Immunoglobulin: production, mechanisms of action and formulations

Marcia Cristina Zago Novaretti
Carla Luana Dinardo

Hemotherapy Service, Instituto do Câncer do Estado de São Paulo – ICESP, São Paulo, SP, Brazil

Human immunoglobulin (Ig) began to be applied in the clinical practice with the treatment of primary immunodeficiencies. Quickly, applications of Ig increased, as its anti-inflammatory and immunomodulatory functions were elucidated. Currently, Ig is the most commonly used blood product. Ig is obtained by processing plasma; methods, in particular, techniques to reduce plasma viral loads have been evolving over the years and include: pasteurization, solvent/ detergent treatment, caprylic acid treatment and nanofiltration. These methods contribute to increased safety and quality of blood products. The mechanisms of action of Ig not only involve the blockade of Fc receptors of phagocytes, but also control complement pathways, idiotype-anti-idiotype dimer formation, blockage of superantigen binding to T cells, inhibition of dendritic cells and stimulation of regulatory T cells (Tregs). There are several formulations of Ig available, each one with its own peculiar characteristics. In Brazil, there is stringent legislation regulating the quality of Ig. Only Ig products that completely fulfill the quality control criteria are released for use. These standards involve different tests from visual inspection to determination of anti-complementary activity. This paper will further review the history and current status of Ig, including its production and mechanisms of action. The formulations available in Brazil and also the criteria of quality control currently applied will be presented.

Keywords: *Immunoglobulins, intravenous/therapeutic use; Immunoglobulins, intravenous/ pharmacokinetics; Plasma; Hemoderivate drugs; Immunoglobulins; Antibodies; Immune system*

Introduction

In 1890, Von Behring and Kitasato proved that serum from rabbits immunized with tetanus toxin had activity against the "poison of tetanus" and thus, when this serum was transferred to healthy rabbits, it protected them against tetanus.[1] This was the first of many studies which showed that many diseases could be prevented or treated using the serum of both animals and humans.

At the beginning of World War II, Cohn and colleagues developed techniques that allowed the separation of plasma proteins in stable individual fractions, with different biological functions.[2,3] Such techniques were improved and are applied until now to prepare blood products. These techniques enable the preparation of human immunoglobulin (Ig).

In 1952, Ig began to be successfully used in patients with primary immunodeficiencies. However, intramuscular Ig caused some disadvantages, such as pain during infusion and a long time to reach peak serum levels.[4] In 1960, the first Ig for intravenous use, prepared by the treatment of plasma with trypsin, was released. Since then, different laboratory strategies have been developed in order to obtain safer, effective and well tolerated blood products.

Ig was first used in the treatment of primary immunodeficiencies; indications for its use have increased greatly over the last 30 years. Therefore, Ig has become the primary or adjuvant treatment for various autoimmune and inflammatory diseases, due to its immunomodulatory and anti-inflammatory properties.

There were problems in the world's supply of Ig in the late 1990s when demand exceeded supplies by 30% and it was difficult to produce blood derivatives in Britain, a leading supplier of Ig. Moreover, there were increases in the number of well established clinical indications and in clinical indications that were not completely evidence based.[5] In Brazil, the annual consumption is estimated at between 500 kg and 1 ton , the equivalent to 0.3 to 0.6 kg/100,000 inhabitants per year. To cater for this demand, Brazil imports more than 90% of human Ig.[6]

Methods of production and safety

Ig is a sterile preparation of concentrated antibodies (immunoglobulins) that derive from large pools of human plasma from healthy donors. While the use of large plasma pools

Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 2/3/2011
Accepted: 3/23/2011

Corresponding author:
Marcia Cristina Zago Novaretti
Serviço de Hemoterapia do Instituto do Câncer do Estado de São Paulo (ICESP)
Av. Doutor Arnaldo 251, Cerqueira César
01246-000 – São Paulo, SP, Brazil
Phone: 55 11 3893-2000
marcitabrz@yahoo.com

www.rbhh.org or www.scielo.br/rbhh

DOI: 10.5581/1516-8484.20110102

for the production of Ig provides a variety of antibodies, it increases the risk of infections, whether viral or prion. This fact has led to an unrelenting pursuit to raise security of Ig while maintaining the tolerability of this blood product.

