravenous immunoglobulin

49
Intravenous immunoglobulin for treatment of recurrent miscarriage

L.A.E.Noeos

MATERNAL-FETAL TOLERANCE

Pregnancy represents a major immunological event in a woman's life. The maternal immune response to the placental and fetal semiallograft largely remains an enigma. The maternal-fetal tolerance is very complex and probably continuously adapting to the different phases of a normal pregnancy. Only some aspects of early pregnancy have been understood, e.g. how a 6-day-old embryo initially attaches to the wall of the uterus by the same mechanism as neutrophils use to attach to the endothelium of the vessel wall in inflammation. This is only the beginning of the pregnancy Implantation is an active process between the blastocyst and the endometrium, and both secrete cytokines that influence each other and ultimately determine the next phase. What happens next is of a high magnitude of complexity. The final result is more than local tolerance. It is well recognized that fetal cells pass into the maternal circulation routinely during the course of pregnancy and that this influx increases at the time of parturition. Fetal-derived stem cells have been found to persist in maternal peripheral blood for decades after childbirth. This persistent micro-chimerism can play an important role in the tolerance of foreign, paternal-derived antigens by the pregnant woman, and has been implicated in some autoimmune disorders in later life[1]. The occurrence of anti-human leukocyte antigen (HLA) alloimmunization in pregnancy is high. Tissue-type antigens and especially HLA determinants have long been suspected of influencing the evolution of pregnancy and the immune response[2]. On the other hand, it has been shown that the child carries an almost lifelong immune memory to the non-inherited maternal HLA molecules (NIMAs) it contacted during embryonic and fetal life. In kidney transplantation programs these NIMAs can be used to select acceptable mismatches in difficult cases.

Table 1 Possible disorders in recurrent spontaneous abortion[3]

Genetic factors (mostly in sporadic miscarriage)
Anatomical factors (uterine septa?)
Cervical incompetence (late miscarriage)
Infective factors (sporadic miscarriage)
Endocrine factors (diabetes, thyroid disease, PCO, ovulatory defects)

Autoimmune factors (primary antiphospholipid syndrome)
Thrombophilic defects (hereditary or acquired)[5]
Alloimmune factors (leukocyte/HLA immunization)
Unexplained recurrent miscarriage: 40% of cases[4]
PCO, polycystic ovary; HLA, human leukocyte antigen

RECURRENT SPONTANEOUS MISCARRIAGE

Recurrent spontaneous abortion (RSA), abortus habitualis, affects a higher number of women than expected by chance alone[3], and the incidence is expected to rise further in the developed world, influenced by the tendency to have pregnancies at a later age. This age-relation is a continuous variable in an otherwise intriguing syndrome. Faced with the failure to explain this RSA phenomenon and with the uncertainty of the outcome of therapeutic, prophylactic or pre-emptive interventions, several groups of clinical investigators have developed diagnostic recommendations and therapeutic strategies. The degree of acceptance of some of these rules and proposals has been rather low. The pressure from the patients involved, and ethical aspects, have forced their physicians to administer treatments of insufficient proven benefit. The diagnostic aspects and evidence-based therapies for some patients with RSA can, however, guide those involved[4].

First, RSA is defined as the loss of three or more mostly first-trimester consecutive pregnancies. These women seem to have a persistent underlying cause for their losses. The probability of a normal third pregnancy is in the order of only 50–60%. So, it seems acceptable to start investigations after at least two unexplained abortions.

The list of underlying disorders is divided into several categories (Table 1).

Immunology may be the key to unexplained pregnancy loss: immune disorders occur at a remarkably high frequency in this population (Table 2), and some immune interventions seem to be therapeutic. These disorders, with autoimmune as well as alloimmune and embryo-derived elements.

Table 2 Immunological abnormalities in 47 study patients[6]

	Positive patients (%)
Antithyroid antibodies	53
Increased natural killer cells	40
Antiphospholipid antibodies	32
Antinuclear antibodies	28
Increased IgM levels	28
Increased CD4/CD8 ratio	15

Antiovarian antibodies	2
IgA deficiency	2

Ig, immunoglobulin

TREATMENT OF RECURRENT MISCARRIAGE

Treatment of RSA has been controversial. Hormonal, antimicrobial or antithrombotic therapies have not been uniformly successful and may be associated with major complications. In specific situations, risk-adapted interventions can be recommended, as in the antiphospholipid syndrome and other thrombophilias[5].

The increasing evidence that immunological factors play an important role in unexplainable RSA, and that alloimmunization and/or immune modulation has been beneficial in many cases, has fuelled discussion considering RSA as an immunological problem[6,7]. Immunomodulatory therapies include immunosuppression with corticosteroids, immunostimulation with allogeneic lymphocyte immunization and modulation by intravenous immunoglobulin (IVIG) concentrates.

The variable success rates with corticosteroids and with complex cellular therapy troubled the outcome of several mostly small-scale trials[8]. Even with the current data on IVIG in RSA, the controversy is not completely settled.

INTRAVENOUS IMMUNOGLOBULIN IN RECURRENT SPONTANEOUS MISCARRIAGE

IVIG has been found to be beneficial in some studies and ineffective in others[7]. This could be expected, faced with such an intricate complex clinical situation. Even small differences in methodology (selection of patients and of measurements) can generate discordant results. The observations of some studies from the past decade illustrate the potential of IVIG (Tables 3–5).

Table 3 Outcome of 61 pregnancies randomized to intravenous immunoglobulin (IVIG)/albumin[7]

Pregnancy outcome	***n***	**IVIG (%)**	**Placebo (%)**	***p* Value**
Delivery	29	62	38	0.04
Abortion	32	34	66	0.04
blighted ovum	15	53	47	NS
intrauterine death	17	18	82	0.004
Total	61	48	52	NS

NS, not significant

Table 4 Pregnancy outcome in 47 study patients[6]

	n	Pregnant *(n)*	IVIG *(n)*	Term *(n)*	*p* Value
IVIG therapy	36	24	>26 weeks, 20	19	
			>10 weeks, 4	3	
No IVIG	11	7	—	0	0.001

IVIG, intravenous immunoglobulin

Table 5 Outcome in 50 pregnancies with three or more first-trimester recurrent spontaneous abortions: study performed at the Department of Internal Medicine, Ghent University Hospital, Belgium

	n	Term *(n)*	Abortion *(n)*	*p* Value
IVIG therapy	42	38	4	
No IVIG	8	6 (3*+3)	2	NS/0.002†

*Pregnancies following IVIG-supported previous successful pregnancy; †untreated pregnancies only; IVIG, intravenous immunoglobulin; NS, not significant

DISCUSSION AND CONCLUSIONS

Important differences exist between the above studies:

(1) Differences in selected study population: inclusion of women with only two spontaneous miscarriages in the first, but three or more in the two other reports;
(2) Differences in study design: randomized in the first, observational in the two others;
(3) Differences in treatment strategies: IVIG started preconception and in high dose or intermediate dose in the first two, and started post-conception and with low dose in the last;
(4) Differences in reporting: in the first and last, only pregnant patients are reported.

We chose only 80–100mg/kg per infusion, every 3–4 weeks, until the 20th week. The other protocols used 500 or 200mg/kg, also every 3–4 weeks, until at least 28 weeks or 26 vs. 10–12 weeks. Caution is needed before any conclusion based on these differences can be drawn. However, IVIG was beneficial in all three studies, and no major side-effects in mother or child were observed, underscoring the safety.

We observed several ‘normal’ pregnancies in women, after prior IVIG supported successful pregnancies.

These observations imply that the IVIG effect could be qualitative rather than quantitative and that this effect could be long-lasting. This is no complete surprise in alloimmunology. In organ transplant immunology, the immunomodulatory effect (T helper cell Th1/Th2 shift) of pretransplant transfusions, even one, has been known for many years, and it has been exploited as such. According to some investigators, this transfusion effect could be attributed to plasma components only, while others have demonstrated that HLA class II sharing (on contaminating leukocytes) is essential.

The shift in Th1/Th2 responses is also well documented after IVIG. Other more or less plausible hypotheses (e.g. anticytokine effects) on the action of IVIG components in early pregnancy are presented in this book.

We need more data, and larger randomized studies, including dosing experiments. Mean-while, we propose to accept/hypothesize a far reaching immunomodulatory effect of components in IVIG preparations, in some (or most) RSA patients.

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Epilepsy and the immune system: is there a link?

L.G.Lagae

INTRODUCTION

Epilepsy is a chronic disease that can be the result of different brain insults such as developmental disorders, brain tumors, hypoxic-ischemic events and trauma (symptomatic epilepsies). However, still in 30–40% of patients no cause for the epilepsy is found (idiopathic epilepsies). Recently, various genetic abnormalities have been described in idiopathic epilepsies. There is also substantial evidence that the immune system can play an important primary or secondary role in the epileptogenic process. This is underscored by the possible antiepileptogenic properties of immunotherapy (especially steroids and immunoglobulins). This chapter provides evidence that some forms of epilepsy have an immunological basis and that there is a higher incidence of epilepsy in well-known immunological disorders. We do not cover the various immunological abnormalities (serum immunoglobulins, cellular immunity, human leukocyte antigen (HLA) types, autoantibodies and cytokines) that have been described in mostly heterogeneous groups of epileptic patients[1–4].

EVIDENCE FOR AN IMMUNOLOGICAL BASIS IN CERTAIN EPILEPSY SYNDROMES

Rasmussen encephalitis

Rasmussen encephalitis (RE) is a relatively rare (<1% of all epilepsies) epileptic syndrome, affecting primarily one hemisphere and occurring in previously healthy individuals. It consists of childhood-onset severe focal seizures and a subsequent progressive evolution towards ‘epilepsia partialis continua’. Seizure activity is refractory to anticonvulsive drug treatment and leads to significant neurological deterioration with hemiparesis and cognitive decline. Neuropathology of affected brain tissue typically shows perivascular lymphocyte cuffing, microglial proliferation with nodule formation, gliosis, neuronophagia (in some cases) and neuron loss in the affected hemisphere. Rasmussen described a first series of 27 patients in the early 1960s, and based on the histopathological findings, a viral etiology was proposed[5]. More recently, other investigators have used techniques such as *in situ* hybridization and the polymerase chain reaction (PCR) to demonstrate the presence of viral sequences (cytomegalovirus (CMV), Epstein—Barr virus (EBV), measles, herpes simplex virus-1 (HSV1)) in surgical

specimens of RE patients[6–10]. Supportive evidence for a viral etiology is the report of four RE patients, of whom three responded favorably to treatment with ganciclovir[11]. In contrast, the fact that RE occurs as a sporadic disease, with no seasonal or epidemic pattern, and without clustering in families, races or geographic regions, argues against a viral etiology.

Should RE indeed be viral in origin, the role played by the virus is not clear. Almost half of RE patients have a history of an infectious or inflammatory event, 1–6 months prior to seizure-onset. Viral agents may therefore act merely as a triggering factor of the inflammatory response. There is increasing evidence indicating that the neuroinflammatory process in RE results from an autoimmune response. Rogers and colleagues reported, in 1994, a correlation in humans between RE and the presence of serum antibodies to the neuronal glutamate receptor subunit GluR3[12]. They showed that immunization of rabbits with GluR3 induced behavior typical of seizures (indicating that anti-GluR3 antibodies can modulate neuronal excitability), associated with histopathological findings mimicking RE. Rogers therefore postulated RE to be an autoimmune disorder. Levite and associates delivered direct evidence of the pathogenicity of GluR3 antibodies by showing that mouse derived GluR3 antibodies bind specifically to cultured neurons and lead to apoptotic neuronal death[13]. GluR3 antibodies, however, have been shown not to be specific for RE. Bernasconi and co-workers demonstrated, using immunohistoblot on rat brain sections, that GluR3 antibodies were present in 82% of RE patients but also in 64% of patients with partial epilepsy[14].

Whitney and associates used immunohistochemistry to demonstrate the presence of focally distributed immunoglobulin G (IgG) and complement-positive neurons, which co-localized with activated astrocytes, in surgical specimens of three of five RE patients[15]. The specimens that stained negative came from two patients with non-active RE and from non-RE epileptic control patients. The authors therefore suggested that the initial active phase of RE is mediated by an antibody-induced, complement-dependent immune destruction of neurons. In this respect, Xiong and colleagues provided direct evidence of the deleterious consequences of the deposition of the ‘membrane attack complex (MAC)’, by showing that sequential infusion of the individual proteins of the membrane attack pathway (C5b6, C7, C8, C9) into the hippocampus of awake, freely moving rats induced behavioral and electrographic seizures, as well as neuronal cell toxicity[16].

Li and colleagues studied the T-cell response in human RE brain lesions. PCR analysis of TCRVβ chain gene expression revealed significant individual biases in TCRVβ usage; moreover, CDR3 size- and sequence-analysis within the predominant TCRVβ families exhibited limited size heterogeneity and extensive repetition of in-frame CDR3 nucleotide motifs[17]. These data indicate that the local T-cell immune response in RE is mediated by a restricted population of T cells, and that it is therefore necessarily initiated by a confined number of discrete (viral?) antigens.

The concept of RE having an immunological background has led clinicians to explore new treatment options. Available experience is limited, but offers further circumstantial evidence for RE as an autoimmune condition. Rogers and colleagues obtained a transient beneficial effect using repeated plasma exchange in a seriously ill child, with improvement of seizure control and neurological function, and with reduced titers of GluR3 antibodies[12]. High-dose corticosteroids offered some improvement in seizure control and neurological deficit in seven of a series of eight RE patients[18] and in ten of a

series of 17 patients[19]. In the former series, those in whom treatment was started early in the disease course experienced a lasting effect, albeit with recurrent episodes of transient relapse[18]. Intravenous immunoglobulin (IVIG) has been used in the past, and was reported to offer some reduction of seizure frequency in eight of a series of nine RE patients[19]. Leach and associates reported successful long-term therapy with IVIG in two adult RE patients, resulting both in clinical improvement and in suppression of inflammatory markers in the cerebrospinal fluid (CSF)[20]. Immunoadsorption (protein A) improved seizure frequency and neuro-psychological deficits in one RE patient, and clinical improvement correlated with reduction in GluR3 antibodies[21]. Two cases were described in whom intraventricular treatment with interferon-α was effective in alleviating seizure activity[22]. The mechanism of action of interferon-α in RE could lie in its immunomodulatory or in its antiviral properties. Alternatively, on the basis of the report by Gahring and associates, who showed that GluR3 antibodies exhibit immuno reactivity against interferon-α, one could hypothesize that interferon-α captures the GluR3 antibodies and prevents them from binding to neuronal antigens and causing disease[23]. Conversely, Gahring and associates hypothesized that the IFN-α receptor-1 may function as an autoantigen and that differential activity of autoantibodies toward heteroclitic antigens may result in variable clinical characteristics of autoimmune diseases.

