

REVIEW ARTICLE

Clinical concerns of immunogenicity produced at cellular levels by biopharmaceuticals following their parenteral administration into human body

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Abstract

Similar to the low molecular weight traditional drugs, biopharmaceuticals are capable of producing not only therapeutic effects but also side effects provided if the dose of these compounds exceeds certain concentration and/or if the exposure duration of these compounds at subtoxic doses is being lengthened. In addition, a major drawback of biopharmaceuticals is the risk of antibody formation. Following the administration of biopharmaceuticals into human body, the formation of antidrug–antibody (ADA) or neutralizing antibody and other general immune system effects (including allergy, anaphylaxis, or serum sickness) are of clinical concern regarding therapeutic efficacy and patient safety. For example, drug-induced neutralizing antibodies to erythropoietin (EPO) result in pure red cell aplasia, whereas drug-induced acquired anti-factor VIII antibodies worsen the pathology associated with hemophilia. Since most of the already developed or under development biopharmaceuticals are to some extent immunogenic, the regulatory agencies insist to conduct potential ADA formation during the drug development process itself. This review encompasses a short overview on the clinical concerns of immunogenicity produced at cellular levels by growth hormone, interferon- α , EPO, factor VIII, and factor IX following their parenteral administration into human body. Clinical concerns related to immunogenicity produced by the biosimilar versions of these drugs are also presented wherever possible.

Keywords: Biopharmaceuticals; biosimilars; clinical concerns; immune response; therapy

Introduction

From the introduction of the first biopharmaceutical/biological products of non-human origin some 100 years ago, to the more recent addition within the past two decades of human recombinant DNA products [e.g., growth hormone (GH), interferons (IFNs), erythropoietin (EPO), and factor VIII], millions of patients with medical conditions such as diabetes, gaucher disease, cancer, and infertility were benefited following treatment with these drugs (Schellekens, 2008a). However, similar to the low molecular weight conventional or traditional drugs, biopharmaceuticals are capable of producing

not only therapeutic effects but also side or untoward effects provided if the dose of these compounds exceeds certain concentration and/or if the exposure duration of these compounds at subtoxic doses is being lengthened (Bruinink, 2008). In addition, a major drawback of biopharmaceuticals is the risk of antibody formation. Following the administration of biopharmaceuticals into human body, the formation of antidrug–antibodies (ADAs) or neutralizing antibody and other general immune system effects (including allergy, anaphylaxis, or serum sickness) are of clinical concern regarding therapeutic efficacy and patient safety. Moreover, many of the recent publications have confirmed the

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role of effector T cells, and begun to explore the role of regulatory T cells in the development of ADAs (De Groot & Moise, 2007; De Groot & Scott, 2007; De Groot et al., 2008; De Groot & Martin, 2009). Several additional factors contributing to immunogenicity were also proposed (Schellekens, 2005), including contaminants or impurities, protein aggregation (Braun et al., 1997; Rosenberg, 2006), chemical degradation, and protein modification, such as differences in glycosylation or oxidation (Shacter, 2000; Wang, 2005) to explain the induction of antibodies.

It should be added that patents for the already developed/approved biopharmaceuticals are either expired or about to expire. This has opened the market to non-innovator versions of these products, called “biosimilars” in the European Union (EU), and “follow-on protein products/biopharmaceuticals” in the United States (Covic & Kuhlmann, 2007). Selected examples of approved biosimilar products along with their innovator product are shown in Table 1. Other terms are also frequently used in medical and pharmaceutical literatures to denote biosimilars which include follow-on protein products, subsequent-entry biologicals, and similar biological medicinal products (Kresse, 2009). Biosimilars may conveniently be defined as biopharmaceuticals that are considered similar in composition to an innovator product, but not necessarily clinically interchangeable (Gottlieb, 2008). Furthermore, using “similar” but not completely “identical” proteins interchangeably raises concerns about potentiating immunogenicity (Krämer, 2008; Schellekens, 2009).

The present review encompasses a short overview on the clinical concerns of immunogenicity produced at cellular levels by GH, IFN α , EPO, factor VIII (fVIII), and factor IX (fIX) following their parenteral administration into human body. Clinical concerns related to immunogenicity produced by the biosimilar versions of these drugs are also presented wherever possible. It should be noted that for clinical trials, the protocol complies with

the particular recommendation and that approval of their protocols was obtained from the concerned regulatory authorities by the author(s) of the reports/papers described in this review.