Ig production begins with the selection of donors for plasma collection. One can deduce from this fact that Ig formulations are not equal, since they depend on the antibody composition of the donor population which varies depending on existent diseases in that population. A report was recently published that stated that antibody levels against hepatitis A were significantly different in various formulations of Ig.[7]

Plasma used for Ig production must come from healthy blood donors with known medical history and absence of known risk factors for blood-borne infectious diseases.[8] Plasma may be collected by apheresis or come from a whole blood donation. It is not necessary to rapidly freeze plasma (less than 24 hours after collection) if it is only used for Ig and albumin production.

Plasma units collected should be negative for laboratory screening of human immunodeficiency virus (HIV) 1 and 2, and hepatitis viruses B and C. Serological methods and also molecular (NAT) methods should be used. In Brazil, plasma must also be negative for syphilis, Chagas' disease and, in endemic areas, for malaria.

The Ig production process involves steps of fractionation and purification of plasma. There are two main techniques of plasma fractionation. The first one involves plasma precipitation by ethanol, used as a nontoxic precipitating agent, and the second is a chromatographic technique, which uses cylindrical columns containing synthetic resins that allow protein separation.[9]

Companies that manufacture blood products use different methods for the purification of plasma and several approved methods for viral removal and inactivation.[10,11] Currently, at least three techniques are being used to manufacture Ig in order to improve tolerability and reduce risks of disease transmission.

Each step in the processing of plasma may cause alterations in its protein structure and its biological activity. As a consequence, commercial preparations of Ig differ in tolerability, and they can also differ in efficacy.[12] For example, some purification methods that include the addition of chemicals or enzymes, in order to inactivate viruses or to reduce the formation of aggregates of Ig, may also alter the structure and function of the Fc fragment of the IgG molecule and, consequently, decrease its biological activity.

The methods commonly used to reduce the viral load are: pasteurization, treatment with solvent/detergent, treatment with methylene blue, treatment with caprylic acid and nanofiltration. Of these, the caprylic acid and solvent/detergent techniques are effective against enveloped viruses, while nanofiltration removes both enveloped viruses and non-enveloped viruses (parvovirus B19 and hepatitis A).[13] There are formulations of Ig in which three methods are dedicated to the removal of pathogens: solvent/detergent treatment, 35-nm nanofiltration and incubation at low pH/high temperature to remove/inactivate viruses/prions.[14]

Recently, it has been shown that methods of inactivation/removal of viruses, such as the use of nanofiltration and caprylate acid, protect the properties of Ig.[12] It should be noted that not only the formulation of Ig should contain high levels of IgG (> 95%), but the molecule must also be intact and without aggregates. The presence of high concentrations of aggregates is correlated to reactions during the infusion of Ig.

In the 1980s, cases of viral transmission caused by Ig infusion were reported and created great concern among both the medical community and users as in many clinical situations there is no substitute for the blood product. However, since the solvent/detergent method and nucleic acid technology (NAT) have been added to the production process, no more cases of viral transmission were reported.[15,16]

Mechanism of action

In primary or secondary immunodeficiency, Ig administration has the aim of replacing antibodies and its mechanism of action is clearly defined: to restore the levels of IgG. However, considering the anti-inflammatory and immunomodulatory properties of Ig, several mechanisms have been suggested to elucidate the effects of this drug in the regulation of the immune system, some of which are shown in Table 1.[17,18] Figures 1 and 2 explain graphically the main mechanisms of action of Ig.

