6 Immunogenicity of Therapeutic Proteins

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INTRODUCTION

The era of the medical application of proteins started at the end of the nineteenth century when animal sera were introduced for the treatment of serious complications of infections such as diphtheria and tetanus. The high doses used, the general lack of quality controls and a regulatory system, and the impurity of the preparations led to many serious and sometimes even fatal side effects. Many of the problems were caused by the strong immune response these foreign proteins induced, especially when readministered. People who had been treated in general had a warning in their passports or identification cards to alert physicians for a possible anaphylactic reaction after rechallenge with an antiserum. Also serum sickness caused by deposits of antigen-antibody complexes was a common complication of the serum therapy.

Also the porcine and bovine insulins introduced after 1922 induced antibodies in many patients. This was again explained by the animal origin of the products, although over the years the immunogenicity became less because of improvements in the production methods and increasing purity.

In the second half of the twentieth century, a number of human proteins from natural sources were introduced such as plasma-derived clotting factors and growth hormone produced from pituary glands of cadavers. These products were given mainly to children with an innate deficiency who therefore lacked the natural immune tolerance. Therefore, their immune response was also interpreted as a response to foreign proteins. The correlation between the factor VIII gene defect and level of deficiency with the immune response in hemophilia patients confirmed this explanation.

Thus, until the advent of recombinant DNA technology, the immunological response to therapeutic proteins could be explained as a classical immune response comparable to that of a vaccine.

THE NEW PARADIGM

In 1982 human insulin was marketed as the first recombinant DNA-derived protein for human use. Since then dozens of recombinant proteins have been introduced and some of these products such as the interferons and the epoetins are among the most widely used drugs in the world. And, although these proteins were developed as close copies of human endogenous proteins, nearly all these proteins induce antibodies, sometimes even in a majority of patients (Table 6.1). In addition, most of these products are used in patients who do not have an innate deficiency and can be assumed to have immune tolerance to the protein.

The initial assumption was that the production by recombinant technology in nonhuman host cells and the downstream processing modified the proteins and the immunological response was the classical response to a foreign protein. However, according to the current opinion, the antibody response to human homologues is based on circumventing B-cell tolerance. This phenomenon is not yet completely understood but is clearly different from the vaccine type of reaction seen with foreign proteins (Sauerborn et al. 2010).

The clinical manifestations of both types of reaction are very different. The vaccine-type response occurs within weeks and sometimes a single injection is sufficient to induce a substantial antibody response. In general high levels of neutralizing antibodies are

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Loss of efficacy	Enhancement of efficacy
Insulin	Growth hormone
Factor VIII	
Interferon alpha 2	Neutralization of endogenous protein
Interferon beta	Megakaryocyte-derived growth factor (MDGF)
Interleukin-2	Epoetin
HCG	
Monoclonal antibodies	General Immune effects
	Allergy
	Anaphylaxis
	Serum sicknes, etc.

Table 6.1 ■ Non-exhaustive list of recombinant proteins showing immune reactions upon administration.

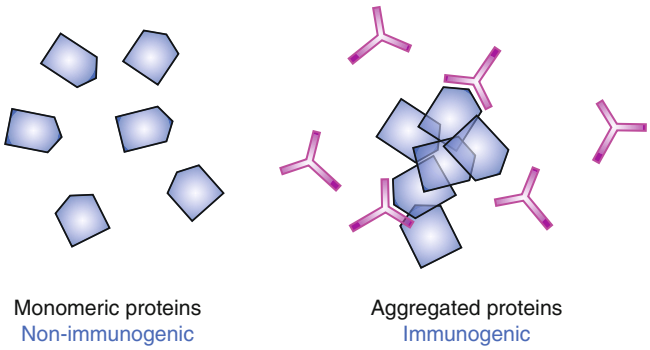

Figure 6.1 ■ Dogma: protein aggregates are immunogenic.

induced and a rechallenge leads to a booster reaction, indicating a memory response.

However, circumventing B-cell tolerance takes in general 6–12 months of chronic treatment and often only leads to the production of binding antibodies with no biological effect. The antibodies often disappear shortly after treatment has been stopped and sometimes even during treatment. This response also appears to have no memory, because rechallenging patients in whom the antibody levels have declined does not induce a response. The lack of a memory response has also been shown in the relevant immune-tolerant transgenic mouse models.

