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 approxi-
mately 20 in 100 000 patient years. Also, it is impor-
tant 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 pro-
ducts were mostly given to children with an innate
deficiency and therefore a lack of immune tolerance.

With the development of recombinant DNA techno-
logy 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 pro-
ducts also induced antibodies, which cannot be
explained by the lack of immune tolerance. Some of
these products such as *Escherichia coli* derived inter-
feron 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 asparginase [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 inter-
ferons, 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

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Table 1. Mechanisms of antibody induction by therapeutic proteins

	Classical immune reaction	Breaking immune tolerance
Properties of the product	Products of microbial or plant origin	Human homologue
Antibody formation	Fast; often after single injection; high incidence; neutralizing antibodies; long duration	Slow, after prolonged treatment; mainly binding antibodies; low incidence; disappear after treatment; sometimes during treatment
Cause	Presence of non-self antigens	Impurities and the presence of aggregates
Consequences	Loss of efficacy in the majority of cases	No consequences in the majority of patients

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 anergetic 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 physiochemical 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

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.

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