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Immunogenicity and Allergenic Potential of Animal and Human Insulins

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Immunological complications of insulin therapy have been evident since animal insulins became available for the treatment of diabetes mellitus in 1922. Insulin allergy has been particularly common, with local symptoms still occurring in ~5% of all patients. Insulin antibodies of high titers were observed in many patients treated with early insulin preparations containing proinsulin, C-peptide, and other peptide contaminants. Immunoglobulin G-insulin antibodies of very high levels can lead to immune-mediated insulin resistance, which is now extremely rare because of the widespread use of highly purified porcine insulin and human insulin preparations. Lipoatrophy, which was reported in 10-55% of patients treated with nonpurified bovine/porcine insulin preparations, has almost disappeared in patients since the advent of exclusive human insulin treatment. In view of the wide spectrum of immune-mediated complications of insulin therapy, much attention has been directed to the reduced immunogenicity and allergenicity of highly purified porcine insulins and the more recently available recombinant and semisynthetic human insulin preparations. Insulin antibodies of the immunoglobulin G and immunoglobulin E type can develop, however, in very low titers in patients treated exclusively with human insulin. Frequency and levels of immunoglobulin G insulin antibodies are identical in patients treated either with biosynthetic or semisynthetic human insulin preparations. Allergic symptoms to human insulin are now found in <1% of de novo-treated patients, but still may occur when human insulin is used in the insulin-allergic patient. In summary, immunological complications of insulin therapy have decreased significantly during the last two decades and are now predominantly observed in patients with interrupted insulin therapy.

Two types of antibodies to insulin are now well defined; insulin antibodies that occur in patients who have been exposed to exogenous insulin treatment and insulin autoantibodies that appear in insulin-naïve subjects. Soon after the introduction of insulin in the 1920s, clinicians became aware of the immunological complications that result from reaction to its therapeutic use (1). Early insulin preparations were very impure and were either single species or

mixtures of bovine and porcine insulin. In the 1950s, Berson et al. (2) used [¹³¹I]insulin to identify and characterize insulin antibodies in vitro and were able to detect antibodies in almost all insulin-treated patients (2). The detection of insulin antibodies was very important, since Berson and Yalow described the kinetics of the interaction between [¹³¹I]insulin and antibody and were able to exploit these observations to measure insulin concentrations over a wide range—the birth of RIA.

Impure insulin preparations may contain several islet-cell peptides, proinsulin, C-peptide, or pancreatic polypeptide, glucagon, and somatostatin all of which are immunogenic (3). Nevertheless, differences in the purity and primary structure of insulin preparations cannot alone account for its immunogenicity, because patients treated with biosynthetic or semisynthetic human insulin also develop antibodies (4,5). It is likely that insulin aggregates (e.g., covalent insulin dimers) and the use of depot preparations of human insulin (e.g., NPH, insulin zinc suspensions) contribute to the continued antibody formation.

IMMUNE RESPONSE TO INSULIN

Insulin injected into the subcutaneous tissue of diabetic patients can elicit various reactions from the immune system. Sensitization of T-cells can induce delayed hypersensitivity, which leads to local delayed insulin allergy. Stimulation of both T- and B-cells can lead to the production of anti-insulin antibodies of the IgG and IgE class, and less frequently of the IgA, IgM, and IgD class. Insulin antibodies are primarily polyclonal IgG, with both κ- and λ- light chains. IgE insulin antibodies are responsible for the immediate type of insulin allergy, whereas very high titers of neutralizing antibodies of the IgG class can lead to either insulin resistance or to other metabolic consequences.

The classification of immunological reactions to insulin are summarized in Table 1, according to the method of

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IgG, immunoglobulin G; IgE, immunoglobulin E; RIA, radioimmunoassay; IgM, immunoglobulin M; IgD, immunoglobulin D; type II diabetes, non-insulin-dependent diabetes mellitus; type I diabetes, insulin-dependent diabetes mellitus.

Table 1—Summary of allergic reactions to insulin using the Gell and Coombs classification (6)

Type I immediate type: mediated by IgE antibodies;
Local reactions
Immediate type
Biphasic (immediate and late reactions)
Generalized reactions: anaphylaxis
Type III serum sickness type: mediated by IgG antibodies (very rare)
Type IV delayed type: mediated by lymphocyte-mediated late local reactions

