2

Clinical Aspects of Immunogenicity to Biopharmaceuticals

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2.1. Introduction

The range of biopharmaceuticals available is steadily increasing. The first generation of products were copies of naturally occurring growth factors, or hormones or cytokines. With the development of new techniques, such as pegylation and glycosylation, second-generation biopharmaceuticals with increased bioavailability and higher therapeutic index are available.

Most biopharmaceuticals induce immune responses. The theoretical basis for immunogenicity to biopharmaceuticals is based either on their foreign nature, being of exogenous origin (neo-antigens or non-self-antigens), or on their similarity to self-molecules (self-antigens). In both cases, clinical manifestation of immunogenicity depends on the activation of antibody-secreting B cells. Besides this, many factors contribute to immunogenicity, such as product-related factors and host-related factors. These are discussed in more detail in Chapter 5.

Immunogenicity can cause a range of consequences, as summarized in Table 2.1. In many cases, antibodies against biopharmaceuticals have little or no consequences. In some cases, they can cause a loss of efficacy of the therapeutic proteins, but the most dangerous effect occurs when autoimmunity is directed against the endogenous molecule (Kromminga and Schellekens 2005). On the next pages, the different effects of antigenicity and immunogenicity will be discussed, and each type of effect will be illustrated by examples.

2.2. Clinical Aspects of Immunogenicity

2.2.1. No Apparent Effect of Antibody Formation: Growth Hormone

Since the 1980s, recombinant forms of human growth hormone (rhGH, *Escherichia coli* derived) have been used as therapy for deficiencies in GH production or response. Most studies have not shown any effect of anti-GH antibodies on growth rate. In a study by Albertsson-Wikland (1987), anti-GH antibodies were found in 1 out of 47 (2.1%) children treated for up to

Table 2.1 Consequences of immunogenicity.

Biopharmaceutical	Clinical use	Consequences of immunogenicity	References
rh-GH	GH deficiency	No evidence of any effect on clinical efficacy	Albertsson-Wikland 1987; Takano et al. 1989
rh-Insulin	Diabetes mellitus	Alteration of the drug pharmacokinetics	Walford et al. 1982; Ishibashi et al. 1986; Van Haeften 1989
GM-CSF	Some cancers	Reduction in drug efficacy	Ragnhammar et al. 1994; Rini et al. 2005
IFNα	Hepatitis C, some cancers	Reduction in drug efficacy	Figlin and Itri 1988; Lok, Lai and Leung 1990; Douglas et al. 1993; Bonetti et al. 1994
IFNβ	Multiple sclerosis	Reduction/loss in drug efficacy	See Table 2.6
Factors VIII and IX	Haemophilia A and B	Cross-reaction with endogenous protein	Lacroix-Desmazes et al. 2002; Lusher 2000; Schellekens and Casadevall 2004.
rh-MDGF		Cross-reaction with endogenous protein	Kuter 2000; Li et al. 2001
rh-EPO	Anaemia	Cross-reaction with endogenous protein	Casadevall 2002; Locatelli et al. 2004
Natalizumab	Multiple sclerosis	Adverse drug reactions and loss of clinical efficacy	AFFIRM study 2006; SENTINEL study 2006
GA	Multiple sclerosis	Unknown effect	Teitelbaum et al. 1996; Brenner et al. 2001; Salama et al. 2003; Farina et al. 2005

Note: rh= recombinant human; GH= growth hormone; GM-CSF= granulocyte-macrophage colony-stimulating factor; IFN= interferon; MDGF= megakaryocyte differentiation and growth factor; GA= glatiramer acetate

six months with recombinant somatotrophin, without observing any adverse effect on growth rate. In another study by Takano (1989), three different kinds of rhGH preparations were administered to 203 patients affected by Turner's syndrome and antibodies were detected in 71.4% and 10.8% of the methionyl-rhGH and methionine-free-rhGH treated patients, respectively; no inhibition of growth rate was observed in these patients.

However, some authors have described a reduction in clinical response in a few patients with high titres of anti-GH antibodies. Kaplan et al. (1986) evaluated the development of antibodies against GH in 36 children who had been treated with methionyl-rhGH intramuscularly for up to four years, finding that the incidence of antibodies was higher than in subjects treated with GH derived from bovine pituitaries. No allergic manifestation or systemic side effects were demonstrable in patients who developed antibodies, but a poor growth was observed in one patient who acquired high-titre, high-binding-capacity antibodies to hGH.

A similar finding has been described in a Japanese study by Okada et al. (1987), in which the authors reported a case of a 10-year-old child treated with methionyl-rhGH for an idiopathic growth hormone deficiency. The child had a decrease in growth rate by the ninth month of therapy, and anti-GH had been detected at two months of treatment, with maximum titre at nine months. A switch from methionyl-rhGH to pituitary-extracted hGH was able to produce a new increase in growth rate, and the cause of growth attenuation was attributed to the high titre of anti-GH antibodies.

It has been observed that antibody titres are inversely related to the purity of the methionyl-rhGH preparation (Takano, Shizume and Hibi 1989). Subsequent studies based on subcutaneous administration and on the use of *N*-methionine-free rhGH were associated with a lower incidence of antibodies.

More recent preparations of rhGH are less immunogenic (antibody incidence of 1.1%) after one year of treatment (Lundin et al. 1991). Massa and co-workers (1993) studied anti-GH antibodies induced by treatment with methionyl-rhGH, finding that antibodies developed in 3 out of 26 patients (12%) who were previously treated with pituitary-extracted hGH, and in 15 out of 20 patients (75%) who were previously untreated. The majority of antibodies positive patients, 15 out of 18, developed antibodies during the first year of treatment, whereas in 3 out of 18 patients, antibodies developed during the second year; they disappeared after the discontinuation of treatment and no effect on growth rate was observed.

It has been 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 (Schellekens and Casadevall 2004).

2.2.2. Change in Drug Pharmacokinetics: Insulin

Insulin replacement therapy is the mainstay pharmacological treatment for patients with type 1 and with advanced type 2 diabetes (DeWitt and Hirsch 2003). Insulin is a 51-amino acid peptide hormone and consists of a 21-amino acid alpha-chain linked by disulphide bonds to a 30-residue beta-chain (Figure 2.1).

