

Antiinflammatory human proteins

Part 2 of a two-part paper

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Immunoglobulins

Immunoglobulin G (IgG class, the four others being IgM, IgA, IgD and IgE) is extracted from pooled normal plasma or hyperimmune plasma by Cohn fractionation. Fractionation is defined as separation of different plasma protein types from each other by creating physicochemical conditions in which one protein type will separate from the other on the basis of its size, isoelectric point and tertiary structure (see Part 1). The plasma pools often contain up to 5000 plasma donations and most production plants start preparing with fractions II and III followed by further purification of fraction II which almost exclusively contains IgG. The conditions for precipitation must be controlled carefully to avoid coprecipitation of proteolytic enzymes, which would lead to IgG fragmentation. During final processing fraction II precipitate may contain some aggregated IgG and prekallikrein activator, which upon i.v. injection gives rise to anaphylactoid reactions. IgG preparations prepared in this way can therefore only be given intramuscularly. To achieve i.v. tolerance, an aggregate-free confection is mandatory.

The discovery of the therapeutic advantages bestowed by IVIG is based on several decades of progress in plasma fractionation on the one hand, and understanding of the clinical pictures associated with quantitative and qualitative IgG deficiencies on the other. The normal range of IgG in humans spans as much as 6–16 g/L plasma, most people living in narrow ranges between these two limits calculated by measurements on thousands of individuals. IgG have the longest half-lives (21 days) of any plasma protein. Costly administration of IgG to any patient at least bears the potential of long-lasting efficacy of infused (better termed transfused) IgG.

The IgG deficiency states were discovered during WWII by *Bruton*, a U.S.

Army surgeon who also kept the health conditions of European “war brides” and their babies in focus, one of them being seen in 1951 as a then 8-year-old boy lacking gammaglobulin in serum.

The boy suffered recurrent bacterial infections while resisting viral infections, and demonstrated normal delayed hypersensitivity responses. The disease now called Bruton’s syndrome was published in June 1952 in *Pediatrics* and *Time* magazine featured the outstanding medical discovery on May 18, 1953. Since all cases discovered were boys, its sex-linked characteristic with women carriers of the trait soon became apparent. Today the Jeffrey Modell Foundation is the key international institution monitoring progress in the field (www.jmfworld.com).

Therapeutic approaches with intramuscular IgG preparations in the 1950s and 1960s and modern trends

Along with the discovery of IgG deficiency states, the biochemical researchers proceeded with their work at the bench and in the animal room. Besides *Bruton*, the other historic names important for IVIG are Paul *Ehrlich* (immunochemist, 1854–1915) and Emil *von Behring* (MD, 1854–1917). Both same-agers, along with Louis *Pasteur* (1822–1895), formed the basis of vaccination with animal and human antitoxins, later to become known as antibody molecules. *Ehrlich*’s work remains the basic pillar of antigen-antibody science (www.immune-complex.ch). Immunodeficiency found its way into textbooks and teaching around 1950, research from bedside to bench and *vice versa* was boosted by classical examples of translational research.

Two decades-long timespans of immunoglobulin therapy may be distinguished:

- substitution era (1950–1982);
- substitution and intervention era (starting 1982).

The i.m. application of 16% IgG preparation has a long history associated with the name of *Charles A Janeway* (see: *J Allergy Clin Immunol* October 2005). Immunodeficient children and (later to be recognised) young adults received i.m. injections, perhaps every week or every month depending on the severity of their condition. The i.v. administration was not an issue since these preparations contained a high proportion of aggregated IgG, which, upon i.v. infusion, precipitously activate complement and make the patient feel sick. Meanwhile it became known that IgG contain four different subclasses: IgG₁, IgG₂, IgG₃ and IgG₄. It was *Andreas Morell* who in the early 1970s published a report that the subclasses’ usual half-life of 21 days varied, IgG₃ expressing a much shorter half-life than the others, with 7 days only. It became evident that i.m. injection leads to destruction of a fraction of the injected material before it can be absorbed and released into the systemic circulation. All this incited doctors to inject IgG by the i.v. route. The Behringwerke were the earliest, using pepsin digestion to produce Fab fragments: the destroyed Fc fragment of the IgG would no longer be able to activate complement. Such safety improvement was paid for by loss in efficacy and shortening of half-life. In contrast, we Swiss aimed at conserving the integral structure of IgG and thus maintaining the recognition Fab end of the molecule as well as the business end, i.e. Fc. The biochemist *Henri Isliker* noted that treatment of purified i.m. IgG preparation at acid pH with traces of pepsin avoids aggregate formation. The then named “immunoglobulin SRC” was registered in the early 1980s by the late *Andreas Gardi* and the author, with several common trips to Washington DC to the North American drug agency (FDA) and the preparation was later to become Sandoglobulin® for use in both European and American patients. It was Paul *Imbach* who found that while substituting im-