THE IMMUNOLOGICAL RESPONSE

The therapeutic proteins currently available cover the whole spectrum, from completely foreign like bacterial-derived asparaginase to completely human-like interferon-α-2b and everything in between. The foreign protein elicits antibodies by the classical pathway which includes ingestion and cleaving of the proteins into peptides by macrophages and dendritic cells, presentation of peptides by the MHC-II system and activation of B cells and boosting, and affinity maturation and isotype switching of the B cells by helper T cells (Stevanovic 2005). Furthermore, memory B cells are induced (see Chap. 22 for details).

It is much less clear how B-cell tolerance is circumvented. There are always autoreactive B cells present. When the receptor on these B cells meets its epitope on the protein in solution, this interaction does not lead to activation. When these B cells meet their epitope in a regularly repeated form, then the B-cell receptor oligomerizes and the cell is activated and starts to divide and produce antibodies. So B cells can recognize three-dimensional repeated protein structures as has been shown experimentally in a number of studies. The explanation why is based on evolution. The only naturally occurring, narrowly spaced repeated protein structures are found on the surface of viruses and some bacterial structures (Bachmann et al. 1993). Apparently, the B-cell system was also selected for its potential to respond to microbial structures independent of the system which discriminates self from nonself.

This explanation of nonself-independent response by repeated protein structures fits nicely with aggregates being recognized as the main driver of an autoreactive response by human therapeutic proteins, because in protein aggregates, certain structures are also presented in a repeated form (Fig. 6.1) (Moore and Leppert 1980).

Thus, the initial activation of B cells by aggregates can be explained and also how these cells start with producing IgM. It is not known how the isotype switching from IgM to IgG occurs. Some studies suggest that aggregates after reacting with the B-cell receptor are internalized. And by internalizing, the B cells become helper cells and start to produce cytokines which will activate other B cells. Others claim that helper T cells are involved. However, studies to show the presence of specific T-cell activity in patients producing antibodies to human therapeutic proteins have failed. In addition, a T-cell-independent mechanism is suggested by the lack of any association with HLA type in some studies and the absence of memory. However, in studies in immune-tolerant transgenic mice, depletion of T cell inhibited the response to aggregated proteins capable of circumventing B-cell tolerance.

FACTORS INFLUENCING ANTIBODY FORMATION TO THERAPEUTIC PROTEINS

Figure 6.2 depicts the different factors that influence immunogenicity (Schellekens 2002; Hermeling et al. 2004).

■ Structural Factors

The degree of nonself and presence of aggregates are the initial triggers of an antibody response to a therapeutic protein. The degree of nonself necessary to induce a vaccine-type response is highly dependent on the protein involved and the site of the divergence from the natural sequence of the endogenous protein. For insulin there are single mutations which lead to a new epitope and an antibody response, while other mutations have no influence at all. Consensus interferon-α in which more than 10 % of the amino acids diverge from the nearest naturally occurring interferon-α subtype shows not more immunogenicity than the interferon-α-2 homologue.

Glycosylation is another important structural factor for the immunogenicity of therapeutic proteins. There is little evidence that modified glycosylation, e.g., by expressing human glycoproteins in plant cells or other nonhuman eukaryotic hosts, may induce an immunogenic response. However, the level of glycosylation has a clear effect. Interferon-β produced in *E. coli* (non-glycosylated) is much more immunogenic than the same product produced in mammalian cells. The explanation is the lower solubility causing aggregation in the non-glycosylated E. coli product.

Antigenicity does not equal immunogenicity (Fig. 6.3). A protein, peptide, or glycan structure with a high affinity for antibodies may not be able to induce antibodies at all. Glycosylation is reported to influence antigenicity by antibody binding. Non-glycosylated epoetin was reported to have a higher affinity to antibodies than epoetin with a normal level of glycosylation; the opposite is true for epoetin engineered to have extra glycosylation. In most subjects who had a hypersensitivity reaction to cetuximab, IgE antibodies against cetuximab were present in serum before therapy (Arnold and Misbah 2008). These antibodies were specific for galactose-α-1,3-galactose and were apparently induced by a microbial organism.