At physiological concentrations, insulin molecules exist in monomeric form, but when stored at commercial therapeutic dose concentrations,

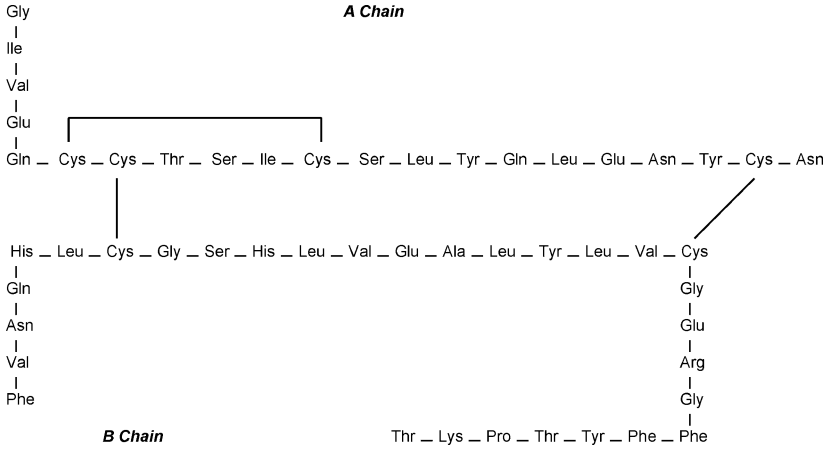

Figure 2.1 Amino acid sequence of human insulin.

individual insulin molecules interact with one another, forming dimers and hexamers (Bristol 1993). Upon subcutaneous administration, an insulin depot is formed, and individual molecules must dissociate before entering the bloodstream. This causes a delayed entry into the blood, and consequently, insulin plasma levels peak 90–120 min after injection. This is why insulin has to be injected up to one hour before food consumption (Walsh 2004).

2.2.2.1. Bovine and Porcine Insulins

In the 1920s, insulin was introduced in the treatment for diabetes; the first insulins were either single species or mixtures of bovine and porcine insulins isolated from pancreata. Bovine, porcine and human insulins differ in their primary structure. Porcine and human insulins have a different amino acid in the beta-chain, whereas bovine insulin differs from porcine and human insulins in two amino acids in the alpha-chain (Smith 1966).

In the 1950s, the in vitro use of radioactive insulin showed that 100% of patients treated with animal insulins produced high levels of circulating anti-insulin antibodies (Berson et al. 1956). Several studies described cutaneous and systemic allergic reactions linked to insulin antibodies (Hanauer and Batson 1961, deShazo et al. 1977, Velcovsky, Beringhoff and Federlin 1978, Deleeuw, Delvigne and Beckaert 1982, Carveth-Johnson, Mylvaganam and Child 1982, Blandford et al. 1982, Altman et al. 1983, Garcia-Ortega, Knobel and Miranda 1984, Gossain, Rouner and Homak 1985, Kumar 1997, Frigerio, Aubry and Gomez 1997) and insulin resistance (Andersen 1973, Witters et al. 1977, Davidson and DeBra 1978, Grammer et al. 1987, Lahtela et al. 1997). In one study by Deckert (1985), hypersensitivity reactions and IgE-mediated responses were described in up to 30% of patients and severe systemic immunological reactions in less than 0.1% of patients treated with insulin of animal origin.

In patients treated with animal insulin, it seems that the development of insulin antibodies correlates with increased insulin requirements. In a study by Walford and co-workers (1982), 40 diabetic patients were switched from bovine to highly purified porcine insulin for a period of six months, and the patients underwent sequential determination of anti-insulin IgG. The authors observed a positive

correlation between percentage change in insulin dose and change in insulin-binding capacity and concluded that the level of circulating antibodies affected the dose of insulin required to maintain stable diabetic control.

Another problem associated with the first animal insulins was that they were contaminated with several other islet cell peptides such as proinsulin, C-peptide and glucagons (Chance, Root and Galloway 1976), which enhanced immunogenicity. The later version of more purified porcine insulin gave a 60% incidence; however, human insulin was lower still at 40–50% incidence (Fireman, Fineberg and Galloway 1982, Fineberg et al. 1983).

2.2.2.2. Human Insulin

During human insulin treatment, both IgG and IgE antibodies have been detected. However, the titres were low and the incidence decreased in patients treated exclusively with human insulin (Velcovsky and Federlin 1982). Antibodies were initially detected 1–2 months after the beginning of therapy and were described at two years in some long-term studies (Schernthaner 1993, Velcovsky and Federlin 1982). They appear not to be specific for variant residues, but rather react with determinants shared by the human protein (Reeves and Kelly 1982, Marshall et al. 1988).

Even though human insulin is less immunogenic than the animal ones, high titres of IgG insulin antibodies were shown to bind and neutralize insulin, leading to insulin resistance by interfering with receptor binding (Van Haeften 1989). It has been described that antibodies alter insulin pharmacokinetics, causing an increase in daily insulin requirements (Ishibashi et al. 1986, Peters et al. 1995). However, in some large-scale trials, the presence of antibodies has not been shown to alter long-term glycaemic control directly (Van Haeften 1989, Chen et al. 2005).

In the last three decades, the prevalence of anti-insulin antibodies has decreased, thanks to the improvements in the purification of insulin preparations and to the development of monocomponent insulins (Fineberg et al. 1983, Van Haeften 1989, Walford, Allison and Reeves 1982). Moreover, the availability of recombinant human forms of insulin contributed to a further decrease of the immunogenicity (Fineberg et al. 2003). However, also recombinant human insulin is immunogenic (Fineberg et al. 1983, Fireman, Fineberg and Galloway 1982), but allergic phenomena are unusual and insulin antibody-mediated insulin resistance is an extremely rare complication of therapy (Fineberg 1994).

In most countries, the use of animal insulins has been largely replaced with recombinant human insulins. Recombinant insulin was the first product of recombinant DNA technology to gain approval in 1982. In the case of insulin, the main focus of engineering was to develop “fast-acting” insulin analogues, which could be administered with meals. These fast-acting analogues were obtained by making amino acid substitutions that increased steric hindrance between individual insulin molecules (Walsh 2005). In addition, various “long-acting” insulin analogues, which have a retarded entry into the blood-stream, have recently been engineered. The immunogenicity of insulin and these novel analogues is discussed in more detail in Chapter 8.

2.2.3. Reduction in Drug Efficacy

The formation of antibodies may reduce the drug efficacy, without these antibodies having any distinct (or known) effects on the pharmacokinetics.

This is exemplified below using granulocyte-macrophage colony-stimulating factor and type I interferons.

2.2.3.1. Granulocyte-Macrophage Colony-Stimulating Factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine which is able to stimulate the production of neutrophilic granulocytes, macrophages and mixed granulocyte-macrophage colonies from bone marrow cells. It can also stimulate some functional activities in mature granulocytes and macrophages. GM-CSF binds to a specific receptor which has significant homologies with other receptors for haematopoietic growth factors, such as interleukin-2b, interleukin-3, interleukin-6, interleukin-7 and erythropoietin. GM-CSF receptors are present in tissues derived from haematopoietic cells as well as in other cell types including those in the nervous system (Antignani and Youle 2007).