Lennox—Gastaut syndrome and West syndrome (infantile spasms)

With regard to intractable idiopathic childhood epilepsies other than RE, convincing evidence to indicate that immune mechanisms are involved is scarce. In this respect, Lennox-Gastaut syndrome (LGS) and West syndrome (WS) have received much attention. LGS and WS are conditions with a specific but different clinical phenotype, which can both be precipitated by various causes, while the exact pathogenic mechanisms remain unknown. Corticosteroids are often used for treatment, and have sometimes been shown to be more effective than treatment with conventional antiepileptic drugs. Moreover, numerous studies, albeit small-scale and uncontrolled, report a positive response to treatment with IVIG[24–26]. The only placebo-controlled study of IVIG treatment in LGS reported a reduction of seizures following IVIG treatment in 20% of patients[27]. Further supportive of an immunological involvement in LGS are the report by Haraldsson and colleagues[28], who noted elevated serum Ig levels in LGS patients, and the reports by van Engelen and co-workers, who found cryptogenic LGS patients to have an impaired humoral response to hemocyanin and elevated total serum IgG levels[29], and who found an association of LGS with HLA DR5[30].

Landau—Kleffner syndrome

In 1957, Landau and Kleffner reported a series of six patients who experienced acquired loss of language during early childhood, suffered from epilepsy and displayed behavioral problems[31]. Since the initial description, numerous hypotheses on the cause and pathogenesis of this syndrome have been proposed. Landau favored the view that aphasia results from seizure-induced dysfunction of cortical (mainly linguistic) regions. Others hypothesized that both aphasia and epilepsy were epiphenomena of the same underlying

cortical dysfunction. In many cases, the onset can be temporally related to an infectious episode, suggesting that an infectious agent plays an etiological role. An immunopathogenic mechanism was suggested following the observations of a dramatic improvement of language function and electroencephalogram (EEG) abnormalities after immunomodulating therapy, namely corticosteroids and/or repeated IVIG infusions[32–34]. Interestingly, in one of these cases, the cerebrospinal fluid IgG index, which was increased prior to therapy, became normal after a first IVIG infusion[33]. In support of an immunological basis is the report by Connolly and associates who demonstrated that 45% of a study group of 13 patients with Landau—Kleffner syndrome or Landau—Kleffner variant had antibrain antibodies[35].

ASSOCIATION OF EPILEPSY WITH CERTAIN PEDIATRIC IMMUNOLOGICAL DISORDERS

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) may be associated with 16 different clinical syndromes affecting the central nervous system (CNS), and, occasionally, it is associated with a specific CNS autoimmune disorder. In a recent literature review paper, the prevalence of epileptic seizures in SLE patients was reported to range between 17 and 37%, constituting a rate significantly higher than in the control population[36,37]. Many patients have onset of epileptic seizures several years before clinical onset of SLE[38]. The pathogenic mechanism of SLE-associated seizures remains unknown. Immune-mediated neuronal damage or neuronal dysfunction, cortical thrombotic events, hypertensive encephalopathy and renal failure have been suggested to play a role in eliciting seizures in SLE, but often no primary event can be identified.

The role of autoantibodies in the pathogenesis of seizures in SLE is debated. Epilepsy is particularly common in SLE patients who also have anticardiolipin (aCL) antibodies, lupus anticoagulant and/or antiphospholipid (aPL) antibodies, although the reports on the incidence of these associations are not unequivocal[24]. Herrantz and colleagues screened a large series of 221 unselected patients with SLE for aPL antibodies and found a statistically significant association between moderate to high titers of IgG aCL antibodies and seizures[39]. Similarly, Liou and co-workers reported that epilepsy was 3.7 times more frequent in SLE patients with aCL antibodies[40]. Evidence for a possible direct role of aPL antibodies in SLE-associated epilepsy was recently provided by Chapman and colleagues, who showed that purified IgG, containing aPL antibodies, was capable of depolarizing rat brain-stem synaptoneurosomes[41]. Furthermore, DeGiorgio and associates have recently shown (in mouse and man) that anti-dsDNA antibodies cross-react with the human glutamate *N*-methy*l*-D-aspartate (NMDA) receptor[42]. The authors revealed a DNA epitope, involved in binding of the anti-DNA antibody, that is homologous to a sequence in two subunits of the NMDA receptor. They subsequently showed that anti-DNA antibodies with this cross-reactivity mediate apoptotic death of neurons *in vivo* and *in vitro*.

Other possible mechanisms by which antibodies can cause seizures include a direct effect on neuronal tissue, trapping of immune complexes within neural tissue or the

generation of microvascular lesions. The possibility of a direct effect of antibodies is supported by studies showing:

(1) That antibodies directed against the brain can directly cause seizures;
(2) That serum from SLE patients with epilepsy and anticardiolipin antibodies can inhibit chloride currents through the γ-aminobutyric acid (GABA) receptor complex;
(3) That the presence of anticardiolipin antibodies correlates with clinical symptoms;
(4) That antiphospholipid antibodies can react directly with CNS tissue[24].

Celiac disease

The prevalence of epilepsy in celiac patients has repeatedly been reported to be higher than that in the control population, suggesting the existence of an etiological—possibly immunological—association[43]. Hadjivassiliou and colleagues investigated a large series of patients suffering from either a specific neurological condition, or neurological disease from unknown origin[44]. They found that gluten sensitivity is significantly more common in patients with neurological disease of unknown etiology, and hypothesized that the antibodies may be either neurotoxic *per se* or participate in a neurotoxic immunological process.

Many patients suffering from celiac disease go unrecognized because this condition often exists with absent, mild or atypical features. This may represent an important bias to all studies of the prevalence of epilepsy in celiac disease. Therefore, several groups have approached this problem by assessing the prevalence of celiac disease in epileptic patients. Fois and associates[45] reported a prevalence of 1 in 87 patients, and Cronin and co-workers[46] reported a prevalence of 1 in 44 epileptics (of which 1 in 37 was in patients suffering from idiopathic epilepsy). According to several studies, the prevalence in the overall population varies between 1/150 and 1/300[47,48].

Interestingly, in recent years there have been several reports suggesting the existence of a specific syndrome of celiac disease, epileptic seizures and cerebral calcifications. Multiple case reports of patients affected with this triad exist, while in larger series, Gobbi and colleagues[49], Bardella and associates[50] and Fois and associates[45] reported that, respectively, 5/12, 4/5 and 3/9 patients with epilepsy and celiac disease had intracranial calcifications.

Stiff man syndrome

Stiff man or stiff person syndrome is a rare disorder characterized by fluctuating but progressive muscle rigidity and spasms. It occurs as an idiopathic disorder or as a paraneoplastic neurological syndrome. In about 60% of patients with idiopathic stiff man syndrome, serum antiglutamic acid decarboxylase (GAD) is found, a cytoplasmic enzyme involved in the synthesis of GABA, and in 80% of patients this is detectable in the CSF[51]. The paraneoplastic form, on the other hand, is asso-ciated with the occurrence of antiamphiphysin antibodies. GAD is located in GABAergic nerve terminals and also in pancreatic β cells. Antibody-positive stiff man syndrome has been reported to be associated with autoimmune diseases, most often insulin-dependent diabetes mellitus. Strikingly, epilepsy has been reported to occur with an increased incidence of 12% in stiff man syndrome. Solimena and colleagues[52] studied 33 such patients, and found that

all of those with epilepsy had GAD antibodies. Clinical reponses to GABAergic antiepileptic drugs and to immunomodulatory treatment (corticosteroids, plasmapheresis) support a pathogenic role of anti-GAD antibodies[24].

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Intravenous immunoglobulin as an immunomodulatory agent in systemic lupus eruthematosus and lupus nephritis

Y.Sherer and Y.Shoenfeld

Systemic lupus erythematosus (SLE) is a multi-systemic autoimmune disease characterized by various clinical manifestations and a huge number of autoantibodies. The World Health Organization (WHO) classification of SLE requires the presence of at least four of 11 criteria in a given patient in order to establish diagnosis[1]. Therefore, whereas one patient could have mild disease manifested by arthritis, photosensitivity, the presence of antinuclear antibodies and mild thrombocytopenia, another patient could experience a life-threatening condition including, for example, rapidly progressive renal failure, pleural effusion, autoimmune hemolytic anemia and high titers of anti-dsDNA autoantibodies. Nonetheless, both patients have the same disease, and this exemplifies the difficulties in new therapy evaluation in SLE. The problem is partially over-come by the use of disease activity scores such as the systemic lupus erythematosus disease activity index (SLEDAI) or Systemic Lupus Activity Measure (SLAM), which combine several clinical and laboratory parameters. Treatment of SLE patients usually consists of non-steroidal anti-inflammatory drugs, corticosteroids and other agents including cytotoxic ones (i.e. cyclophosphamide). These treatment options are often associated with serious adverse effects.

Intravenous immunoglobulin (IVIG) is used in SLE as an immunomodulatory agent. The evidence suggesting a beneficial role of IVIG in SLE is based on case reports and usually small case series. Various clinical manifestations have been reported to be successfully treated with IVIG (Table 1). We have reported a 28-year-old SLE patient who had persistent fever for 3 months, and developed within a few hours motor and sensory aphasia, rotator nystagmus with deviation of the eyes and severe nuchal rigidity Extensive series of imaging and laboratory tests were interpreted as normal, except for an elevated opening pressure at lumbar puncture, cerebrospinal fluid inflammatory findings and asymmetrical cortical perfusion on single-photon emission computed tomography. The patient with

Table 1 Clinical manifestations of systemic lupus erythematosus treated successfully with intravenous immunoglobulin

Pericarditis
Psychosis
Autoimmune hemolytic anemia

Lupus nephritis
Pancytopenia
Pneumonitis
Myelofibrosis
Thrombocytopenia
Polyradiculoneuropathy
Pleural effusion
Encephalitis
Pure red cell aplasia
Cardiogenic shock
Acquired factor VIII inhibitor

cerebritis received one course of high-dose IVIG and within 5 days her condition returned to that of 3 months before admission[2]. Another interesting case is that of an SLE patient with severe clinical presentation which included pericarditis, pleural effusion, nephrotic range proteinuria, leukopenia and lymphopenia. The patient received one course of high-dose IVIG (2.8g/kg body weight), and within a week post-IVIG therapy her condition significantly improved. One month post-IVIG there were decreased proteinuria, elevated leukocytes and lymphocytes count, a decrease in antinuclear and anti-dsDNA antibodies and the disappearance of pericarditis and pleuritis. This case further demonstrates the efficacy of IVIG in severe SLE with various clinical manifestations[3]. We also described a 59-year-old SLE patient who, while being treated with steroids, developed severe cardiac dysfunction with a left ventricular ejection fraction of 20%. Coronary angiography demonstrating normal coronary arteries supported the diagnosis of myocarditis. High-dose IVIG treatment was started, followed by improved cardiac function a few days later and normalization of the ejection fraction (50%) 1 month later[4].

Case series provide better evidence for the beneficial role of IVIG in SLE. We have reported 20 SLE patients treated with high-dose (2g/kg) IVIG monthly, in a 5-day schedule. Each patient received between one and eight treatment courses. They were evaluated for the clinical response, Systemic Lupus Activity Measure (SLAM) score before and after IVIG, levels of antinuclear antibody, dsDNA, SS-A or SS-B, ENA (extractable nuclear antigens), C3 and C4 levels before and after the treatment, and before and after each treatment course[5]. A beneficial clinical response following IVIG treatment was noted in 17 out of 20 patients (85%). A few clinical manifestations responded more to treatment: arthritis, fever, thrombocytopenia and neuropsychiatric lupus. The mean SLAM score was significantly decreased in nine patients evaluated before and after IVIG treatment. There was a tendency towards abnormal levels of complement and antibodies before IVIG courses among the treatment responders, compared with the non-responders, and similarly the former tended to have normalization of their abnormal levels more than the latter. These differences were found to be statistically significant only with respect to C4 and SS-A or SS-B levels before IVIG courses[5]. We also have preliminary results from a study of an additional 45 SLE patients treated with low-dose IVIG, in whom the treatment resulted in a significant decrease in SLEDAI score (unpublished data).

Other case series evaluated thrombocytopenia in SLE. Arnal and colleagues[6] retrospectively examined the response to treatment of SLE patients having thrombocytopenia. Among 31 patients treated with IVIG, 12 had a complete response and an additional eight had a partial response to treatment. These responses were transient. In the small series of Maier and associates[7], five of seven SLE patients had a beneficial response to IVIG, with sustained platelet counts for more than 6 months in four patients. The response rate in the series of Schroeder and co-workers[8] was 75% (nine of 12 patients), and in that case the patients had SLE manifested as arthritis, cytopenia, nephritis, erythema and vasculitis. In another study, 11 of 12 (92%) patients having various manifestations of SLE responded beneficially to IVIG[9].

One of the adverse effects associated with IVIG use is acute renal failure, usually transient but might be severe[10]. However, this is usually related to the solvent sucrose used in some IVIG preparations. Our data support the use of IVIG also in lupus nephritis. We described the clinical response of seven patients with treatment-resistant membranous and membranoproliferative lupus nephritis[11]. They were treated with six courses or one or two courses of high-dose IVIG. Plasma levels of albumin, total cholesterol, urea, creatinine, dsDNA antibody titers and daily proteinuria were measured just before the IVIG therapy, immediately on completion and 6 months later. All seven patients had a beneficial response to IVIG. In one patient, a decrease in proteinuria was evident 2 weeks after IVIG was started, the nephrotic syndrome gradually disappeared and she had no proteinuria in a 3-year follow-up. A decline in proteinuria was evident in another patient after the fourth IVIG course, but proteinuria reached the pretreatment level 4 months after the therapy ended. In the rest, the mean daily proteinuria before IVIG decreased after one or two IVIG courses, and further decreased when measured 6 months later. Similarly, the plasma cholesterol level decreased while the plasma albumin level increased after IVIG. Several other reports also support the use of IVIG in lupus nephritis, the most important being the one describing 14 patients with proliferative lupus nephritis who were randomized to monthly IVIG or cyclophosphamide as maintenance therapy[12]. The maintenance of remission over 18 months was similar in both groups.