Clinical concerns of immunogenicity produced by biopharmaceuticals

Little or no consequences due to antidrug-antibodies formation

Growth hormone

Recombinant forms of human GH (rhGH, *Escherichia coli* derived) are used as therapy for deficiencies in GH production or response. After intramuscular and subcutaneous (SC) administrations of different kinds of rhGH preparations in GH-lacking children, anti-rhGH antibodies were formed. However, little or no inhibition in the growth rate of the children was observed (Kaplan et al., 1986; Albertsson-Wikland, 1987; Okada et al., 1987; Wilton & Gunnarsson, 1988; Takano, Shizume, & Hibi, 1989). Recent preparations of rhGH were less immunogenic (antibody incidence of 1.1%) after 1 year of treatment (Lundin et al., 1991). In addition, the anti-rhGH antibodies formation was dependent on the rhGH products used. When compared to the treatment with pituitary-extracted rhGH, methionyl-rhGH treatment resulted in higher incidence of anti-rhGH antibodies formation in patients (Massa, Vanderschueren-Lodeweyckx, & Bouillon, 1993). To find out the reason why the ADAs formation did not inhibit growth rate of patients, Schellekens and Casadevall (2004) suggested that antibodies and GH receptor bind to different epitopes on the GH molecule, which can explain the fact that anti-rhGH antibodies generally do not influence growth.

Two biosimilar somatotropins (human GH), Omnitrope®, and Valtropin® (marketed by Sandoz and Biopartners, respectively) were approved by

Table 1. Selected examples of approved biosimilar products along with their innovator product

Innovator product	Biosimilar products
Genotropin® (rhGH) (manufactured by Pfizer)	Omnitrope® and Valtropin® (marketed by Sandoz and Biopartners, respectively)
Recombinant glucagons for injection	GlucaGen®
Recombinant human hyaluronidase	Hylenex®
Recombinant calcitonin salmon	Fortical® Nasal Spray
Eprex® (rhEPO) (but the INN for these products is epoetin- α)	Abseamed®, Binocrit®, and Epoetin- α HEXAL® (all produced by Rentschler Biotechnologie GmbH but marketed by three different companies)
Eprex® (rhEPO) but the INN for these products is epoetin- ζ rather than epoetin- α	Retacrit® and Silapo® (manufactured by Norbitec GmbH)
Neupogen® (filgrastim products for the treatment of neutropenia)	Ratiograstim® and Filgrastim ratiopharm® (Ratiopharm GmbH), Biograstim® (CT Arzneimittel GmbH), and Tenvagrastim® (Teva Generics GmbH)

rhGH, recombinant human growth hormone; rhEPO, recombinant human erythropoietin; INN, international nonproprietary name.

the European Medical Evaluation Agency (EMA). Omnitrope® is a biosimilar version of the reference product, Genotropin® (manufactured by Pfizer). Similar to Genotropin®, Omnitrope® is a recombinant form of human somatotropin that is manufactured with rDNA technology in *E. coli*. During the clinical development program, various formulations of GH were used: Somatotropin Sandoz powder for solution for injection (Covance); Somatotropin Sandoz powder (Sandoz), a formulation to be marketed as Omnitrope; and Somatotropin Sandoz liquid (Sandoz). The presence of excess host cell protein in Covance injection as contaminant resulted in the formation of ADAs in GH-lacking children (Omnitrope®—EMA, 2007). Therefore, additional purification steps were processed for other formulations of GH by the manufacturers in order to control the excess host cell protein contamination (Omnitrope®—EMA, 2006; Romer et al., 2007; Schneider & Kalinke, 2008). Valtropin® (synthesized in *Saccharomyces cerevisiae*), a biosimilar version of Humatrope® (*E. coli*-derived) manufactured by BioPartner, was shown to have efficacy and safety similar to the reference product in a 12-month randomized controlled trial involving 149 children lacking GH (Valtropin®—EMA, 2006a, 2006b).

In 2007, US Food and Drug Administration (USFDA) approved two biosimilar somatotropins Omnitrope (Sandoz) and Valtropin (LG Life Sciences, Biopartners). Omnitrope is being marketed in the US since 2007 for GH deficiency (GHD) in children and adults (Dudzinski & Kesselheim, 2008). Valtropin is not yet on the market, except in Korea.