GM-CSF is mostly used to accelerate marrow recovery after cancer chemotherapy. Moreover, GM-CSF may enhance the immunogenicity of tumour cells by facilitating tumour antigen presentation (Hill et al. 1993, Charak, Agah and Mazumder 1993, Hooijberg et al. 1995). Studies made in animal models (Hill et al. 1993, Charak, Agah and Mazumder 1993) showed that GM-CSF, either alone or in combination with other therapeutic agents, is able to reduce the growth of tumour cells by the activation of macrophages. These data, together with results obtained in vitro (Masucci et al. 1989, Ragnhammar et al. 1994a), support the use of GM-CSF alone (as has been used widely for therapy of malignant diseases) or in combination with tumour-specific antibodies or immunomodulatory cytokines (e.g. interleukin-2) as an immunotherapeutic approach in patients with cancer.

Recombinant human GM-CSF produced in *E. coli* is a single, non-glycosylated, polypeptide chain containing 127 amino acids and with a molecular mass of 14477 dalton. The therapeutic administration of GM-CSF causes the development of antibodies in patients (Ragnhammar et al. 1994a). There are few data about the induction of antibodies in patients treated with GM-CSF because this cytokine is especially used in patients who are immuno-suppressed.

Antibodies against yeast-derived recombinant human GM-CSF as well as *E. coli*-derived rhGM-CSF have been described. Gribben et al. (1990) observed antibodies in 31% of patients affected by chemotherapy-resistant solid tumours treated with yeast-derived rhGM-CSF. In another study by Ragnhammar et al. (1994b), 95% of cancer patients treated with *E. coli*-derived rhGM-CSF subcutaneously, at 250 μg/m^2/day for 10 days every month for four months, developed binding antibodies after the second cycle. The same percentage of antibody-positive patients has been reported in a study by Wadhwa et al. (1996) on 20 colon carcinoma affected patients treated with a combination therapy (GM-CSF plus a colon carcinoma reactive antibody); in 40% of patients, these antibodies had neutralizing activity in an in vitro bioassay.

Results from a study by Rini and co-workers (2005) conducted on 15 prostate cancer patients demonstrated that antibodies against GM-CSF developed in all patients. In 87% of the patients they developed within three months, while in the other patients antibodies developed after additional cycles of GM-CSF. Sixty percent of the patients developed GM-CSF antibodies which neutralized the biological activity of GM-CSF in vitro in a cell-based

bioassay. These antibodies also recognized GM-CSF from different expression systems, indicating that they are directed towards the amino acid backbone of the protein. In fact, it has been suggested that antibodies recognize an epitope of the protein backbone, which in the native protein is normally masked by a carbohydrate residue, whereas it is exposed in the *E. coli*-derived as well as in the yeast-derived rhGM-CSF (Gribben et al. 1990, Mellstedt 1994).

Antibodies appear to modify GM-CSF pharmacokinetics, but this phenomenon is not clear. It is suggested that the mitogenic activity of the drug leads to an increase in target cells and their mitogen receptors. This phenomenon has led to observation of an inverse relationship between neutrophil count and serum levels of GM-CSF (Petros et al. 1992). Thus, changes in pharmacokinetics may not be directly attributed to circulating antibodies.

2.2.3.2. Type I Interferons

Interferons (IFNs) are a family of regulatory proteins, the majority of which are composed by 166 amino acid residues with molecular weight of 16–26 kilodalton. Based on the homology in their sequences, IFNs are classified into two groups: type I and type II.

Type I IFNs include seven families with different antigenic characteristics: alpha (α), beta (β), delta (δ), kappa (κ), epsilon (ϵ), omega (ω) and tau (τ) (Pestka et al. 2004). They are encoded by a cluster of genes located on chromosome 9, are produced in response to a viral stimulation and recognize the same membrane receptor, IFNAR (Pestka, Krause and Walter 2004; Bekisz et al. 2004).

Currently, only one member of type II IFNs is known: IFNγ, which is encoded by a gene located on chromosome 12 and which is produced by T lymphocytes and natural killer cells in response to non-self-antigen presentation. Its receptor is different from IFNAR. Here, attention is focused on type I IFNs, in particular to IFNα and IFNβ.

2.2.3.2.1. Interferon-Alfa: Interferon alfa (IFNα) is a protein, composed of 166 amino acids. Several genes of IFNα have been identified which share a high degree of homology. These proteins have antiviral activity, inhibit viral replication, increase class I MHC, stimulate Th1 cells and inhibit proliferation of many cell types.

IFNα is used for the treatment of many malignant diseases and for chronic hepatitis C and B. Several different recombinant preparations of IFNα exist. The most commonly used include IFNα-2a and IFNα-2b, which differ in the amino acid in position 23 (lysine in IFNα-2a and arginine in IFNα-2b); both preparations are glycosylated. Many studies have reported the development of antibodies in patients chronically treated with the two preparations, with different percentages, ranging from 19 to 61% and with a higher incidence in patients treated with IFNα-2a compared with patients treated with IFNα-2b (Quesada et al. 1985, von Wussow et al. 1987, Steis et al. 1988, Spiegel, Jacobs and Treuhaft 1989, Freund et al. 1989; Berman et al. 1990). As the gene for IFNα-2a is not present in the population, it is possible that this type of IFN carries a neo-antigen, but extensive studies have shown that the structural differences between the two products may not be the reason for the difference in immunogenicity (Kromminga and Schellekens 2005).

Discordant data exist about the role of anti-interferon antibodies on the drug clinical efficacy. In the study by Berman et al. (1990) on hairy cell leukaemia-affected subjects, 19% of patients with antibodies showed a resistance to treatment. In another study of IFNα-treated patients affected by hairy cell leukaemia (Steis et al. 1988), antibodies were reported in 16 out of 51 (31%) subjects, but a clinical resistance was observed in 6 out of 16 (38%) of these antibody-positive patients. Freund et al. (1989) evaluated IFNα-2b-treated patients affected by myelogenous leukaemia, finding antibodies in 8 out of 27 (30%) subjects and observing a resistance to therapy in all the antibody-positive patients.

Different data arise from other studies: Figlin and Itri (1988) described anti-interferon antibodies in 12 out of 19 renal carcinoma patients treated with IFNα-2a intramuscularly, reporting that 6 out of these 12 patients developed neutralizing antibodies, but no correlation between antibody presence and clinical response to therapy nor clinical toxicity was found. A review by Jones and Itri (1986) evaluated more than 1300 cancer patients who had received IFNα-2a treatment. The authors reported that about 27% of patients developed antibodies, but they did not find any adverse clinical manifestation associated with them.

The kinetics of antibody development seems to be linked to the type of disease. For example, in patients affected by renal cell carcinoma, antibodies have been described after a median time of eight weeks, while in patients affected by hairy cell leukaemia they have been reported to arise after an average of seven months (Figlin and Itri 1988).