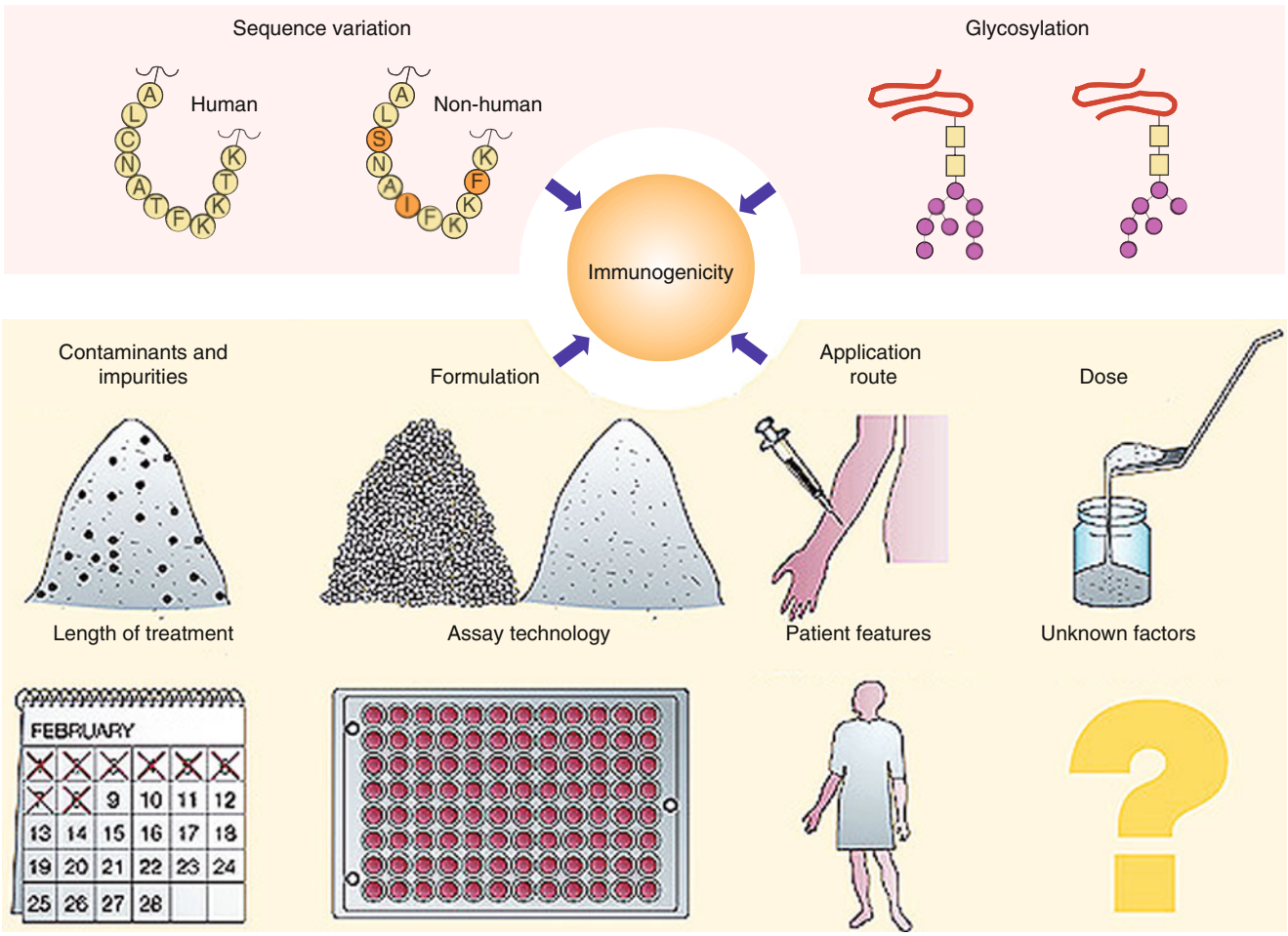

Figure 6.2 ■ Factors relevant in immunogenicity.