Regarding the issue of switching patients receiving a GH preparation to a biosimilar medicinal product, it is of interest that in a recent position paper by the Arzneimittelkommission (Drug Review Board) of the German Medical Association (2009) voices no concern with the following conditions. It is mentioned that switching is to be accompanied by close monitoring of the patient and it has to be checked by the physician if changes of dosing, dosing intervals, or administration routes are necessary. The situation is comparable to the prescription of a new entity in a known ATC (Anatomical Therapeutic Chemical)-class. After complying with these conditions, the Board does not see any reason not to switch patients receiving a biopharmaceutical to a biosimilar medicinal product if so desired or requested because of lower cost. In other countries, including the United States, third party payers increasingly demand similar changes as a cost-saving measure. It should also be mentioned that substitution of biopharmaceutical and biosimilars on pharmacy level is regulated on a national basis and prohibited by law in some European countries.

Reduction in drug efficacy

The role of ADAs on reducing the drug clinical efficacy was exemplified below using IFN α as a case study.

Interferon- α

IFN α is a protein composed of 166 amino acids. Several genes of IFN α have been identified which share a high degree of homology. IFN α possesses antiviral activity, inhibit viral replication, increase class I major histocompatibility complex (MHC), stimulate Th1 (also known as Type 1 helper T cells), and inhibit proliferation of many cell types. IFN α is used for the treatment of many malignant diseases and for chronic hepatitis C and B. Several different recombinant preparations of IFN α exist. The most commonly used IFN include IFN α -2a (Roferon A) and IFN α -2b (Intron A/Reliferon), which differ in the amino acid in position 23 (lysine in IFN α -2a and arginine in IFN α -2b) and both preparations are glycosylated. When compared with IFN α -2b, higher ADAs occurrence was noted in patients after chronic treatment with IFN α -2a (Steis et al., 1988; Freund et al., 1989; Berman et al., 1990). Since the gene for IFN α -2a is not present in the population, it is possible that the IFN α -2a also carries a neo-antigen too. However, according to Kromminga and Schellekens (2005), the structural differences between IFN α -2a and IFN α -2b may not be the reason for the observed difference in immunogenicity.

Discordant data exist about the role of ADAs on the drug clinical efficacy. Furthermore, the kinetics of ADAs development seems to be linked to the type of disease. For example, in patients affected by renal cell carcinoma, antibodies were described after a median time of 8 weeks, while in patients affected by hairy cell leukemia they were reported to arise after an average of 7 months (Figlin & Itri, 1988). After treatment with IFN α in patients affected by hairy cell leukemia, a drug resistance to therapy was seen in the antibody-positive patients (Steis et al., 1988; Berman et al., 1990). Similar observation of drug resistance to therapy was also noticed in IFN α -2b-treated patients affected by myelogenous leukemia (Freund et al., 1989). In contrast, intramuscular injection of IFN α -2a to patients affected by renal cell carcinoma resulted in the development of neutralizing antibodies, but no correlation between antibody presence and clinical response to therapy or clinical toxicity was found (Jones & Itri, 1986; Figlin & Itri, 1988).

IFN α -2a is also widely used for the treatment of chronic hepatitis C. Here too, within 6 months of treatment, ADAs were formed and after their formation, no therapeutic response to IFN α -2a was seen in patients affected by chronic hepatitis C (Bonetti et al., 1994). After comparing responder and non-responder patients,

a higher percentage of antibodies positive subjects were found in the group of non-responders (40% vs. 14%, respectively) (Douglas et al., 1993). Low dose versus high dose treatment with IFN α -2a in hepatitis B-affected patients resulted in the formation ADAs only in the group of patients treated with a low dose. The high antibody titer was also correlated with a failure in treatment response (Lok, Lai, & Leung, 1990). On the other hand, treatment with recombinant IFN α -2b in 175 patients showed ADAs formation in only one patient and thus no correlation was made on the presence of antibodies with a reduction in clinical response (Dianzani et al., 1989).

In January 2001, the FDA approved the use of PEGylated IFN α in the United States. Initially, the drug was used for the production of PEGylated IFN α -2a (Pegasys in USA and Reiferon Retard in Egypt), approval for PEGylated IFN α -2b (PegIntron) followed in October 2002. In comparison to conventional IFN α , the PEGylated version of these drugs reduced the injection frequency from three times per week to once in a week. It should be added that with the introduction of PEGylated IFN α , the prevalence of neutralizing antibodies is only about 1–2% (Frost, 2005). This corresponds well with the hypothesis that the backbone is the antigenic epitope and that shielding thereof by glycosylation or PEGylation reduces unwanted antibody formation. In combination with antiviral drug, ribavirin, the IFN α -2b is used in Canada (Pegetron) for effective treatment of hepatitis C. Some case reports were described paternal exposure to ribavirin-IFN α -2b within 6 months of conception; however, no adverse fetal effects were noted (Taguchi & Ito, 2005).

