

MINIREVIEW

Human Immune Response to Recombinant Human Proteins

SETH PORTER

Cerus Corporation, 2411 Stanwell Drive, Concord, California 94520

Received 27 March 2000; revised 15 August 2000; accepted 24 August 2000

ABSTRACT: Delivery of pharmacological doses of proteins to people has raised concerns of inducing immune responses, especially when the protein is provided in multiple doses over an extended period of time. Immune responses could impact the therapeutic exposure and efficacy of the protein itself. In addition, there have been fears of anaphylaxis or autoimmunity. This review summarizes the available literature regarding the measurement and evaluation of immune responses observed during clinical assessment of recombinant human proteins. Immune responses have ranged from none at all to inactivation and/or accelerated clearance. Presence of antibodies does not necessarily impact therapeutic viability. While responses are related to frequency and route of delivery, there is no clear relationship that enables one to predict the clinical experience. © 2001 Wiley-Liss, Inc. and the American Pharmaceutical Association *J Pharm Sci* 90: 1–11, 2001

Key Words: recombinant protein; immune response; immunoglobulin; therapeutic protein; biotechnology; monoclonal antibody; antibody; insulin; growth hormone; factor VIII; DNase; interleukin; growth factor; colony stimulating factor; interferon

INTRODUCTION

The development and use of recombinant human proteins has represented the greatest hope and the most tangible success of biotechnology over the last 20 years. Nearly 20 recombinant human protein pharmaceuticals have been approved for clinical use.

From the start, the prospect of delivering pharmacological doses of a protein to people has raised concerns of inducing immune responses, especially when that protein is provided in multiple doses over an extended period of time. Whereas

an immune response to an administered protein could impact therapeutic exposure and efficacy, the greater concern has been the induction of a severe hypersensitivity or anaphylactic response posing a potentially substantial risk to patients. Furthermore, induction of an immune response to recombinant protein could result in an autoimmune reaction to the endogenous form of that protein, leading to long-term detrimental effects. This review is intended as a summary of the actual reported observations regarding human immune response to administered doses of recombinant human proteins. For the most part, immune response will be defined as detectable serum antibodies specific for the protein of interest. Because some proteins can have profound and diverse biological effects, apparent immunologic responses could be based on the biological activity of

Correspondence to: S. Porter (Telephone: 925-603-9071; Fax: 925-603-1360; E-mail: seth.porter@ceruscorp.com)

Journal of Pharmaceutical Sciences, Vol. 90, 1–11 (2001)
© 2001 Wiley-Liss, Inc. and the American Pharmaceutical Association

the protein and not on an immune response against that protein per se. For that reason, this assessment of immune response is limited to an overt presence of detectable antibodies.

The ability to detect and measure antibodies specific for recombinant protein therapeutics is an inherently flawed analytical exercise for several reasons. First, there are rarely appropriate positive controls. In most cases, it is impossible to find human immune serum specific for a particular human protein. Therefore, development and validation of assays for such antibodies are usually based on extrapolation from use of monoclonal or polyclonal antibodies prepared in non-human species. Second, the presence of circulating therapeutic protein or the endogenous form of that protein can interfere with one's ability to detect antibodies because detection of serum antibodies is usually based on the ability of the antibodies to bind to recombinant protein used as an assay reagent. Third, methods for expressing the quantity of specific antibody can vary substantially. Most often, the amount of antibody is expressed as a titer value. However, a titer value for any one sample can vary based on the assay format and reagents. Furthermore, because there usually are no appropriate standards or controls, it is difficult, if not impossible, to normalize results in a meaningful way. Finally, a principle concern with regard to serum antibodies to therapeutic protein is their ability, or inability, to neutralize the biological activity, and thus efficacy, of the therapeutic. Assessment of neutralizing activity of serum antibody is usually based on its ability to impact a quantifiable response in an *in vitro* bioassay. Most cell-based bioassays are quite susceptible to variations in the assay conditions as well as the duration of continued culture of the cells used. For all of these reasons, it is difficult to make quantitative comparisons between results obtained in different studies with the same protein, and certainly comparison of results obtained with different proteins is qualitative at best.

Although there is a robust scientific literature providing detailed descriptions of immune responses to recombinant proteins used as vaccines, the literature and information related to immunoreactivity of recombinant proteins used as therapeutics is far less extensive. In general, there is a desire to minimize the perceived adverse response to a new therapeutic. Often in reports of clinical trial results, information relative to immunoreactivity of recombinant proteins is contained in brief statements to the effect that no

antibodies were detected, with little information regarding the details of the sensitivity or selectivity of the analytical methods used. Sometimes, a report will indicate only that no neutralizing antibodies were detected. Although such a statement clearly indicates that no antibodies were detected that could block a biological activity of the protein under consideration, it remains unclear whether or not there were detectable antibodies that were not neutralizing. Such information is important because it would indicate whether the protein is at all immunogenic in humans.

In this review, consideration of the available literature related to immune responses to therapeutic recombinant human proteins will be organized according to a broad categorization of proteins: hormones, enzymes, cytokines, growth factors, antibodies, receptors, and antagonists. The review will not attempt to be comprehensive, but rather cover those recombinant human proteins with the most extensive clinical experience.

