THE POTENTIAL OF HUMAN PLASMA AND HUMAN BLOOD PRODUCTS FOR IMMUNE PROTECTION

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1. Plasma proteins - history

Human plasma is the most obtainable and clinically valuable blood derivative[1]. As compared to traditional pharmaceutical products and other biopharmaceuticals, human plasma is a source of essential therapeutic products with unique features due to their human origin.

Plasma protein therapies (PPTs) are a unique set of biologic therapies for innate life-threatening conditions. Nearly 74 years have passed since Ogden C. Bruton's has treated an 8-year-old boy with recurrent streptococcal pulmonary bacteremia whose plasma contained few gamma globulins (IgG). The regular intramuscular (i.m.) injections of from human plasma IgG led to an impressive reduction in the number of serious bacterial infections[2]. Other innate life-threatening conditions as alpha-1 antitrypsin deficiency (alpha-1), hereditary angioedema (HAE), and bleeding disorders due to lack of different haemostatic proteins have been also successfully managed. In addition, products prepared from the plasma of donors with high titres of anti-rhesus antigen D provided a therapeutic revolution with the prevention of Rh isoimmunisation and the consequent haemolytic disease of the newborn. Delays in diagnosis of plasma protein deficiencies can cause disease progression and fatal patient health outcomes. Thus, individuals with severe haemophilia who rarely lived past 13 years of age in the beginning of the 20th century, nowadays approach a normal life expectancy due to both plasma-derived and recombinant therapies[3]. In 1971, only 37% of individuals with variable immune deficiency, the most common PID, survived until 10 years of age. Due to PPTs, that survival rate has increased to 90%[4]. In addition to direct quantitative benefits, PPTs improve patient outcomes by reducing disease-related disabilities. Individuals with a plasma protein deficiency can suffer from significant activity limitations such as immobility, and other nerve system disorders[5].

Human plasma contains several groups of high-abundance proteins that seriously interfere with the low-abundance protein components. Thus, albumin comprises 51–71% and immunoglobulin G 8–26% of the total protein content in human plasma[6]. Immunoglobulin G (IgG) is one of the most abundant plasma proteins with concentrations of 6.6 to 14.5 mg/mL[7].

Cohn's different separation methods[8] pioneered the plasma fractionation. A 5-variable system has been established for the separation of plasma proteins based on pH, conductivity, ethanol content, protein concentration and temperature. The five most abundant proteins were enriched in Fractions I to V by sequential precipitation in increasing concentrations of ethanol as follows: fibrinogen in Fraction I, γ-globulins in Fraction II, lipid-bearing ß-globulins in Fraction III, α-globulins in Fraction IV and albumin in Fraction V.

Fractions II+III from Cohn's method 6 have become the starting material for isolation of intravenous immunoglobulins (IVIG). Fractionation of Fraction II+III may continue further using ethanol or other precipitation agents like PEG or caprylate. Deutsch et al. (1946) increased the yield of IgG in Fraction II by lowering the ionic strength for precipitation of Fraction III[9]. Oncley's (1949) separation method for separation of Fraction III from Fraction II + III was based on the work of Deutsch et al.[10] A detailed separation of Fraction III into 4 subfractions containing isoagglutinins (III-1), prothrombin (III- 2), plasminogen (III-3) and certain lipoproteins (III-0) led

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to a satisfactory isolation of Fraction II with antibodies only. The difference between IgG content in Fraction II + III (7 g/L) and in Fraction II (5.4 g/L) indicated IgG loss of 22.9%. This loss was further reduced by using filtration technology instead of centrifugation for removal of Fraction III[11]. Nowadays, Fraction III is considered as a waste in large scale industry. Further attempts to increase the yield were made by modifying certain precipitation steps. Kistler and Nitschmann (1962) changed the fractionation scheme of IgG by reducing the ethanol content from 17% to 12% [12].

Albumin was the first industrial plasma product. With some exceptions, most manufacturers fractionate albumin according to the original Cohn method which is also used in Bulgaria for production of human injectable albumin.

For many years, immunoglobulins have been produced in a concentrated formulation suitable for intramuscular administration only. Immunoglobulin aggregates generated over the fractionation process were thought to provoke severe systemic reactions in hypogammaglobulinaemic patients. The possibility to deliver large amounts of immunoglobulin to immunodeficient individuals was therefore limited. However, the intramuscular preparations from donors with high plasma concentrations of specific immunoglobulins, were largely applied for prevention and treatment of a range of infections.

2. Intravenous Immunoglobulins

In the 1970s, the fractionation industry developed immunoglobulin products that were tolerated intravenously (IVIG). This has led to the market dominance of this product.