It should also be noted that Alpheon[®], a biosimilar version of Roferon-A[®] (IFN α -2a), was rejected by the EMEA. The reasons for the rejection by the EMEA included quality and clinical differences between Alpheon[®] and the reference product, inadequate data on the stability of the active substance, inadequate validation of the process for the finished process, and finally insufficient validation of immunogenicity testing (Alpheon[®]—EMEA, 2006). Thus, the regulatory agencies insist to conduct potential ADAs formation during the drug development process itself.

Cross-reaction with endogenous protein

The most dangerous consequence of the development of antibodies occurs when the endogenous protein is neutralized. This effect was exemplified later with EPO case study.

Erythropoietin

EPO is a 165-amino-acid glycoprotein that stimulates erythropoiesis. Endogenous EPO and most recombinant human EPOs (rhEPO) variants [e.g., epoetin- α (Eprex[®], Epogen[®], and Procrit[®]), epoetin- β (NeoRecormon[®]), methoxy polyethylene glycol-epoetin- β (MIRCERA[®]),

and darbepoetin- α (Aranesp[®])] have minor differences in glycosylation, with variation in the sialic acid composition of the oligosaccharide groups (Sasaki et al., 1987; Skibeli, Nissen-Lie, & Torjesen, 2001).

As the erythropoiesis-stimulating agents (ESAs) are injected through intravenous (IV) or SC routes, the rhEPO products were used successfully for more than 17 years to treat millions of patients with anemia secondary to chronic kidney disease (CKD), cancer, chemotherapy, or human immunodeficiency virus infection (Locatelli et al., 2004; Bokemeyer et al., 2007). Although IV- and SC-administered EPOs have significantly improved the quality of life and longevity of, especially, the CKD population, the formation/production of antibodies directed against rhEPO as well as against the endogenous EPO causes the development of pure red cell aplasia (PRCA) (Casadevall et al., 2002; Locatelli et al., 2004; Schellekens & Jiskoot, 2006). According to the FDA's definitional revision, the PRCA can be redefined as "anaemia associated with neutralizing antibodies includes both PRCA and severe anaemia, with or without a decrease in white blood cells or platelets associated with neutralizing antibodies." Without any EPO stimulation, red blood cell precursors in the bone marrow perish, PRCA leaves the patient with refractory anemia and thus patients routinely become transfusion dependent. EPO antibodies produced in response to Eprex[®] were also cross-reacted with other epoetin formulations and endogenous EPO (Casadevall, 2002; Swanson et al., 2004). The antibodies are usually of subtype immunoglobulin-G (IgG), IgG1, or IgG4 and are directed against the protein part of the EPO molecule. EPO antibodies are identified by either a radio-immune precipitation assay (RIPA) or an enzyme-linked immunosorbent assay (ELISA). Neutralizing effects of antibodies are confirmed in bioassays involving growth of primary marrow culture or an erythroleukemic cell line (Casadevall, 2002; Swanson et al., 2004).

Alterations in delivery route (IV to SC) and stability-providing ingredient [human serum albumin (HSA) to polysorbate 80 and glycine] for the epoetin- α products were initially recommended especially in Europe purely because of the possible economical and etiological (HSA might transmit variant Creutzfeldt-Jakob disease) reasons, respectively (Kaufman, 1998; PRCA case report, 2008). It should be noted that in Canada, Singapore, and Australia, both HSA-free and HSA-containing Eprex[®] are available, and other epoetin or darbepoetin products have not undergone any formulation changes. The causation of epoetin-associated PRCA was attributed to formulations without HSA, subcutaneous administration, and uncoated rubber stoppers (Casadevall et al., 2002; Anand & Nissenson, 2003). A class effect or an improper product handling is the most likely cause. Therefore, the EPO antibodies formation was due to the exposure of rubber

leachates in HSA-free and prefilled Eprex[®] syringes with uncoated rubber stoppers, although proof of the role of leachates remains elusive (Boven et al., 2005a, 2005b). Additional studies did not identify leachates when Teflon coating was added to the stoppers and/or when the amount of silicone in the prefilled syringes was decreased. The Canadian PRCA Focus Group and The Research on Adverse Drug Events and Reports (RADAR) group simultaneously reported estimates of the exposure-adjusted incidence rates (Bennett et al., 2004; Cournoyer et al., 2004). It is recommended to store the prefilled syringes containing Eprex[®] in 2–8°C and shielded from sunlight during shipment as well.