HORMONES

Insulin

Insulin was one of the first recombinant human proteins to gain regulatory approval and widespread use. Of course, the path had been blazed by years of use of insulin purified from animal sources. Generation of anti-insulin antibodies in diabetic patients receiving animal insulin was quite common, yet in general, the antibodies did not prevent efficacy and the therapeutic benefits far outweighed the somewhat adverse response. For these reasons, the first assessments of recombinant insulin (rh-insulin; *Escherichia coli* derived) compared the immunoreactivities of human insulin to the other forms that had been used. An entire supplement of *Diabetes Care* (Volume 5, Supplement 2, 1982) was devoted to the then new subject of immune response to rh-insulin. One of the first studies¹ assessed the immune response to rh-insulin administered by continuous subcutaneous (sc) infusion in two diabetic patients who had previously been treated with porcine or bovine insulin. The investigators observed a gradual decrease in circulating anti-insulin antibodies detectable over the course of 3 months, indicating a reduced immunoreactivity of the recombinant protein. A more extensive study² assessed the immunogenicity of rh-insulin in diabetic patients who had never received insulin treatment and in diabetic patients who had already been treated with monocomponent (single

animal species) insulin. In both studies, responses to rh-insulin were compared with matched control groups receiving other forms of insulin treatment. After 6 months of treatment, the amount of anti-insulin antibodies in those patients who were treated with just rh-insulin were significantly lower than those observed in control patients treated with monocomponent or conventional insulin. No significant difference in the amount of insulin antibodies or immune complexes was observed between patients who were switched to rh-insulin and those who continued monocomponent insulin. The authors conclude that the immunogenicity of rh-insulin was lower than that of monocomponent insulin. A larger study compared immune response to rh-insulin or porcine insulin in diabetic patients who had received no prior insulin therapy.³ After 12 months, the frequency of antibody positive subjects was 44% for those who receive rh-insulin and 60% for those who received porcine insulin. Even in the subjects treated with recombinant protein who were positive for antibodies, the extent of insulin binding was generally less than that seen with serum antibodies to porcine insulin. In children, the frequency of subjects who were positive for anti-insulin antibodies was the same, whether they received human or porcine insulin.⁴ However, the affinities of antibodies for binding to radiolabeled insulin (log insulin binding values) were somewhat lower in the human insulin-treated group. In summary, rh-insulin elicits anti-insulin antibodies, even in those subjects that had not received previous insulin therapy. It is possible that the frequency of dosing (at least once per day) contributes to the ubiquity of the immune response. In general, compared with those who received animal insulin, antibodies to human insulin have a lower affinity or binding capacity for insulin than do the antibodies generated against animal insulin. Furthermore, even when anti-recombinant human insulin antibodies do occur, they are rarely in the form of immunoglobulin E (IgE). Fireman et al.⁵ reported that 2 of 31 subjects treated with rh-insulin developed IgE antibodies, but their presence was transient and no apparent allergic response developed.

One factor in the production and formulation of insulin, and probably other proteins as well, is the presence of protein aggregates that have been reported to increase the frequency of elicited antibodies.⁶

Growth Hormone

In the mid-1980s, the new recombinant form of human growth hormone (r-hGH, *E. coli* derived) was evaluated in children and other subject populations as therapy for deficiencies in GH production or response. In a study of 36 children treated with methionyl r-hGH administered intramuscularly (im), 0.1 mg/kg three times per week for up to 4 years, Kaplan et al.⁷ found that the incidence of anti-GH antibodies was higher in subjects receiving r-hGH than it was in those receiving GH derived from bovine pituitaries. However, they also showed that when a more highly purified preparation of recombinant protein was used, the result was a reduction in the incidence of antibodies. A high incidence of anti-GH antibodies was also observed in a study that compared three different preparations of methionyl GH delivered im.⁸ Interestingly, they observed that the antibody titers were inversely related to the purity of the methionyl GH preparation. In later studies, r-hGH was typically delivered sc, several times per week, and use of *N*-methionine-free r-hGH resulted in a much lower incidence of anti-GH antibodies.⁹⁻¹² In a large study of 395 children with either GH deficiency or Turner syndrome treated for up to 54 months, a systematic evaluation was conducted of the anti-GH status every 2-3 months.¹² Nine of the 304 growth deficiency subjects developed anti-GH antibodies within the first 24 months; however, only two of those had antibodies for longer periods of time. There was no indication that antibodies impacted clinical efficacy. Although comparison of the immunogenicity of different sources of the same recombinant protein is usually difficult, due to different dosing regimens as well as assays, a study was conducted specifically to compare six different preparations of r-hGH.¹³ The investigators used both a solution-phase and a solid-phase assay to assess immunoreactivity over the course of 24 months. Of the 260 GH-deficient children treated, anti-GH antibodies were detected in subjects treated with three of the preparations. Most antibodies had low binding capacities; however, one particular source of GH yielded antibodies in 16% of its recipients, and those antibodies tended to have higher binding capacities. A biochemical comparison of the different preparations would certainly be helpful in understanding the apparent differences in immunogenicity. As with insulin, the presence of protein aggregates in the formulation

of growth hormone has been correlated with increased frequency of immune response.¹⁴

ENZYMES

Factor VIII

Recombinant factor VIII (mammalian cell expressed) has been developed to enhance the blood coagulation capacity of hemophiliacs. Hemophiliacs often have antibodies (inhibitors) against endogenous factor VIII, so it would not be surprising to observe antibodies that recognize exogenous recombinant protein. Shima et al.¹⁵ characterized the immunoglobulin G and M (IgG and IgM, respectively) antibodies generated in three previously untreated hemophilia A patients who generated anti-factor VIII antibodies after treatment with recombinant human factor VIII by infusion. Only low levels of immunoreactivity and neutralizing activity were measurable. In a clinical comparison of recombinant factor VIII from two different sources, no allergic reactions or inhibitory activity were observed.¹⁶ However, in a larger study that lasted for 3 years,¹⁷ 23.9% of subjects developed inhibitors. The authors modeled their results and predicted that in the study population of severe hemophilia A, the fraction of inhibitor-free subjects treated with recombinant factor VIII would be 88.4% after 8 days of exposure to recombinant factor VIII, 73.6% after 10 days, and 61.6% after 25 days. In general, the antibodies are of low titer and are comparable to the response obtained with human plasma-derived factor VIII.

DNase

Recombinant human DNase (mammalian cell expressed) has been delivered to cystic fibrosis sufferers to reduce the pulmonary symptoms of that disease. The drug is delivered as an aerosol to the lungs. In a study designed to evaluate both positive and adverse responses to DNase,¹⁸ 184 patients were treated with aerosol doses of DNase twice a day for 14 days followed by a 14-day wash-out period. This treatment regimen was repeated for a total of six treatment cycles. Detectable anti-DNase antibodies occurred in 16 of the 184 patients. The antibodies were all IgG, appeared to have no impact on symptoms or efficacy, and did not appear to cause an allergic response. In a small phase I clinical trial of DNase in systemic lupus erythematosus patients, subjects received

recombinant human protein by an initial intravenous (iv) dose followed by 10 sc doses.¹⁹ Although measurable levels of serum DNase were obtained, no neutralizing anti-DNase antibodies were detected. Because the sizes of the studies were very different, as were the doses, it is difficult to make any clear conclusion regarding the different routes of delivery and the resulting immunogenicity.