Recently, the action of Ig on regulatory T cells (Tregs) of $CD4^+$, $CD25^+$ and $FoxP3^+$ has been described. Tregs have a significant role in the maintenance of non-immune response to self antigens and prevention of immune aggression and autoimmune diseases.[19] Ig has been shown to promote expansion and enhancement of the Treg suppressive function, but the mechanism is not known yet.[20]

Table 1- Mechanisms of action of human immunoglobulin[18]

Interaction with Fc fragment specific receptor (FcR)
Control of complement pathways and activation of mechanisms inducing solubilization of circulating immune complex
Formation of idiotype-anti-idiotype dimers
Modulation of some cytokines and production of their antagonists
Apoptosis of B and T cells through the activation of Fas receptor
Blocking binding between T cells and superantigens
Control of self reactivity and tolerance induction
Inhibition of differentiation and maturation of dendritic cells

Besides the relationship between IV Ig and Tregs, there is a hypothesis that there is an interaction between Ig and dendritic cells. These cells are antigen-presenting cells and are involved in the induction of immunogenic or tolerogenic immune response. It is believed that the effects of Ig on T cell activation may be mediated by dendritic cells.[21]

Recently, it was shown that Ig formulations have an inhibitory effect on the differentiation and amplification of TH17 cells. These cells correspond to a subpopulation of T cells that, besides protecting against extracellular pathogens (such as *Klebsiella* and *Candida*) play a critical role in the pathogenesis of various autoimmune, allergic and inflammatory diseases. Inhibition of TH17 cells reduces the production of a series of inflammatory cytokines and other pro-inflammatory mediators, thereby interfering in the maintenance of chronic inflammatory response.[22]

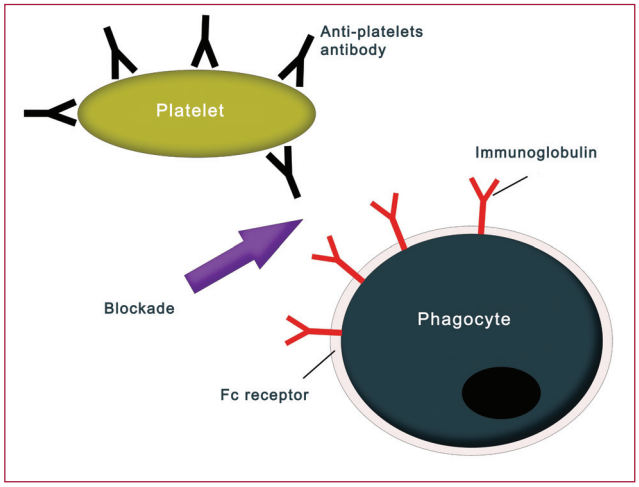

Figure 1 – Fc receptor blockade of phagocytes by Ig in a patient with immune thrombocytopenic purpura (presence of anti-platelet antibodies).