In conclusion, IVIG is probably beneficial in SLE, both in the treatment of various clinical manifestations, and in general in decreasing disease activity as well as in lupus nephritis. Controlled studies are still needed to establish this efficacy, but it seems that IVIG can immunomodulate SLE, and thus should be considered in its management.

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52
Intravenous immunoglobulin for fibrosis, atherosclerosis and malignant conditions

I.Krause and Y.Shoenfeld

During recent years, we have treated a large number of patients (more than 200) with various autoimmune conditions using intravenous immunoglobulin (IVIG). This chapter reviews our experience with IVIG treatment in fibrotic conditions, and describes our findings and experience regarding the effect of IVIG therapy to prevent metastases in malignancy.

TREATMENT OF ATHEROSCLEROSIS WITH INTRAVENOUS IMMUNOGLOBULIN

The past decade has brought about a revolution in our attitude towards the etiology and pathogenesis of atherosclerosis. In addition to the conventional risk factors (e.g. smoking, diabetes, obesity, etc.), infections and inflammatory and immune involvements have been found to be detrimental in causation of atherosclerosis. Accelerated atherosclerosis has also been reported in many classical autoimmune rheumatic diseases, e.g. systemic lupus erythematosus (SLE), rheumatoid arthritis, vasculitis and others.

The autoimmune aspects of atherosclerosis have been delineated, and seem to involve both the humoral and cellular arms of the immune system, demonstrated by passive transfer of activated lymphocytes into naive mice leading to accelerated atherosclerosis. In contrast to many of the classical autoimmune diseases, several autoantigens/autoantibodies seem to be implicated, e.g. heat-shock protein 65[1], oxidative low-density lipoprotein (LDL), α_2-glucose phosphate isomerase[2,3], lipoprotein(a), prothrombin, anti-endothelial cell antibodies (AECAs) and more.

Being an immune/autoimmune-mediated disease, atherosclerosis is subject to immunomodulation with anti-CD40, IVIG, cytokines, chemokines and other immunomodulating agents. The mechanisms by which IVIG can influence atherosclerosis include an effect on matrix metalloproteinase-9 (MMP-9), and anti-idiotypes to anti-oxidative LDL[4,5]. It seems that all the above-mentioned immunomodulations lead to reduced atherosclerosis in animal models. It is conceivable that, in the near future, novel therapeutic approaches (e.g. anti-tumor necrosis factor (TNF)-α) will be incorporated into the routine therapy of subjects with atherosclerosis (for example, for restenosis prevention after angioplasty), and specifically when associated with rheumatic autoimmune diseases.

INTRAVENOUS IMMUNOGLOBULIN IN FIBROSIS

Deposition of collagen, laminin, fibrinogen, and other molecules is fundamental to the process of inflammation and healing. However, the accumulation of excessive amounts of these extracellular proteins may lead to malfunction of vital organs, resulting in myelofibrosis, cirrhosis, pulmonary fibrosis, extraperitoneal fibrosis or skin thickening. Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterized by microvascular damage, extracellular matrix deposition and fibrosis, involving mainly the skin, lungs and gastrointestinal tract. No effective therapy has so far been described for the diffuse fibrotic changes. Blank and colleagues[1] assessed the effect of IVIG on skin fibrosis in tight skin (Tsk/+) mice. The Tsk/+ mouse represents a murine model of scleroderma-like disease, with heritable fibrosis resembling the skin fibrosis seen in human SSc patients. The excessive fibrosis in these mice is the result of increased synthesis and accumulation of collagen in the skin. Tsk/+ mice received IVIG beginning at the age of 4 weeks, administered twice weekly for 4 weeks. Control mice were infused with 2% maltose. Collagen expression was decreased in the skin of Tsk/+ mice treated with IVIG, compared with that in control mice. The decreased collagen expression after exposure to IVIG was associated with decreased type I collagen gene expression. The reduction in skin fibrosis upon IVIG treatment was accompanied by inhibition of transforming growth factor-β (TGF-β) and interleukin-4 (IL-4) secretion by splenocytes. Levy and colleagues[6] were the first to report a response of SSc patients to treatment with IVIG. Three patients with progressive and rapidly deteriorating disease (mainly affecting the skin) were scheduled to receive 6 monthly courses of high-dose IVIG (2g/kg). Two of the three patients received six IVIG courses as planned, and no adverse effects or disease progression occurred during the therapy. The third patient received three courses, after which he developed renal failure, and later died of sepsis. All three patients had a large decrease in their skin score after treatment compared with that before treatment. It seems, therefore, that IVIG may have a role in the treatment of SSc patients with rapidly deteriorating skin disease, and possibly other target-organ fibrosis. Recently, we have expanded our experience to an additional 14 patients[7].

Myelofibrosis has been reported as a rare cause of pancytopenia in patients with autoimmune diseases. Aharon and colleagues[8] described a 54-year-old female patient who was admitted with severe anemia subsequently found to be due to marrow fibrosis. During the course of her hospitalization, the diagnosis of SLE was established. The patient was treated with high-dose steroids, but improvement of her clinical symptoms as well as normalization of her peripheral blood count were achieved only after high-dose therapy with IVIG was instituted. Along with the improvement in peripheral blood parameters, normalization of the bone marrow architecture was recorded on repeated bone marrow biopsy We suggest that IVIG therapy may be considered in extreme cases of bone marrow suppression in SLE.

ANTIMETASTATIC EFFECTS OF INTRAVENOUS IMMUNOGLOBULIN (Patents no. 5.562.902 and 5.965.730)

There is a bidirectional relationship between autoimmunity and cancer. Malignant conditions are frequently associated with autoimmune phenomena. Examples include: an increased incidence of Eaton-Lambert myasthenia-like syndrome in patients with small-cell carcinoma of the lung, thymoma in patients with myasthenia gravis, different types of epithelial or lymphoproliferative malignancies in patients with autoimmune hemolytic anemia, thrombocytopenia or neutropenia, and melanoma associated with vitiligo. Conversely, there is an increased risk of cancer in autoimmune conditions as exemplified by the emergence of ovarian carcinoma in patients with dermatomyositis, lymphoproliferative diseases in patients with rheumatoid arthritis, SLE and Sjogren's syndrome, lung cancer in scleroderma patients and thyroid papillary carcinoma in patients with autoimmune thyroid diseases. The cancer may appear at the time of diagnosis of the autoimmune disease or several years later. Since the two diseases are similarly treated, we studied the efficacy of IVIG as a treatment for malignant conditions.

The administration of IVIG to mice inoculated intravenously with melanoma or sarcoma cells induced a statistically significant inhibition of metastatic lung foci and prolongation of survival time[9]. Similar results were seen with severe combined immune deficiency (SCID) mice inoculated with SK-28 human melanoma cells. In a different model, a lower number of melanoma recurrences and prolongation of survival time were demonstrated in the IVIG-treated groups. *In vitro* studies revealed that IVIG was found to stimulate the production of interleukin-12 (IL-12), an antitumor and antiangiogenic cytokine. Moreover, it enhanced natural killer (NK) cell activity, thus explaining its beneficial effect in SCID mice (which lack B and T but possess NK cells)[3,9]. The results indicate that IVIG acts as an antitumor agent, and may be considered as a potential therapy for the prevention of tumor spread in humans.

In another experiment we studied the effect of purified IVIG on MMP-9 secretion and mRNA expression by *in vitro* differentiated human monocytic cells. Degradation of the extracellular matrix (ECM) is essential for the progression and metastasis of cancer cells. The ECM-degrading enzymes, matrix metalloproteinases (MMPs), are produced mainly by intratumor monocytes/macrophages. MMPs, particularly MMP-9, are reported to be of crucial significance for both growth and tumor invasiveness. Inhibition of the expression of MMP-9 may prevent tumor development. We found that IVIG dose-dependently and significantly reduced the amount of secreted MMP-9 and its mRNA expression. F(ab)2, but not Fc fragments, led to suppressed MMP-9 activity. However, competitive experiments demonstrated that Fc, but not F(ab)2 fragments, reversed the IVIG-induced inhibitory effects. Our results suggest that the whole immunoglobulin G (IgG) molecule may be needed for pertinent IVIG-induced MMP-9 down-regulation.

Our study points to an additional new mechanism whereby IVIG may play a beneficial role in the prevention of tumor spread in humans. We also tried to determine whether F(ab)2 prepared from IVIG binds to cellular structures of different tumor tissues. Direct immunohistochemistry using a streptavidin peroxidase staining method was performed on biopsy samples of 18 different tumor tissues. Positive staining of the cytoplasm, cell

membrane and nuclear membrane of several types of malignant tumors by F(ab)2 from IVIG was immunohistochemically demonstrated[10]. Nuclear staining of tumor cells by IVIG was rare. IVIG bound to various tumors of epithelial origin, especially colon carcinoma, breast carcinoma and squamous cell carcinoma of the lung. Malignant tumors of mesenchymal origin such as leiomyosarcoma have also demonstrated positive staining by IVIG. Hence, IVIG contains antibodies to the cytoplasm, nuclear membrane and cell membrane of various malignant tumors, especially of epithelial origin. This binding might provide a basis for the assumption that IVIG treatment of cancer patients may induce antibody-dependent cell-mediated cytotoxicity response against tumors, and implies that it can be potentially beneficial as adjuvant treatment of malignant diseases.

Merimsky and colleagues[2] observed a patient with a malignant peripheral nerve sheath tumor (MPNST) who was treated with IVIG for multiple sclerosis. Her MPNST course was remarkably longer and more indolent than expected, and she achieved a disease-free interval (DFI) of 30 months. Seven other patients, who were not treated by IVIG, had a relatively aggressive course (median DFI 3 months). These results led to examination of the effect of IVIG on the growth of sarcoma *in vitro* and *in vivo* in an experimental model of MCA-bearing mice. When added to MCA-105 sarcoma cell cultures, IVIG produced a dose-dependent inhibitory effect on [H^3]thymidine incorporation. The results demonstrate that the antiproliferative activity results from an apop totic effect of IVIG on the tumor cells. In a second set of experiments, we evaluated the capability of IVIG, when administered orally or subcutaneously, to inhibit the growth of MCA-105 sarcoma lung metastases. A decrease in the mean lung weight was observed in the mice that were treated by subcutaneous or oral administration, the latter being more effective. The results point to a potential role for IVIG in the treatment of MPNST and other soft-tissue sarcomas.

Recently, we reported a patient with superficial spreading melanoma with liver and lung metastases. The patient refused chemotherapy and was treated with monthly high-dose IVIG (2g/kg). Six months after the initiation of IVIG therapy the liver metastases regressed significantly, while the lung metastases remained the same[11]. After about 9 months from the initiation of IVIG therapy, new subcutaneous and bony metastases appeared, which continued to grow in the next 6 months, although there was no significant change in the lung and hepatic lesions. The patient died in a septic state 14 months after the start of IVIG. This is the first report pointing to the probable efficiency of high-dose IVIG in cases of unresponsive widespread metastases of melanoma, and points to the safe administration of IVIG in some patients with tumor metastases.

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Summeries of poster sessions

Posters are indicated in the text by numbers in parentheses

53
Intravenous immunoglobulin for immune deficiencies and passive immunization

I.Quinti, M.Cagliuso and F.Aiuti

The first indication for the use of gammaglobulins was the treatment of a primary immuno-deficiency, Bruton's or X-linked agamma-globulinemia. Since then, the efficacy in numerous open-label studies has been so clear that few clinicians would question its use in any of the severe primary humoral immuno-deficiencies. Most studies concluded that early intravenous immunoglobulin G (IgG) replacement therapy achieving residual IgG levels >5g/l is effective in preventing severe bacterial infections and pulmonary insufficiency. It could then be assumed that, concerning the treatment of primary immunodeficiencies, the story was over, but the presentations of the four posters in this session demonstrate that many problems still exist and require a better understanding. In particular, controversies exist regarding the need for a more intensive therapy required to prevent fully the onset of bronchiectasis, chronic sinusitis and non-bacterial infections, particularly enteroviral infections, in all cases, and the use of gamma globulin replacement in incomplete immunodeficiency states.

Two posters dealt with case reports. The first poster reported a successful liver transplantation in a patient with common variable immunodeficiency (CVID) and concomitant liver cirrhosis (1). A 13-year-old patient with liver cirrhosis, chronic sinusitis and candidiasis was treated for 3 years with intravenous immunoglobulin (IVIG). At the age of 16, due to liver failure, she underwent a liver transplantation which was successful. Since then, she has been treated with tacrolimus and IVIG. Important points of the discussion related to the wide variety of clinical conditions associated with CVID, the problems of diagnosis and, more importantly, the treatment opportunities. It should be stressed that primary immunodeficiency is not a condition that contraindicates immunosuppressive treatment, if this is required, in patients with CVID and concomitant autoimmune diseases or neoplasias, or in transplanted patients.

The second poster reported the clinical and immunological history of a 16-year-old male with chronic fatigue syndrome related to a PCR-confirmed parvovirus infection treated with high doses of IVIG (2g/kg) (2). The patient developed aseptic meningitis. He was then shifted to a subcutaneous route of gammaglobulin administration without clinical benefit. IVIG therapy at high doses was then reintroduced with a steroid pre-infusion pre-medication. This therapy was well-tolerated, viremia disappeared and the patient improved clinically without further adverse reactions. This poster addressed the question of severe adverse reactions in patients treated with high doses of IVIG. Severe headache in some patients has been attributed to aseptic meningitis, which has been observed in 1–11% of patients with myasthenia gravis or other diseases. The patient

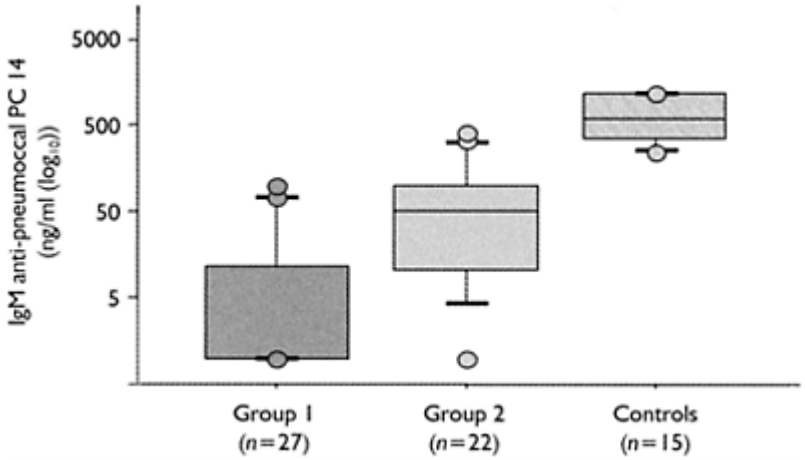
presented in the poster complained about headache, meningismus, photophobia, nausea, vomiting and fever which began within 24 h after IVIG and lasted for 3–5 days. The spinal fluid showed pleocytosis and increased concentration of protein and immunoglobulin, but the cause of aseptic meninigits is not known. This report showed that a severe adverse effect may be controlled by the concomitant use of steroid pre-medication.