Tissue Plasminogen Activator

Recombinant human tissue plasminogen activator (rt-PA, mammalian cell expressed) is delivered as an iv infusion for acute treatment of myocardial infarction. Several investigators have evaluated the immune response to rt-PA.²⁰⁻²² Reed et al.²⁰ evaluated subjects treated with rt-PA using two different antibody assays. Using an immunoprecipitation assay, they found 3 out of 932 treated subjects became positive for low titer antibodies against rt-PA. After a 2-year follow-up, all subjects were negative. Another 754 subjects were evaluated using an ELISA assay, and none were found to be antibody positive. Cugno et al.²² demonstrated that previous treatment with the recombinant protein can enhance the immune response upon re-exposure. One subject, previously treated with rt-PA was retreated 3 years later. That subject yielded the highest and longest lasting anti-t-PA antibody titers in their study.

CYTOKINES

Interleukin 2

Interleukin 2 (IL-2, *E. coli* derived) has been developed for its anti-tumor activity. Because it is itself immunostimulatory, it might be expected, when delivered as a therapeutic, to promote immune response against itself. Ardizzoni et al.²³ evaluated efficacy and adverse response to recombinant human IL-2 administered by continuous infusion. Cancer patients received continuous infusion for a week at a time with 1-day to 1-week breaks in between doses. Not unexpectedly, IL-2 induced multiple indications of an activated immune system; however, no anti-IL-2 antibodies were reported. In a larger phase I/II trial, 217 cancer patients were evaluated after IL-2 treatment.²⁴ They received IL-2 by continuous iv infusion or by sc injection. Of those receiving infusions, 52% developed antibodies, whereas 47% of those receiving sc injections developed antibodies. Of those generating antibodies, 6% of continuous infusion patients and 10% of sc patients evi-

denced neutralizing activity, as assessed by an IL-2-dependent cell proliferation assay, but only after a second cycle of treatment. The development of antibodies did not appear to impact the clinical course of test subjects. This study is one of the most thorough evaluations of induced antibodies. The investigators used a full spectrum of assays to detect antibodies, assess neutralizing activity, confirm immunoreactivity in an absorption study, and finally to isotype the detected antibodies. They found that of all the subjects with detectable antibodies to IL-2, 88–100% had IgG antibodies and 24–38% had IgM.

Interleukin 3

Interleukin 3 (IL-3) has also been developed for potential anti-cancer activity. In a study of 31 refractory cancer patients,²⁵ recombinant human IL-3 was administered daily for 15 days by the sc route, and dosing cycles were repeated at intervals of 28 days. Eight percent of patients developed anti-IL-3 antibodies by day 29 of cycle 1, but the antibodies were not neutralizing. The occurrence of antibodies did not increase with subsequent cycles of treatment. Recombinant human IL-3 has also been constructed as a fusion protein with granulocyte-macrophage colony stimulating factor (PIXY321) for cancer therapy. PIXY321 was administered sc for 18 consecutive days of a 28-day cycle with or without carboplatin treatment.²⁶ After two cycles, 92% of the 49 subjects treated with PIXY321 had developed anti-PIXY321 neutralizing antibodies that suppressed *in vivo* biological activity. Immunoreactivity to the components of the construct was not synchronous. After cycle 1, >80% of subjects had antibodies to IL-3, but not to GM-CSF. However, by the end of cycle 2, 64% had antibodies to GM-CSF as well. This result represents one of the most glaring examples of potent immunoreactivity in a recombinant protein product and one of the rare cases in which immunoreactivity undermined the therapeutic potential of that product. It is likely that the construction of the fusion protein created unique epitopes not represented in either of the constituent proteins.

Interleukin 12

Interleukin 12 (IL-12) was evaluated in a phase I/II clinical study of 60 hepatitis C patients.²⁷ Patients were treated sc weekly for 10 consecutive weeks. The authors report that “significant anti-

rhIL-12 antibody titers were not detectable in any patient.”

ANTIBODIES

Anti-CD3

OKT3 is a murine anti-CD3 monoclonal antibody that acts as an immunosuppressant by blocking human T-cell proliferation. Being a murine antibody, it is highly immunogenic when administered clinically.²⁸ A humanized version of OKT3, denoted hOKT3 γ 4, has been evaluated in a phase I clinical trial, administered as three biweekly iv infusions, using doses of 50–1600 μ g/injection.²⁹ Of the 24 subjects in the trial, six developed anti-idiotypic antibodies in a nondose-dependent manner. Four of six patients receiving multiple cycles of therapy developed antibodies. None of the subjects appeared to experience interference in the T-cell activation by the antibody.

Anti-Her2

A humanized antibody, trastuzumab (mammalian cell expressed), that blocks the Her2 receptor, has been developed as therapy for breast cancer. In a phase II clinical trial, anti-Her2 was evaluated for efficacy and toxicity in 46 metastatic breast cancer subjects.³⁰ After an iv loading dose of 250 mg, the antibody was administered as 10 weekly 100-mg doses. No antibodies were detected in any of the subjects.

Anti-IgE

A humanized antibody against human IgE (rhu-Mab-E25, mammalian cell expressed) is being developed as therapy for allergic asthma. In a phase II clinical trial, no subjects developed antibodies against the humanized antibody.³¹

Anti-Respiratory Syncytial Virus (RSV)

MEDI-493 is a recombinant humanized monoclonal antibody (mammalian expression) to respiratory syncytial virus. The antibody has been tested in several clinical trials. Unlike the other humanized monoclonal antibodies, MEDI-493 evidenced a substantial frequency of elicited antibodies during clinical trials.³² Ten of 62 subjects in one trial and 13 or 56 subjects in another developed anti-MEDI-493 antibodies with low titers of 1:10 to 1:40 after im administration. In the largest placebo-controlled phase III clinical trial, 14 of 500 placebo-treated and 12 of 1002 drug-treated subjects developed low titer antibodies. Thus, the frequency was higher in the placebo group. In this

case, it is possible that the antibody assay was not sufficiently discriminating.