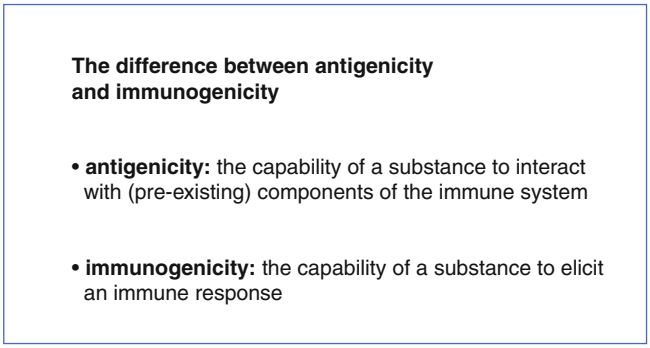

Figure 6.3 ■ Immunogenicity versus antigenicity.

■ Impurities

Impurities are considered to be important factors in the immunogenicity of therapeutic protein products. Substances like host cell components, resins from chromatographic columns, or enzymes used to activate the product and monoclonal antibodies used for affinity purification may end up in the final product. Impurities may also be introduced by the components of the formulation, may leak from the container and sealing of the product, or may be introduced during the fill and finish steps. These impurities may boost an immune response different than an immune response to the therapeutic protein. These products may be immunogenic by themselves. Antibodies induced by impurities may lead to general immune reactions as skin reactions, allergies, anaphylaxis, and serum sickness. Antibodies to impurities also raise quality issues concerning the product and therefore need to be monitored. Interestingly, there are a number of examples of products declining in immunogenicity over the years because of improvements in the purification and other downstream processing steps.

Impurities may enhance immunogenicity via different mechanisms. Endotoxin from the bacterial host cells has been reported to cause the immunogenicity of the first recombinant DNA-derived human growth hormones. G-C-rich bacterial host cell DNA and denatured proteins are capable of activating Toll-like receptors and can also act as adjuvants. The activity of these impurities, however, is restricted to the nonhuman proteins which have a vaccine-like activity.

Adjuvants are incapable of boosting the immune response based on circumventing B-cell tolerance. However, impurities that are modified human proteins such as clipped and oxidized variants which can be present in therapeutic protein products could indirectly induce antibodies which react with the unmodified protein. This immunological mimicry has been described in dogs treated with human epoetin. The degree of nonself of this protein for dogs is sufficient to induce an immune response. But there is still enough homology between the human and canine erythropoietin for the antibodies to neutralize the endogenous, canine erythropoietin and causing severe anemia.

■ Formulation

Human therapeutic proteins are often highly biologically active and the doses may be at the μg level, making it a technological challenge to formulate the product to keep it stable with a reasonable shelf-life and to avoid the formation of aggregates and other product modifications. The importance of formulation in avoiding immunogenicity is highlighted in two historical cases (Fig. 6.4). In the case of interferon-α-2a, a large difference was noted among different formulations. A freeze-dried formulation containing human serum albumin as a stabilizer that according to its instructions could be kept at room temperature was particularly immunogenic. It appeared that at room temperature interferon-α-2a became partly oxidized. And the oxidized molecules formed aggregates also with the unmodified interferon and human serum albumin, and these aggregates were responsible for the immune response.

More recently an antibody-mediated severe form of anemia (pure red cell aplasia; PRCA) occurred after the formulation of an epoetin-α product was changed (Casadevall et al. 2002). Human serum albumin was replaced by polysorbate 80. How this formulation change induced immunogenicity is still not certain. However, the most likely explanation is a less stable new formulation resulting in aggregate formation when not appropriately handled.

■ Route of Administration

There is a clear effect of route of administration on the immunogenicity. The subcutaneous route is the most immunogenic and the intravenous the least immunogenic one. However, immunogenicity can be seen after any route of application including the mucosal and the intrapulmonary route.

■ Dose

The effect of the dose is not quite clear. There are studies with the lowest incidence of antibody formation in the highest-dose group. However, such data should be interpreted with caution. In the highest-dose group, there may be more products in the circulation interfering with the assay or the antibody level may be lower by increased immune complex formation.