The last two posters underlined the importance of testing specific antibody responses in patients with recurrent infections and in patients with well-defined primary immunodeficiencies. Both posters addressed the clinical and immunological advantages of the evaluation of anti-polysaccharide antibody responses. Both concluded that the response to polysaccharide antigens has a good inverse correlation with the risk of developing infections due to encapsulated bacteria and in particular to *Streptococcus pneumoniae*.

The first poster addressed the point of the diagnosis of possible selective antibody deficiency in patients with recurrent infections, low or no IgG antibody titers to pneumococcal polysaccharides and no other identified immunological defect (3). In these patients, the levels of specific IgG and IgM to capsular polysaccharide antigens before and after immunization with vaccines containing 23-valent capsular polysaccharides alone followed by vaccination with a seven-valent conjugated pneumococcal vaccine were assessed. All the patients investigated showed an impaired response to both vaccines. The authors concluded that specific IgG and IgM responses to pneumococcal vaccines are good predictors for the susceptibility to develop bacterial respiratory infections, even better than the simple measurement of the immunoglobulin serum levels. More importantly, the results of this poster demonstrated that the use of a conjugated vaccine did not overcome the defective response to a polysaccharide vaccine.

The last poster of this section (4) analyzed 60 patients with CVID: 27 with a clinical history of recurrent bacterial pneumonias and a pulmonary TC scan positive for the presence of bronchiectasis (Group 1), and 22 who had never had pneumonias and showed a negative pulmonary TC scan (Group 2). The two groups were comparable for the following characteristics: current mean age, age at onset of symptoms, age at diagnosis, time under IVIG therapy and mean delay of diagnosis. The impaired IgM response to pneumococcal capsular polysaccharide antigens correlated with the clinical presentation of the disease. CVID patients with and without severe pulmonary infections differed significantly in

Figure 1 Residual capacity of B cells to produce antibodies to polysaccharide antigens in patients with common variable immunodeficiency with (Group 1) and without (Group 2) severe pulmonary infections



the residual capacity of B cells to produce antibodies to polysaccharide antigens (Figure 1). It has recently been demonstrated that, despite early immunoglobulin replacement therapy, some patients develop lung disease and bronchiectasis. To overcome this complication it has been proposed that the IVIG dosage should be increased, although antibody quality might be more important than concentration. The goal of immunoglobulin substitution therapy in a CVID patient is to prevent morbidity and mortality resulting from infectious diseases. IVIGs substitute the function of serum IgG, corresponding to the high-affinity antibodies. The role of IgM antibodies, that represent most of the antipolysaccharide opsonizing antibodies directed to capsular antigens cannot be replaced by IVIG. This measurement of anti-pneumococcal polysac charide IgM antibodies represented a useful approach to predict the clinical outcome of the CVID disease and to address a more aggressive strategy for therapy, which beside IVIG, should include other therapeutic options such as prophylaxis of infectious episodes with antibiotics and respiratory rehabilitation.

POSTERS

1. Macura-Biegun A, Kowalczyk D, Siedlar M. Successful liver transplantation in a patient with common variable immunodeficiency concomitant with liver cirrhosis—case report
2. Stiehm ER, McGhee S, Kaska B, Liebhaber M. Prolonged IVIG in the treatment of parvovirus-related chronic fatigue syndrome (CFS) in a 16 year old male
3. Wolf HM, Artaker G, Samstag A, Sacher M, Eibl MM. Antibody response to pneumococcal vaccination in children with recurrent infections and impaired IgG-antibody response to pneumococcal polysaccharide
4. Quinti I, Donnanno S, Guazzi V, Aiuti F. B cell abnormalities in CVID patients: immunological mechanisms for an adequate IVIG treatment

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The use of intravenous immunoglobulin in hematology and transplantation

D.Provan

There were nine posters in this category, covering a wide range of topics including hemorrhagic scores in childhood idiopathic thrombocytopenic purpura (ITP), use of intravenous immunoglobulin (IVIG) in chronic immune neutropenia, hemolytic disease of the newborn, accelerated hemolysis in sickle cell disease and other hematological diseases, IVIG pre-solid organ transplantation and cytomegalovirus (CMV) prophylaxis in patients undergoing hematopoietic stem cell transplantation.

BLEEDING MANIFESTATIONS IN CHILDHOOD IDIOPATHIC THROMBOCYTOPENIC PURPURA

Dr Aronis' group from Athens, Greece have assessed bleeding severity and the use of IVIG in childhood ITP (1). This disorder is generally acute (lasting <6 months) and requires little therapy. However, as they pointed out, bleeding may occur in children with both acute and chronic forms of the disease. Their retrospective study involved 795 children (538 acute, 222 chronic and 35 recurrent), presenting to the Aghia Sophia Children's Hospital between 1975 and 2002. The authors showed clearly that children who bleed generally do so at the time of onset of their disease. Bleeding included epistaxis (20%), melena (5%), hematuria (3%) and bleeding from other sites. Intracranial hemorrhage occurred in 0.2% of their group. When treatment was administered, IVIG was most commonly used (131 out of 229 patients treated). In keeping with previous studies using IVIG, although the therapy was effective in most cases, no lasting responses were seen.

IVIG IN CHRONIC IDIOPATHIC NEUTROPENIA

Immune neutropenia is a disorder analogous to ITP in which antibody-opsonized neutrophils are removed prematurely by the reticuloendothelial system. Systematic studies of neutropenia are lacking, and most studies have focused on the use of granulocyte colony-stimulating factor (G-CSF) for elevation of the neutrophil count. The authors reported one case involving a 50-year-old woman with recurrent mouth ulcers and a neutrophil count that ran between 0.3 and 1.8×10^9/l (2). Strong evidence for an immune immunoglobulin G (IgG)-mediated etiology came from the observation that both of her children were neutropenic until they were 6 months of age, around the time when

maternal antibodies would be expected to disappear from their circulations. G-CSF could not be given to this patient for medical reasons and the authors elected to use IVIG. She was treated with IVIG at 2g/kg body weight, and this resulted in elevation of the neutrophil count which was maximal on day 8 and lasted for 3 weeks. This observation is interesting, and suggests that IVIG is a reasonable treatment for antibody-mediated neutropenia. Larger studies would be useful in disorders like this, since there are other anecdotal reports suggesting a lack of response to IVIG in autoimmune neutropenia. However, there is doubtless disease heterogeneity, and we cannot read too much into single case reports. However, the results in this patient would suggest that IVIG offers a reasonable alternative to standard therapies.

IVIG IN HEMOLYTIC DISEASE OF THE NEWBORN

This was a literature-based survey looking at the use and efficacy of IVIG in rhesus or ABO fetomaternal incompatibility (3). The author surveyed the literature between 1986 and 2000. In brief, the analysis showed that IVIG is effective in reducing the need for exchange transfusion, and a single dose of 0.5g/kg on day 1 was effective. When hemolytic disease of the newborn was treated with IVIG and phototherapy there was an overall reduction in the degree of hemolysis and hence reduction in the need for exchange transfusion.

USE OF A HEMORRHAGIC SCORE TO DETERMINE TREATMENT OF SEVERE AUTOIMMUNE THROMBOCYTOPENIC PURPURA

This study comes from Dr Godeau's group who have evaluated the use of a hemorrhagic score to guide treatment for patients with severe autoimmune thrombocytopenia (4). The authors assessed the hemorrhagic score at the onset of the patient's disease (Table 1).

Patients scoring ≥8 and who had platelets $\leq 20\times10^9$/l were treated with IVIG. If the total hemorrhagic score was <8, the patients received oral prednisone or high-dose methylprednisolone (HDMP). Seventy-six patients were recruited into the study with a mean age of 45 years; 51 patients (67%) had platelets $\leq 20\times10^9$/l and 14 (27%) of these had a hemorrhagic score of >8. Thirteen received IVIG and one received HDMP Fifteen of 37 received IVIG even if the hemorrhagic score was ≤8, because steroids had failed or were contraindicated. The authors reported that of the 51 patients with platelets $\leq 20\times10^9$/l, the use of the hemorrhagic score obviated the need for IVIG in 22 (43.1%) patients. In conclusion, the authors felt that patients with a mild hemorrhagic score ≤8) can safely be treated with steroids rather than IVIG even if the platelet count is very low, and therefore basing treatment decisions using clinical criteria (e.g. hemorrhagic score) rather than the platelet count is a reasonable approach. This would appear to agree with pediatric practice where the management emphasis is very much on the symptoms rather than the absolute platelet count. Other hemorrhagic scores have been developed by Dr Bolton-Maggs in the UK and Dr Buchanan in Texas; their scores, aimed at treatment of pediatric patients, are similar to that proposed by Dr Godeau's group.

Table 1 Hemorrhagic score

Age over 60 years	1
Cutaneous purpura localized	1
Unilateral epistaxis	2
Bilateral epistaxis	3
Extensive purpura, progressive or both	3
Purpura associated with large ecchymoses	4
Hemorrhagic oral bullae, spontaneous gingival bleeding or both	4
Major menorrhagia, metrorrhagia or both	4
Macroscopic hematuria	5
Overt gastrointestinal hemorrhage	5
Bleeding on the fundus oculi in the absence of other causes	5

IVIG IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTS

Dr Mühleisen and colleagues provided data from the University of Basel (5). The use of IVIG has been a standard component of allogeneic stem cell therapy for some years as CMV prophylaxis. This treatment predates the use of ganciclovir. There have been few studies looking at the efficacy and cost-effectiveness of such regimens. The study involved 160 transplant patients and detailed analysis was carried out in 104 patients. Cohort 1 was given IVIG on days −7, −5, −3 and −1 and +10, +20 and +30. The second cohort was given IVIG only if hypogammaglobulinemia or graft versus host disease (GVHD) occurred. Their findings showed that transplant-related mortality was the same in both groups, and the type and frequency of infection was identical in both cohorts 1 and 2. Similarly, the rate of GVHD did not differ between the two groups. Their modified schedule reduced cost, and they recommended that the routine use of IVIG in allogeneic stem cell transplantation be abandoned. Replacement therapy with IVIG should be used if the IgG level falls to <4g/l.

ADMINISTRATION OF INTRAVENOUS POLYCLONAL IMMUNOGLOBULIN IN RENAL TRANSPLANT PATIENTS

This small study aimed at evaluating the efficacy of IVIG in renal transplant patients in terms of reducing the incidence of anti-human leukocyte antigen (HLA) antibodies prior to transplantation (6). Ultimately this should result in an increase in negative cross-matches in planned renal transplant recipients. Only four patients have been studied so far, three of whom received IVIG 0.4g/kg on a 3-monthly basis; two of these also received IVIG 1g/kg on three occasions. Because of the small number of patients included it is difficult to draw meaningful conclusions. However, the IVIG appears to be

safe, but at the lower dose (0.4g/kg) does not reduce the number of positive B-cell cross-matches. Clearly further study is required of the dose used and regimen involved.

IVIG IN ACCELERATED HEMOLYSIS AFTER TRANSFUSION IN LYMPHOMA

Accelerated red cell destruction has been documented in sickle cell disease as well as other disorders. In some studies, red cell kinetic assays have shown normal survival of recipient red cells but rapid destruction of incoming donor red cells. From radionuclide imaging the site of destruction appears to be splenic. Examination of the patient's serum often fails to show the presence of red cell antibodies. Win and colleagues reported three patients with lymphoma who suffered recurrent hemolytic transfusion reactions following fully compatible red cell transfusions (7). The direct antiglobulin test was negative in all cases, and there were no detectable alloantibodies in pre-or post-transfusion samples. Hemolytic markers (bilirubin, lactate dehydrogenase and others) were strongly positive. The patients were pretreated with IVIG and further transfusions were administered with no evidence of hemolysis. This would appear to suggest that, among other mechanisms, IVIG is able to suppress macrophage Fc receptors thereby preventing accelerated hemolysis.

HYPERHEMOLYSIS SYNDROME IN SICKLE CELL DISEASE

Dr Win and others also reported two sickle cell disease patients who had similar hyperhemolysis associated with blood transfusion (8,9). Once again, the transfused units were compatible. The patients were pretreated with IVIG and corticosteroids and further transfusions were uneventful, in much the same way as for the lymphoma patients.

Hyperhemolysis is not common, but when it occurs in patients such as those with sickle cell anemia, the resulting major drop in hemoglobin may have severe adverse effects. It is therefore useful to know that IVIG may be one strategy for the ongoing management of these patients.

POSTERS

1. Aronis S, Platokouki H, Koussiafes D, Avgeri M, Pergantou H, Kaloudi A. Bleeding manifestations in childhood idiopathic thrombocytopenic purpura and necessity for therapeutic intervention
2. Carne E, Chua I, Gupta S, Williams PE. Intravenous immunoglobulin in chronic idiopathic neutropenia
3. Gottstein R, Cooke RWI. Intravenous immunoglobulin in hemolytic disease of the newborn: a systematic review
4. Khellaf M, Michel M, Bierling P, Godeau B. Is the use of a hemorrhagic score more effective than platelet count to determine the treatment for patients with severe autoimmune thrombocytopenic purpura?

5. Mühleisen B, Passweg J, Tichelli A, *et al.* Intravenous immunoglobulins in allogeneic hematopoietic stem cell transplants: a quality control study
6. Villard J, Binet I, Buhler L, Berney T, Martin P-Y. Administration of intravenous polyclonal immunoglobulins to allow kidneys to be successfully transplanted for immunized patients
7. Win N, Madan B, Gale R, Matthey F. Trial of intravenous immunoglobulin in accelerated hemolysis after transfusion in lymphoma (three cases)
8. Win N, Yeghen T, Chen FE, Okpala I. Hyperhemolysis syndrome in sickle cell disease: use of intravenous immunoglobulin and steroids
9. Win N, Clark M, Pollard C, Bevan D. Prevention of recurrence of hyperhemolytic transfusion reaction (hyperhemolysis syndrome) in sickle cell disease

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The of intravenous immunoglobulin in neurology

W.K.Engel

I have only a few pages to comment on the 12 assigned posters involving about 50 authors. They are interesting, and their variety illustrates the broad current and potential therapeutic applications of intravenous immunoglobulin (IVIG).