Anti-IL-2 Receptor

Daclizumab (mammalian expression) is a humanized monoclonal antibody against the α subunit of the IL-2 receptor. By blocking the receptor and preventing IL-2-induced T-cell proliferation, it functions as an immunosuppressant. In two phase III clinical trials, 12 and 18% of treated subjects developed anti-idiotypic antibodies. However, there was no evidence to indicate that antibodies impacted pharmacokinetics, saturation of IL-2 receptors or immunosuppressive efficacy.³³

RECEPTORS AND ANTAGONISTS

CD4

Soluble human CD4 was evaluated in a phase I clinical trial for efficacy in 11 children suffering from human immunodeficiency virus (HIV).³⁴ The protein was administered at doses of 100, 300, and 1000 $\mu\text{g/kg}$ per day for 12 weeks, followed by another 12 weeks of dosing in combination with dideoxyinosine. Two of the 11 subjects developed "low-level anti-CD4 antibodies". As an alternative approach to blocking CD4 receptors on HIV virus, a construct of recombinant CD4-IgG was evaluated in a phase I clinical trial with 16 subjects suffering from AIDS or AIDS-related complex.³⁵ The drug was administered weekly for 12 weeks at doses as high as 1 mg/kg by iv or im routes. No antibodies were detected. In another study of the fusion protein,³⁶ CD4-IgG was coadministered with zidovudine for 12 weeks. CD4-IgG at 300 or 3000 $\mu\text{g/kg}$ twice per week plus zidovudine daily were administered to 41 AIDS patients. After 12 weeks, no antibodies to the fusion protein were detected. In this case, it is possible that the immunocompromised status of the study populations may have impacted their ability to generate an immune response to the administered protein.

TNF Receptor

Lenercept, a recombinant TNF receptor p55-IgG fusion protein, has been evaluated in a phase II clinical trial with 168 multiple sclerosis patients.³⁷ Subjects received iv doses of 10–100 mg every 4 weeks for up to 48 weeks. More than 88%

of subjects developed anti-lenercept antibodies that appeared to lead to enhanced clearance of the drug. Antibody titers correlated with dose of lenercept, particularly at the earliest time points. In contrast, only 5% of placebo-treated subjects had detectable immunoreactivity with lenercept.

Etanercept (mammalian expression) is a dimeric fusion protein composed of the p75 portion of the TNF receptor linked to the Fc portion of IgG₁. In clinical trials evaluating its utility in rheumatoid arthritis, adult subjects received 10–25 mg of the protein twice a week by sc injection, with or without methotrexate, for up to 6 months. Pediatric subjects received doses of 0.4 mg/kg. Overall, 16% of subjects developed anti-etanercept antibodies, although none were considered neutralizing.³⁸ This is another case where direct biochemical and immunochemical comparison of the two protein constructs could lead to insights into the immunoreactivity of protein drugs.

Interleukin 1 Receptor

A recombinant form of truncated human interleukin 1 (IL-1) receptor was tested in a phase I trial with 11 acute myeloid leukemia patients.³⁹ Subjects received iv loading doses of 500–2000 $\mu\text{g/m}^2$ followed by 13 daily sc doses of 250–1000 $\mu\text{g/m}^2$. No antibodies were detected after the course of treatment.

GROWTH FACTORS

PDGF

Recombinant PDGF-BB (yeast expression) plus recombinant IGF-1 were tested in 38 human subjects to assess the ability of those proteins to promote periodontal regeneration.⁴⁰ Each growth factor at 50 or 150 $\mu\text{g/mL}$ were applied to the periodontal lesion sites. No antibodies to either protein were detected.

G-CSF

G-CSF (*E. coli*) stimulates hematopoiesis and is used in patients suffering from neutropenia. Laricchia-Robbio et al.⁴¹ characterized pre-existing and therapy-induced antibodies to human G-CSF in untreated subjects as well as lymphoma patients. Fifteen of 135 untreated subjects contained low concentrations of anti-G-CSF antibodies, as did 5 of 40 umbilical cord blood samples. Treatment with G-CSF led to a rise in anti-G-CSF antibody titers of both IgG and IgM types in

the majority of subjects treated. However, the antibodies did not appear to affect drug efficacy.

GM-CSF

Ragnhammar et al.⁴² investigated the induction of antibodies in patients receiving recombinant GM-CSF (*E. coli*). They found that, when administered to 28 cancer patients sc at 250 $\mu\text{g}/\text{m}^2/\text{day}$ for 10 days every month for 4 months, 95% of patients not receiving other chemotherapy generated anti-GM-CSF antibodies after the second cycle. The antibodies appeared to alter GM-CSF pharmacokinetics in the third and fourth cycles and impact ability of the drug to enhance numbers of granulocytes and monocytes. The authors looked for serum immune complexes but found none. It is important to recognize that for GM-CSF, and perhaps other proteins with mitogenic activity, the activity of the drug could impact its pharmacokinetics. The mitogenic activity of the drug leads to an increase in target cells and their mitogen receptors. This phenomenon has led to observation of inverse relationships between neutrophil count and serum levels of G-CSF⁴³ or GM-CSF.⁴⁴ Thus changes in pharmacokinetics may not be directly attributable to circulating anti-drug antibodies. In another study,⁴⁵ 20 colon carcinoma patients were treated with GM-CSF plus a colon carcinoma reactive antibody. The dosing regimen was similar to that used in a previous report,⁴² and as in that case, 95% of subjects generated anti-GM-CSF antibodies. However, only 40% of those with antibodies had neutralizing antibodies, as assessed by an *in vitro* bioassay. Although those with neutralizing antibodies evidenced a reduced expansion of leukocytes, neutrophils, and eosinophils, no such abrogation of efficacy was seen in those without neutralizing antibodies.