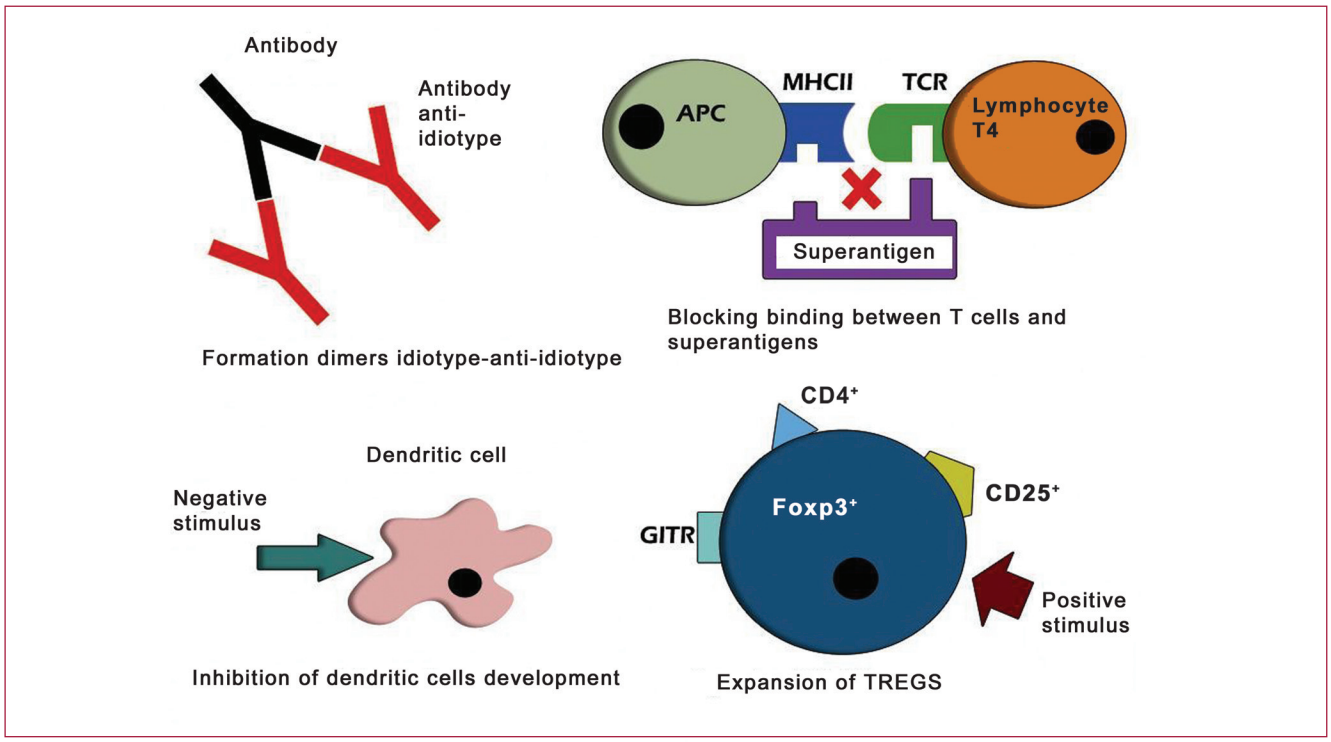

Figure 2 – Multiple actions of Ig: formation of idiotype-anti-idiotype dimers, blocking the binding of superantigens to T cells, inhibition of dendritic cells, stimulation of Regulatory T cells (Tregs)

Formulations of human immunoglobulin

Ig preparations differ in stabilizers and diluents. Such variations make each product unique and, consequently, its effectiveness and tolerability are also specific. This explains, in part, why some patients have reactions to certain products and not to others.

Some characteristics of Ig formulations must be carefully observed. The first one is the presence of latex; 0.3 to 1% of the population is sensitive to this antigen and may have allergic reactions, sometimes severe, following exposure.[23] The other one is the presence of sorbitol, which is contraindicated for patients with hereditary fructose intolerance. Table 2 shows the main Ig formulations available in Brazil.

Table 2 - Formulations of human immunoglobulin available in Brazil[24]