My comments are based on my personal experience with IVIG, which has involved my treatment of about 500 neuromuscular patients, many successfully. They encompass patients with CIDP (chronic immune dysschwannian/dysneuronal polyneuropathy; my preferred definition), including ones with a CIDP-like neuropathy in type-2 diabetes, as well as patients with myasthenia gravis, polymyositis, dermatomyositis, inclusion body myositis, chronic progressive multiple sclerosis and human T-cell leukemia virus (HTLV)-1 myelopathy. IVIG can produce truly remarkable improvement in dysimmune diseases, and for many patients it can have a better benefit/side-effect profile than any other drug.

Use of IVIG often has to be customized for the individual patient. Fix-doses in controlled trials are not ideal, but are commonly used. Regarding IVIG schedules, I consider that dosing only once a month, for 1 or 2 consecutive days, is usually too infrequent, because my patients typically show return of symptoms within 2–3 weeks after the last dose of IVIG. Evaluating patients 4 weeks after the last IVIG infusion can miss earlier peak benefit, now waning. For older hypertensive or younger migraine patients, to obviate those side-effects, often occurring increasingly on days 3–5 of a 5-day 0.4g/day schedule, I developed a safer schedule of 0.4g/kg on 2 non-consecutive days every week. To try to prevent thrombotic events, I give all IVIG patients daily aspirin 325mg or 81mg as tolerated, if they are not already taking coumadin, clopidogrel or another anticoagulant. I also require the blood pressure and general condition of the patient to be monitored at least every 30min—if the pressure rises above 155/90, the infusion is slowed or stopped. To try to protect fragile veins, I prefer the infused IVIG concentration to be not over 7.5%. Longer-term problems with IVIG include phlebitis or occlusion at venous infusion sites, especially in women, which can necessitate a subcutaneous vascular access port. The ports can function well for several years, but sometimes result in occlusion of a central vein.

As part of my personal experience with the use of IVIG, and before commenting on the posters, I would like to take this opportunity to address a personal note on neuropathy in type-2 diabetes mellitus (D2). ‘Diabetic neuropathy’ is commonly considered untreatable, and probably of metabolic or ischemic cause[1–3]. An example is the recent factual, but elliptically misleading and discouraging, statement that there is ‘no treatment approved by the US FDA’ for ‘diabetic peripheral neuropathy’[3]. To the contrary, I have

found[4–8], and continue to find (and others have confirmed[9–11]), that the motor and small- and large-fiber sensory components of peripheral neuropathy occurring in my patients with D2 or pre-D2 are, off-label, often very treatable with antidysimmune measures, especially IVIG, thereby resembling CIDP. (This is not to say that neuropathies in D2 are entirely dysimmune. Other potentially detrimental factors in D2 being proposed include advanced glycosylation end-products, ischemia at the capillary level, and polyolpathway abnormalities[1,12–14]. However, for the sake of the patients, it is imperative to emphasize the treatable dysimmune aspect.)

Eighty-one per cent of my 48 recently summarized D2 polyneuropathy patients adequately treated with IVIG were relieved, sometimes completely, of symptoms such as: moderate to severe distal burning or 'hurting' pain; feeling of walking on rocks, needles or broken glass; lancinating electric-like feet pains; pain-impaired walking; necessity to soothe feet in ice-water nightly; numbness, tingling, or 'tightness' levels to ankles, knees or mid-thighs; imbalance and frequent falling; distal and proximal weakness, including inability to rise from a chair or ascend stairs, walk without foot-drops, turn keys or sign checks legibly; prominent neuromuscular fatigability; or nocturnal painful cramps, restless legs, or jerks. For example, some patients have become completely free of distal burning pain that had required narcotics or nightly ice treatments; had prominent lowering of the degree and intensity of numbness from mid-thighs to only the toes; increased their walking from 6 m to six blocks; or stopped falling, regained ability to climb steps, squat or brush teeth. Symptomatic pain medication, previously inadequate, sometimes could be reduced or stopped. These responses are considered to indicate a dysimmune component of the pathogenesis that is treatable with IVIG.

Accurate evaluation of the benefit obtained with IVIG treatment of neuropathy must include thoughtful subjective reports, which are more sensitive to subtle improvements or deteriorations than so-called objective testings. The latter are not completely objective; evaluation of a drug cannot depend solely on them, and they must not be exclusively the gold-standard.

Initial IVIG benefit can be rapid, becoming evident in 1–8 weeks. Regular re-treatments can produce cumulative improvement over the first 4–10 months or so and then sustain moderate to dramatic benefit, sometimes for at least 8 years. Attribution of benefit to the IVIG was confirmed by a gradual increase of symptoms (waning of benefit) beginning 1–3 weeks after the last IVIG treatment, followed by improvement usually commencing within 1–4 days of resuming the next infusion of IVIG (glycemia management being unchanged). Accordingly, continuing improvement of IVIG-responsive patients is dependent upon continuing their IVIG treatment. (Note—in D2 neuropathy, to determine a similar or better efficacy of a new drug, '3–5 years' for a trial[3] would not be necessary.)

The IVIG-responsive D2 polyneuropathy, like CIDP, is usually dysschwannian, but is sometimes mainly dysneuronal. While it can be argued, taxonomically, that such improved D2 patients have a concurrent but unrelated CIDP, our D2 patients were deemed by various clinicians to have 'diabetic neuropathy'. Not everyone agrees with my proposals (not yet statistically confirmed) that: (a) there is a higher incidence of CIDP-like neuropathy in D2 patients and in patients with a strong family history of D2[6]; and (b) that the IVIG-treatable dysimmune neuropathy in D2 is brought on by the genetico-diabetoid-2 state. Nevertheless, therapeutically, a significant proportion of the early and

mid-stage D2 neuropathy patients respond to IVIG and merit treatment, as do some chronic patients. Higher protein values in cerebral spinal fluid seem to augur a better IVIG response. Irrespective of their taxonomic designation, the dysimmune treatability of these D2 neuropathy patients is commonly overlooked. The treatable neuropathy of our patients had been progressive for 0.25–18 years; for example, sustained improvement has occurred with IVIG initiated even after 18 years of neuropathy[5].

Comments on specific posters are discussed below.

IVIG given to multiple sclerosis (MS) patients during pregnancy and postpartum resulted in fewer relapses during pregnancy and the postpartum period (1). The dose was not stated. This seems to reaffirm that IVIG is safe to give during pregnancy. Whether all pregnant MS patients should receive prophylactic IVIG and if so, what dosage and frequency, is now an interesting question. This reported safety and efficacy might be applicable to pregnancy-related exacerbations in myasthenia gravis and CIDP

In 28 amyotropic lateral sclerosis (ALS) patients, IVIG 0.4g/kg 1×per month was reported to have a therapeutic effect on the clinical course of ALS (2). Half of the patients were given IVIG, half placebo, and all were on riluzole and vitamins. Treated ALS patients reportedly had slower progression. This minimal benefit has not, unfortunately, been the experience of others.

One man with acquired sensory motor neuropathy (MADSAM) responded for 4 months to periodic 5-day infusions of IVIG (3). The dosage and frequency were not stated. Distal conduction block also improved.

Chronic neurocognitive dysfunction in four patients having systemic lupus erythematosus (SLE) with the antiphospholipid syndrome responded to IVIG (4). All four also had abnormal ANF, anti-DNA antibodies, low C3, C4, and elevated anti-Ro (without evidence of Sjogren's syndrome). It was not stated if the dysimmune parameters were measured before or after IVIG treatments, because IVIG itself might have produced their positivity. I have had to assure several IVIG-treated patients that they do not have 'lupus' based on blood test results that their rheumatologists used for stating firmly that they had 'lupus', but which I have found to be producible by IVIG, such as a positive ANA. Their patients benefited from IVIG, which was given after they had been on prednisone 60mg daily for a few months. The SLE went into remission, but anticardiolipin antibodies remained elevated. The persisting anticardiolipin antibodies may have been due to the administered IVIG and not related to the basic disease. One patient had a seizure attributed to the IVIG and one a pulmonary embolus. A number of questions arise. Were those two patients on aspirin, coumadin or other prophylactic anticoagulation during the IVIG months to try to prevent thrombotic events? Was the infusion rate more than 100 ml/h? How high did the blood pressure rise during the infusions?

One 49-year-old woman had a lung small cell carcinoma-related paraneoplastic neuromyelopathy responsive to IVIG (5). She had also had 'insulin-dependent diabetes mellitus' (IDDM) since age 44. The IDDM was probably type-2 diabetes, and possibly was the basis of her sensory motor neuropathy, which may have been at least partially dysimmune and thereby responsible for at least some of the IVIG-induced improvement. The authors suggest that diabetes might predict a higher rate of IVIG side-effects. If so, they might be obviated if IVIG is given slowly, at concentration not over 7.5%, on the schedule of 1 or 2 non-consecutive days weekly and, if kidney function is impaired, at a dosage half or less of the usual.

IVIG was useful in ten myasthenia gravis patients and had a ‘steroid-sparing’ effect, given as 0.4 g/kg 1×per month (6). Five of the ten patients could eliminate their initial corticosteroid. This was a medically worthwhile accomplishment, because of the corticosteroid side-effects. The four IVIG-treated ‘seronegative’ myasthenics still required corticosteroids.

Multicenter treatment of 65 childhood Guillain-Barré patients with IVIG was examined using two different single courses, 2g/kg over 2 days versus 5 days (7). When IVIG was given before walking was lost, there was no apparent benefit from the 2-day regimen (the only one used). IVIG given after walking was lost produced some benefit from both regimens. There were some relapses. Possibly, retrospectively, they might have been obviated if more courses of IVIG had been given. The authors stated that there were very few patients and the statistical power was low.

Isoardo and Cocito reported that the benefit in 38 CIDP patients treated with IVIG was more likely if the patients had conduction blocks or did not have anti-(MAG) antibodies (8). However, I have seen anti-MAG antibody neuropathy patients who do respond very well to IVIG, some rather dramatically, so they should not be excluded from IVIG therapy.

Six months of IVIG, monthly 2g/kg, with high-dose corticosteroid, benefited six adults with Rasmussen’s severe autoimmune epileptogenic encephalitis (9). Seizure control was achieved within weeks, and functional recovery began after a latency of 2–3 months. These good results are a reason to delay or omit radical surgery. Possibly, functional recovery could have begun sooner with more frequent IVIG treatments, such as every third week.

One 47-year-old man who benefited from IVIG had stiff person syndrome with very high anti-GAD antibodies, type-2 diabetes from age 43, polyneuropathy, elevated thyroid antibodies and Hodgkin’s lymphoma in remission (10). Possibly his concomitant neuropathy was dysimmune CIDP-like and related to his type-2 diabetes; if so, a CIDP IVIG-responsiveness may also have contributed to his improvement.

Of patients with regional chronic pain syndromes occurring after major or minor trauma, 30–80% benefited from low-dose IVIG (dosage not given) (11). Sera binding to rodent tissues, viz. peripheral nerves, endothelia or cell nuclei, identified a subgroup with strong staining and a history of only minor preceding trauma. The authors plan to evaluate usefulness of this finding in predicting IVIG responsiveness of these syndromes and we await the results.

In a pilot study, IVIG was evaluated in chronic pain syndromes, specifically in 14 women with ‘fibromyalgia’ (12). In five non-atopic patients, expression of tumor necrosis factor (TNF)-α was down-regulated by IVIG, associated with parallel decrease in pain scores. In nine atopic patients, TNF-α was up-regulated by IVIG, paralleled by pain relief. This is an interesting but very preliminary study, without placebo infusions.

POSTERS

1. Achiron A, Chapman J, Kishner I, Dolev M, Stern Y, Dulitzky M, Schiff E, Achiron R. Effect of intravenous immunoglobulin treatment on pregnancy and postpartum-related relapses in multiple sclerosis

2. Karageorgiou CE, Korakaki D, Sakkou V, Karageorgiou E. A comparative study of the IVIG therapeutic effect in the course of ALS
3. Larrodé P, Salgado F, Iñiguez C, Santos S, Fabre O, Pascual LF. Clinical and electrophysiological correlation of the response to treatment with IVIG in multifocal acquired demyelination sensory and motor neuropathy (MADSAM)
4. Zandman-Goddard G, Levy Y, Chapman J, Shoenfeld Y, Langevitz P. Chronic neurocognitive dysfunction in SLE patients responds to IVIG therapy
5. Bunyan RF, Swenson MR. Paraneoplastic neuromyelopathy responsive to intravenous immunoglobulin
6. Reddy S, Small GA. Utility and steroid sparing properties of IVIG in a myasthenia gravis clinic
7. Korinthenberg R, Schessl J, Kirschner J, Schulte Mönting J. A prospective multicentre study on treatment of childhood Guillain-Barré syndrome with IVIG
8. Isoardo G, Cocito D. Predicitve value of clinical, electrophysiological and immunological features for response to IVIG in patients with CIDP
9. Hart IK. Long-term hIVIg treatment of adult-onset Rasmussen's syndrome
10. De Weweire M, Meire V, De Bleecker J. A patient with stiff person syndrome treated with intravenous immune globulin
11. Goebel A, Vogel H, Sprotte G. Serum-autoantibodies: potential targets for the establishment of predictive factors for IVIG efficacy in complex regional pain syndrome (CRPS)
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The use of intravenous immunoglobulin for dermatological disorders

M.Sticherling

Intravenous immunoglobulins (IVIG) are used clinically for both substitution and immunomodulatory therapy. Whereas they are only licensed for a limited number of diagnoses, a large array of empirical applications is available which includes various inflammatory and autoimmune disorders of different human organs[1,2]. In dermatology, dermatomyositis and autoimmune bullous skin diseases have been studied most extensively[3–9]. However, controlled studies at a higher evidence-based level are only available for dermatomyositis. Most other data have been compiled on a limited number of patients, within case reports or the retrospective analysis of published data as recently supplied for autoimmune bullous skin diseases[8]. The diseases studied are both B-and T-cell mediated and induced by grossly divergent pathogenetic mechanisms. The obviously broad spectrum of IVIG functional activities is reflected by its therapeutic effects on this array of divergent diseases. Within most single-disease subsets, individual mechanisms of action of IVIG which induce clinical response are, however, mostly speculated upon. These different items are reflected by the posters presented during the Interlaken meeting which addressed both clinical and pathogenetic aspects of the diseases studied.