The administration of IVIG has not only improved the quality of life but has also saved lives in various indications. Humoral immunodeficiency can be caused by different pathophysiological mechanisms and can be either primary or secondary to other diseases, or treatments [13]. Plasma-derived intravenous immunoglobulin (IVIG) preparations have been successfully applied as substitution therapy for prevention of infectious diseases in immunodeficient patients. In the decades following Bruton's report, the development of simple and reliable IgG assays permitted the identification of additional hypogammaglobulinemic patients. IVIG preparations are known to modulate autoimmune diseases through several F(ab')(2)- and Fc-dependent mechanisms. Nikolova KA et al. [14]showed that the *in vitro* and *in vivo* exposure of B lymphocytes from lupus-prone and from healthy mice to IVIG results in an increased expression of their surface inhibitory FcgammaIIB receptors. This study described a good approach with clinical relevance for modulating B lymphocyte activity using IVIG.

Decreased immunoglobulin levels were documented in preterm neonates, in certain otherwise healthy infants with transient low IgG levels in the first years of life, as well as in older infants, children and adults with primary or acquired hypogammaglobulinemia[15]. Although prophylaxis with high-titer human IVIG does not significantly reduce the incidence of infections, it reduced the severity of infections in very young high-risk patients who became infected. Reductions in hospitalization rates and significantly shorter hospital stays compared to well-matched control patients were observed[16]. At the same time in certain types of immune deficiency as is the most frequent isolated IgA deficiency, infusion with foreign IgA may provoke undesirable allergy reactions.

Infusion of IVIG has also immunomodulatory effects and is therefore applied for the treatment of increasing number of autoimmune, inflammatory and immune-mediated pathologies[17]. However, these effects are not very well defined mostly because of the variable content, and characteristics of the individual plasma pools.

Therefore, there is a great need of high-throughput manufacturing processes for plasma-derived and well characterized immunoglobulins.

3. Applications of immunoglobulins as prevention and replacement therapy

a) bacterial infections

The effects of immunoglobulins on bacterial infections are thought to involve bacterial cell lysis via complement activation, phagocytosis via bacterial opsonization, toxin neutralization, and antibody-dependent cell-mediated cytotoxicity. Nowadays IVIG is recommended as an additional therapy for

severe Salmonella, Clostridium, Staphylococcal and Streptococcal infections. The ability of S. aureus to elaborate a series of potent cytolytic toxins and peptides is an essential mechanism to evade the host's immune response and plays a key role in pathogenesis[18].

The pathogenesis of Staphylococcus aureus is mediated by several important virulence factors, including the two-component family of leukocidin toxins.

The two-component leukocidins—gamma-hemolysins (HIgAB and HIgCB), Panton-Valentine leukocidin (PVL), and LukED and LukAB (also known as LukGHare a group of pore-forming toxins capable of degrading human phagocytic cells[19]. LukAB, a recently discovered staphylococcal leukocidin, has been shown to be the dominant toxin in vitro[20]. Recent studies showed that LukAB is abundantly produced by invasive disease isolates, is recognized by the host response during invasive human infection and is critical for the pathogen's ability to subvert the human innate immune response. Previous studies have investigated the ability of IVIG to neutralize S. aureus toxins, including Panton-Valentine leukocidin (PVL), hemolysin, and toxic shock syndrome toxin-1 (TSST-1)[21]. Deep et al. showed that IVIG was protective against death in a rabbit model of necrotic pneumonia and characterized two specific IVIG antibodies that neutralized the toxic effects of hemolysin and PVL[22]. Commercial batches of IVIG containing LukAB-specific antibodies capable of neutralizing toxin-dependent phagocytic lysis *in vitro*. Adjusted for total IgG per sample, the concentration of anti-LukAB-binding antibodies in IVIG was similar to that observed in healthy uninfected pediatric controls and significantly lower than in pediatric patients with invasive S. aureus infection. The functional neutralization capacity of IVIG was significantly lower than in pediatric patients with invasive S. aureus disease and healthy controls[23] Further characterization of functional antibodies will be important given the frequent clinical use of IVIG for severe S. aureus infections.

b) In Fungal infections

Fungi are among the most common microbes encountered by mammalian hosts. Approximately, 1-10 fungal spores are ingested with each breath, making it a natural route of infection for most filamentous fungal pathogens. Medically important fungi include *Aspergillus*, *Blastomyces, Candida, Coccidioides, Coccidioides*, *Cryptococcus*, *Histoplasma*, *Malassezia, Paracoccidioides* and *Pneumocystis*[24]. Innate immune responses are the first line of defence against fungal infections that lay foundation for the long lasting, more specific and effective adaptive immune responses. The fungal pathogen-associated molecular patterns (PAMPs) such as heatshock protein 60 (Hsp60), β-glucans, phospholipomannan, O-linked mannans, zymosan and fungal DNA are recognized by various pattern recognition receptors that include toll-like receptors (TLRs) (such as TLR 2, 4 and TLR9) and C-type lectin receptors (such as Dectin-1 and DC-SIGN)[25]. Antibodies provide protection against fungal infections through several, possibly interdependent, mechanisms. Indeed, antibodies are well-known effector molecules of the adaptive immune system and neutralize pathogens and their derivative molecules in part through complement activation. Furthermore, they also exert a regulatory role in the activation of innate immune cells by signaling through various Fc receptors.