■ Patient Features

There are also a number of patient-related factors which influence the incidence of antibody formation. The biological effect of the product can either enhance or inhibit the antibody formation as can the concomitant

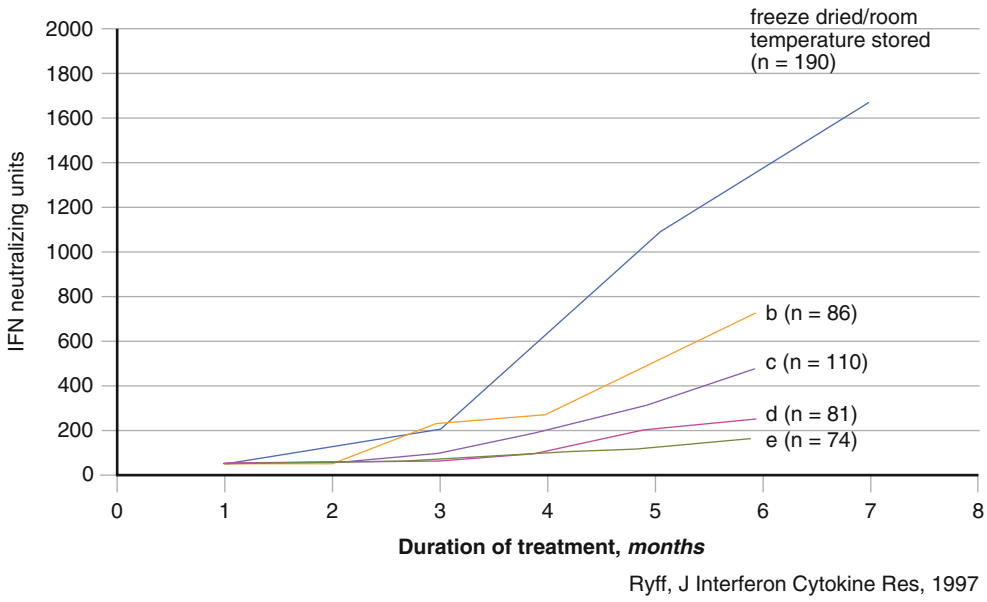

Figure 6.4 ■ Immunogenicity differences between interferon (IFN)-α formulations in patients (Ryff 1997).

treatment of patients. Sometimes the concomitant treatment is administered to inhibit antibody formation, e.g., methotrexate treatment to inhibit antibody formation to TNF inhibitors.

The underlying disease for which the patients are being treated is also important. Patients treated with interferon-α-2 for chronic hepatitis are more likely to produce antibodies than patients with solid tumors. The upsurge of epoetin-α-induced PRCA after the formulation change was only seen in patients with chronic kidney disease and not in patients with cancer.

As discussed before there are many indications that circumventing B-cell tolerance is independent of HLA type. Indeed a number of clinical studies have failed to show an association between antibody response and HLA type.

■ Assays for Antibodies

Assays are probably an important factor influencing the reported incidence of antibody induction by therapeutic proteins. In the published studies with interferon-α-2 in patients with viral infections, the incidence of antibody induction varied from 0 % to more than 60 % positive patients. This variation must be assay related. Evaluations of the performance of different test laboratories with blind panel testing showed a more than 50-fold difference in titers found in the same sera. Thus, any reliable comparison between different groups of patients when looking for a clinical effect of antibodies or studying factors influencing immunogenicity can only be done if the antibody quantification is done with in a well-validated assay in the same laboratory.

There is obviously a lack of standardization of assay methodology. There are also only a few reference and/or standard antibody preparations available. A number of white papers have appeared mainly authored by representatives of the biotechnology industry in the USA (Mire-Sluis et al. 2004). Although the area of biotechnology-derived therapeutics is still too much in development to formulate a definite assay methodology, there is a growing consensus on the general principles.

There is an agreement that a single assay is not sufficient to evaluate the immunogenicity of a new protein drug, but a number of assays need to be used in conjunction. Most antibody assay strategies are based on a two-tier approach: a screening assay to identify the antibody-positive sera followed by further characterization such as whether the antibodies are binding only or neutralizing and what is the titer, affinity, and isotype.

In general, the screening assay is a binding assay, mostly an ELISA type of assay (see Chap. 2) with the radio-immune-precipitation methodology as an alternative. Binding antibodies have mostly no biological consequences. However, assays for the more biologically important neutralizing antibodies are in general cumbersome and expensive. Thus, screening with a binding assay to select the positive sera for the neutralizing assay saves time and money.