The first poster within the dermatology section was presented by Howard Amital and co-workers from Tel-Hashomer, Israel and Florence, Italy (1). They studied the modulatory effects of IVIG on the cutaneous involvement and skin fibrosis in systemic sclerosis within an open study. Previous experimental work from the group has demonstrated the reduction of skin fibrosis in TSK mice with a decrease of collagen I gene as well as transforming growth factor (TGF)-β and interleukin (IL)-4 expression after infusion of 2g/kg body weight IVIG twice weekly over 4 weeks[10,11]. In contrast, interferon (IFN)-γ expression remained unchanged. Based on these promising experimental data in a scleroderma-like murine model, 15 patients with scleroderma of both limited (*n*=8) and diffuse type (*n*=7) and a disease course over 4–20 years were studied. The clinical effects were monitored by changes of the modified Rodnan score pre- and post-application of three to six cycles of 2g/kg body weight IVIG at 4-week intervals. The average Rodnan score decreased by 35% (10±5.9 units). However, no information was given on the duration of the beneficial effects after stopping the infusions. The authors concluded from their data that the decrease of skin fibrosis results in an improved quality of life in patients with systemic sclerosis.

The second poster by Danieli and co-workers from Ancona, Italy examined the long-term effectiveness of IVIG in 21 patients with polymyositis (*n*=9) and dermatomyositis (*n*=12) within a retrospective study covering the years 1995–2001 (2). All patients were treated with prednisolone and cyclosporin. Patients with refractory and relapsed disease

received IVIG at 1g/kg body weight monthly for 6 months and three times bimonthly thereafter. They were compared to seven patients in a control group who were treated with prednisolone and cyclosporin only. All patients on IVIG improved and had a statistically significant longer disease-free period at long-term follow-up when compared to the control group (Figure 1). The authors postulated a beneficial combinatory effect of immunomodulatory therapy comprising corticosteroids, cyclosporin A (CSA) and IVIG. Thus, the poster added further information on the long-term effects to earlier studies which showed beneficial effects of IVIG on dermatomyositis[3,12–14].

The third contribution to this session was made by Maurizio Pietrogrande and co-workers from Bergamo and Milano in Italy (3). They studied the effects of plasmapheresis and IVIG in cryoglobulinemic syndrome[15,16] within a pilot study on 13 patients. The patients had been treated with IFN, ribavirin, prednisolone or colchizin before. They presented with cutaneous necrotizing vasculitis with ulcers, severe sensory motor neuropathy or signs of renal function worsening, or combinations thereof. After a first cycle of plasmapheresis and IVIG at 2g/kg body weight, apheresis and IVIG at 1g/kg body weight were

Figure 1 Dermatomyositis: response at long-term follow-up

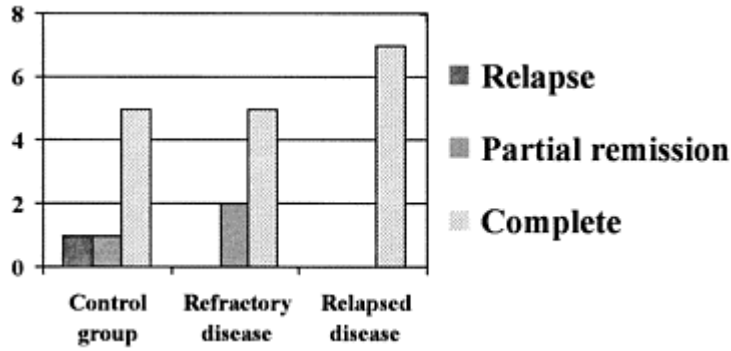
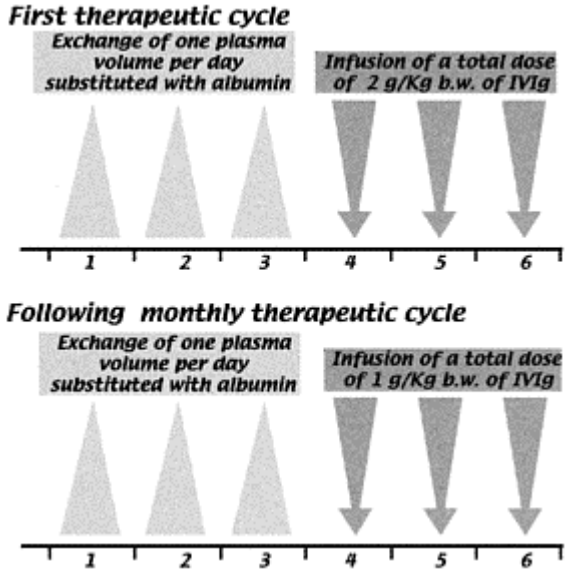


Figure 2 Protocol for plasmapheresis and IVIG infusion



applied for up to six cycles (Figure 2). This therapy was well-tolerated, apart from transitory renal insufficiency in one case, and resulted in improvement of skin ulcers (7/9 patients), proteinuria (1/4), pain score (7/9), muscle strength (3/6) and cryocrit (3/6). The authors conclude from their data that this combinatory therapy had beneficial effects with regard to severe complications of cryoglobulinemic syndrome. Special attention should, however, be paid to possible renal involvement as has been pointed out by other authors as well[17–19].

In the last presentation within the dermatological section, the clinical case of a 50-year-old female patient with recalcitrant subacute cutaneous lupus erythematosus was presented by Sticherling and co-workers from Leipzig, Germany (4). The 20-year course of major skin involvement was followed by recent exacerbation without proper response to high-dose oral glucocorticoids and azathioprine. After only two cycles of IVIG at 2g/kg body weight major improvement of skin symptoms could be induced and maintained for the following 12 months with daily oral methylprednisolone at 8mg and 100mg azathioprine. The authors concluded that in cases of recalcitrant disease, IVIG may represent an alternative and supportive therapy for cutaneous lupus erythematosus[20–25].

This session on dermatological diseases reflects the effective use of IVIG in cases of refractory or relapsing disease, with contraindications of classical first-line therapy or severe side-effects. However, IVIG is not licensed for any of the diseases represented here. With regard to increasing legal and economic constraints on medical therapy worldwide, further controlled studies at a high-evidence level are needed on the clinical effectiveness of IVIG, as well as detailed examination of pathogenetic mechanisms.

POSTERS

1. Amital H, Levy Y, Langevitz P, Nacci F, Righi A, Conforti L, Generini S, Cerinic MM, Shoenfeld Y. Intravenous immunoglobulins modulate cutaneous involvement and reduce skin fibrosis in systemic sclerosis—an open study
2. Danieli MG, Malcangi G, Calcabrini L, Cappelli M, Calabrese V, Logullo F. Long-term effectiveness of intravenous immunoglobulins in polymyositis and dermatomyositis
3. Pietrogrande M, Fusi A, Vozzo N, Invernizzi F. Intravenous immune globulin (IVIG) plus plasmapheresis for severe cryoglobulinemic syndrome
4. Sticherling M, Beckheinrich P, Wandel E. Recalcitrant subacute cutaneous lupus erythematosus (SCLE)—clinical response to intravenous immunoglobulins

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The use of intravenous immunoglobulin in collagen vascular and heart diseases

A.H.Lazarus

INTRODUCTION

Intravenous immunoglobulin (IVIG) is prepared from large pools of plasma, typically from more than 3000 healthy blood donors. The first description of the treatment of individuals with an autoimmune disease with IVIG was provided by Imbach in 1981; he found that high-dose administration of IVIG promoted a rapid recovery of idiopathic thrombocytopenic purpura (ITP) in children. Since this landmark discovery, IVIG has also been found to have beneficial effects in many autoimmune states, including some patients with collagen vascular and heart diseases.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is a chronic multisystem disease that displays a wide variation in its clinical expression and course. As the immunopathogenesis of SLE is quite complex, the issue of the efficacy of IVIG treatment in this disease is not simple to address.

Work presented by Manger and colleagues sought to gain an understanding of which allotypes of the Fcγ receptors (FcγR) were related to the disease process in 115 SLE patients (1). They observed that the frequency of clinical and serological markers of SLE was higher and the onset occurred earlier in patients with the homozygous allotype FcγR IIA-R/R131, IIIA-F/F158, and the combined genotypes IIA-R/R131, IIIA-F/F158, I and IIA-R/R131, IIIA-F/F158, IIIB-NA2/2. In the group with the combined genotypes IIA-R/R131, IIIA-F/F158, disease onset occurred 13 years (median) earlier. Patients with elevated anti-dsDNA of immunoglobulin IgG_2 and IgG_3 (but not IgG_1) subclasses also had earlier disease onset, while high levels of anti-dsDNA IgG_3 were associated with an increased relative risk for heart involvement.

Blank and colleagues sought to increase the efficacy and specificity of IVIG preparations in the treatment of SLE (2). These authors have constructed a recombinant chromatography column (using a selected peptide phage display library that resembles anti-dsDNA) to select and enrich anti-idiotypic antibodies from IVIG that would neutralize anti-dsDNA antibodies. IVIG that was enriched for neutralizing anti-dsDNA antibodies is referred to by the authors as peptide-specific IVIG (psIVIG). This psIVIG

inhibited the binding of patient anti-dsDNA antibodies to dsDNA. In addition, psIVIG was introduced to mice with experimental SLE, and found to have efficacy in ameliorating disease activity.

Work from Sherer and associates examined the clinical response to low-dose IVIG therapy in a large cohort of SLE patients (3). It was found that low-dose IVIG is effective in SLE, resulting in SLE daily activity index (SLEDAI) score decrease and amelioration of several manifestations of the disease in most patients. The clinical manifestations most likely to respond rapidly to low-dose IVIG therapy were mucosal ulcers, fever, urinary casts, new rash, pleurisy and pericarditis.

CHURG-STRAUSS SYNDROME

Churg-Strauss syndrome is a necrotizing vasculitis of medium- and small-sized vessels. Despite treatment with corticosteroids and cytotoxic drugs, a high rate of relapse and substantial drug-dependent morbidity are still problems. Danieli and co-workers evaluated 24 patients with Churg-Strauss syndrome who were treated with either steroids only (n=12) or steroids in combination with 2g/kg IVIG (per month for 6 months followed by 2g/kg every 2 months) (4). After 12 months, all patients who received IVIG were in remission, compared with 4/12 steroids-only patients.

OTHER SYSTEMIC MANIFESTATIONS OF VASCULITIS

Peripheral neuropathy can be a prominent feature of the systemic and secondary vasculitides. Levy and co-workers evaluated patients who exhibited different inflammatory diseases accompanied by vasculitic peripheral neuropathies, for which IVIG was used for therapy (5). These diseases included Sjogren's syndrome, SLE, vaccination-induced vasculitis, Churg-Strauss syndrome, mixed cryoglobulinemia associated with hepatitis C infection or sarcoidosis. Subjects were treated with high-dose IVIG (2g/kg) and followed for 1–5 years after this treatment. In four patients (Sjogren's syndrome, Churg-Strauss vasculitis, SLE and vaccination-induced vasculitis), the neuropathy resolved after IVIG therapy.

Ito-Ihara and colleagues evaluated 13 patients with myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA)-associated glomerulonephritis who were treated with IVIG (400mg/kg/day for 5 days) (6). The effects of IVIG were evaluated by the Birmingham Vasculitis Activity Score (BVAS), serum C-reactive protein (CRP), serum creatinine (Cre) levels, change of reciprocal Cre (1/Cre) rate and plasma tumor necrosis factor-α (TNF-α) levels. IVIG treatment significantly decreased CRP values and BVAS score, and improved the change of 1/Cre rate. Plasma TNF-α values, which were increased compared with normal controls, significantly decreased after IVIG treatment, and all patients under-went remission of the disease without any severe infectious complications. The authors suggest that IVIG may be of benefit in helping to reduce the dosage of immunosuppressive agents, for patients with ANCA-related glomerulonephritis/vasculitic syndrome.

KAWASAKI DISEASE

Kawasaki disease is an acute childhood vasculitis which results in aneurysms of the coronary arteries (CAA) in about 20% of patients. The risk of CAA is decreased with IVIG.

Pietrogrande and colleagues prospectively evaluated 128 patients with Kawasaki disease (7). CAA developed in 34/128 children (26.5%). CAA were seen in 52% of untreated patients, while treatment with IVIG reduced CAA occurrence to 19%. These data help confirm the effectiveness of IVIG in Kawasaki disease.

DILATED CARDIOMYOPATHY

Dilated cardiomyopathy (DCM) is a leading cause of heart failure and a common cause of heart transplantation. Despite recent improvements in therapy, DCM-associated mortality remains high. Based upon the hypothesis that multiple secondary autoimmune mechanisms may be involved in progressing DCM, Dubiel and co-workers investigated the influence of IVIG on cardiopulmonary function and exercise performance (8). Seven patients with DCM were given IVIG (2g/kg) on 2 consecutive days. Although one patient needed temporary dialysis, the other patients were treated without severe side-effects. An improvement of cardiopulmonary function and exercise performance was achieved in most patients. The authors conclude that, although IVIG may serve as a potential adjunctive therapy for DCM, the therapeutic potential of IVIG in DCM requires further evaluation.

In summary, IVIG appears to have efficacy in many subjects with collagen vascular and heart diseases.

POSTERS

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4. Danieli MG, Cappelli M, Malcangi G, Calcabrini L, Calabrese V, Logullo F. Intravenous immunoglobulin for the Churg-Strauss syndrome
5. Levy Y, Amital H, Uziel Y, *et al.* Intravenous immunoglobulin (IVIG) in peripheral neuropathy associated with vasculitis
6. Ito-Ihara T, Suzuki K, Ono T, *et al.* Beneficial effect of intravenous immunoglobulin for patients with myeloperoxidase antineutrophil cytoplasmic antibody (MPO-ANCA)-associated rapidly progressive glomerulonephritis
7. Pietrogrande MC, Laicini E, Fesslova V, *et al.* Kawasaki disease: revision of guidelines for early IVIG treatment?
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Intravenous immunoglobulin: machanisms of action

P.E.Williams

INTRODUCTION

Analysis of immunomodulation by intravenous immunoglobulin (IVIG) at the clinical level is simple, involving observation of straightforward clinical parameters. At a molecular level, it is more difficult to discern the ways in which molecules and processes are significantly, consistently and reliably perturbed by infusion of the molecular mean of >20 000 allogeneic extracellular fluid proteomes.

OVERVIEW

Cells constituting a multicellular organism constantly communicate by secreting chemicals to each other and catching them on receptors, which mediate the biochemical signals to the cytoplasm and nucleus. It appears to be essential for cells to take part in these biochemical conversations to stay alive and avoid death by apoptotic neglect. The fluid that lies between cells will thus contain multiple secreted molecules and shed receptors, variably inactivated and fragmented by proteolysis. The immune system may respond to some degree or other to this molecular noise from the host's proteome by making antibodies against the molecules concerned.