Product	Formulation	Presentation	Use	Excipient	Latex	Origin
Blauimuno ® (Blausiegel)	Lyophilized	0.6, 3, and 9.0 g	IV	Glucose, Sodium Chloride	No	CLB - Central Laboratory of the Netherlands
Flebogamma 5% ® (Grifols Brasil)	Injectable solution	0.5, 2.5, 5 and 10 g	IV	D-sorbitol, Water for injection	No	Grifols Institute, Spain
Gamaglobulina IM Grifols ® (Grifols Brasil)	Injectable solution	320 and 800 mg	IM	Glycine, sodium chloride, Water for injection	No	Grifols Institute, Spain
Gamunex ® (Meizler Biopharma)	Injectable solution	1, 2.5, 5, 10 and 20 g	IV	N/D	N/D	Talecris Biotherapeutics, INC (United States)
Imunoglobulin® (Blausiegel)	Injectable solution	0.5, 1, 2.5, 3, 5 and 10 g	IV	Maltose 100 mg/mL	N/D	Greencross Plasma Derivatives - Korea
Imunoglobulin® (Blausiegel)	Lyophilized	0.5, 1.0, 2.5 and 5.0 g	IV	Albumin, glucose and Sodium Chloride	N/D	Greencross Plasma Derivatives - Korea
Imunoglobulina humana Normal® (Blausiegel)	Lyophilized	0.6, 3.0, 6.0 and 9.0 g	IM	Glycine, sodium chloride and sucrose	No	Finnish Red Cross, Helsinki - Finland
Imunoglobulina humana Normal® (Blausiegel)	Lyophilized	0.6, 3.0, 6.0 and 9.0 g	IM	Glycine, sodium chloride and sucrose	No	Finnish Red Cross, Helsinki - Finland
Keyven ® (Macrofarma Química e Farmacêutica Industrial)	Injectable solution	50 mg/mL flasks with 20, 50, 100 and 200 mL	IV	N/D	N/D	Kedrion SPA, Italy; Hardis SPA, Italy
Kiovig ® (Baxter)	Injectable solution	1, 2.5, 5, 10 and 20g	IV	Glycine	No	Baxter - Europe and North America
Pentaglobin® (Marcos Pedrilson Produtos Hospitalares ltda)	Injectable solution	Ampoules: 10 and 20 mL, Flasks: 50 and 100 mL	IV	Glucose 27.5 mg; Sodium Chloride: 78 mmol	N/D	Biotest Pharma GmbH, Germany
Sandoglobulina® (Meizler Comércio Internacional S/A)	Lyophilized	1.0, 3.0, 6.0 and 12.0 g	IV	Sucrose: 1.67 g/g IgG, Sodium Chloride: 0.02 g/g protein	N/D	ZLB - Bioplasma AG, Switzerland
Sandoglobulina ® (CSL Behring Comércio de Produtos Farmacêuticos)	Lyophilized	1, 3, 6, and 12 g	IV	Sucrose, Sodium Chloride	No	CSL Behring AG - Switzerland; CSL Behring LLC- United States
Vigam®-Liquid (Meizler Comércio Internacional S/A)	Injectable solution	1, 2.5 and 5 g	IV	Albumin: 20mg, Sucrose: 24 mg, Glycine: 5 mg, In acetate: 3 mg, n-octanoate, Na: 0.5 mg	N/D	BPL - Bio Products Laboratory Dagger Lane, Elstree - United Kingdom
Tegeline ® (LFB-Hemoderivados e Biotecnologia)	Lyophilized	0.5, 2.5, 5 and 10 g	IV	N/D	N/D	LFB-Biomedicaments-France
Venimmuna N ® (CSL Behring)	Lyophilized	50 mg/mL with diluent flasks of 10, 50, 100 and 200 mL	IV	Water for injection	N/D	ZLB Behring GMBH-Germany

IV= intravenous, IM=intramuscular, N/D=not declared

In Brazil, the quality parameters of Ig for therapeutic use were defined by the National Agency of Sanitary Surveillance (ANVISA) in 2000 and are explained in Table 3.

Injectable Ig solution should be stored refrigerated between 4-8 degrees and has a shelf life of two to three years. Lyophilized Ig should be stored at room temperature (up to 25 degrees) and has a shelf life of up to five years.[25]

Recently, there has been a tendency to produce Ig solutions with higher protein concentrations, such as 100 mg/mL solutions (10%) and use a low pH that favors product stability (pH = 4.3 to 5.0). The increase in IgG concentration (from 5 to 10%) reduces the time of infusion,

Table 3 - Key features of the quality of Ig[25]