IFNα-2a is also widely used for the treatment of chronic hepatitis C. A study by Bonetti et al. (1994) evaluated antibodies against therapeutic IFNα-2a in 60 patients affected by chronic hepatitis C and observing 61% of antibody-positive patients within six months of treatment. Interestingly, they found that 75% of patients who showed no therapeutic response to IFN had detectable antibodies. Similarly, in one study by Douglas (Douglas et al. 1993) on IFNα-2a for hepatitis C, antibodies were present in 32% of treated patients, and when comparing responder and non-responder patients, a higher percentage of antibodies positive subjects were found in the group of non-responders (40% versus 14%, respectively). In an Italian study by the group of Dianzani et al. (1989), the authors analysed 175 patients treated with recombinant IFNα-2b, trying to correlate the presence of antibodies with a reduction in clinical response, but antibodies were found in only one patient. A Chinese trial of IFNα-2a in hepatitis B-affected patients reported that 39% of patients developed antibodies. They were more likely to develop in the group of patients treated with a low dose than in the group treated with a high dose of drug. A high antibody titre correlated with a failure in treatment response (Lok, Lai and Leung 1990).

With the introduction of pegylated-IFNα, the prevalence of neutralizing antibodies is 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.

2.2.3.3. Interferon Beta

Interferon beta (IFNβ) is used in the treatment of multiple sclerosis. Clinical aspects of the formation of neutralizing antibodies against IFNβ will be

discussed further below in Section 2.5, and immune responses to IFNβ are discussed in great detail in Chapter 7.

2.2.4. Cross-Reaction with Endogenous Protein

2.2.4.1. Megakaryocyte Differentiation and Growth Factor

The most dangerous consequence of the development of antibodies occurs when the endogenous protein is neutralized. This effect has been described for megakaryocyte differentiation and growth factor (MDGF) and for erythropoietin (EPO). The development of first-generation thrombopoietic growth factor (human recombinant thrombopoietin, TPO, and pegylated recombinant human MDGF) has been stopped due to the development of antibodies against endogenous TPO, causing severe thrombocytopenia in 13 out of 325 healthy volunteers and in 4 out of 650 cancer patients (Kuter 2000). In a study by Li and co-workers (Li et al. 2001), the authors evaluated three of these thrombocytopenic subjects (two volunteers and one cancer patient). They found that that in all of the patients thrombocytopenia was due to the development of antibodies which cross-reacted with endogenous TPO, neutralizing its biological activity. All the subjects underwent bone marrow examination that showed a marked reduction in megakaryocytes. All anti-TPO antibodies were IgG and most of the anti-TPO were IgG4. The biological activity of endogenous TPO was inhibited by the binding of anti-TPO to the first 163 amino acids of TPO, preventing TPO from binding to its receptor. In two subjects endogenous TPO level were elevated, but biologically inactive, as it formed an immune complex with IgG. A study of the time course of anti-TPO development was performed in all the three subjects, showing that no subject had antibodies before the first injection of PEG-rhMDGF and that there was no IgM response. In one subject, IgG appeared on day 56 after the drug injection, when the platelet count had already begun to fall and was maximal on day 147; these antibodies progressively decreased and disappeared. In another subject, anti-TPO rapidly disappeared after treatment with cyclosporine, and in the third subject (the oncologic patient), antibodies persisted at high titre despite plasmapheresis.

New non-immunogenic second-generation thrombopoietic growth factors have been developed; they have been tested in healthy humans, producing a dose-dependent rise in platelet count, without adverse effects (Kuter 2007).

2.2.4.2. Erythropoietin

Recombinant human erythropoietin (rhEPO) has been successfully used for the treatment of anaemia due to chronic renal failure. Several patients affected by chronic kidney disease and treated with recombinant epoetin-α developed pure red cell aplasia (PRCA), due to the production of antibodies directed against recombinant EPO as well as against the endogenous EPO (Locatelli et al. 2004). The immune response to EPO is discussed in detail in Chapter 6.

PRCA is a rare disorder, which can arise either spontaneously or in association with thymoma, lymphoid proliferation or an immune-related disorder, such as lupus erythematosus or rheumatoid arthritis. It can also occur secondary to viral infections or to drugs. In adults it is usually an autoimmune disorder, in which antibodies of cytotoxic T lymphocytes are directed against erythroid progenitors (Casadevall 2002).

The use of recombinant EPO started in 1988, initially by the intravenous route, and since 1990, by the subcutaneous route. The molecular mass of the peptidic part of both endogenous EPO and rhEPO is 18 kilodalton. The erythropoietin molecule folds into four alpha helices stabilized by two disulphide bonds, which are essential for biological activity. EPO is a glycosylated molecule, causing an increase in the molecular mass to about 30 kilodalton, and the glycosylation is essential to the biological activity of the molecule. Therefore, rhEPO is produced in mammalian cell lines, as bacterial cell lines are not able to produce this post-translational modification. Endogenous EPO and most rhEPO variants have minor differences in glycosylation, with variation in the sialic acid composition of the oligosaccharide groups (Sasaki et al. 1987, Skibelli, Nissen-Lie and Torjesen 2001). Various rhEPO variants exist (for example, epoetin-α, epoetin-β, darbepoetin-α), which differ from each other and from endogenous EPO in the carbohydrate groups (Skibelli, Nissen-Lie and Torjesen 2001).

During the first 10 years of its therapeutic use, three cases of PRCA have been reported in patients treated with rhEPO (Bergrem et al. 1993, Peces et al. 1996, Prabhakar and Muhlfelder 1997). By 1999, a sudden increase in the number of PRCA was observed. In 2002, Casadevall and co-workers described 21 cases of PRCA, which occurred in patients treated with rhEPO to correct anaemia due to chronic renal failure. They were observed between 1998 and 2001 in different centres throughout Europe (Casadevall et al. 2002). In all these patients, anaemia was secondary to PRCA and neutralizing antibodies were present. All EPO-related PRCA patients showed an initial normal response to rhEPO and then developed severe anaemia, which was resistant to increasing dose as well as to a switch to another EPO. Anaemia appeared at different time points from the beginning of EPO treatment, with a median time of nine months (Bennett et al. 2004). It has been found that the majority of PRCA cases were associated with the subcutaneous administration (Casadevall 2002, Eckardt and Casadevall 2003). The study of the first cases described by Casadevall showed that sera from these patients recognized the endogenous molecule as well as the deglycosylated EPO: in fact, when sera of patients were incubated with the glycosylated and the deglycosylated epoetin, antibodies bound to both forms with the same efficiency. The depletion of IgG in the sera was associated with a reversion of Ab-mediated inhibition of erythroid cell proliferation in vitro. In the majority of PRCA cases, an immunosuppressive treatment after the discontinuation of EPO therapy was required to stop antibody development (Verhelst et al. 2004).