Screening assays are designed for optimal sensitivity to avoid false negatives. For new proteins, defining an absolute sensitivity is impossible because of the lack of positive sera. An alternative approach is to set the cut-point for the assay at a 5 % false-positive level using a panel of normal human sera and/or untreated patient sera representative of the groups to be treated.

The assay for neutralizing antibodies is in general a modification of the potency assay for the therapeutic protein product. The potency assay is in most cases an

in vitro cell-based assay. A predefined amount of product is added to the serum and a reduction of activity evaluated in the bioassay.

An important caveat in interpreting the neutralization assay results is the possible presence of inhibitors of the products other than antibodies (e.g., soluble receptors) in human serum or factors stimulating the bioassay which may compensate for the neutralizing activity. To overcome these problems, patient serum should be tested as control. IgG-depleted serum should also be tested for neutralizing activity to identify neutralizing factors other than antibodies. Further characterization of the antibodies may include evaluation of Ig isotype and affinity.

ISSUES SPECIFICALLY RELATED TO MONOCLONAL ANTIBODIES

The thinking about the immunogenicity of monoclonal antibodies went through the same paradigm shift as occurred with therapeutic proteins in general. The first generation of monoclonal antibodies was of murine origin. They induced an immune response in the majority of patients as foreign proteins should trigger a classical vaccine-type immune response. This so-called HAMA response (human antibodies to murine antibodies) was a major restriction in the clinical success of these murine antibodies. Over the years, however, methods were introduced in different stages to humanize monoclonal antibodies (cf. Chap. 1). Recombinant DNA technology was used to exchange the murine constant parts of the immune globulin chains with their human counterparts resulting in chimeric monoclonal antibodies. The next step was to graft murine complementarity-determining regions (CDR"s), which determine the specificity, into a human immune globulin backbone creating humanized monoclonal antibodies. And the final step was the development of transgenic animals, phage display technologies, and other developments allowing the production of human monoclonal antibodies. The assumption that human monoclonal antibodies would have no immunogenicity proved to be wrong. Although humanization has reduced the immunogenicity, even completely human monoclonal antibodies have been shown to induce antibodies. The introduction of chimeric antibodies by the exchange of the murine constant regions with their human counterparts has resulted in a substantial reduction of the induction of antibodies. Whether further humanization has resulted in an additional decrease is less clear. As discussed, the presence of aggregates has been identified as a major cause of immunogenicity of human therapeutic proteins. It is likely that with human monoclonal antibodies, aggregates are also responsible for antibody induction. In fact in the classical studies of B-cell tolerance done more than 40 years ago, aggregated immunoglobulin preparations were used to break tolerance (Weigle 1971).

Monoclonal antibodies have properties which may contribute to their immunogenicity. They can activate T cells by themselves and may boost the immune response by their Fc functions such as macrophage activation and complement activation. Indeed removal of N-linked glycosyl chains from the Fc part of the immunoglobulin may reduce Fc function and lead to a diminished immunogenicity.

What the antibodies are binding is also influencing their immunogenicity. Monoclonal antibodies targeting cell-bound antigens induce a higher level of antibody formation than those with circulating targets. Monoclonal antibodies directed to antigens on immune cells with the purpose of inducing immune suppression also suppress an immunogenic response.

Although more injections and higher doses are associated with a higher immune response, in some cases chronic treatment and higher doses were reported to be less immunogenic than episodic treatment and lower doses. The interpretation of these data is difficult because under these treatment conditions, the level of circulating product is higher and more persistent and the presence of circulating monoclonal antibodies during the time of blood sampling may mask the detection of induced antibodies. Only a few studies were performed in which the subcutaneous and intravenous route of administration of monoclonal antibodies were compared showing little difference in immunogenicity.

The immune status of the patients influences the antibody response as with other protein therapeutics. Many of the patients receiving monoclonal antibodies are immune compromised by diseases such as cancer or by immune suppressive treatment and are less likely to produce antibodies than patients with a normal immune status. Sometimes immune suppressive agents such as methotrexate are given to patients with the purpose of inhibiting an antibody response.