Gell and Coombs (6). Type I reactions are immediate-type, anaphylactic reactions to insulin. These are IgE mediated and result in local or systemic reactions. The typical history involves patients thought to require insulin initially. Insulin therapy was started and then stopped. Subsequently, after months or years, insulin therapy was resumed because of clear medical indication. After insulin therapy is reinstituted, several days of treatment go by without allergic reactions, followed by immediate-type local reactions. Type II reactions are cytotoxic reactions that occur when an antibody is directed either against a cell-surface antigen or against a hapten fixed to a cell, resulting in destruction of that cell. We are not aware of any evidence for this type of reaction causing insulin hypersensitivity. Type III reactions are antigen-antibody complex reactions that result in complement fixation and leukocyte attraction with resultant inflammatory response. The classic type III reaction is serum sickness attributable to the administration of foreign serum. Such a reaction is possible with insulin therapy. The major immunological reactants in type III reactions are usually antibodies of the IgG class. These antibodies may also result in immunological insulin resistance. Type IV reactions are lymphocyte-mediated reactions. Such reactions have been described using *in vitro* stimulation of lymphocytes of patients treated with insulin and may be involved in some local reactions in humans treated with insulin (7). Local reactions of this type attributable to zinc have also been reported (8).

FACTORS INFLUENCING THE IMMUNE RESPONSE TO INSULIN

The immune response to exogenous insulin is determined by both the insulin administered and the individual receiving it. Among the factors to be considered are differences in the state of purity, in the primary structure of the insulin, in the physical properties, and in the mode of insulin administration (Table 2). Pork and human insulin differ only in a single amino acid residue at the end of B-chain at position B30, whereas both pork and human insulin also differ from beef insulin at the position A8 and A10. Therefore, bovine insulin is more immunogenic for diabetic patients than porcine insulin, and insulin of human sequence is less immunogenic than porcine insulin (9).

In addition to insulin-related factors, the pH, solubility, the concentration of zinc (8) and protamine (10), and other retarding agents seem to be of critical importance. Cross-sectional studies indicate that 60–90% of patients receiving

Table 2—Factors influencing the immune response to insulin

Insulin factors
Purity
Species (bovine > pork > human)
Physical properties (pH)
Retarding agents (zinc, protamine, surfen)
Individual factors
Age
Immunogenetic background (HLA type)
Presence of insulin autoantibodies
Mode of insulin administration
Subcutaneous > intravenous
Insulin pumps
Interrupted insulin therapy

ing NPH bovine/porcine insulin treatment develop protamine antibodies (10). Because insulin antibodies are also found in the majority of patients, Kurtz et al. (10) suggested that the immunogenicity of insulin and protamine antibody formation might be linked. In a prospective follow-up study (11), antibodies to protamine and IgG-insulin antibodies were not significantly associated in type II diabetic patients who received NPH human insulin. However, the findings of the follow-up study (11) clearly indicate that the protamine-antibody formation that occurs with NPH insulin treatment will not be reduced by using NPH human insulin instead of NPH bovine/porcine insulin.

The immunogenicity of newer insulin delivery techniques has also been investigated. Two reports (12,13) demonstrate an increase in insulin antibody titer during intensive treatment with either insulin pumps or multiple daily injections compared with conventional insulin treatment, whereas one study (14) could not confirm this finding. Attempts to prevent precipitation of insulin in the reservoirs of implantable insulin delivery devices have demonstrated that polymers of insulin and circulating insulin fragments can lead to insulin antibody formation (13). Because insulin antibodies increased in all patients, Micossi et al. (13) concluded that glycerol insulin is not an adequate insulin preparation for use in implanted devices.

The anti-insulin immune response also depends on patient-related factors, such as age, sex, and the immunogenetic background of the patient (15–17). All these variables are important when comparing the immunogenicity of beef, human, and porcine insulins. There is almost uniform agreement that low or non-insulin-antibody response is significantly associated with the histocompatibility antigen HLA-DR3—a finding that we first reported in 1976 (15). The link between low immune response to insulin and HLA DR3 has been confirmed by many groups as summarized

previously (4). High levels of insulin antibody formation were found to be associated with the HLA gene DR4 in some but not all studies (4). Patients with insulin autoantibodies show a significantly higher insulin antibody response to injected insulin compared with those negative for insulin autoantibodies at diagnosis (18).

Very limited information is available concerning immunogenicity of insulin administration in nondiabetic subjects. Remarkably, only 2 of 27 nondiabetic patients who received many insulin-shock treatments for psychiatric disorders had detectable insulin antibodies in a retrospective study of Danish patients (19). Because the risk of diabetes was not increased by the use of many injections of poorly purified insulin of bovine/porcine origin, the authors concluded that clinical trials on prevention of type I diabetes by prophylactic insulin treatment can be regarded as safe. In contrast, in another prospective study (20), administering only 6 injections of human insulin resulted in a significant production of insulin antibody levels in all nondiabetic subjects tested, which was comparable to levels observed in type I diabetic patients receiving chronic insulin replacement therapy.