Parameter analyzed	Expected result
Visual inspection	- Preparation lyophilized: powder or solid mass crispy white or slightly yellowish - Solution: color between pale yellow and colorless, free of particles
Volume	Should range up to 5% of declared on the label
pH	- Intramuscular: 6.4-7.2 - Intravenous: 4-7.4
Protein concentration	- Intramuscular: 100-180 g/L - Intravenous: at least 30 g/L
Electrophoretic purity	- Intramuscular: gamma globulin corresponds to 90% of the total protein sample - Intravenous: gamma globulin is 95% of the total protein present in the sample
Determination of polymers and aggregates	- Intramuscular: less than 10% of polymer aggregates - Intravenous: less than 3% of polymer aggregates
Proof of Identity	Reactivity only with anti-human serum
Determination of antibodies against surface antigens of hepatitis B virus	More than 0.5 IU/g should be detected
Test of normal Ig power	- Intramuscular: at least 10 times more potent than the initial mixture - Intravenous: at least 3 times more potent than the original mixture
Determination of pre-kallikrein activator	Activity less than 35 IU/mL (solution of 30g/L)
Determination of anti-complementary activity	Complement consumption below 50% (1 mg of CH50 per mg of Ig)
Determination of hemagglutinin anti-A and anti-B	No agglutination when dilution equal to 1:64
Osmolality	Above 240 mosmol/kg

which is very important for patients with primary immunodeficiency who receive blood products every 21-28 days.[26]

Dosage and administration of immunoglobulins

There are several recommended Ig dosages according to clinical indication. The replacement dose of Ig in immunodeficiency must be individualized for each patient.[5] For other situations, the dose commonly used in adults is 2 g/kg, which can be split over five days of infusion (0.4 g/kg/day) or over two days of infusion (1 g/kg/day). The infusion over two days is particularly indicated in patients with acute and severe conditions.

The usual speed of Ig infusion varies from 4 mL to 8 mL/kg/h depending on formulation (whether 5% or 10%) and patient tolerability. At the beginning of infusion, however, the speed should be slower at around 0.4 to 0.6 mL/kg/h that is the equivalent to 0.01 mL/kg/min.[6] It is recommended to accompany the first 20 minutes of infusion of this blood product.

Ig is usually infused intravenously either in peripheral veins or central catheters. Subcutaneous administration has been used in patients without venous access and/or home infusions with the help of specially designed infuser. In exceptional circumstances, Ig can be administered orally or intrathecally.[6]

Conclusions

Ig is the most commonly used blood product in clinical practice. Over the years, there has been considerable progress in its production from the processing of plasma, which guarantees better product safety, especially considering the reduction in viral transmission.

Despite the various formulations of Ig available on the market, all of them must follow quality parameters spelled out by Brazilian legislation, which seeks to ensure the quality of this blood product.

The mechanisms of action of Ig are multiple and go far beyond simply blocking Fc receptors of phagocytes. It is believed that many mechanisms are yet to be clarified and will certainly contribute to better clinical indications of this blood product.

References

1. Von Behring E, Kitasato S. Über das zustandekommen der diphtherie-immunität und der tetanus-immunität bei tieren. Dtsch Med wochenschr. 1890;16:1113-4.
2. Cohn EJ. Blood proteins and their therapeutic value. Science. 1945;101(2612):51-6.

3. Cohn EJ, Hughes WL Jr, Weare JH. Preparation and properties of serum and plasma proteins; crystallization of serum albumins from ethanol water mixtures. J Am Chem Soc. 1947;69(7):1753-61.

4. Eibl MM. History of immunoglobulin replacement. Immunol Allergy Clin North Am. 2008;28(4):737-64, viii.

5. Provan D, Nokes TJ, Agrawal S, Winer J, Wood P, Group IGDGotIEW. Clinical guidelines for immunoglobulin use. 2nd ed. London: Department of Health; 2008.

6. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Diretriz para transfusão de imunoglobulinas [Internet]. Brasília: ANVISA; 2004. [cited 2008 Jan 12]. Available from: http://www4.anvisa.gov.br/base/visadoc/CP/CP%5B7489-5-0%5D.PDF

7. Farcet MR, Planitzer CB, Stein O, Modrof J, Kreil TR. Hepatitis A virus antibodies in immunoglobulin preparations. J Allergy Clin Immunol. 2010;125(1):198-202.

8. Radosevich M, Burnouf T. Intravenous immunoglobulin G: trends in production methods, quality control and quality assurance. Vox Sang. 2010;98(1):12-28.

9. Buchacher A, Iberer G. Purification of intravenous immunoglobulin G from human plasma--aspects of yield and virus safety. Biotechnol J. 2006;1(2):148-63. Comment in: Biotechnol J. 2006;1(2):111.