Antibodies made during such responses may in theory bind, block, aggregate, enhance or inhibit the action of a multitude of glycoprotein and possibly other molecules, thus offering the potential to interfere with and modulate communications between cells. In a healthy person the sum total of the immunoglobulins present (the individual's humoral immune network) can reasonably be expected to be in optimal balance with the individual's proteome, set so as not to detract from the normal intercellular communications that enable health.

When such antibodies are harvested from many different individuals and pooled together in IVIG, an immunomodulatory effect emerges. The pooled product has the ability favorably to influence aberrant immunological activity in recipients. In many instances the precise nature of the aberrations in cellular cross-talk that underlie the diseases in the recipient remain unknown, as do the precise mechanisms by which IVIG confers therapeutic benefit to such recipients.

The actions of IVIG in persons who have disturbed immunity (usually autoimmunity) may be likened to the actions of a buffer in controlling the pH of a solution in which chemical reactions occur. It appears to act as if returning and maintaining biochemical and/or autoimmune processes to within a narrow range of (what might be) the population mean. This might occur because the mixture of a sufficiently large number of immune networks may cause molecular buffering via many mechanisms, including:

(1) Idiotype-anti-idiotype antibody interaction;
(2) Receptor-antireceptor antibody interaction;
(3) Agonist-antagonist molecules interaction;
(4) Equalization of the influence of different alleles of key molecules towards a population mean;
(5) Interaction of antibodies with allogeneic target molecules;
(6) Other mechanisms.

When IVIG preparations have been assayed for various molecules, it is not surprising that they have been found to contain so many molecules with potentially significant biological reactivity. When supernatants from *in vitro* systems exposed to IVIG or blood from recipients of IVIG have been assayed for soluble receptors, receptor fragments, cytokines, chemokines, anti-idiotype antibodies, etc., it is not surpris ing to find that IVIG administration may be associated with changes in their assayed concentrations. Attributing to these observations cause and effect, and deducing mechanisms of action, however, are more difficult. In order to draw credible conclusions and reach plausible views it is necessary to understand the (immuno)pathogenesis of each disease at a molecular level. Even then, IVIG might modulate a disease in many different ways at the same time, and in different ways at different stages of the disease.

This summary is not intended to review mechanisms of action of IVIG, which have been recently reviewed[1,2], but to abstract from the posters presented some additional points which may usefully extend knowledge, improve perspectives or raise awareness of views on how IVIG might act. A brief reference to each of the posters follows.

Main points from the posters presented

Altznauera and colleagues used flowcytometric competitive labelling and apoptosis assays to demonstrate that IVIG gave dose-dependent inhibition of the apoptosis caused by an anti-CD95 monoclonal antibody at 1–10 mg/ml concentration, but dose-dependent neutrophil death and apoptosis at 20–50 mg/ml concentration, concluding that IVIG contains both agonistic and antagonistic antibodies against CD95 (1). That this effect could only be partially blocked by soluble CD95 receptors allows the possibility that other mechanisms may be involved in these effects of IVIG in this system.

Bayry and associates found that peripheral blood monocyte-derived dendritic cells (DCs) matured with lipopolysacclaride (LPS) showed more interleukin-10 (IL-10) and less IL-12 production when exposed to 0.15 mmol/l IVIG, with diminished expression of co-stimulatory molecules and inhibition of auto- and alloreactive T-cell activation and proliferation (2,3). The anergizing effects of IVIG may thus occur in part via modulation of DC function. They also found that 0.15 mmol/l IVIG could inhibit the interferon-α (IFN-α)-dependent action of sera from systemic lupus erythmatosus (SLE) patients,

causing normal monocytes to differentiate into DCs. IVIG also inhibited the uptake of nucleosomes by DCs, indicating a functional effect of IVIG on DCs.

Bruley-Rosset and co-workers found that the anti-inflammatory actions of IVIG in collagen-induced arthritis and experimental allergic encephalitis in rats seemed not to be predominantly mediated by the anti-idiotype antibody IVIG fraction, but by the IVIG fraction enriched in natural polyreactive autoantibodies (4).

The role of autoantibodies directed against the high-affinity immunoglobulin E (IgE) receptor on basophils in causing urticaria is uncertain. Fux and colleagues found that the binding to basophils of such autoantibody clones caused their activation (5). Binding and activation was inhibited by receptor occupation by IgE. The presence of low total serum IgE concentrations in urticaria is consistent with this.

Microarray gene expression analysis of blood incubated with IVIG revealed changes in expression of mRNA in 96 of 8793 genes. These 96 genes were associated with a whole host of cell biological functions, and the authors postulated a role for IVIG in re-establishing anti/pro-inflammatory balances (6).

Matrix metalloproteinase (MMP) inhibition, especially of MMP-9, has been reported to be crucially important for growth and invasiveness of tumors. In a mouse melanoma model, Gilburd and co-workers found that high-dose IVIG gave a significantly reduced amount and activity of MMP-9, and fewer lung metastases in treated animals, thus suggesting this additional mechanism of action for IVIG in this situation (7). The formation and fate of immune complexes was displayed on Macromedias Flash™ software by Nydegger and Landel (8), and anti-inflammatory actions of IVIG were described on endothelial cells (9).

Raju and Dalakas used microarray analysis to look at gene expression in serial muscle biopsies of three patients with dermatomyositis who had responded to IVIG therapy, and found that the expression of 4484 genes changed by >1.8-fold, with significant and consistent changes in the expression of 267 of these genes, notably IFN-γ, C3 and human leukocyte antigen (HLA) class II (10).

High-dose IVIG tends to reduce mitogenstimulated CD69 expression but not intracellular IFN-γ or tumor necrosis factor-α (TNF-α) expression in CD4+ or CD8+ T cells in patients with eczema. Replacement IVIG at 400 mg/kg in patients with common variable immunodeficiency (CVID) or X-linked agammaglobulinemia (XLA) has no effect on IFN-γ, TNF-α, IL-2, CD69 or IL-12 expression (11).

Trawinski and colleagues found that increasing doses of IVIG from 2.5 to 10mg/ml caused inhibition of normal lymphocyte proliferative responses to phytohemagglutinin (PHA), tetanus toxoid and IL-2 (12).

Atherosclerosis is associated with immune activation, and Vandaele and associates found that IVIG therapy was associated with reduced fatty streaks and fibrofatty plaques in an apoliprotein (apo) E knock-out mouse model fed a ‘Western’ diet (13). This is consistent with what is known about the etiology of atherosclerosis.

Van Mirre and colleagues hypothesized that monomeric IgG might act as a low-affinity Fc fragment γ receptor (FcγR) antagonist (14). Experiments on human neutrophils and FcγRIIa-transfected IIa1.6 cells using dimeric and aggregated IgG as FcγR antagonists showed that monomeric IgG purified from IVIG, at concentrations similar to IgG in plasma, inhibited binding of dimeric IgG and aggregated IgG-induced calcium influx but had no action of its own, and no effect on processes induced by fMLP.

Their hypothesis seemed proven by the data presented. Varambally and co-workers found that natural, normal, unmutated IgM may maintain homeostasis by inducing apoptosis in human lymphocytes, using mechanisms inhibited by soluble Fas(CD95) (15).

POSTERS

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2. Bayry J, Lacroix-Desmazes S, Delignat S, Kazatchkine MD, Kaveri SV Induction of tolerogenicity of dendritic cells by therapeutic immunoglobulins
3. Bayry J, Lacroix-Desmazes S, Mouthon L, Kazatchkine MD, Kaveri SV Intravenous immunoglobulin abrogates the dendritic cell differentiation induced by interferon-α present in sera of patients with systemic lupus erythematosus
4. Bruley-Rosset M, Mouthon L, Chanseaud Y, Dhainaut F, Lirochon J, Bourel D. Polyreactive autoantibodies purified from human intravenous immunoglobulins prevent the development of experimental autoimmune diseases
5. Fux M, Bobrzynski T, Vogel M, Gautschi E, Stadler BM, Miescher SM. Frequency estimation and characterization of natural anti-FcεRIα-chain antibodies in healthy donors and autoimmune urticaria patients
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10. Raju R, Dalakas MC. Changes in gene expression profile assessed by microarray analysis in the muscles of patients with dermatomyositis after successful therapy with intravenous immunoglobulin
11. Sewell WAC. Immunomodulatory effects of intravenous immunoglobulin studied *in vivo* depend on the condition treated and the dose used
12. Trawinski H, Wandel E, Sticherling M. *In vitro* effects of intravenous immunoglobulins on lymphocyte activation
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Pathogen safety and tolerability of intravenous immunoglobulin

F.Rossi

The increase in intravenous immunoglobulin (IVIG) use has led to new side-effects coming to light. Most of them are now quite well recognized and can be divided under two headings, as for any plasma-derived or biological product from human origin: infectious pathogen safety and clinical tolerability.

PATHOGEN SAFETY

Intravenous immunoglogulin, like albumin, exhibits the highest safety margin of all plasma-derived products. Two papers, however, addressed the issue of pathogen safety.

Functional integrity of IVIG following irradiation with a virucidal dose of γ radiation

Tran and colleagues at Clearant and ZLB showed that γ irradiation at 50 kGy as an additional safety measure does not induce changes in conformation, polymerization or functional antigenic recognition and cytokine induction of purified immunoglobulins from the intermediate product (1). It is not clear whether the tests were performed on the intermediate or the final product, however. A real benefit of increasing the already excellent viral safety record of IVIG, even with regard to parvovirus B19, was not supported.

Improving IVIG safety or checking its level might be a more sensible goal if looking at the theoretical presence of prions in the starting material of IVIG, human plasma.

Partitioning of TSE infectivity during ethanol fractionation of human plasma

Gregori and associates, from Robert Rohwer's laboratories in association with ZLB, used either scrapie sheep brain homogenate or PrP fibrils tested by an infectivity titer in hamster or by reduction of proteinase K resistance, respectively (2). Without providing figures, the abstract states that 'substantial removal' was achieved. The log reduction data are given in the poster for each successive step of the process: 2.2, 3.0, 3.5, 4.5, 2.8, 7.2, 4.2, respectively. The size of the filters used during nanofiltration is not provided. The

poster indicates correlation between the two materials/methods for each step, except for step 1, cryoprecipitation. The conclusion states that plasma products are 'safe', but there is no argument allowing a link between infectivity levels in the intermediate products after each key step and lack of residual risk for the recipients of the final product.

Cleaning of manufacturing equipment by sodium hydroxide

Käsermann and Kempf from ZLB studied cleaning procedures of material in contact with potential transmissible spongiform encephalopathy (TSE) material during the IVIG manufacturing process and contaminated medical equipment (3). Kinetic studies allowed a faster than currently assumed elimination of TSE infectious material, as there was an almost 4 log reduction after 15min for a range 0.025–0.1mol/l NaOH. The conclusion states that more than 4 log reduction does not equal complete disappearance of cross-contamination by TSE material, but a substantial lowering, if ever present.

CLINICAL TOLERABILITY

Characterization of adverse reactions related to IVIG administration: a 10-year perspective

A 10-year survey from one center in Columbus by Siegel and Sierawski documented in the abstract only 125 patients followed for the past year (4). Overall results for 1462 patients are, however, provided in the poster, i.e. adverse events (AEs) occurring in 29% of the patients related to 7.6% of injections with a range of 4–11%, depending on the year. They found a product relationship of AE incidence (4–14%) which was not biased by the market share of the various products (nearly 0–60%). This survey adds large numbers to the known experience of a higher risk of side-effects of high doses of IVIG used for immunomodulatory purposes, and highlights the greatest incidence of AEs in patients suffering from neuropathies. 'Changes in practices' not described in the abstract included the dilution of IVIG in water instead of saline, which divided by 2 the osmolar concentration of the solution and brought the AE incidence to 3.6% instead of 9% (7.6%?). It is stated that the appropriate use level was 98%, but no reference is provided for this, although variability in interpretation is high.

Therapy with IVIG: complications and side-effects

A prospective study was performed by Wittstock and colleagues in Rostock, DE, in 117 patients with various neurological diseases, receiving 0.4g/kg body weight of IVIG, for 408 therapy courses (5). A higher incidence of AEs (including two cases of deep vein thrombosis) were observed: 42.7% versus 32% in a previous study, most likely due to asymptomatic laboratory changes (glycosuria, neutropenia, blood sedimentation) in addition to more common AEs such as chills, headache, skin reactions, fever and gait disturbance.

SPECIFIC RISKS

Three posters focused on specific IVIG risks, renal toxicity, thrombotic events and anti-globulin/hemoly sis.

Hemolytic anemia following IVIG: a cautionary tale

Cleary and associates described true hemolytic anemia in a child receiving IVIG for Kawasaki disease, owing to anti-D antibody found in a specific batch (6). This batch, used in another patient suffering from systemic juvenile idiopathic arthritis, led to a biological antiglobulin test without clinical impact. The authors, from the UK, suggested that the individual batch number should be recorded in the patient's file. This recommendation is part of the IVIG European core Summary of Product Characteristics (SPC), CPMP/BPWG/859/95 rev 1: 'In the interest of patients, it is recommended that, whenever possible, every time that [name of the product] is administered to them, the name and batch number of the product is registered.'

IVIG and the kidney: indications and complications

The risk of renal toxicity in patients with glomerulonephritis was studied by Orbach and co-workers, in Jerusalem (7), as assessed using the literature and pharmacovigilance cases collected by the Food and Drug Administration (FDA):

(1) IVIG treatment was provided for lupus nephritis/immunoglobulin A (IgA) nephropathy and Henoch-Schonlein purpura/idiopathic membranous nephropathy/antineurophil cyto-plasmic antibodies.
(2) More reports (88 patients) and more severe reports were notified to the FDA (25 reports in 64 patients) than were published—and this is fortunate.
(3) Sucrose-containing products were more prone to induce renal toxicity in patients with nephrology pathology (70% literature, 90% FDA: 81% of cases), in accordance with 24 histological verifications showing signs of osmotic injury.

Regulatory agencies (French first, then the FDA, then the European Medicines Evaluation Agency (EMEA)) have provided warnings and guidance for the use of IVIG in patients at risk, since 2001, as worded in the CPMP/BPWG/859/95 rev 1 IVIG core SPC: 'Cases of acute renal failure have been reported in patients receiving IVIG therapy. In most cases, risk factors have been identified, such as pre-existing renal insufficiency, diabetes mellitus, hypovolemia, overweight, concomitant nephrotoxic medicinal products or age over 65… While these reports of renal dysfunction and acute renal failure have been associated with the use of many of the licensed IVIG products, those containing sucrose as a stabiliser accounted for a disproportionate share of the total number. In patients at risk, the use of IVIG products that do not contain sucrose may be considered.' The authors concluded that 'IVIG and the kidney is a two edged sword'.