According to the pharmacovigilance data, a sudden increase (a 25-fold increase) in the incidence of PRCA with Eprex[®] use from 1999 to May 31, 2002 was noticed in Europe (Janssen-Ortho communication to Australian health care professionals, June 2002). On the other hand, no significant increase in antibody-proven PRCA with epoetin- α use was seen in the United States (Amgen, personal communication, November 2002). The observed sudden increment in the epoetin-associated PRCA was explained at that time on the basis of the stabilizer alteration made from HSA to polysorbate 80 and glycine on the epoetin formulation available especially in Europe. But this explanation has become rapidly unrealistic when seeing the clinical results of a very few cases of PRCA obtained with epoetin- β (NeoRecormon[®]) formulation. If the lack of HSA was an important factor *per se* in the pathogenesis of the PRCA, one would expect epoetin- β (NeoRecormon[®]), manufactured without HSA, to be associated with more cases of PRCA. In fact, NeoRecormon[®] was also stabilized with polysorbate 20 and glycine. At this juncture, questions concerning micellar formation and stabilization efficacy need to be considered/answered for polysorbate 80-based epoetin α - and β -formulations. The polysorbate 80 may have increased the immunogenicity of Eprex[®] by eliciting the formation of epoetin-containing micelles or by interacting with leachates released by the uncoated rubber stoppers of prefilled syringes. A situation of protein denaturation or aggregate formation due to the susceptibility of the stress conditions like insufficient attention to the cold chain may also be occurred if the stabilization efficacy of polysorbate 80 is lowered in epoetin formulations. Apart from the abovementioned two formulation-related questions, animal models are also being developed to obtain interpretative answers of why antibody-proven PRCA occurred with epoetin- α use. Of particular interest, an EPO antibody-mediated PRCA rat model was recently developed by administering rhEPO through SC route to rats three times weekly for 4 weeks. The rats that developed PRCA were rescued by a synthetic EPO receptor agonist in contrast to those receiving vehicle injections of EPO (Woodburn et al., 2007).

Irrespective of the manufacturing and storage alterations that have been reasoned for antibody-proven PRCA cases, the change in drug delivery route is also considered. Because, the majority of patients who have developed PRCA following Eprex[®] administration received the drug subcutaneously. For instance, from 1998 to 2002, the incidence of SC Eprex[®]-associated PRCA climbed to 20.06 cases per 100,000 patient-years of SC Eprex[®] exposure. At the same time, PRCA cases linked to IV Eprex[®] use remain low, around 0.67 cases per 100,000 patient-years of IV Eprex[®] exposure. Thus, whatever manufacturing or storage problems that occurred with Eprex[®] after 1998, it appears that they are potentiated when the drug is given by the SC route. Since extensive immune defense system and slow rate of absorption are connected with the subcutaneous tissue, minor alterations in the manufacturing and storage conditions may result in the recognition of rhEPO as foreign to elicit greater immunogenicity against one particular epoetin product (Porter, 2001). Consistent with this hypothesis is the case report of a patient with PRCA who repeatedly developed wheals at former SC administration sites following IV administration of Eprex[®] (Weber et al., 2002).

To control the increasing number of new cases of epoetin-associated PRCA and to share emerging safety findings, manufacturers, academic researchers, and national regulatory authorities are focused on disseminating safety warnings and evaluating etiologic hypotheses (McKoy et al., 2008). Nevertheless, international collaborations in 2004 and 2005 facilitated collaborative reporting of consensus diagnostic criteria, treatment recommendations, and long-term follow-up assessments (Casadevall et al., 2002; Hynes et al., 2002). In 2003 and 2004, regulatory authorities in Canada, Singapore, and Australia finally mandated administration of Eprex[®] IV to hemodialysis patients or switching to SC darbepoetin for non-dialyzed CKD patients. In 2004, faced with continuing reports of Eprex[®]-associated PRCA cases among hemodialysis patients, Health Canada advised that HSA-free Eprex[®] be administered IV and that if HSA-containing multiuse Eprex[®] vials were used, IV administration was preferred (Important Drug Safety Update EPREX, 2002). In 2005, PRCA incidence rate for patients who received Eprex[®] from syringes with polysorbate 80 and uncoated rubber stoppers was 3.4 per 10,000 patient-years versus 0.2 per 10,000 patient-years for products with polysorbate 80 and coated stoppers (Boven et al., 2005a, 2005b). Of note, in 2005 and 2006, Australia and Canada, respectively, became the first countries to reauthorize SC administration of the HSA-free formulation of Eprex, after the addition of a Teflon coating to the rubber stopper (Australian Drug Evaluation Committee recommendations, 2005). After changes in the route of administration, formulation,