ALLERGIC REACTIONS TO ANIMAL INSULIN

Allergic reactions, including urticaria and anaphylaxis, occurred with early insulin preparations (1,3,9,16), but as these were very impure, the antigen response may not necessarily have been insulin. Immediate-type, systemic hypersensitivity reactions to insulin were previously a rare complication and have virtually disappeared since the introduction of highly purified insulins. In affected subjects, high insulin-specific IgE levels can be detected in serum, and the allergy can be identified by a skin-prick test using insulin preparations (21–23). In insulin allergic patients treated with conventional insulin preparations, the insulin specific IgE values are often 10- to 20-

fold higher than in patients without allergy (24). A major problem is the cross-reactivity that occurs between anti-insulin antibodies and the various animal and human insulin preparations in patients presenting with allergy to animal insulin. In nonallergic cases, positive skin tests to human insulin do not necessarily have any clinical significance, because as many as 40–50% of patients receiving conventional insulin therapy showed wheal-and-flare responses on intradermal testing (25,26).

Another manifestation of insulin allergy, which is also now relatively rare, is a delayed local reaction to injected insulin. This presents as a tender subcutaneous lump that develops at the injection site ~30 min after injection and lasts for 12–24 h. This is a local Arthus-type reaction, mediated by IgG rather than IgE, and is attributable to the complement activation by insulin-IgG immune complexes. It often responds to the addition of hydrocortisone to injected insulin.

The exact frequency of allergic reactions to animal insulin preparations is unknown. Hannauer and Batson (27) reviewed the literature in 1961 and reported that 5–10% of all patients receiving insulin exhibited some kind of allergic reaction. However, most of the reactions were mild and harmless. A steady decline in the prevalence of insulin allergic reactions was observed in Germany, decreasing from 30% of all insulin-treated diabetic patients in 1930 to ~5% in 1980 (28).

ALLERGIC REACTIONS TO HUMAN INSULIN

In view of the wide spectrum of immune-mediated complications of insulin therapy, much attention has been directed to the reduced immunogenicity of highly purified forms of animal insulins and the more recently available recombinant and semi-synthetic forms of human insulin. Delayed-type insulin allergy and especially immediate-type insulin allergy were extremely rare in both type I and type II

diabetic patients who were treated exclusively with human insulin in our center (Table 3).

Because human insulin preparations are not totally nonimmunogenic (4,5,29,30), local and acute systemic responses to exogenous human insulin have occasionally been reported (31–33). Grammer et al. (32) described a patient with anaphylaxis to beef-pork insulin but no systemic or local reactions to human (rDNA) insulin. By contrast, in other patients with anaphylaxis to animal insulins, the binding of antibodies often react to bovine, porcine, and human insulin (32). Recently, Ganz et al. (34) described a type II diabetic patient who manifested both severe insulin resistance and persistent systemic allergy despite treatment with recombinant human insulin. However, in this case, symptoms of insulin allergy had already emerged several months after initiating therapy with mixed beef-pork insulin. After switching to recombinant human insulin, generalized urticaria with pruritus, significant eosinophilia, and diffuse lymphadenopathy reminiscent of the serum sicknesslike response occurred. This case illustrates that a wide array of clinically significant immunological responses to human insulins occur when it is used in the insulin allergic patient.

Patients, who are intermittently exposed to insulin because of irregular administration appear to be at a higher risk for more persistent and severe allergic reactions. Table 3 summarizes our experience concerning the relatively high rate of insulin allergic reactions after human insulin administration in patients with intermittent insulin therapy. Of the 36 patients, 34 had a history of previous treatment with animal insulins, whereas only 2 patients used human insulin in the pretreatment. Delayed-type allergy was seen in 3 (8%) of 36 patients who received intermittent insulin therapy. In addition, immediate-type allergy was observed in one patient and lipatrophy in another patient who had intermittent therapy with human insulin at surgical

Table 3—Immunological findings in patients treated exclusively with human insulin and in patients treated with intermittent insulin therapy

	Type I diabetic patients (n [%])		Type II diabetic patients (n [%])	Patients with intermittent insulin therapy (n [%])
	<20 yr	20–35 yr		
n	65	140	271	36*
Delayed-type allergy	1 (1.5)	None	4 (1.5)	3 (8)
Immediate-type allergy	None	None	None	1 (3)
Lipoatrophy	None	None	1 (0.3)	1 (3)
Insulin resistance (>2.5 U/kg body weight)	None	None	3 (1.1)	None
IgG-insulin antibodies†				
No antibodies (<0.05 U/L)	20 (31)	87 (62)	157 (57)	3 (8)
Low antibodies (0.05–1.0 U/L)	44 (68)	92 (37)	112 (41)	20 (56)
High antibodies (>1.0 U/L)	1 (1.5)	1 (1)	2 (1)	13 (36)

*Pretreatment with animal insulins in 34 patients.

†After one year of treatment.

interventions three times. Thus, intermittent insulin therapy seems to be a potent stimulus for the immunogenicity and allergenicity of most insulin preparations. At present, it is unknown whether human insulin has a significant advantage in this particular situation.

