Immunogenicity of therapeutic proteins

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Introduction

A year ago Nicole Casadevall of the Hotel-Dieu in Paris and her colleagues published their first 13 cases of pure red-cell aplasia (PRCA) associated with the use of erythropoietin (Epo) in patients with chronic renal failure [1]. As of November 2002, the number of antibody-mediated reported cases in Europe, Canada and Australia has increased to more than 175. The most likely explanation for this serious side effect is a subtle change in the Epo molecule that may occur during the manufacturing and formulation process, or in the handling and distribution processes.

Apparently a change in the product leads to the induction of antibodies neutralizing the endogenous Epo in these patients causing a complete block in the differentiation of red blood cells. The increased s.c. use and self administration with inappropriate use, handling and storage have been suggested as cofactors enhancing the immunogenic potential introduced by the change in the product. Recently, most European regulatory agencies have contraindicated the s.c. use of one specific Epo product in dialysis patients.

Although PRCA is a serious clinical condition, which requires the patients to be treated by frequent blood transfusions, the incidence is rare and approximately 20 in 100 000 patient years. Also, it is important to realise that most, if not all, therapeutic proteins are immunogenic, sometimes even in the majority of patients [2].

History of the use of therapeutic proteins

The medical use of proteins has a long history. It started more than a century ago when immune sera from animal origin introduced for the prevention or treatment of infections, followed with the use of insulin of porcine and bovine origin some decades later. These products were immunogenic in patients, sometimes even leading to serious anaphylactic reactions [3]. These side effects were easily explained by the foreign nature of the proteins leading to a classical immune reaction.

The introduction of human-derived proteins such as growth hormone and factor VIII was also associated with the induction of antibodies [4,5]. But these products were mostly given to children with an innate deficiency and therefore a lack of immune tolerance.

With the development of recombinant DNA technology the large-scale production of human homologues like the interferons, growth factors and hormones became feasible resulting in the application in a large number of patients. It was a surprise that these products also induced antibodies, which cannot be explained by the lack of immune tolerance. Some of these products such as Escherichia coli derived interferon beta and interleukin-2 induce these antibodies even in the majority of patients [2].

Immunization or breaking tolerance

It is now clear that nearly all biopharmaceuticals induce antibodies. The frequency of these antibodies varies widely, from common to rare, as is the case with Epo. These antibodies are induced by two mechanisms as depicted in Table 1.

There is the classical reaction to foreign proteins as caused by the biopharmaceuticals of bacterial or plant origins such as streptokinase [6] and asparaginase [7]. The reaction to these proteins is comparable with an immune reaction to a vaccine. Neutralizing antibodies appear in the majority of cases, often even after a single injection. The antibodies persist for a long time and they inhibit the efficacy of the product. The reaction can be easily explained as a normal reaction to a foreign protein.

The other mechanism by which antibodies are induced is based on breaking immune tolerance existing normally to self-antigens. This is the mechanism leading to the antibodies to human homologues like the interferons, IL-2, GM-CSF and Epo. These antibodies are mainly only binding, in general appear after prolonged treatment and often only in a minority of patients. The
antibodies disappear after stopping treatment and sometimes even during treatment. In the majority of cases the antibodies have no consequences. The mechanisms by which tolerance is induced or broken are not completely understood. An important way to break tolerance is to present the self-antigens in a repetitive way [8]. A periodicity of these antigens as present in aggregates of proteins is apparently very efficient in activating ignorant or anergic B cells that are responsible for tolerance [9].

Factors influencing the incidence of antibody induction

An important issue when assessing the immunogenicity of biopharmaceuticals is assays. There are in principle two types: the RIA and ELISA-like assays, which determine binding antibodies, and the bioassays identifying the presence of neutralizing antibodies. These assays are used in conjunction. Sera are first screened for the presence of binding antibodies and, if positive, the presence of neutralizing antibodies is assayed with the more cumbersome bioassay. In most cases, patients start by producing binding antibodies and may ultimately develop neutralizing antibodies. There are, however, no standardized assays available and there are no reference standards which make it difficult to compare results obtained from different laboratories and different studies [10].

As is the case with biopharmaceuticals from plant or microbial origin, the structure of the protein and the presence of foreign epitopes may cause immunogenicity. Also, the lack of glycosylation of glycoproteins produced in prokaryotes, such as GM-CSF and interferon beta, may induce antibodies because such molecules are less soluble or by the exposition of epitopes which are normally hidden by the glycosylation [11,12].

Impurities and contaminants have been identified as the main cause of immunogenicity of human growth hormone and insulin [13,14]. The presence of aggregates by suboptimal production or formulation has been associated with the induction of antibodies [15].

Patient’s characteristics are also important. In cancer patients with an impaired immune system the incidence of antibodies is lower than in patients with viral infection [16]. In haemophilia patients the type of the genetic defect in the patients Factor VIII gene influences the frequency of immunogenicity [17].

Route of administration is also a factor. In studies in which the routes of administration were compared the i.v. and local routes showed a lower incidence of antibodies than the groups treated subcutaneously or intramuscularly [18].

But, there are also a number of unknown factors influencing immunogenicity. The same product produced at different sites showed considerable difference in immunogenicity without showing differences in physicochemical characterization (S. Goelz, personal communication).

Consequences of antibodies

In the majority of cases the presence of antibodies has no clinical consequences. The most common biological effect is the loss of efficacy. Sometimes increasing the dose restores efficacy. General immune effects such as anaphylaxis and allergic reactions, which were relatively common, historically have become rare in the highly purified products currently used.

The most dramatic effect of antibodies occurs if a natural protein with an essential biological activity is neutralized. Such a consequence has been described for megakaryocyte-derived growth factor (MDGF) some years ago. This thrombopoietin-like protein induced antibodies neutralizing endogenous TPO leading to severe thrombocytopenia in volunteers and cancer patients [19].

This effect is comparable with the Epo-associated PRCA.

Conclusion

The antibodies associated with Epo treatment in a small number of patients are not an uncommon event as most biopharmaceuticals induce antibodies in patients. In the majority of cases these antibodies have no clinical effects. However, in the case of Epo the antibodies cross-react with the residual natural erythropoietin resulting in PRCA.

Although the cause of the immunogenicity of Epo is unclear, a subtle change in the molecule was probably introduced by the manufacturing and/or formulation

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changes in 1998. The current physicochemical characterization methods do not allow us to fully predict the biological and clinical properties of biopharmaceuticals. This puts further emphasis on the quality and the consistency of the production process to ensure the safety of therapeutic proteins. Shortly, the first patents of biopharmaceuticals will expire, opening the market for copy products [20]. Clinicians need to be more aware that the source of the product and the reliability of the manufacturer matter. Only clinical studies and careful monitoring of the market can be used to conclusively demonstrate rates of immunogenicity in humans for protein therapeutics. This is probably the most important lesson to learn from this incident with Epo.

References