and packaging of the Eprex® formulation without HSA, antibody-mediated PRCA is now a rare class-related toxicity that occurs after extended periods of SC administration of EPOs (Epogen, Procrit, darbepoetin, Eprex, or epoetin- β) to CKD patients (Richard et al., 2003). Rare instances of PRCA with long-term use of Epogen, Procrit, or darbepoetin were also identified in the United States following the FDA's definitional revision of PRCA, as appeared in "Dear Doctor" letters (Stravitz et al., 2005; PRCA updates 2007). In 2005, a collaboration of investigators with the Canadian PRCA Focus Group, the European PRCA Working Group, and the RADAR project described long-term outcomes for 170 hemodialysis patients with epoetin-associated PRCA (Bennett et al., 2005). Of 19 PRCA patients who received a renal transplant and subsequent administration of cyclosporine or tacrolimus, transfusion independence was obtained by all patients except one (95%). Among 89 non-transplantation PRCA patients who received immunosuppressive therapies, 49% achieved hematologic recovery, with higher recovery rates being associated with cyclosporine.

Three non-innovator products for epoetin- α are now manufactured in Korea (Eporon®, Dong-A Pharmaceutical Company Ltd; Espogen®, LG Life Sciences; Epokine®, CJ Corporation). However, all three brands have been shown to differ from the reference epoetin- α product manufactured by Amgen (Epogen®), with variations in the activity, concentration, and isoforms of the products (Park et al., 2006). For example, isoelectric focusing demonstrated the presence of additional isoforms in all three products from Korea compared with Epogen®, despite claims of their substitutability and bioequivalence. An *in vitro* bioassay showed that both Eporon® and Espogen® had a higher bioactivity than was listed on their respective labels. In addition, ELISA indicated that both of these products had higher concentrations of epoetin- α than stated on the product labels.

In another study, differences in structure and stability were also found between IV formulations of epoetin- α produced by two different manufacturers. A biophysical comparison of Epogen® (Amgen) with Eprex® (manufactured by Ortho Biologicals LLC and distributed by Johnson & Johnson) showed that the two products were not structurally identical. Small differences were found in the hydrodynamic structure, the degree of α -helicity and the stability of these products (Deechongkit et al., 2006). Combe, Tredree, and Schellekens (2005) evaluated literature reports of studies conducted with non-innovator epoetin products marketed outside the United States and the European Union. The authors found that analytical studies failed to demonstrate comparability of non-innovator epoetins to the reference product. Furthermore, an *in vivo* bioassay in mice demonstrated that bioactivity was

higher than specifications in four samples (137–226%) and below specifications in two samples (71–75%) (Schellekens, 2004; Combe et al., 2005).

It is also important to be aware of the different immunogenic profiles that regulatory approved biosimilars have versus their "similar biological medicinal products" (SBMPs). For example, a biosimilar epoetin- ζ product [SB309 (Retacrit®)] manufactured by Hospira showed a marked difference in immunogenicity in dogs compared with epoetin- α (its SBMP), although the antibodies were non-neutralizing and were not associated with any deterioration in the case of the animals (EMA Internet Access). While there is no hint that SB309 will be more immunogenic than epoetin- α (at least with respect to clinically relevant neutralizing antibodies), only longer term clinical data can fully address the relevance of this difference in immunogenic profile in animals.

A recent case report has documented an instance of PRCA in a hemodialysis patient with CKD ~6 months after treatment with the follow-on epoetin- α Wepox® (Wockhardt Ltd, Mumbai, India) (Keithi-Reddy, Kandasamy, & Singh, 2008). Though the precise mechanism for the development of PRCA in this patient has not been elucidated, the increase in immunogenicity could have been due to problems in the manufacturing and storage of Wepox®.