Another important aspect when studying the immunogenicity of monoclonal antibodies is timing of the blood sampling of patients. These products may have a relative long half-life (several weeks) and the circulating product may interfere with the detection of induced antibodies and may lead to false-negative results. Sampling sera up to 20 weeks after the patient has received the last injection may be necessary to avoid the interference of circulating monoclonal antibodies. Also natural antibodies, soluble receptors, and immune complexes may interfere with assays and lead to either false-positive or false-negative results (as explained above).

CLINICAL EFFECTS OF INDUCED ANTIBODIES

Despite the methodological drawbacks, the list of protein products with clinically relevant immunogenic side effects is growing. The most common consequence is loss of efficacy. Sometimes this loss can be overcome by increasing the dose or changing to another product.

The most dramatic and undisputed complication occurs when the antibodies to the product cross neutralize an endogenous factor with an important biological function. This has been described for a megakaryocyte growth and differentiation factor which induced antibodies cross-reacting with endogenous thrombopoietin (see Table 6.1). Volunteers and patients in a clinical trial developed severe thrombocytopenia and needed platelet transfusions. Because of this complication, the product was withdrawn from further development.

More recently the upsurge of PRCA (see above) associated with a formulation change of epoetin-α marketed outside the USA occurred. The antibodies induced by the product neutralized the residual endogenous erythropoietin in these patients resulting in a severe anemia which could only be treated with blood transfusions.

Antibodies can also influence the side effects of therapeutic proteins. The consequences are dependent on the cause of the side effects. If the adverse effects are the results of the intrinsic activity of the products, antibodies may reduce the side effects, as it is the case with interferon-α-2. Sometimes the mitigation of the side effects is even the first clinical sign of the induction of antibodies.

With some products the side effects are caused by the antibody formation. This is in general the case when the product is administered in relatively high doses, like with some monoclonal antibodies. Symptoms caused by immune complexes like delayed-type hypersensitivity and serum sickness are related to the level of antibodies induced.

The general effects caused by an immune reaction to a therapeutic protein such as acute anaphylaxis, hypersensitivity, skin reaction, and serum sickness are relatively common when large amounts of nonhuman proteins are administered. These effects are relatively rare for modern biotechnology-derived products which are highly purified human proteins administered in relatively low amounts. However, these side effects caused by an immune response are currently still relatively common during treatment with high doses of monoclonal antibodies.

PREDICTING AND REDUCING IMMUNOGENICITY

As discussed the mechanisms leading to antibody induction by therapeutic proteins are still not completely understood. As a consequence it is impossible based on our current knowledge to fully predict the immunogenicity of a new product in patients. For nonhuman proteins which induce the classical immune response, the level of nonself is a relative predictor of an immune response. However, it is not an absolute predictor. Sometimes a single amino acid change is sufficient to make a self-protein highly immunogenic. With other proteins, substantial divergence from the natural sequence has no effect. For foreign proteins a number of in vitro stimulation and binding tests and computational models are advertised as predictors of immunogenicity. However, all these tests have their limitations. T-cell proliferation assays, for example, have the drawback that many antibodies are capable of inducing some level of T-cell activation or inhibit cell proliferation. The computational algorithms which predict binding of antigens to HLA class II only give limited information on the interaction of the proteins with the immune system and also under-detect epitopes (Stevanovic 2002). These limitations are also evident when these assays or algorithms are used to reduce immunogenicity: there is hardly any convincing evidence of a clinically relevant reduction of antibody induction.

For human homologues the best predictor of immunogenicity is the presence of aggregates and to a minor degree the presence of impurities. Thus, the quality of the therapeutic protein and its formulation are important factors. There is also evidence about immunogenicity introduced by a change in formulation and a reduction in immunogenicity by avoiding aggregation and improving purification and formulation (see above).

Although animal studies are helpful in obtaining control sera and may provide insight in the possible clinical effects of immunogenicity, they are not very good predictors of immunogenicity in patients. All proteins, including the human homologues, will be in principle immunogenic in animals. Sometimes animal studies may help to study the relative immunogenicity of different products or formulations, although their predictive value for the clinic is questionable. Even monkey studies do not completely predict immunogenicity in patients. Some products which are immunogenic in nonhuman primates do not induce antibodies in humans and vice versa.