2.3. Immunogenicity of Biopharmaceuticals in Gene-Defective Hosts

2.3.1. The Case of Haemophilia 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 2.2). The clinical effects of immunogenicity will vary, depending on the extent of gene mutation: the absence of the gene, as

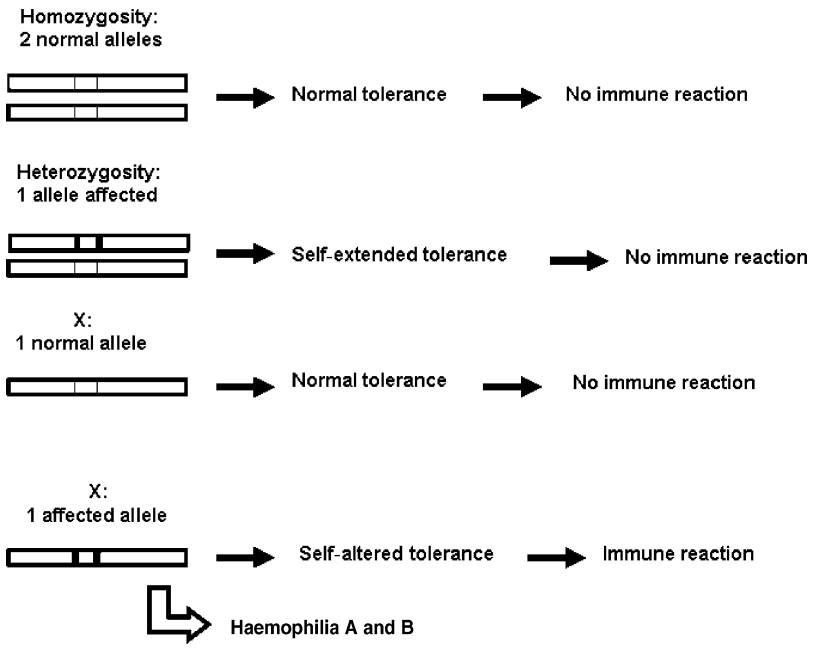

Figure 2.2 Relationship between genome and immune responses.

well as large gene deletions or a premature stop-codon, will have more severe consequences than point mutations.

Haemophilia 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 factor VIII. The disease results from mutations or deletions that alter the expression and secretion of factor VIII. The malfunction of factor VIII causes a reduced coagulation power, with prolonged post-traumatic bleeding times and spontaneous haemorrhages. Replacement therapy with factor VIII has been successfully used, and initial preparations of factor VIII were natural preparations derived from donated blood. Today, a large number of recombinant DNA-derived factor VIII preparations are available (Kromminga and Schellekens 2005). As expected, substitution therapy results in antibody formation, as the patient's immune system recognizes parts of the factor VIII as a foreign antigen. The immune response is a classical one, with a switch from IgM to IgG and affinity maturation (Opdenakker et al. 2003), discussed in more detail in Chapter 9.

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

Recent studies on antibodies to factor VIII show that neutralization may be due to a proteolytic activity (Lacroix-Desmazes et al. 2002), and a correlation between the neutralizing activity of IgG in plasma and the hydrolysis rate of factor VIII by IgG in vitro was observed. Factor VIII can be completely absent in patients with severe haemophilia A. These patients do not benefit from the administration of factor VIII because factor VIII represents a foreign antigen and patients develop an immune response against it. The same occurs in those patients who have factor VIII 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 and Schellekens 2005).

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

The experience on haemophiliac 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).

2.3.2. The Case of Pompe Disease

Pompe disease is a rare autosomal recessive disorder caused by deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA). The incidence is about 1/40,000. The genetic defect leads to lysosomal glycogen accumulation in different tissues, the most severely affected being the skeletal and cardiac muscles. Different forms of the disease are known, depending on the residual GAA activity. The classical form is the infantile type, characterized by a progressive cardiomyopathy, muscular weakness, respiratory insufficiency and death in the first year of life. The adult form is less severe, as cardiac muscle is usually not affected. Recombinant precursor human acid alpha-glucosidase has been produced in CHO cell cultures and from milk of transgenic rabbits (Van Hove, Yang and Wu 1996, Bijvoet et al. 1999). There are still few data about replacement therapy: in a phase II trial on two affected infants (Klinge et al. 2005) treated with rhGAA for 48 months, results are encouraging, as the therapy was well tolerated and there was an overall improvement in left-ventricular mass and in cardiac and skeletal muscle functions. The two patients developed antibodies against rhGAA, but, according to the authors, no reduction in muscle function was observed. Data from a multicentre, multinational open-label study (Kishnani et al. 2007) on 18 infants affected by Pompe disease, who began treatment with intravenous infusion of rhGAA prior to six months of age, have been published. This study represents the largest cohort of patients with Pompe disease treated

with replacement therapy. The study showed that the treatment was safe and effective. One of the 18 patients developed antibodies, which inhibited rhGAA activity in vitro. As the total number of treated patients is limited, no conclusive observations on the effect of antibodies are available. Further long-term studies are required to evaluate the potential of this therapy and the consequences of immunogenicity.

2.4. Adverse Drug Reactions

Drug reactions include all adverse events related to drug administration, regardless of aetiology. They can be classified into two groups: immunologic aetiology and non-immunologic aetiology (Table 2.2). About 20–25% of adverse drug reactions are caused by unpredictable effects, both immune and non-immune mediated, whereas 75–80% of adverse reactions are caused by predictable non-immunological events (Riedl and Casillas 2003). The predominant immune mechanisms leading to drug hypersensitivity are described in the classification by Gell and Coombs (Table 2.3). The risk of drug hypersensitivity can be increased by some patient-related factors, which include female gender (Barranco and Lopez-Serrano 1998), asthma, use of beta-blockers (Lang et al. 1991), specific genetic polymorphism, as well as by some drug-related factors, which include the chemical properties, the molecular weight of the drug and the route of administration. It is known that drugs with great structural complexity are more likely to be immunogenic. However, drugs with a small molecular weight (less than 1,000 daltons) may become immunogenic by coupling with carrier proteins, such as albumin, forming complexes (Riedl and Casillas 2003). Moreover, the route of administration affects the immunogenicity, the subcutaneus route being more immunogenic than the intramuscular and the intravenous routes.

An example of adverse drug reactions presumably secondary to immunogenicity is represented by antibodies to natalizumab, an alfa4-integrin antagonist, used in the treatment of aggressive forms of relapsing multiple sclerosis. In the AFFIRM study (Polman et al. 2006) on 627 patients receiving natalizumab, 37 (6%) had persistent antibodies (detectable on at least two occasions) to the drug, which were associated to an increase in infusion-related adverse events and a loss of clinical efficacy. Comparable findings were obtained in the SENTINEL study (Rudick et al. 2006) conducted on

Table 2.2 Immunological and non-immunological drug reactions.