10. Gelfand EW. Critical decisions in selecting an intravenous immunoglobulin product. J Infus Nurs. 2005;28(6):366-74.

11. Shah SR. A newer immunoglobulin intravenous (IGIV) - Gammagard liquid 10%: evaluation of efficacy, safety, tolerability and impact on patient care. Expert Opin Biol Ther. 2008;8(6):799-804.

12. Siegel J. The product: All intravenous immunoglobulins are not equivalent. Pharmacotherapy. 2005;25(11 Pt 2):78S-84S.

13. Oh DJ, Lee YL, Kang JW, Kwon SY, Cho NS, Kim IS. [Evaluation of the virus-elimination efficacy of nanofiltration (Viresolve NFP) for the parvovirus B19 and hepatitis A virus]. Korean J Lab Med. 2010;30(1):45-50. Korean.

14. Poelsler G, Berting A, Kindermann J, Spruth M, Hammerle T, Teschner W, et al. A new liquid intravenous immunoglobulin with three dedicated virus reduction steps: virus and prion reduction capacity. Vox Sang. 2008;94(3):184-92.

15. Lane RS. Non-A, non-B hepatitis from intravenous immunoglobulin. Lancet. 1983;2(8356):974-5.

16. Dichtelmuller HO, Biesert L, Fabbrizzi F, Gajardo R, Groner A, von Hoegen I, et al. Robustness of solvent/detergent treatment of plasma derivatives: a data collection from Plasma Protein Therapeutics Association member companies. Transfusion. 2009; 49(9):1931-43.

17. Liumbruno GM, Bennardello F, Lattanzio A, Piccoli P, Rossettias G; Italian Society of Transfusion Medicine and Immuno-haematology (SIMTI). Recommendations for the use of albumin and immunoglobulins. Blood Transfus. 2009;7(3):216-34.

18. Ephrem A, Misra N, Hassan G, Dasgupta S, Delignat S, Duong Van Huyen JP, et al. Immunomodulation of autoimmune and inflammatory diseases with intravenous immunoglobulin. Clin Exp Med. 2005;5(4):135-40.

19. Miyara M, Sakaguchi S. Natural regulatory T cells: mechanisms of suppression. Trends Mol Med. 2007;13(3):108-16.

20. Maddur MS, Othy S, Hegde P, Vani J, Lacroix-Desmazes S, Bayry J, et al. Immunomodulation by intravenous immunoglobulin: role of regulatory T cells. J Clin Immunol. 2010;30 Suppl 1:S4-8.

21. Crow AR, Brinc D, Lazarus AH. New insight into the mechanism of action of IVIg: the role of dendritic cells. J Thromb Haemost. 2009;7 Suppl 1:245-8.

22. Maddur MS, Vani J, Hegde P, Lacroix-Desmazes S, Kaveri SV, Bayry J. Inhibition of differentiation, amplification, and function of human TH 17 cells by intravenous immunoglobulin. J Allergy Clin Immunol. 2011;127(3):823-30.e 1-7.

23. Navarrete MA, Salas A, Palacios L, Marin JF, Quiralte J, Florido JF. [Latex allergy]. Farm Hosp.2006;30(3):177-86. Spanish.

24. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Bulário eletrônico: imunoglobulina humana. [Internet]. Brasília: ANVISA. [cited 2010 Jan 12]. Available from: http://www4. anvisa.gov.br/base/visadoc/BM/BM[34162-1-0].PDF

25. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução RDC n°46 de 18 de maio de 2000. Aprova regulamento técnico para a produção e controle de qualidade de hemoderivados de uso humano. Brasília: Anvisa. [cited 2010 Jan 12]. Available from: http://www.anvisa.gov.br/legis/resol/2000/46_00rdc.htm

26. Hooper JA. Intravenous immunoglobulins: evolution of commercial IVIG preparations. Immunol Allergy Clin North Am. 2008;28(4): 765-78, viii.

xxx