Erythropoietin

Erythropoietin (mammalian) has been developed to stimulate erythropoiesis in anemic patients. Multiple doses have been administered both iv and sc to normal volunteers as well as sufferers of renal disease. The lack of detectable anti-erythropoietin antibodies has been reported in several studies.⁴⁶⁻⁴⁸ Nonetheless, several papers exist that report the development of assays for anti-erythropoietin antibodies, suggesting that they can occur.⁴⁹ In addition, a few communica-

tions have reported sporadic, infrequent observations of anti-erythropoietin antibodies.^{50,51}

INTERFERONS

Interferon α

Interferon α -2a (IFN α -2a; *E. coli*) and interferon α -2b (IFN α -2b, *E. coli*) appear to be the most notably immunogenic recombinant human proteins. IFN α -2a has been developed for treatment of hepatitis C and IFN α -2b has been developed for leukemia therapy. Many papers have reported on antibodies generated in subjects receiving chronic treatment with IFN α -2a. Bonetti et al.⁵² looked in detail at antibodies generated against therapeutic IFN α -2a in 60 patients with chronic hepatitis C. Of the treated subjects, 61% generated anti-IFN α -2a within 6 months of treatment, and the majority of the subjects with antibodies evidenced a neutralizing effect of those antibodies in an *in vitro* bioassay. Of those subjects that showed no therapeutic response to interferon therapy, 75% had detectable antibodies. In another trial of interferon α -2a for hepatitis C,⁵³ antibodies were detectable in 32% of the treated subjects. Forty percent of nonresponders had antibodies versus 14% of the responders, demonstrating a correlation between presence of antibodies and lack of efficacy. In a small study of metastatic renal cell carcinoma patients treated with IFN α -2a plus prednisone,⁵⁴ 8 of 26 patients developed antibodies to IFN. Two of those patients had high levels of neutralizing antibodies that coincided with low serum IFN levels, and in one patient, the loss of responsiveness. In contrast to these results, Dianzani et al.⁵⁵ specifically evaluated 175 patients undergoing recombinant IFN α -2b (rIFN α -2b) therapy in an attempt to correlate antibody production to disease progression. IFN α -2b differs from the 2a variant by one amino acid. IFN α -2a has Lys at position 23, whereas IFN α -2b has Arg at that position. They used a bioassay to quantify neutralizing antibodies and found only one patient producing antibodies. The authors conclude that rIFN α -2b has minimal antigenicity, even when administered for prolonged periods of time. In comparison, other investigators conclude that long-term treatment of CML with rIFN could be problematic because of the induction of neutralizing antibodies.⁵⁶ rIFN α -2b was tested for treatment of malignant carcinoid tumors.⁵⁷ Although good efficacy in tumor size reduction was observed, the authors conclude that it is important to recognize

that antibodies neutralizing IFN may develop in some patients, with concomitant loss of anti-tumor effects. Figlin et al.⁵⁸ evaluated 19 renal carcinoma patients treated with IFN α -2a im beginning at 3×10^6 units and escalating to 36×10^6 units, 5 days/week for a total induction period of 14 weeks. Twelve patients developed measurable anti-IFN antibodies and six of those were neutralizing. Yet, the presence of neutralizing antibodies did not correlate with duration of response to treatment, nor to clinical toxicity. In a review of clinical experience with IFN α -2a in cancer patients, Jones and Itri⁵⁹ reported that of the >1300 patients receiving treatment, ~27% developed antibodies to IFN α -2a. However, no adverse clinical manifestations appeared to have been associated with antibody development. Itri et al.⁶⁰ include a table summarizing the reported incidences of anti-IFN α -2a antibodies through the late 1980s. Subjects had typically been treated with a regimen of three im doses per week for 8 to 24 weeks. Of 781 subjects, 24% had neutralizing antibodies. However, there was no statistically significant difference in antibody incidence between responders and nonresponders across the studies. In fact, on average, patients with antibodies survived longest.

These data and others suggested that IFN α -2a was more immunogenic than was IFN α -2b; a surprising observation given the minimal structural difference between them. Another possibility, however, was that the apparent differences in immunogenicity were a result of the differences in assays used to assess immunoreactivity. Blood samples from subjects treated with IFN α -2a were typically assayed by an enzyme immunoassay (EIA) or neutralizing assay that directly quantified antibodies that bound to IFN α -2a or blocked its activity. Samples for antibodies to IFN α -2b were typically assayed by an immunoradiometric assay (IRMA), a competitive immunoassay that assesses the ability of a sample to compete with a radiolabeled monoclonal antibody for binding to the recombinant protein.

Itri et al.⁶⁰ compared responses in two different antibody assays. They found that of 100 subjects treated with IFN α 2a that were antibody positive by both an EIA as well as a neutralizing assay, only 52 were positive by an IRMA assay. Furthermore, of 50 subjects that were antibody positive, but neutralizing negative, all 50 were negative by the IRMA. Because the IRMA assay is based on a competition between serum antibodies and a labeled anti-IFN α -2 monoclonal antibody, the as-

say would not detect antibodies that recognize epitopes distant from the epitope for the monoclonal antibody. However, these findings were contradicted in part by Spiegel et al.⁶¹ who evaluated the serum samples from subjects treated with IFN α 2b in three different assays [IRMA, enzyme-linked immunosorbent assay (ELISA), and bioassay]. They found that the incidence of antibodies to IFN α -2b was consistently lower. Immunoreactivity of IFN α -2b was even lower when administered intranasally or intralesionally,⁶² and in those with neutralizing activity, there was no discernible difference in the incidence or severity of side effects or of clinical benefit. Hayden et al.⁶³ observed antibodies and neutralizing antibodies when IFN α -2a was administered intra-nasally. Bekisz et al.⁶⁴ directly compared treatment of hairy cell leukemia subjects with either IFN α -2a or IFN α -2b. They found the same low incidence of antibodies, regardless of interferon variant.