Immunological	Non-immunological
Type I reaction (IgE mediated)	*Predictable*
Type II reaction (cytotoxic)	Pharmacological side effect
Type III reaction (immune complex)	Secondary pharmacological side effect
Type IV reaction (delayed, cell mediated)	Drug toxicity
Specific T-cell activation	Drug–drug interactions
Fas/Fas ligand-induced apoptosis	Drug overdose
Other	*Unpredictable*
	Pseudoallergic
	Idiosyncratic
	Intolerance

Table 2.3 Drug hypersensitivity reactions: Gell and Coombs classification.

	Type I	Type II	Type III	Type IV
Immune reaction	IgE mediated	Cytotoxic	Immune-complex	Delayed, cell mediated
Mechanism	Drug-IgE complex, with release of histamine from mast cells	Specific IgM or IgG antibodies directed at drug-hapten coated cells	Drug–antibody complexes with complement activation and tissue deposition	MHC presentation of drug molecule to T cells
Timing	Minutes to hours	Variable	1–3 weeks	2–7 days

1171 subjects, in which persistent antibodies to natalizumab were detected in 6% of patients and their presence was associated to a loss of clinical efficacy, as well as an increase in infusion-related adverse events.

2.5. Unknown Effects: Glatiramer Acetate

Glatiramer acetate (GA, also known as copolymer-1, copaxone) is a synthetic random polypeptide of the amino acids alanine, glutamate, tyrosine and lysine in a defined molar ratio and with a molecular weight ranging between 5000 and 9000 dalton.

It was first synthesized more than 30 years ago, in an attempt to mimic the encephalitogenic properties of myelin basic protein (MBP). Surprisingly, it was observed that GA was able to block the induction of experimental autoimmune encephalomyelitis, EAE (Arnon and Aharoni 2004). Subsequently, it was tested in therapeutic trials in patients with MS (Johnson et al. 1995, 1998, 2000), and it has gained marketing approval for the treatment of relapsing-remitting multiple sclerosis, showing a significant reduction in the number of new and enhancing lesions on MRI (Wolinsky et al. 2001, Comi et al. 2001), in relapse rate and a lower rate of disease progression (Johnson et al. 1995, 1998, 2000, 2005).

Development of anti-GA antibodies in treated patients is a known phenomenon, but the clinical meaning of anti-GA is not clear yet. Data from different studies give inconclusive results. The study by Brenner and coworkers (Brenner et al. 2001) showed that anti-GA antibodies developed only in treated patients and not in the placebo group and that antibodies titres peaked after three months, then slowly decreased but remained higher than baseline values. Farina and colleagues (2005) not only observed the presence of anti-GA antibodies in treated patients but also found naturally occurring anti-GA antibodies in some untreated individuals. The authors suggested that these naturally occurring anti-GA antibodies might belong to the low-affinity, poly-reactive pool and observed that the difference between treated and untreated patients was not the mere presence or absence of antibodies, but rather their isotype profile, as treated individuals frequently produced IgG4 antibodies, while unexposed subjects had anti-GA IgM, IgG1 and IgG2.

In a study by the group of Teitelbaum et al. (1996), the authors did not observe any neutralizing activity in serum of GA-treated patients. In contrast, Salama and co-workers (2003) observed neutralization of GA-specific T-cell reactivity in a study which included 42 patients treated with GA for a mean time of three years; 48% of the patients developed anti-GA antibodies, which were expressed at high titre in 33%. The study did not have clinical endpoints, but the authors nonetheless tried to correlate clinical response to GA with the presence of antibodies and observed that patients with high-titre antibodies tend to have a higher relapse rate and a more rapid progression than patients with low antibodies titres. In the study by Brenner and co-workers (2001), all the patients (n = 130) developed anti-GA antibodies, which declined after six months, persisting at low titre, but they did not seem to interfere with copolymer activity.

In Theiler's virus model of demyelinating disease (Ure and Rodriguez 2002), anti-GA antibodies seemed to promote remyelination. However, no clinical studies to date have shown either a neutralizing or a beneficial effect of anti-GA antibodies in patients with multiple sclerosis. Depending on route and frequency of administration, anaphylactic reactions may occur. Rauschka and colleagues (2005) described a systemic anaphylactic reaction to GA in a patient who had a history of atopic allergy and high serum IgE concentrations. The patient had a positive skin test to GA and an unusually high concentration of IgG4 antibodies to the drug. These observations suggest that caution is needed in administrating GA to atopic patients with multiple sclerosis.

2.6. Treatment of Multiple Sclerosis Patients Who Have Developed Antibodies Against Interferon-β

Antibody formation against interferon-β (IFNβ) in patients with multiple sclerosis is used here as a good illustration of the clinical aspects of antibody formation against biopharmaceuticals, and includes a discussion on how to prevent or reduce antibody formation. Some of the aspects in this section are discussed in more detail in Chapter 7.

2.6.1. Description of Interferon-β

The gene of IFNβ shows 45% homology with IFNα genes. The protein is composed of 166 amino acids and has a molecular weight of 22,500 dalton. It has several functions, which include antiviral, antiproliferative and immunomodulant activities, such as the regulation of antigen presentation to T lymphocytes (Goodbourn, Didcock and Randall 2000), the stimulation of T helper 2 and the activation of natural killer cells (Nguyen et al. 2002).

It is one of the first-line drugs for the treatment of relapsing-remitting multiple sclerosis (RR-MS). Two types of recombinant IFNβ exist: IFNβ-1a, which is available in two different commercial preparations (Avonex by Biogen-Idec, Cambridge, and Rebif by Serono, Geneva) (Table 2.4) and is obtained from Chinese hamster ovary, and IFNβ-1b (Betaferon, Schering, Berlin), which is generated in *E. coli*. IFNβ-1b and IFNβ-1a have the same receptor binding region, but IFNβ-1b is a non-glycosylated molecule that has a Met-1 deletion and a Cys-17 to Ser mutation (Mark et al. 1984), while IFNβ-1a is a glycosylated polypeptide with the predicted natural amino acid sequence

Table 2.4 Different commercial preparations of IFNβ.

	Avonex	Betaferon	Rebif
Number of amino acids	166	165	166
Production	CHO	*E. coli*	CHO
Dosage/injection	6 MIU (30 μg)	8 MIU (250 μg)	6–12 MIU (22–44 μg)
Route of administrations	i.m.	s.c.	s.c.
Frequency	Once/week	Every other day	Thrice/week

Note: CHO= Chinese hamster ovary; i.m.= intramuscular; s.c.= subcutaneous

(Holliday and Benfield 1997). These differences affect the pharmacokinetic, pharmacodynamic and immunological properties. In particular, the absence of glycosylation causes the formation of aggregates and a reduction of in vitro activity (Runkel 1998).