However, early studies to understand the role of antibodies in anti-fungal immunity were largely inconclusive due to the insufficient quantity of protective anti-fungal antibodies found in serum. On the other hand, that could be due to the presence of inhibitory antibodies neutralizing the effects of the protective ones[26]. Several reports suggest that natural antibodies play an important protective role against fungal infections. In fact, the administration of normal mouse serum to μMT (B cell-deficient) mice was shown to restrict fungal growth in various models[27]. Natural antibodies are polyreactive, generally germ-line encoded and are characterized by low to medium affinity. Natural antibodies belong to IgM, IgA and IgG classes and are produced mainly by B1 cells[28]. A substantial fraction of serum antibodies from naive mice recognizes fungal antigens including C. albicans[29].

The increased incidence of fungal infections in immunocompromised individuals and allergic and

inflammatory fungal diseases in immunocompetent individuals cause serious concern. Therefore, immunoglobulins are considered as an important tool for limiting the fungal load and treatment of fungal infections and inflammations.

Antibodies provide protection against fungal infections through a variety of mechanisms that include direct neutralization of fungi, inhibition of fungal growth, alteration of gene expression, signaling and lipid metabolism, induction of iron deficiency, inhibition of polysaccharide release, and biofilm formation. The anti-inflammatory and immunomodulatory role of antibodies in mycosis is one of the potential applications of IVIG[30]. Although, humoral immunity might not have a major role in conferring protection against fungal infections in human, passive administration of specific protective antibodies could prove to be beneficial in drug-resistance cases, to reduce the dosage and associated toxic symptoms of anti-fungal drugs.

c) viral infections and COVID-19

Towards the end of World War II, Edwin Cohn's pooled human plasma "Fraction II" was injected intramuscularly to control outbreaks of red measles and infectious hepatitis in U.S.[31]. Soon after, fractionated IgG became generally available for i.m. use. At that time, human IgG treatment was recommended to modify the course of measles and hepatitis A, but other indications for its clinical use were not fully defined. As IgG could be safely given only by the i.m. route, doses were limited to about 100 to 150 mg/kg of body weight/month. Larger doses were too painful.

Nowadays IVIG is a well-known treatment for a variety of viral infections. The anti-viral effects include preventing viral penetration into the host cells and activating the innate immune cells and complement pathways. IVIG usually present significant effects against different viruses like Crimean-Congo hemorrhagic fever (CCHF)[32], cytomegalovirus (CMV), varicella-zoster virus (VZV), herpes simplex virus (HSV), hepatitis A virus (HAV), respiratory syncytial viral (RSV), Epstein-Barr virus (EBV), measles, mumps, rubella, parvovirus B19, and polyomavirus BK. Several case reports have described the successful use of IVIG in the treatment of anemia caused by chronic parvovirus B19 infection. IVIG therapy was to clear viremia and to improve symptoms and cytokine dysregulation in parvovirus B19–associated chronic fatigue. Since parvovirus B19 infection is highly prevalent in the general population, IVIG contain a significant concentration of anti–parvovirus B19 antibodies and are considered as the only specific treatment of this infection. The so-called "hyperimmune preparations" i.e. immunoglobulins collected from convalescent donors with high titers of desired antibodies, are still used for the treatment of a variety of viral infections and infection-related disorders (CMV, hepatitis B, rabies, Crimean-Congo haemorrhagic fever)[33]. Pathogen-specific immunoglobulins (Ig) are used to prevent infection with rabies virus, and during pregnancy – to prevent varicella-zoster and congenital cytomegalovirus infection[34].