In a Chinese trial of IFN α -2a in chronic hepatitis B patients, 39% of the treated subjects developed antibodies. Interestingly, antibodies were more likely to develop in the low-dose group rather than the high-dose group. High titer antibodies were often associated with failure to respond to therapy.⁶⁵ In another study of IFN α -2a in renal cell carcinoma patients,⁶⁶ appearance of anti-IFN antibodies occurred with a higher frequency in patients receiving oligomeric protein versus those who received monomeric protein. In a more recent communication, McSweeney et al.⁶⁷ observed anti-IFN α -2a antibodies in CLL patients treated sc three times per week for several months. Over half of the patients developed antibodies, some at high titers, which appeared to impact clinical efficacy.

The basis for the discrepancy in apparent immunoreactivities between IFN α -2a and IFN α -2b remains unresolved. Although the assays and patient populations are likely to be factors, direct comparisons have suggested that at least to some degree the differences are real. It would be surprising for the Lys to Arg substitution to be the cause. Formulation or contaminant profiles may be playing a role in this case.

Interferon β

Interferon β (IFN β ; mammalian expression) has been approved for treatment of multiple sclerosis and has been evaluated as a chemotherapeutic. In clinical studies with cancer patients receiving recombinant IFN β , im administration was far more immunogenic than iv administration.⁶⁸ Twenty-

four of 30 patients receiving both im and iv doses developed serum antibodies to the recombinant protein. However, only one of those had neutralizing activity. Antibodies did not appear to alter the pharmacokinetics of IFN after iv administration, and the antibody levels decreased after discontinuation of IFN administration. When IFN β administration was limited to im or iv routes, the authors observed antibody development in only 2 of 36 iv patients versus 20 of 25 patients who received im administration. No antibody-related clinical manifestations were detected. They observed a modest correlation between antibody titers and cumulative dose of IFN β . Also, somewhat uniquely, the authors attempted to correlate a titer value with a concentration value. They estimated that their highest titer subject had a titer of 15,000, equal to 750 $\mu\text{g/mL}$ of serum IgG. Furthermore, they took the added measure of looking for anti-*E. coli* protein antibodies in an effort to fully assess the sources of immunoreactivity against administered therapeutic. They found no anti-*E. coli* immunoreactivity within the constraints of their assay. Larocca et al.⁶⁹ treated 335 patients by the iv route and 136 patients by the sc route, three times per week. The frequency of induced antibodies was 10 times greater in the sc group. Furthermore, they determined that anti-IFN β antibodies detectable by ELISA always appeared sooner than neutralizing activity by an average of 3 months. In a rather unique trial,⁷⁰ malignant melanoma patients were treated with natural IFN β plus recombinant interferon γ (IFN γ). IFN β was delivered by either the iv or sc route, whereas IFN γ was always administered by the sc route. Ninety-two percent of the sc patients and 30% of the iv patients developed antibodies to IFN β . In contrast, no subject developed antibodies to recombinant IFN γ .

SUMMARY

Overall, antibodies to recombinant human proteins have not been a major impediment to their use as a class. The presence of antibodies has not necessarily been detrimental to the clinical efficacy of a protein. Certainly, some proteins have proved to be more immunogenic than others, and a minority have proved to be nonviable as therapeutics because of their immunoreactivity. However, early fears of significant anaphylactic responses to large doses of proteins have not been borne out. No particular property of a protein has

arisen as an obvious predictor of immunogenicity in humans. The generation of potentially unusual epitopes, such as *N*-methionine for GH or the fusion site for PIXY321, has been associated with enhanced immunoreactivity. Yet the fusion protein etanercept has not suffered from the same effect. The likelihood of an immune response is increased following administration via sc, im, and intranasal delivery routes compared with via the iv route. Often, but not always, repeated dosing leads to enhanced opportunity for an immune reaction, yet it has not compromised the therapeutic viability of recombinant human insulin and growth hormone. There is no apparent correlation between the expression system, *E. coli* or mammalian cell, used for production of the protein and its immunoreactivity.

Elicited antibodies could effectively sequester a protein from metabolic pathways and thereby reduce the clearance of the therapeutic agent. Such mechanisms have been inferred for naturally occurring autoantibodies to some cytokines and growth factors, although the actual cause or role for those antibodies has not been clearly elucidated.⁷¹⁻⁷³ No clear evidence of such an effect on therapeutic proteins has been reported.

The occurrence and extent of immunogenicity has been a matter of empirical observation. When an immune response to administered human proteins does occur, it appears to be largely in the form of IgG antibodies. An assessment of neutralizing activity has been the best predictor of the impact of elicited antibodies on pharmacokinetics or efficacy, but there is no model or method that can substitute at this time for direct clinical assessment. A good indication of current thinking on immunoreactivity of recombinant proteins in the clinical setting can be gleaned from the transcript of the FDA Biological Response Modifiers Advisory Committee meeting entitled "Immune Reactions Against Therapeutic and Diagnostic Biological Products" that occurred on July 15, 1999.⁷⁴ The committee recognized that:

- (1) it is not possible at this time to standardize antibody assay formats;
- (2) the occurrence of antibodies, even neutralizing antibodies, does not necessarily mitigate against the therapeutic viability of a recombinant protein drug; and
- (3) the most important criterion for assessing the significance of antibodies against therapeutic proteins is the correlation of antibody incidence and amount with clinical

cal pharmacokinetics, pharmacodynamics, efficacy, and safety.