munodeficient children with IVIG some of the thrombocytopenic patient group corrected their blood platelet count. Thus was the intervention era opened, because one now treated low platelet counts with IVIG, even in the absence of overt immunodeficiency. The antiinflammatory activity of IVIG is based on three major working points. IVIG deviates complement activation to innocuous targets and therefore keeps such reactants as C3a and C5a away from the site of inflammation (see pipette 01/2006). Excess monomeric IgG surrounding the Fc receptors of inflammatory cells protects these cells and IVIG preparations contain an array of anti-cytokine antibodies downregulating local mediators of inflammation such as the potent IL-6 or pyrogens.

In summary, IVIG are a major plasma protein fraction of human donor plasma. They are the driving force for purchase of plasma by fractionation plants on a market which is clearly dominated by blood transfusion services worldwide.

The side-effect spectrum of IVIG is large and goes from slight headache on the first treatment to severe renal failure or a meningitis-like syndrome. However, these side effects are very rare. Since the last update by the author and the clinical neurologist *Sturzenegger*, no new aspects have emerged.

C1 inhibitor

The antiinflammatory activity of this protein cuts complement activation at the early stage of C1q assembly with its cofactors C1r and C1s. In conditions with excessive complement activation, C1 inhibitor (the most notable commercial preparation being Berinert® [www.zlbbhehring.com]) becomes beneficial for the patient. The product was initially developed for patients deficient in C1 inhibitor, i.e. those suffering from angioneurotic oedema. As it transpired, the product may be of interest for patients in sepsis or for recipients of an ABO-histo-blood group incompatible allograft.

The effect of C1 inhibitor administration on circulating elastase-alpha(1)-antitrypsin complex (EA) and lactoferrin (LF) levels in these patients was studied to gain further insight into ag-

onists involved in the activation of neutrophils in human sepsis. Elevated levels of EA and LF were found in 65 and 85% of the septic patients respectively. Patients with elevated EA levels had higher organ dysfunction scores, higher levels of cytokines, and higher levels of complement activation products than patients with normal EA levels. C1 inhibitor therapy reduced EA as well as complement activation and IL-8 release in the patients with elevated EA on admission. It was concluded that neutrophil activation in human sepsis correlates with the severity of organ dysfunction and involves complement and interleukin-8 as agonists. The favourable effect of C1 inhibitor administration on neutrophils may provide an explanation for the beneficial, although mild, effects of this treatment on organ dysfunction in sepsis.

Activated protein C

As soon as the potentially antiinflammatory function of activated protein C (APC) was recognised, therapeutic endeavours were directed at providing deficient patients with APC. APC is a good example of how fresh frozen plasma for its source was replaced by affinity purified APC and eventually recombinant human APC.

The protein C pathway serves as a modulating system with both antiinflammatory and anticoagulant properties and is involved in the pathophysiology of inflammation and sepsis.

It has been recognised that the protein C pathway is a link between the inflammation and coagulation cascades. APC was originally identified on the basis of its antithrombotic properties, which result from the inhibition of activated factor V and VIII. In the early 1990s any potential antiinflammatory properties of APC were thought to relate primarily to its inhibition of thrombin generation, but a few years later, with the identification of endothelial protein C receptor, APC was also seen as a cofactor in enhancing the generation of such receptors. In the recombinant human activated protein C worldwide evaluation in severe sepsis (PROWESS) study, APC was apparently associated with improved cardiovascular function, respiratory function and preven-

tion of haematological dysfunction. The main findings were a significant reduction in mortality and an increase in the incidence of serious bleeding.