The animal model to study the factors important for circumventing tolerance is transgenic mice carrying the gene for the human protein (Hermeling et al. 2005). These animals have an immune tolerance comparable to the immune tolerance in patients. These animals have also been successfully used to identify new epitopes in modified products. Although transgenic animals have been proven to be important scientific tools to identify the factors important to circumvent

tolerance and study the immunological processes involved, also this animal model will never be able to serve to predict the human response.

■ Reducing Immunogenicity

Several strategies are being applied to reduce immunogenicity besides changing the amino acid sequence of the product. Linking proteins to polymers such as polyethylene glycol (see Chap. 21) and low molecular weight dextran reduces immunogenicity. However, these modifications also make the molecules less active, necessitating higher doses. This and their increased half-life extend their exposure to the immune system, which may increase the immunogenic potential. Another approach is to reduce the immunogenic response by immunosuppressive treatments. In addition, tolerance induction is being applied, e.g., in hemophilia patients with antibodies to factor VIII.

CONCLUSIONS

The most important points of this chapter are summarized in the following bullet points:

- The immunogenicity of therapeutic proteins is a commonly occurring phenomenon.
- The clinical consequences can vary.
- Validated detection systems are essential to study the immunogenicity of therapeutic proteins.
- The prediction of immunogenicity in patients based on physicochemical characterization and animal studies is not easy.
- There is still a lot to be learned about why and how patients produce antibodies to therapeutic proteins.

The growing awareness of the importance of immunogenicity of therapeutic proteins is illustrated by the adoption of a standard requirement in regulatory dossiers for new proteins and biosimilars to evaluate their immunogenicity in clinical trials (cf. Chap. 11)

SELF-ASSESSMENT QUESTIONS

■ Questions

1. Which factors contribute to unwanted immunogenicity of therapeutic proteins?
2. What are possible clinical consequences of antibody formation against biopharmaceuticals in patients?
3. Why do aggregates of recombinant human proteins induce antibodies that cross-react with the (non-aggregated) drug?
4. Explain the fundamental difference between (a) antibody formation in children with growth hormone deficiency treated against recombinant human growth hormone and (b) antibody formation against rh-erythropoietin in patients with chronic renal failure.
5. Give an example of a case that demonstrates that the formulation of a biopharmaceutical can affect the immune response.
6. Give at least 3 approaches that can be followed to reduce the immunogenicity of a biopharmaceutical.
7. Why is standardization of assays for detection of antidrug antibodies important?
8. Why are antidrug-antibody titers against a monoclonal antibody more difficult to determine accurately than antibodies against interferon?

■ Answers

1. See Fig. 6.2.
2. Reduction of therapeutic efficacy, (seldom) enhancement of efficacy, anaphylactic reactions, cross-reactivity with endogenous protein.
3. Aggregates can circumvent B-cell tolerance against native (–like) epitopes (repetitive epitopes); the more 'native-like" the aggregate, the more likely cross-reactivity with the monomer will occur.
4. (a) is the classical immune response versus (b) circumventing B-cell tolerance.
5. The examples given in the text re erythropoietin and interferon-α.
6. Design another formulation, remove aggregates, pegylate or change the glycosylation pattern of the protein or use amino acid mutants, use human(ized) versions of the proteins, or select another route of administration. NB some of these approaches will lead to a new bioactive drug molecule and that has implications for the way authorities will judge the procedure to be followed for obtaining marketing approval ((see Chap. 11) on Regulatory Issues).
7. Different assay formats and blood sampling schedules give different answers and thus hamper direct comparison between studies. Therefore, it is difficult to compare the results obtained with different products that are tested for immunogenicity in different labs.
8. Monoclonal antibodies are often administered in high doses and have a long circulation time (days/weeks). This will likely cause interference with the assay by the circulating drug (resulting in false negatives or underestimation of antibody titers). Another possibility for interference is the occurrence of cross-reactivity of the reagents in the test for the induced antibodies and the original drug-antibody. With interferon a different situation is encountered – interferons are rapidly cleared and administered in low doses (microgram range); therefore, interferons will less likely interfere with the measurement of anti-IFN antibodies.

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