REFERENCES

1. Spijker AJ, Poortman J, Thijssen JH, Erkelens DW. 1982. *Diabetes Care* 5 Suppl 2:171–174.
2. Di Mario U, Arduini P, Tiberti C, Lombardi G, Piétravalle P, Andreani D. 1986. *Diabetes Res Clin Pract* 2:317–324.
3. Fineberg SE, Galloway JA, Fineberg NS, Rathbun MJ, Hufferd S. 1983. *Diabetologia* 25:465–469.
4. Marshall MO, Heding LG, Villumsen J, Akerblom HK, Baevre H, Dahlquist G, Kjaergaard JJ, Knip M, Lindgren F, Ludvigsson J, et al. 1988. *Diabetes Res* 9:169–175.
5. Fireman P, Fineberg SE, Galloway JA. 1982. *Diabetes Care* 5 Suppl 2:119–125.
6. Kahn CR, Rosenthal AS. 1979. *Diabetes Care* 2:283–295.
7. Kaplan SL, Underwood LE, August GP, Bell JJ, Blethen SL, Blizzard RM, Brown DR, Foley TP, Hintz RL, Hopwood NJ, et al. 1986. *Lancet* 1:697–700.
8. Takano K, Shizume K, Hizuka N, Okuno A, Umino T, Kobayashi Y, Kusano S, Nakajima H, Irie M, Hibi I, et al. 1986. *Endocrinol Jpn* 33:589–596.
9. Albertsson-Wikland K. 1987. *Acta Paediatr Scand Suppl* 331:28–34.
10. Buzi F, Buchanan CR, Morrell DJ, Preece MA. *Clin Endocrinol* 30:531–538.
11. Takano K, Shizume K, Hibi I. 1989. *Acta Endocrinol* 120:559–568.
12. Zeisel HJ, Lutz A, von Petrykowski W. 1992. *Horm Res* 37 Suppl 2:47–55.
13. Rougeot C, Marchand P, Dray F, Girard F, Job JC, Pierson M, Ponte C, Rochiccioli P, Rappaport R. *Horm Res* 35:76–81.
14. Moore WV, Leppert P. 1980. *J Clin Endocrinol Metab* 51:691–697.
15. Shima M, Sawamoto Y, Nakai H, Kamisue S, Murakami T, Morichika S, Tanaka I, Hayashi K, Yoshioka A. 1995. *Int J Hematol* 62:35–43.
16. Brackmann HH, Aygoren E, Scharrer I, Schwaab R, Hammerstein U, Oldenburg J. 1993. *Blood Coagul Fibrinolysis* 4:421–424.
17. Bray GL, Gomperts ED, Courter S, Gruppo R, Gordon EM, Manco-Johnson M, Shapiro A, Scheibel E, White G 3rd, Lee M. 1994. *Blood* 83:2428–2435.
18. Eisenberg JD, Aitken ML, Dorkin HL, Harwood IR, Ramsey BW, Schidlow DV, Wilmott RW, Wohl ME, Fuchs HJ, Christiansen DH, Smith AL. 1997. *J Pediatr* 131:118–124.
19. Davis JC Jr, Manzi S, Yarboro C, Rairie J, Mcinnes I, Averthelyi D, Sinicropi D, Hale VG, Balow J, Austin H, Boumpas DT, Klippel JH. 1999. *Lupus* 8:68–76.
20. Reed BR, Chen AB, Tanswell P, Prince WS, Wert RM Jr, Glaesle-Schwarz L, Grossbard EB. 1990. *Thromb Haemost* 64:276–280.
21. Jang IK, Vanhaecke J, De Geest H, Verstraete M, Collen D, Van de Werf F. 1986. *J Am Coll Cardiol* 8:1455–1460.
22. Cugno M, Cicardi M, Colucci M, Bisiani G, Merlini PA, Spinola A, Paonessa R, Agostoni A. 1996. *Thromb Haemost* 76:234–238.
23. Ardizzoni A, Bonavia M, Viale M, Baldini E, Mereu C, Verna A, Ferrini S, Cinquegrana A, Molinari S, Mariani GL, et al. 1994. *Cancer* 73:1353–1360.
24. Scharenberg JG, Stam AG, von Blomberg BM, Roest GJ, Palmer PA, Franks CR, Meijer CJ, Scheper RJ. 1994. *Eur J Cancer* 30A:1804–1809.
25. Bukowski RM, Olencki T, Gunn H, McLain D, Budd GT, Sandstrom K, Tuason L, Redovan C, Rayman P, Tubbs R, Resta D, Elson P, Finke J. 1996. *Clin Cancer Res* 2:347–357.
26. Miller LL, Korn EL, Stevens DS, Janik JE, Gause BL, Kopp WC, Holmlund JT, Curti BD, Sznol M, Smith JW 2nd, Urba WJ, Donegan SE, Watson TM, Longo DL. 1999. *Blood* 3:3250–3258.
27. Zeuzem S, Hopf U, Carreno V, Diago M, Shiffman M, Grune S, Dudley FJ, Rakhit A, Rittweger K, Yap SH, Koff RS, Thomas HC. 1999. *Hepatology* 29:1280–1287.
28. Chatenoud L. 1993. *Clin Transplant* 7:422–430.
29. Richards J, Auger J, Peace D, Gale D, Michel J, Koons A, Haverty T, Zivin R, Jolliffe L, Bluestone JA. 1999. *Cancer Res* 59:2096–2101.
30. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L. 1999. *Semin Oncol* 26 Suppl 12:78–83.
31. Milgrom H, Fick RB Jr, Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ. 1999. *N Engl J Med* 341:1966–1973.
32. Center for Biologics Evaluation and Review, Product Approval Information—Licensing Action, Palivizumab, <http://www.fda.gov/cber/review/palimed061998r2.pdf>
33. Center for Biologics Evaluation and Review, Product Approval Information—Licensing Action, Daclizumab, <http://www.fda.gov/cber/review/dac1hof121097r1.pdf>
34. Husson RN, Chung Y, Mordenti J, Butler KM, Chen S, Duliege AM, Brouwers P, Jarosinski P, Mueller BU, Ammann A, et al. 1992. *J Pediatr* 121:627–633.
35. Hodges TL, Kahn JO, Kaplan LD, Groopman JE, Volberding PA, Amman AJ, Arri CJ, Bouvier LM, Mordenti J, Izu AE, et al. 1991. *Antimicrob Agents Chemother* 35:2580/2586.
36. Meng TC, Fischl MA, Cheeseman SH, Spector SA, Resnick L, Boota A, Petrakis T, Wright B, Richman