2.6.2. Prevalence of Neutralizing Antibodies

Randomized, double-blind, placebo-controlled studies have demonstrated the efficacy of IFNβ in the treatment of RR-MS on both clinical and MRI measures (The IFNB MS Study Group 1993, Jacobs et al. 1996, PRISMS 1998, PRISMS-4 2001, Kappos et al. 2005). A percentage of IFN-treated patients can develop neutralizing antibodies (NAbs) against IFNβ during the course of treatment. The percentage of Nabs-positive (NAbs+) patients varies depending on the IFNβ product, the frequency and route of administration and the type of assay used, with Betaferon the most immunogenic and Avonex the least immunogenic (Table 2.5). Factors influencing the development of anti-IFNβ antibodies are not clearly defined as yet. A cross-reactivity of NAbs has been demonstrated (Khan and Dhib-Jalbut 1998, Antonelli et al. 1999, Bertolotto et al. 2000); thus, once NAbs develop, the switch to a different commercial preparation is not clinically useful.

2.6.3. Dynamics of NAbs

The majority of patients become NAbs+ within 6–18 months of treatment, while clinical impact of NAbs is delayed and is not seen until 24 months of therapy (Figure 2.3). It has been suggested that patients on IFNβ-1b tend to become antibody-positive earlier than those on IFNβ-1a and that in NAbs+ patients, the probability of reverting to NAb-negative status is significantly higher in patients treated with IFNβ-1b than in patients treated with IFNβ-1a (Rebif) (Gneiss et al. 2004, Sorensen et al. 2005). Several studies have

Table 2.5 IFNβ immunogenicity.

Type of drug (phase III trials)	Percentage of NAbs+ (2–3 years)
Avonex (Jacobs 1996)	22
Avonex (Rudick 1998)	6
Rebif 22 (PRISMS)	24
Rebif 44 (PRISMS)	12
Rebif 44 (SPECTRIMS)	15
Betaferon (1996)	38
Betaferon (Polman 2003)	28

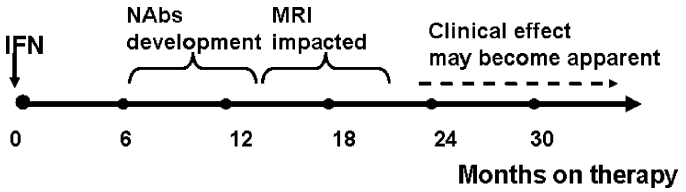

Figure 2.3 Dynamics of NAbs and clinical consequences.

demonstrated that NAbs persist for a long time, despite IFNβ discontinuation. In the study by Polman et al. (2003), 37% of NAbs+ patients reverted to seronegative over three years. In the PRISMS study, 22% reverted to seronegative by four years and 37% by six years, and in a recent Danish study (Petersen et al. 2006), it was shown that patients with NAbs titres above 200 tended to maintain NAb+ status for a median time of 22 months. In a study by our group (Malucchi et al. 2005), spontaneous disappearance of NAbs was only observed in those NAbs+ patients which had low titre of antibodies (<100 TRU), whereas NAbs persisted in patients showing higher titre, despite immunosuppressive treatment.

2.6.4. Effects of NAbs on IFNβ Efficacy

Several studies have demonstrated that the presence of NAbs causes a reduction in IFNβ bioavailability as measured by reduced levels of biologic markers such as neopterin, β2-microglobulin (Rudick et al. 1998), myxovirus resistance protein A (MxA) (Deisenhammer et al. 1999, Kracke et al. 2000, Vallittu et al. 2002) and M×A messenger RNA (Bertolotto et al. 2001, 2003). Besides that, a number of studies have demonstrated that the presence of NAbs is associated with a loss of IFNβ clinical and radiological efficacy (Table 2.6). Betaferon was the first IFNβ approved in the United States for the treatment of RR-MS, as it demonstrated to reduce clinical activity as well as radiological activity in a large study, which involved 372 patients (The IFNβ MS study Group 1993), randomly assigned to receive placebo or the active drug at two different dosages (1.6 MIU and 8 MIU, respectively). Thirty-five percent of patients receiving 8 MIU became NAbs+, and in these patients, the relapse rate in the second and third year of treatment was comparable

Table 2.6 Studies demonstrating a loss of IFNβ clinical and radiological efficacy in NAbs+ patients.

Type of drug	Clinical effect of NAbs relapse rate	MRI effect of NAbs
Avonex (Jacobs 1996)	–/–	–
Avonex (Rudick 1998)	NS/NS	Reduced*
Rebif 22 (PRISMS-4)	Reduced^{p}	Reduced^{p}
Rebif 44 (PRISMS-4)	Reduced^{p}	Reduced^{p}
Rebif 44 (SPECTRIMS)	Reduced*	Reduced^{p}
Betaferon (1996)	Reduced^{p}	Reduced^{p}
Betaferon (Polman 2003)	Reduced^{p}	Reduced^{p}

Note: NS= not significant; *= suggestion; ^{p}= statistically significant

to one of the placebo group (The IFNβ MS Study Group and the University of British Columbia MS/MRI Analysis Group 1996). The loss of efficacy was also radiologically evident, as NAbs+ patients had a significantly higher number of MRI lesions by the second and third years. A negative effect of NAbs was also present in the evaluation of disability progression, even if there was no significant difference between NAbs+ and NAbs negative (Nabs–) patients.

Comparable results are also evident in the Betaferon study on 718 secondary-progressive MS patients (Polman et al. 2003), in which NAbs+ patients had a significantly higher relapse rate and a significantly higher increase in MRI lesion burden than NAbs– patients. Similar evidence arises from other studies in which the other commercial preparations of IFNβ have been used: in the PRISMS-4 study (PRISMS-4 2001) conducted on more than 500 patients treated with Rebif 22 μg or 44 μg for 4 years (or with placebo for two years and then randomly assigned to Rebif 22 or 44), the negative effect of NAbs on clinical and, even more, radiological activities is clearly evident. By the third year of treatment, NAbs+ patients had a significant higher relapse rate than Nabs– (0.81 and 0.5, respectively), as well as about five-fold increase in the mean number of MRI active lesions (Figure 2.4) and three-fold increase in the burden of disease compared to Nabs–. A post hoc analysis by Francis et al. (2005) focused on a subgroup of patients (368 subjects treated with Rebif 22 or 44 from the beginning) of the PRISMS-4. The authors studied two markers of IFN biological activity, represented by β2-microglobulin and neopterin, and the clinical and radiological effects of IFNβ. Interestingly, at one year, the mean increase in the level of these markers from baseline was significant only in Nabs– patients. In addition, upon analysis of the relapse rate during the four years, the authors observed a comparable relapse rate between Nabs– and NAbs+ groups in the first and second years, whereas in the third and fourth years NAbs+ had a significant higher relapse rate. The impact of NAbs was even more evident on MRI results. Also the SPECTRIMS study (2001) on SP-MS patients showed the negative effect of NAbs on clinical and radiological parameters (Li et al. 2001). In the more recent study by Kappos and co-workers (2005) on 802 patients randomly assigned to Avonex 30 or 60 μg for four years, 1.8% and 4.8%, respectively, were NAbs+. Despite the low percentage of NAbs+