Plasma from donors recovered from COVID-19 (regardless of their vaccination status) may contain antibodies against SARS-CoV-2 that can help suppress virus replication[35]. In August 2020, the Food and Drug Administration (FDA) issued an emergency use authorization (EUA) for COVID-19 convalescent plasma (CCP) for the treatment of hospitalized patients with COVID-19. The EUA was subsequently revised. The current EUA restricts the approval of hyperimmune plasma products that contain high levels of antibodies against SARS-CoV-2. The testing criteria used to identify high-titer CCP products have also been revised[36]. Immunocompromised patients are at risk of reduced antibody response to SARS-CoV-2 infection and vaccination against COVID-19, suboptimal control of viral replication, and development of severe disease[37]. Although definitive evidence is lacking, there is a physiological rationale for the use of SARS-CoV-2 antibody therapy in these patients. Under the revised EUA issued on 27 December 2021, CCP is authorized for the treatment of COVID-19 in non-hospitalized or hospitalized patients with immunosuppressive disease or immunosuppressive therapy[38].

To investigate the presence of specific anti-SARS-CoV-2 IgG and IgA antibodies in plasma from donors who recovered from COVID-19 and in IVIG

batches produced from plasma collected during COVID-19 pandemic, we compared samples from 90 convalescent donors after SARS-CoV-2 infection (collected in the period 01.2021 - 06.2021), and 10 batches of IVIG derived from human plasma (collected from 10.2021 to 03.2022). Anti S1-IgG and anti S1-IgA were tested semiquantitatively. RBD-IgG was detected in 50/90 samples (55.5%), mean (min-max) level 5.19 (1.12 - 8.37), mean. RBD- IgA was also detected in 50/90 samples (55.5%), mean (min-max) 5.84 (1.19 - 9.41). Notably, 40/90 tested sera did not contain one or both SARS-CoV-2 specific immunoglobulins. At the same time, RBD-specific IgG weredetected in all 10 batches of IVIG with levels ranging between 4.3 and 12.5, mean 9.3. The effective use of CCP for passive immunization requires their preliminary examination for the presence and titter of SARS-CoV-2 specific IgG and IgA antibodies. The presence of specific Anti S1-IgG in Intravenous immunoglobulin enables their reliably effective use for passive immunization[39].

Standardization of IVIG

Each batch of IVIG is produced by fractionation of a plasma pool obtained from at least 10,000 healthy donors. The assumption underlying the applications of IgG formulations was that plasma collected from a large number of donors would ensure lots containing comparable levels of antigen-specific antibodies. However, significant batch-to-batch differences in neutralizing antibody (NT) levels for respiratory syncytial virus (RSV) and specific opsonizing antibodies against group B streptococci (GBS) have been demonstrated[40]. The quantity of pathogen-specific antibodies in each production batch of IgG is never granted.

A recent report by the International Neonatal Immunotherapy Study Collaborative Group is the account of the last in a series of clinical trials that have failed to show an effect of IVIG administration on sepsis outcome. All these studies have used different immunoglobulin preparations assuming that their properties were identical as the IgG repertoire in them is representative for a large population of healthy plasma donors[41].

The Bulgarian Immunovenin-intact 5% IgG 50 g/l infusion solution produced in BulBio-NCIPD Ltd. is used as replacement therapy for primary and secondary immunodeficiencies, such as: congenital agammaglobulinemia and hypogammaglobulinemia, common variable immunodeficiency, severe combined immunodeficiency states, Wiskott-Aldrich syndrome, myeloma or chronic lymphocytic leukemia with severe secondary hypogammaglobulinemia and recurrent infections, in children with congenital AIDS and recurrent infections, as well as immunomodulation therapy in idiopathic thrombocytopenic purpura (ITP), Guillain Barre syndrome and Kawasaki disease. Lately, an increasing demand is registered for treatment of unexplained infertility and repeated reproductive failure. Immunovenin has a documented approximate distribution of IgG subclasses: IgG 1 ≥ 56.5% IgG 2 ≥ 35.1% IgG 3 ≥ 5.7% IgG 4 ≥ 2.7% and IgA content of 1500 micrograms/ml which reduces to minimum the probability of intolerance in the rare cases of IgA deficiency. However, the successful application of this valuable blood product requires more detailed analysis and standardization for the content and ratio of isotypes and subclasses, the content of specific immunoglobulins of major importance, the possible content of autoantibodies or other substances with a potentially negative effect, as proinflammatory or inhibitory cytokines that are released in the course of severe infections.

CONCLUSION

As IVIG is prepared from multiple donors, it contains numerous antibodies directed against a wide range of antigens. IVIG contains a broad spectrum of antibody specificities against bacterial, viral, parasitic and mycoplasma antigens, that are capable of both opsonization and neutralization of microbes and toxins. In addition to their initial use as replacement therapy in primary and secondary immunodeficiencies, IVIG are increasingly recommended for a large spectrum of inflammatory diseases. IVIG use has rapidly grown in during the COVID-19 pandemic. In this context, an unmet need is to broaden the scope of IVIG standardization protocols, including precise quantitative characteristics of Ig classes, isotypes; and specific antibodies content alongside undesirable components tracing.

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