Five biosimilar rhEPOs that are manufactured by two companies have been approved by the EMA. Abseamed®, Binocrit®, and Epoetin- α HEXAL® are epoetin- α products and they are biosimilar versions of the reference product Eprex®, all produced by Rentschler Biotechnologie GmbH but marketed by three different companies. By using immunoaffinity chromatography (HEXAL®—EMA, 2007; Binocrit®—EMA, 2007; Abseamed®—EMA, 2007), comparability exercises between these biosimilars and their reference product Eprex® were performed. There, it was demonstrated that although the active substance of the biosimilars was representative of the active substance isolated from Eprex®, there was a difference in glycosylation levels. The biosimilars contained higher levels of high-mannose-type structures, but this difference was not thought to be clinically significant (Schellekens, 2008b).

Two additional biosimilar versions of Eprex®, Retacrit®, and Silapo® are manufactured by Norbitech GmbH (EMA-Silapo: European Medicines Agency, 2007; EMA-Retacrit: European Medicines Agency, 2008). Although this biosimilar manufacturer also used Eprex® as a reference product, the international non-proprietary name (INN) for these products is epoetin- ζ rather than epoetin- α . Because, differences were noted for the glycosylation profile with respect to glycoforms without an O-glycan chain and variants of sialic acid, and a different immunogenicity profile was observed in

dogs (EMA-Silapo: European Medicines Agency, 2007; EMA-Retacrit: European Medicines Agency, 2008). Like biosimilar epoetin- α , epoetin- ζ was approved for indications in renal anemia, chemotherapy-induced anemia, and for pre-donation of blood before surgery for autologous transfusion (Silapo[®] SmPC, STADA Arzneimittel, Retacrit[®] SmPC, HOSPIRA Enterprises). Because of its unique INN, epoetin- ζ is more readily distinguished from other epoetin products. Unique INNs for biopharmaceuticals may help to facilitate accurate prescribing and dispensing of biopharmaceuticals, as well as pharmacovigilance (Mellstedt, Niederwieser, & Ludwig, 2008).

Immunogenicity of biopharmaceuticals in gene-defective hosts

The case of hemophilia A and B

From the immunological point of view, X-linked disorders represent a special situation because the mutated or deleted gene segments cause an altered immune repertoire, and replacement with the wild-type protein is expected to be immunogenic (Figure 1). The clinical effects of immunogenicity will vary, depending on the extent of gene mutation: the absence of the gene, as well as large gene deletions or a premature stop-codon, will have more severe consequences than point mutations.

Hemophilia A is an X-linked genetic disease, and thus males are mainly affected, whereas their mothers

are carriers of the defective gene. It is a bleeding disorder caused by a deficiency of blood coagulation fVIII. The disease results from mutations or deletions that alter the expression and secretion of fVIII (Hoyer, 1993, 1995). The malfunction of fVIII causes a reduced coagulation power, with prolonged post-traumatic bleeding times and spontaneous hemorrhages. Replacement therapy with fVIII has been successfully used, and initial preparations of fVIII were natural preparations derived from donated blood. Today, a large number of recombinant DNA derived fVIII preparations are available (Kromminga & Schellekens, 2005). As expected, substitution therapy results in antibody formation, as the patient's immune system recognizes parts of the fVIII as a foreign antigen. The immune response is a classical one, with a switch from immunoglobulin-M (IgM) to IgG and affinity maturation (Opdenakker et al., 2003; Oldenburg et al., 2004).

The reported incidence of antibodies to plasma-derived fVIII is about 20–25% (Ehrenforth et al., 1992; Lusher et al., 1993; Bray et al., 1994). For hemophilia A, low titer and transient antibodies were seen in about one-third of patients who develop antibodies to fVIII, and their blocking effect can be overcome by increasing the dosage of fVIII (Lusher, 2000). In contrast, in those patients who develop antibodies at high titer, bleeding is a significant problem which requires alternative treatments, such as the use of recombinant factor VIIa or activated prothrombin complex concentrate (APCC).