- DD. 1995. *J Acquired Immune Defic Syndr Hum Retrovirol* 8:152–160.
37. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. 1999. *Neurology* 53:457–465.
38. Center for Biologics Evaluation and Review, Product Approval Information—Licensing Action, Etanercept, <http://www.fda.gov/cber/label/etanimm110298lb.pdf>.
39. Bernstein SH, Fay J, Frankel S, Christiansen N, Baer MR, Jacobs C, Bloesch C, Hanna R, Herzig G. 1999. *Cancer Chemother Pharmacol* 43:141–144.
40. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE. 1997. *J Periodontol* 68:1186–1193.
41. Laricchia-Robbio L, Moscato S, Genua A, Liberati AM, Revoltella RP. 1997. *J Cell Physiol* 173:219–226.
42. Ragnhammar P, Friesen HJ, Frodin JE, Lefvert AK, Hassan M, Osterborg A, Mellstedt H. 1994. *Blood* 84:4078–4087.
43. Takatani H, Soda H, Fukuda M, Watanabe M, Kinoshita A, Nakamura T, Oka M. 1966. *Antimicrob Agents Chemother* 40:988–991.
44. Petros WP, Rabinowitz J, Stuart AR, Gilbert CJ, Kanakura Y, Griffin JD, Peters WP. 1992. *Blood* 80:1135–1140.
45. Wadhwa M, Bird C, Fagerberg J, Gaines-Das R, Ragnhammar P, Mellstedt H, Thorpe R. 1996. *Clin Exp Immunol* 104:351–358.
46. Nielsen OJ, Thaysen JH. 1989. *J Intern Med* 226:89–94.
47. Mohini R. 1989. *Semin Nephrol* 9 Suppl 1:16–21.
48. McMahon FG, Vargas R, Ryan M, Jain AK, Abels RI, Perry B, Smith IL. 1990. *Blood* 76:1718–1722.
49. Urrea JM, de la Torre M, Alcazar R, Peces R. *Clin Chem* 43:848–849.
50. Peces R, de la Torre M, Alcazar R, Urrea JM. 1996. *N Engl J Med* 335:523–524.
51. Casadevall N, Dupuy E, Molho-Sabatier P, To-belem G, Varet B, Mayeux P. 1996. *N Engl J Med* 334:630–633.
52. Bonetti P, Diodati G, Drago C, Casarin C, Scacabarozzi S, Realdi G, Ruol A, Alberti A. 1994. *J Hepatol* 20:416–420.
53. Douglas DD, Rakela J, Lin HJ, Hollinger FB, Taswell HF, Czaja AJ, Gross JB, Anderson ML, Parent K, Fleming CR, et al. 1993. *Dig Dis Sci* 38:601–607.
54. Fossa SD, Lehne G, Gunderson R, Hjelmaas U, Holdener EE. 1992. *Int J Cancer* 50:868–870.
55. Dianzani F, Antonelli G, Amicucci P, Cefaro A, Pintus C. 1989. *J Interferon Res* 9 Suppl 1:S33–S36.
56. Freund M, von Wussow P, Diedrich H, Eisert R, Link H, Wilke H, Buchholz F, LeBlanc S, Fonatsch C, Deicher H, et al. 1989. *Br J Haematol* 72:350–356.
57. Oberg K, Alm G, Magnusson A, Lundqvist G, Theodorsson E, Wide L, Wilander E. 1989. *J Natl Cancer Inst* 81:531–535.
58. Figlin RA, deKernion JB, Mukamel E, Palleroni AV, Itri LM, Sarna GP. 1988. *J Clin Oncol* 6:1604–1610.
59. Jones GJ, Itri LM. 1986. *Cancer* 57 Suppl:1709–1715.
60. Itri LM, Sherman MI, Palleroni AV, Evans LM, Tran LL, Champion M, Chizzonite R. 1989. *J Interferon Res* 9 Suppl 1:S9–S15.
61. Spiegel RJ, Jacobs SL, Treuhart MW. 1989. *J Interferon Res* 9 Suppl 1:S17–S24.
62. Spiegel RJ, Spicehandler JR, Jacobs SL, Oden EM. 1986. *Am J Med* 80:223–228.
63. Hayden FG, Mills SE, Innes DJ, Darragh A, Just M, Linder-Chiccolunghi SN, Gauci L. 1987. *Antiviral Res* 8:139–149.
64. Bekisz JB, zur Nedden DL, Enterline JC, Zoon KC. 1989. *J Interferon Res* 9 Suppl 1: S1–S7.
65. Lok AS, Lai CL, Leung EK. 1990. *Hepatology* 12:1266–1270.
66. Steineck G, Strander H, Carbin BE, Borgstrom E, Wallin L, Achtnich U, Arvidsson A, Soderlund V, Naslund I, Esposti PL, et al. 1990. *Acta Oncol* 29:155–162.
67. McSweeney EN, Giles FJ, Worman CP, Jewel AP, Tsakona CP, Hoffbrand AV, Mehta AB, Newland AC, Cawley JC, Galvani D, et al. 1993. *Br J Haematol* 85:77–83.
68. Konrad MW, Childs AL, Merigan TC, Borden EC. 1987. *J Clin Immunol* 7:365–375.
69. Larocca AP, Leung SC, Marcus SG, Colby CB, Borden EC. 1989. *J Interferon Res* 9 Suppl 1:S51–S60.
70. Dummer R, Muller W, Nestle F, Wiede J, Dues J, Lechner W, Haubitz I, Wolf W, Bill E, Burg G. 1991. *Cancer* 67:2300–2304.
71. Bendtzen K, Svenson M, Jonsson V, Hippe, E. 1991. *Immunol. Today* 11:167–169.
72. Wadwa M, Meager A, Dilger P, Bird C, Dolman C, Das RG, Thorpe R. 2000. *Immunology* 99:113–123.
73. Dicou E, Nerriere VJ. 1997. *Neuroimmunology* 75:200–203.
74. Center for Biologics Evaluation and Review (<http://www.fda.gov/ohrms/dockets/ac/cber99.htm>).