For many years, protein C deficient patients were treated with fresh frozen plasma, prothrombin concentrates, heparin and oral anticoagulants. Protein C was then produced from cryopoor plasma by a chromatographic procedure. Despite the concern of posttranslational modifications between plasma derived and recombinant protein, the latter method of producing APC has gained over the former (Ceprotin, Baxter) and Ely Lilly is now on the market with its drotrecogin alfa (activated) preparation (Xigris®), which, however, is extremely expensive. One treatment costs approximately 14.000 Swiss francs, which is why many intensivists use it with reluctance and only when the patients fail to improve with other measures.

Drotrecogin alfa (activated) is recommended for adult patients who

- have severe sepsis that has resulted in multiple organ failure (two or more major organs not functioning properly) and
- are being given the most appropriate intensive care support for their condition.

The use of drotrecogin alfa (activated) is now supervised by a specialist consultant with skills in intensive care and experience in the care of patients with sepsis. It is not impossible that on closer investigation the purification of APC from plasma fractions may compete price-wisely with the genetically engineered form.

Further prospects; in the pipeline: high-density lipoprotein and parotid secretory protein

High-density lipoprotein

Reconstituted high density lipoprotein (rHDL) is a molecule that is chemically and biologically similar to nascent HDL. rHDL consists of apolipoprotein A-I (the major protein moiety of HDL) isolated from a human plasma fraction by *Peter Lerch*, and phosphatidylcholine (lecithin) from soybean. Apolipoprotein A-I and phosphatidylcholine are combined in the presence of a detergent, and form upon dialysis disc-

shaped, non-covalently associated particles resembling nascent HDL. The final product is stabilised with sucrose and lyophilised (Lerch et al., 1996). rHDL has undergone extensive pre-clinical and clinical testing that demonstrates a wide spectrum of biological activities relevant not only to atherosclerosis, but also to inflammatory diseases such as gram-negative sepsis: rHDL binds lipopolysaccharide (LPS) and inhibits its biological activity, such as the expression of adhesion molecules, with, as a consequence, inhibition of leucocyte adherence. Furthermore, rHDL suppresses pro-inflammatory cytokine production and inhibits LPS-induced clinical symptoms (Pajkrt et al., 1996). In view of rHDL's antiinflammatory activities it should be considered a potential agent in the prevention and treatment of inflammatory diseases.

Parotid secretory protein

Parotid secretory protein (PSP) and palate-lung-nasal epithelium clone (PLUNC) are recently discovered secretory proteins expressed in the oral cavity and upper airways. Both proteins are related to bactericidal/permeability increasing protein (BPI). Cationic peptides derived from BPI exhibit antiinflammatory activity. To test whether PSP (C20orf70 gene product) also contains antiinflammatory peptides, 3 cationic peptides were designed on the basis of the predicted structure of PSP and known active regions of BPI. Each peptide inhibited the lipopolysaccharide (LPS)-stimulated secretion of TNF α from RAW 264.7 macrophage cells. PSP peptides directly inhibited the binding of LPS to LPS-binding protein. The cationic peptide Substance P had no inhibitory effect in these assays, confirming the specificity of the PSP peptides. These findings suggest that PSP peptides can serve as templates for the design of novel antiinflammatory peptides. Anti-inflammatory agents copied from nature and found not only in plasma but also in secretions may be useful in the fight against noma, an orofacial gangrene which during its fulminating course mutilates malnourished children, mainly in the sub-Saharan Africa region (www.lamaison.ch).

The safety of plasma-derived antiinflammatory proteins

Plasma-derived proteins follow the safety requirements of blood products (see Part 1). Their infectious disease transmitting capacity is theoretically possible, but for practical purposes, the hurdles of registration requirements are so high that any preparation now on the market in developed countries is safe. Questions remain on prion transmission, but the problem is unresolved and will remain so as long as there is no adequate screening test for TSE.

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Further reading

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