The issue of unwanted immunogenicity of therapeutic biologicals has recently received much attention by manufacturers, users, recipients, and regulatory organizations concerned with such products. However, the problem is not new. Induction of antibodies by coagulation factors, hormones, monoclonal antibodies, and cytokines in patients receiving these for therapy has been noted and published many times over the past two decades. Development of antibodies is sometimes apparently benign in effect, causing limited if any undesirable effects on patients receiving therapy. Unfortunately, in other instances antibodies are associated with undesirable consequences. These can manifest as mild to severe adverse “anaphylactoid” reactions, but more often compromise therapy by neutralizing (or blocking) the biological action of the therapeutic product.

Clearly, protein therapeutics with nonhuman sequences are likely to induce immune responses in non-immunocompromised individuals, particularly if they are administered repeatedly. However, it is now well established that at least some human-sequence biologicals can be immunogenic in some recipients. It is also evident that prediction of the unwanted immunogenicity, its characteristics and clinical significance, is very difficult, if not impossible. Some proteins seem somewhat intrinsically to be immunogenic (e.g., GM-CSF, IFNα, IFNβ), whereas others may be less immunogenic, such as IL-2 and growth hormone. Interestingly, some (such as IFNγ and GCSF) seem incapable of inducing detectable immune responses.

The nature and clinical significance of antibodies induced also varies. In some cases — for GM-CSF for example — antibodies can be induced that may or may not neutralize the biological activity of the cytokine, but only neutralizing...
responses impair clinical efficacy of therapy. In other cases, induced antibodies impair therapy whether or not they neutralize biological activity, whereas with other proteins no apparent adverse effects are associated with induced antibodies of any type. The situation is further complicated by product-related immunogenicity. Thus, some products based on a particular biological may be immunogenic, whereas others based on the same protein (even of the same sequence) can be less immunogenic or non-immunogenic. Again the clinical consequences can be product dependent.

**Immunogenicity of Erythropoietin**

Erythropoietin (EPO) has been used for therapy for a long period. Adverse events associated with its use appear rare, and clinical responses are impressive. Unwanted immunogenicity associated with EPO products was until recently regarded as relatively infrequent, with little or no clinical significance. The situation changed dramatically in 1998 when three patients in France undergoing treatment with EPO developed pure red cell aplasia (PRCA), which was caused by induction of neutralizing antibodies against EPO. The first confirmed diagnosis of PRCA by bone marrow examination was made by Casadevall et al in 1999. Since then, more EPO-treated patients presented with PRCA in the United Kingdom, Switzerland, Australia, Canada, Germany, Italy, Spain, Finland, and Norway. By September 2002, 155 cases had been reported, with 112 of these confirmed as positive for antibodies against EPO. Despite the considerable clinical use of EPO in the United States, the PRCA problem seemed limited to other countries.

An assessment of the EPO products used to treat the patients who developed antibody-mediated PRCA showed that they had all received the Jansen-Ortho (Johnson & Johnson) product Eprex (Epociitin alpha), although some patients had also received other EPO products. The link between development of PRCA and Eprex was further strengthened by its geographical incidence: Eprex is licensed for use in all the countries where cases were noted, but not in the United States where Eprex is not approved for use. PRCA also seemed predominantly associated with subcutaneous rather than intravenous administration of Eprex (possibly but not conclusively explaining the relatively large number of cases in France, where subcutaneous injection is often used), and it also developed mainly in renal-disease rather than cancer patients.

Development of PRCA results in such patients is becoming refractory to further EPO treatment. Although antibody development seems solely restricted to treatment with Eprex, the antibodies induced cross-react...
and neutralize all EPO products (including the murein Arancst) and naturally occurring EPO. This clearly prohibits switching patients to non-Eprex products as a mechanism for restoring EPO responses. EPO antibodies diminish in patients when EPO treatment is discontinued, but blood transfusions, immunosuppressive therapy, and plasmapheresis were required in many cases.

CAUSE(S) OF IMMUNOGENICITY

No clear factor responsible for the dramatic increase in the immunogenicity of Eprex has been positively identified. The situation is made even less clear by the fact that Eprex was widely used for several years before any significant immunogenicity was noted. Most surprising, Eprex and Epogen, which clearly showed very different unwanted immunogenicity profiles, were developed from the same rDNA derived EPO construct. However, several changes to production appear to have been made to Eprex that have obviously led to differences important for antibody development. These include removal of human serum albumin (HSA) from the formulation and other modifications, some of which have been proposed, but not proven to be involved in the altered immunogenicity. It has been suggested that subtle differences in glycosylation may explain the immunogenicity of Eprex, but because the induced antibodies equally bind deglycosylated or nonglycosylated EPO, this would require a novel and unusual mechanism for action.

CONSEQUENCES OF IMMUNOGENICITY: DEVELOPMENT OF PRCA

Following the initial reports of PRCA in patients receiving EPO, the immediate concern that it was a “generic” problem for all EPO products was addressed in some detail. The early evidence that almost all cases could be linked to a single product was widely promulgated to avoid concern over EPO therapy as a clinical option. Fortunately, the manufacturers of EPO products approached the problem from a scientific perspective, with clear aims of resolving the problem and establishing its causes. However, as outlined above, that has been far from easy and is still not clarified.

Responses from regulatory agencies have been compromised by the unexpected and unexplained nature of the problem. Also, the non-USA occurrence of the PRCA cases limited the responses from the US FDA. Reaction to the Eprex immunogenicity differed throughout Europe and elsewhere. In the United Kingdom, the Committee on Safety of Medicines recommended switching exclusively to intravenous administration for renal failure patients. Health Canada provided health professionals with information concerning the problem and screening for EPO antibodies in recipients of Eprex. However, a centralized EU response has not been forthcoming, probably because the problem is regarded as the prime responsibility of national regulatory agencies.

The EPO immunogenicity problem has highlighted the unwanted immunogenicity issue at a time when it was already receiving considerable attention. Immunogenicity of β-interferon had already been an ongoing issue in patients receiving various products for treatment of multiple sclerosis. Similar, but less controversial immunogenicity problems are ongoing with α-interferon, other cytokines, hormones, monoclonal antibodies, coagulation factors, and other biologicals. Immunogenicity issues for “generic” biologicals are clearly very significant and perhaps even more complex than for conventional biologicals.

All of that has almost certainly contributed to the importance attributed to unwanted immunogenicity in the guidelines relating to licensing of biologicals produced in the European Union, the United States, and elsewhere. Such documents stress the importance of appropriate screening for immunogenicity (including as part of comparability studies) but recognize the problems associated with this. The important concept that unwanted immunogenicity cannot be predicted — hence necessitating careful assessment during clinical trials and so on — has been stressed. Equally important, the relatively low incidence of immunogenicity with some products has been recognized in EU guidelines as indicating the ongoing need to assess products for immunogenicity postlicensing. The EPO situation clearly shows that this is necessary and highlights the unexpected and unpredictable aspects of the intriguing immunogenicity problem with biological products.

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