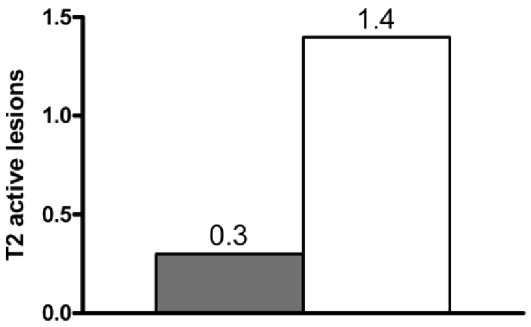

Figure 2.4 Impact of NAbs on MRI: median number of T2-active lesions reported in the PRISMS-4 study (2001). Grey square: Nabs– patients; white square: NAbs+ patients; the difference is statistically significant ($p<0.001$).

patients, the impact of NAbs was dramatically evident, as the annualized relapse rate was 39% higher in NAbs+ than in Nabs– over 12–48 months, time to three-month sustained disability progression was shorter in NAbs+ than in Nabs–, and mean number of MRI active lesions was significantly higher in NAbs+ than in Nabs– patients.

More generally, NAbs have been demonstrated to have a prognostic value, as NAbs+ patients have a higher risk to relapse than Nabs– patients (Sorensen et al. 2003, Malucchi et al. 2004, Tomassini et al. 2006).

As MS is characterized by an unpredictable course, in which, up to date, no clinical or laboratory markers are available, the prognostic character of the detection of NAbs is a useful instrument for the neurologist. Recently, after evaluating NAbs literature, European guidelines regarding antibodies against IFN in MS have been written, giving as an "A level" recommendation that therapy with IFNβ should be discontinued in patients with titres of NAbs >100 (Sorensen et al. 2005).

2.6.5. Possible Strategies to Eliminate NAbs

Up to date no predictive factors of the development of NAbs are known and no guidelines exist about the management of NAbs+ patients. However, as it is evident that NAbs abolish IFNβ efficacy and have a prognostic meaning, some strategies have to be undertaken in NAbs+ patients. As already written above, it is possible that a spontaneous seroreversion occurs, especially in those patients with low titres of antibodies, but several studies (Bellomi et al. 2003, Petersen et al. 2006) showed that NAbs can be a long-lasting phenomenon. Thus, the continuation of IFN therapy after NAbs development, hoping in a spontaneous seroreversion, makes the patient be at risk of taking a useless therapy for many years. Similarly, the switch to a less immunogenic preparation of IFN has no positive effect, as NAbs cross-react with any type of IFNβ.

Hypothetic strategies to eliminate NAbs include the use of plasmapheresis and intravenous immunoglobulins (Sorensen et al. 2005). At present, IgG and plasma exchange are used in the treatment of autoimmune diseases (Yamamoto, Takamatsu and Saito 2007, Zinman, Ng and Bril 2007), but they have no effect on memory or plasma cells, so these treatments could help the elimination of circulating NAbs, but not avoid their development.

Another hypothetic strategy is to increase the dosage of IFN, with the aim of overcoming antibody neutralization, but probably this strategy fails if the NAbs titre is high. Finally, a monoclonal antibody used for the treatment of lymphoma, rituximab, is available. This drug is directed against B cells; thus from a theoretical point of view, it could be used to reduce NAbs titres, but no data exist on this.

2.6.6. Possible Strategies to Prevent Formation of NAbs

An Italian study by Pozzilli and co-workers (2002) evaluated about 160 MS-affected patients, who were treated with IFNβ1a alone or in combination with short pulses of steroids for one year. The authors observed that in the group treated with the combination therapy there was a 50% reduction in the incidence of NAbs development. However, steroids were not able to reduce the

titre in NAbs+ patients. In other clinical trials performed on RR- as well as SP-MS-affected patients, a combination therapy (IFN plus immunosuppressive agents) has been used (Fernandez et al. 2002), but no definitive conclusions about the ability in reducing NAbs formation are available. Surely, the modifications in the technical preparation of the drug are of extreme importance in determining drug immunogenicity. This has been demonstrated for Avonex, which in the first study by Jacob and co-workers (1996) induced 22% of NAbs+ patients, whereas in the study by Clanet et al. (2002) induced only 2–3% of NAbs+ patients.

2.6.7. The Costs of Ineffective IFNβ Treatment

As previously stated, IFNβ modifies the natural course of multiple sclerosis, significantly reducing the relapse rate and slowing disease progression. In addition, several studies showed the beneficial effect of IFNβ in delaying the onset of defined multiple sclerosis in patients affected by clinical isolated syndrome (CIS). Thus, there is a large consensus to start IFNβ treatment early.

In Italy, about 52,000 subjects are affected by multiple sclerosis, and about one-third of them receive IFNβ therapy. Consequently, the cost of treatment is about 170 millions euro/year. If we consider that about 15% of treated patients develop NAbs, we can estimate that 2,500 patients are receiving a useless drug and, thus, about 25 millions euro/year are ill spent. This refers only to the Italian situation, whereas, considering the situation in Europe as a whole, the cost obviously increases.

While on one side IFNβ treatment tends to be initiated very early in the disease course, on the other side a percentage of patients are at risk of receiving, for a long period, a useless drug because the identification of IFN non-responder patients by clinical observation often requires several years. Thus, it is clear that the availability of a biological assay which can rapidly identify this group of patients leads to a considerable reduction in therapeutic costs. It also has to be considered that NAbs+ patients would benefit from alternative therapies that have comparable (glatiramer acetate) or lower cost (mithoxantrone, azathioprine) compared to IFNβ.

2.7. Conclusions

At present, proteins are widely used as therapeutic agents; with the development of recombinant DNA techniques, highly purified proteins have been produced. However, even if they are nearly identical to the endogenous proteins, they still may show immunogenicity.

Antibodies against these drugs can have a neutralizing activity which inhibits the efficacy or they can induce changes in pharmacokinetic properties or lead to hypersensitivity reactions. Antibody production seems to be related to many different mechanisms, such as the post-translational modifications, the development of drug aggregates and the use of adjuvants. Antibodies which cause the loss of clinical efficacy or which are directed against the endogenous molecule should be prevented, but at present no failsafe strategy to prevent neutralizing antibodies formation is known. An interesting approach to reduce immunogenicity has been described in a recent work by Tangri

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 HLA class II molecules; the authors found that the modified forms of EPO were non-immunogenic in vitro; so this strategy could represent one way to limit the immunogenicity of protein drugs. 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. Intravenous immunoglobulin and plasmapheresis can be used to reduce the antibody burden, but they do not affect memory cells. Considering the potential high costs of such therapies, as well as those adverse events and reduction of drug efficacy, early detection of antibody formation is an important issue in treatment with biopharmaceuticals. Assays for determining antibody formation and type of antibodies are discussed in detail in the Chapter 3.

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