The clinical features of 16 cases of stroke associated with administration of IVIG

Caress and colleagues presented in their poster results obtained from 498 patients during the years 1998–2002, with 16 cases of strokes associated with IVIG in 13 patients having additional risk for stroke (8). The French Safety Health Product Agency (AFSSAPS) French pharmacovigilance currently proposes to include in the CPMP/BPWG/859/95 rev 1 IVIG core SPC a warning statement on thrombotic events.

NOVEL PREPARATION

A novel IVIG preparation is very much in fashion, and two abstracts addressed the assessment of efficacy and safety of the caprylateprepared IVIG from Bayer.

Safety and efficacy of a novel immunoglobulin (IGIV-C, 10%) for treatment of patients with idiopathic thrombocytopenia

The first was presented by Gatterman and Enriquez for the European Ig-IV-S in idiopathic thrombocytopenic purpura (ITP) study investigators in an open study of acute or chronic adult ITP analyzing 18 patients (9). Efficacy criteria were in accordance with the CPMP/BPWG/388/95 rev 1 note for guidance on the clinical investigation of IVIG, and were met in 83% of patients. Overall, efficacy and safety in ITP were demonstrated. This study could be compared with a randomized, double-blind international trial in 97 children and adults with acute and chronic ITP versus IGIV-S/D (International Society on Thrombosis and Haemostasis (ISTH) 2003). Both showed similar results.

Rapid infusion of a novel IVIG preparation liquid-formulated with glycine (IGIV-C, 10%)

The rate of infusion of IVIG is known to be linked with AEs. Gelfand and Hanna with Bayer in Denver studied increased rates of administration of IGIV-C, 10% in 21 ITP patients, randomized to three arms of 0.08, 0.11 or 0.14mg/kg/min (10). Results were expressed as 23–36% of patients exhibiting related AEs, and the authors reported no relationship between AE incidence and infusion rate. Headache was the only drug-related AE in >10% patients (4–23%). This wording can lead to uncertainties. For example, were the 21 patients included those recruited to a previous study? If so, why were data on administration rate available for all patients, but data on efficacy missing for three of them? The expression 23–36% could be understood as 23% in the 0.08-mg/kg/min group versus 36% in the 0.14-mg/kg/min group, where a lack of statistical significance would have to be provided. The poster, however, indicates a repartition of 23% (0.08mg/kg/min), 36% (0.11mg/kg/min), 33% (0.14mg/kg/min). The same remark could apply to headache (4–23%), which was actually 4% (0.08mg/kg/min), 18% (0.11mg/kg/min), and 8% (0.14mg/kg/min). Urticaria occurred in 4%, 5%, 8% of

patients, and rash in 4%, 0%, 8% of the patients, respectively. Unless the results are more clearly expressed, no conclusion can be appreciated.

DIMERS

Two posters addressed the hypothesis that dimers present in IVIG preparations could be responsible for a number of AEs in patients receiving IVIG.

Tolerability of IVIGs in human volunteers: determination of relevant laboratory parameters and dependence on IgG dimer content

Spycher at ZLB injected 0.4g/kg of IVIG into healthy volunteers and observed transient changes in inflammatory laboratory parameters (11):

(1) Increase in proinflammatory cytokines including tumor necrosis factor-α (TNF-α) concentrations;
(2) Decrease of total leukocyte count, especially monocytes;
(3) Decrease of Fc receptor expression on circulating phagocytes associated with less-tolerated products, as assessed by laboratory parameters and clinical score, only described in the poster, grading AE intensity as 1 for mild, 2 for moderate and >2 for severe.

Complement activation was not modified. The increase of TNF-α and decrease in monocyte count paralleled the concentration of IgG dimers. The infused IVIG preparations were not described, nor the number of healthy individuals studied. This interesting opening study exploring the role of IgG dimers in IVIG preparations, associated with short-term inflammatory reactions involving Fc receptor expression rather than C‘ activation, does not truly correlate with the clinical score in healthy individuals. The authors suggested controlling the IgG dimer content in IVIG.

Small amphilphillic compounds effectively inhibit IgG dimer formation in liquid IVIG preparations and improve their i.v. tolerability

Bolli and colleagues from ZLB provided a way to decrease this amount, based on the above hypothesis (12). Small amphiphilic compounds effectively inhibit IgG dimer formation in liquid IVIG preparations and improve their intravenous tolerability. Results showed that small hydrophobic compounds, such as vitamin nicotinamide and its derivatives, were better dissociators of IgG dimers than large hydrophilic compounds. Neutral amino acids were more efficient, correlating with an increase of their side-chain hydrophobicity. Dissociation was more efficient at neutral than at acidic pH, and was reversible when the compounds were removed. A correlation between a blood pressure decrease model in the rat and IgG dimer content was shown, with a threshold of 13% of IgG dimers, leading to a 40% blood pressure decrease, in IVIG preparations considered to be ‘not well tolerated’. However, such ‘considered’ ‘well’ or ‘not well’ tolerated IVIG preparations were not defined.

CONCLUSION

To summarize abstracts as such, before having access to posters, is actually a very difficult exercise which highlights the various degrees of precise wording capabilities of the authors.

Three main topics were reviewed: pathogen safety, clinical tolerability and the role of dimers in adverse events.

γRadiation of IVIG to eliminate classical infectious agents showed conformational and functional integrity of IVIG. Nanofiltration and sodium hydroxide exhibited elimination of or a virucidal effect on TSE theoretical infectivity of plasma and IVIG production or medical equipment.

However, it must be pointed out that a certain amount of relativity needs to be brought to this accumulation of safety steps in the plasma-derived production processes of IVIG, which exhibits the highest safety records and margin with regard to classical infectious agents.

There was not a great deal of novelty in the abstracts addressing clinical tolerability. The higher risk of adverse drug reactions in neurological conditions was confirmed by two posters, as well as renal toxicity in patients with glomerulonephritis linked to the presence of sucrose, stroke and the presence of antiglobulin in one batch of IVIG, with or without clinical expression. In the latter, the responsibility of very nature of antiglobulins has still to be anti-D IgG was clear. In other similar cases, the explored. The tolerability of Bayer's new caprylate Ig-IV-S in an ITP study was highlighted in this session. Efficacy was in accordance with the European note for guidance. Its rate of infusion has also been explored, and assessed as well tolerated.

Finally, an interesting novelty, the role of dimers in the occurrence of AEs, was reported. It was brought to light that short-term inflammatory reactions involving Fc receptor expression rather than C‘ activation might correlate with the amount of dimers in the preparation, but was not truly correlated with clinical score in healthy individuals. Based on this hypothesis, the addition to IVIG of small hydrophilic compounds which reversibly dissociated dimers could be a way to decrease IVIG content. The impact of this dissociation of IgG dimers, which have been shown to be idiotype-anti-idiotype complexes, on IVIG immunomodulatory properties remains to be explored.

POSTERS

1. Tran H, Marlowe K, McKenney K, *et al.* Functional integrity of IVIG following irradiation with a virucidal dose of γ radiation
2. Gregori L, Maring J-A, MacAuley C, *et al.* Partitioning of TSE infectivity during ethanol fractionation of human plasma
3. Käsermann K, Kempf C. Cleaning of manufacturing equipment: sodium hydroxide renders the prior PrP^{sc} proteinase K sensitive
4. Siegel J, Sierawski S. Characterization of adverse reactions related to IVIG administration: a 10-year perspective
5. Wittstock M, Benecke R, Zettl UK, *et al.* Therapy with IVIG: complications and side-effects
6. Cleary AG, Brown BJ, Minards J, Sills A, Bolton-Maggs PHB. Hemolytic anemia following IVIG: a cautionary tale

7. Orbach H, Tishler M, Shoenfeld Y. IVIG and the kidney: indications and complications
8. Caress JB, Cartwright MS, Donofrio PD, Peacock JE Jr. The clinical features of 16 cases of stroke associated with administration of IVIG
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Pharmacoeconomics and evidence-based medicineG.Antonini and A.Di Pasquale

The increasing use of intravenous immunoglobulin (IVIG) in the past two decades has stimulated an interest in pharmacoeconomics and evidence-based medicine with regard to this drug.

IVIG IN SPONTANEOUS ABORTION

Gooi and colleagues presented an open study on the efficacy of IVIG in the treatment of spontaneous recurrent miscarriage based on 13 years' experience (1). The success rate in 82% of 16 women with recurrent pregnancy loss encourages the setting up of a randomized, controlled trial (RCT) of IVIG in this clinical problem. Nevertheless, despite these and other positive results, the overall outcome of IVIG in recurrent spontaneous abortion reported in various studies remains contradictory.

IVIG IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

Mead and co-workers reported the efficacy of IVIG in idiopathic thrombocytopenic purpura (ITP) (2). The authors presented some useful guidelines for treatment decisions in ITP

ITP in adults

As a first-line treatment, IVIG is effective in 75% of patients, particularly when there are significant symptoms or signs, or when the platelet count needs to be increased rapidly. Different dose schedules can be used. Repeated infusions may help to maintain remission. IVIG is also a useful therapy in chronic refractory ITP, but responses are short lived.

ITP in children

IVIG can raise the platelet count rapidly, but should be reserved for:

(1) The emergency treatment of serious bleeding symptoms;
(2) Children refractory to steroids;
(3) Children undergoing procedures likely to induce bleeding.

IVIG is effective given as a single dose of 0.8g/kg.

ITP during pregnancy

IVIG is an appropriate first-line therapy in ITP during pregnancy with a response rate of 80%.

There are no convincing data on the effect of corticosteroids or IVIG on the fetal/neonatal platelet count. Only 10% of neonates of mothers with ITP develop significant thrombocytopenia and only a small proportion of these will require treatment. IVIG 1g/kg is indicated in these rare cases.

IVIG IN CIDP AND MNN

Neurological diseases represent an interesting field of application of IVIG. Van Schaik presented two systematic Cochrane reviews of RCTs with IVIG in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and multiple motor neuropathy (MMN) (3,4). In CIDP, IVIG therapy (six RCTs) was more efficacious than placebo, increasing the chance of improvement from 15% to 47%, with an absolute risk difference of 32%. This means that if ten patients are treated with IVIG, one can expect an improvement due to IVIG in three, a spontaneous improvement in two and no improvement in five. When compared to plasmapheresis and prednisone, IVIG showed the same efficacy in CIDP. Therefore, the choice of treatment (IVIG or plasmapheresis) is mainly based on patient characteristics and logistic factors.

A series of open studies (including at least 100 patients) support the efficacy of IVIG in MMN. There are three RCTs including 31 patients, comparing IVIG and placebo in MMN in a cross-over trial design. Approximately half the patients showed an improvement of disability after IVIG and 7% after placebo, but the statistical significance of these results is weak and does not rule out the possibility that this effect is not significant. However, the authors state that IVIG treatment definitely has an effect on impairment in MMN, as it significantly improves muscle strength in a majority of cases, and they conclude that, because of the lack of alternative therapies and the low score of side-effects, a full course of IVIG is indicated in MMN patients.

ECONOMIC IMPACT OF IVIG

Simon and colleagues gave a picture of the use of IVIG and its economic impact at the regional university hospital group of Paris (5). In this group of hospitals, 366 kg of IVIG have been infused in 2001 and 2002. IVIG was used for accepted indications (77.4%), indications currently being assessed (17.9%) and unwarranted indications (4.7%). Even though the consumption was stable in these 2 years, the total expenditure increased to € 9.41 million (+4.4%), due to an increase in the average price of IVIG by 7.4%.

COST-EFFECTIVENESS AND COST-UTILITY OF IVIG

Two posters considering cost-effectiveness and cost-utility of IVIG treatments were presented by McCrone (6,7).

One poster dealt with a study on the economic evaluation of IVIG in relapsing-remitting multiple sclerosis (MS). The other poster depicted a cost-utility analysis of IVIG and prednisolone for CIDP. The meta-analysis of five placebo-controlled trials of IVIG versus placebo in relapsing-remitting MS, gave an average reduction in relapses of 0.7 per year. Annual average cost, based only on IVIG cost and hospital costs, came to €17 000. Cost-effectiveness was calculated by dividing the 'extra cost' of IVIG by the 'extra number' of relapses avoided. Average cost-effectiveness ratio was €24 000 which is favorable compared to a figure for β-interferon use over 5 years €45 000). The cost-utility analysis, based on the number of quality-adjusted life years (QALYs) gained by avoiding one relapse, also showed a similar figure to that for interferon, for which the reported cost per QALY was around €1.5 million. Therefore, even if there are strong cost differences among different centers, mainly due to the use of different dosages of IVIG, cost-effectiveness and cost-utility of IVIG in relapsing-remitting MS seem to be similar to those of β-interferon.

For an incremental cost-effectiveness analysis comparing IVIG and prednisolone treatment for CIDP, data from the INCAT study were used. McCrone and colleagues performed an economic study on a 6-week use of IVIG and prednisolone in 25 patients with CIDP, adopting a Client Service Receipt Inventory for measuring service use and costs and using the gained QALYs as the main outcome measure. The cost in the prednisolone group fell by 34% following treatment, while in the IVIG group there was an increase of 217%. The cost difference between the two treatments was €3754 over the 6-week period. The quality of life increased more in the IVIG group but the difference was not statistically significant. The authors concluded that the cost-effectiveness of IVIG in CIDP is strongly influenced by the price and the amount administered. The impact of later side-effects of prednisolone on long-term costs and QALYs are likely to reduce the cost per QALY of IVIG.

In a final poster, McCrone reviewed the discipline of health economics for decision-making processes and the role of health economics in the evaluation of IVIG (8). In this poster he highlighted the importance of incorporating health economics in evaluations of IVIG, summarized the main methods that can be used when conducting economic evaluations and identified the key challenges that IVIG presents to economic evaluation.

In conclusion, some points emerge from this poster session:

(1) The evaluation of the efficacy of IVIG in new therapeutic strategies must be based on randomized trials;
(2) Economic studies should be designed and performed in parallel to the clinical trial, to obtain a comprehensive evaluation;
(3) The price of IVIG is a crucial factor influencing the analysis cost-utility. Studies on price and cost-utility relationships as well as studies on alternative dosages of IVIG should be encouraged.

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