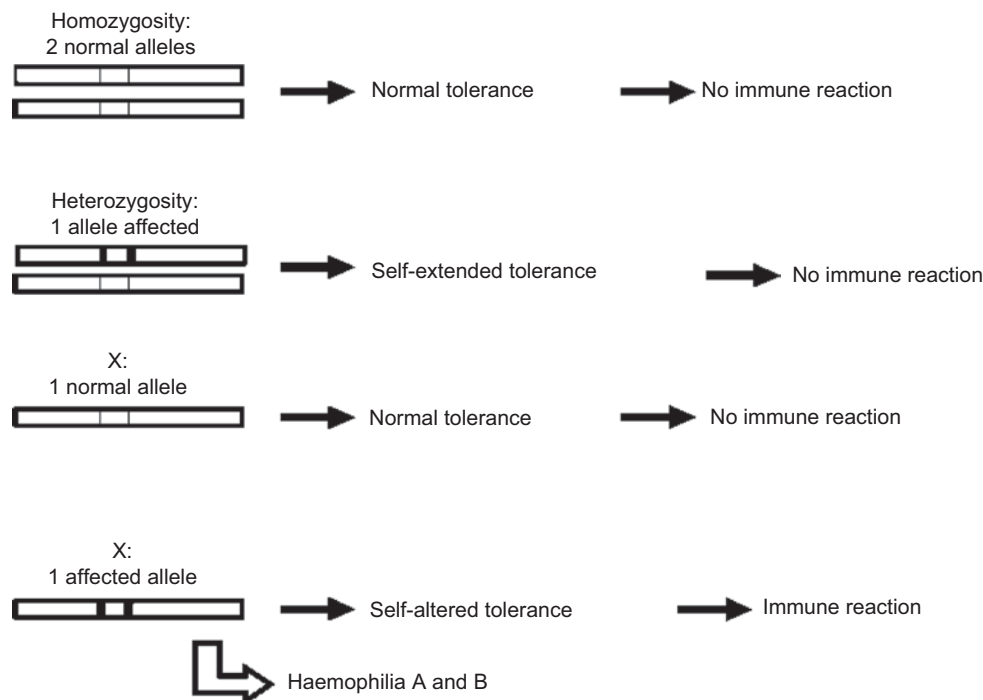


Figure 1. Relationship between genome and immune responses.

Recent studies on antibodies to fVIII show that neutralization may be due to a proteolytic activity (Lacroix-Desmazes et al., 2002), and a correlation, *in vitro*, between the neutralizing activity of IgG in plasma and the hydrolysis rate of fVIII by IgG was observed. Factor VIII can be completely absent in patients with severe hemophilia A. These patients do not benefit from the administration of fVIII because fVIII represents a foreign antigen and patients develop an immune response against it. The same occurs in those patients who have fVIII gene with large deletions or nonsense mutations that cause premature termination, whereas antibody development rarely occurs in patients with only frameshift or missense mutations (Kromminga & Schellekens, 2005).

Hemophilia B is another X-linked bleeding disorder caused by a deficiency of blood coagulation factor IX (fIX). The prevalence of this disease is much lower than that of hemophilia A, and the incidence of antibodies against fIX is about 1–3% (Lusher, 2000). In contrast with fVIII, the immune response to fIX leads to severe allergic reactions and anaphylaxis in about 50% of patients (Lusher, 2000). For patients with anti-fIX antibodies, other treatments are required, such as factor VIIa and APCC (Schellekens & Casadevall, 2004).

The experience on hemophilic patients teaches that with the extent of the gene defect and the severity of the phenotype, the probability of developing neutralizing antibodies increases. Thus, for patients with the most severe disease, substitution therapy or gene therapy will have the highest risk of failure because of the production of neutralizing antibodies, and therapy has to be accompanied by strategies of immunological tolerance induction or immunosuppression (Opdenakker et al., 2003; Oldenburg et al., 2004).

Conclusions

Although identical to the endogenous proteins, biopharmaceuticals and biosimilars have the potential to cause antibody formation following parenteral administration into the human body. The formed ADAs can have a neutralizing activity which inhibits the therapeutic efficacy of the drug or lead to hypersensitivity reactions. Several options aiming to decrease potential ADA reactions of biopharmaceuticals include careful formulation (to avoid contaminants and aggregates), reduction of immunogenicity by glycosylation and PEGylation, in some cases, linkage of the therapeutic protein to a human Ig Fc fragment and reduction of T-cell epitopes by sequence modification (De Groot & Scott, 2007). An interesting approach to reduce immunogenicity has been described by Tang et al. (2005), in which the authors tried to reduce EPO immunogenicity

by engineering modified forms of the protein with substitutions in the regions containing the epitopes binding to human leukocyte antigen (HLA) class II molecules. Other strategies include the use of combination therapy, adding steroids to temporarily suppress the immune system. When antibodies are already present, they may sometimes disappear spontaneously when the treatment is continued, but this process may take years. Whatever the “final” solution might be, it is important to remember that the goal of this research is to accelerate the development of new compounds or novel formulations that are safe for use in human subjects. The bright new future of therapeutic proteins will arrive when, and if, developers remember to consider immunogenicity.

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