EXPERIENCE WITH HUMAN INSULIN IN INSULIN-ALLERGIC PATIENTS

Does human insulin have any advantage in the treatment of the insulin-allergic patients? In 1982 we investigated insulin-specific IgE in newly diagnosed type I diabetic patients treated with human insulins or conventional insulins (4). IgE insulin antibodies were very low in the human insulin-treated patients compared with patients on non-monocomponent insulin preparations (4). Falholt et al. (35) studied the binding of insulin-specific IgE to bovine, porcine, and human insulin in patients with clinically significant insulin allergy. The binding of the preformed IgE antibodies was highest for bovine insulin and lowest for human insulin. Interestingly, a progressive fall in IgE antibody binding was seen in the patients after transfer from monocomponent porcine insulin to semisynthetic human insulin. The lower affinity of human insulin to insulin-

specific IgE (24) may explain the advantage of using human insulin in the treatment of insulin allergy. The study by Bruni et al. (36) confirms the advantage of human insulin in the long-term treatment of diabetic patients with immunological reactions to heterologous insulin. In their study, 8 type II diabetic patients, intermittently treated with conventional insulins, presented with allergic reactions to porcine and mixed-species monocomponent insulins. Allergy was systemic and locally delayed in 2 subjects and locally immediate or biphasic in 6 subjects. After treatment with human semisynthetic insulin, systemic allergy disappeared. Local allergy disappeared in 5 subjects and was reduced in 3 subjects. The findings suggest that patients with intermittent insulin administration should have preferential treatment with human insulins to prevent the well-known booster effect.

LIPOTROPHY—The phenomenon of lipoatrophy, in which there is loss of fat at insulin injection sites, was previously quite common, being reported in 10–55% of patients treated with conventional bovine/porcine insulin (37). Patients with lipoatrophy usually have moderately high circulating insulin anti-

body titers. An immune basis for this condition has been suggested by the immunohistochemical demonstration of deposits of both insulin and IgG in subcutaneous tissue biopsied from lipoatrophic areas (38). Interestingly, purified pork or human insulin at the edge of the sites can be used for treatment of lipoatrophy. With the development of purified pork or human insulin preparations, lipoatrophy has become very rare and was found in only 1 of 467 cases with de novo treatment of human insulin (Table 3).

IMMUNOLOGICAL INSULIN RESISTANCE

Immunological insulin resistance has been described as being caused by high titers of high-avid IgG-antibodies to animal insulins (39). Affected patients with an insulin requirement of several thousand units a day have been reported (40). Antibody-mediated resistance to insulin may be defined as an insulin requirement of >1.5 U/kg/day in patients with type I diabetes who show no apparent endocrine abnormalities nor other explanation (41). In this respect, it should be mentioned that many type I diabetic patients with very poor glycemic control have a high insulin requirement that can-

Table 4—Frequency and mean levels of IgG-insulin antibodies in diabetic patients after a 2-yr treatment with insulin preparations of different purity and species of origin

Type of insulin	Species	Degree of purity	Patients (n)	IgG insulin antibody	
				Positive (%) (>0.05 U/L)	Mean $\pm$ SD level (U/L)
Kornb-Insulin*	Beef	Impure	30	30/30 (100)	7.6 $\pm$ 2.4
Depot-Hoechst klar CR*	Beef	Purified	38	33/38 (87)	4.9 $\pm$ 2.2
Depot-Hoechst klar CS*	Pork	Purified	42	33/42 (76)	3.7 $\pm$ 2.1
Desphe 015/025*	Pork	Purified	23	13/23 (56)	0.20 $\pm$ 0.3
Monotard monocomponent/Actrapid monocomponent†	Pork	Highly purified	108	73/108 (68)	0.28 $\pm$ 0.10
Monotard monocomponent/Actrapid monocomponent†	Human†	Highly purified	132	64/132 (48)	0.11 $\pm$ 0.04
Humulin Basal/Normal§	Human	Highly purified	85	43/85 (51)	0.12 $\pm$ 0.03
Insuman Basal/Rapid*	Human†	Highly purified	120	56/120 (47)	0.14 $\pm$ 0.04

*Hoechst, Kornb insulin is a nonpurified mixture of short- and long-acting insulin; Depot Hoechst Klar is a mixture (30%/70%) of chromatographed long- and short-acting insulin.

†Novo Nordisk.

‡Semisynthetic insulin.

§Eli Lilly.

||Biosynthetic insulin.

not be explained by the presence of insulin antibodies. In fact, with the increasing use of highly purified insulin preparations, immunologically conditioned insulin resistance is now very rare. Affinity studies have demonstrated that sera of insulin-resistant type I diabetic patients, which contains very high IgG-insulin immunoglobulins, showed a significantly lower binding of [¹²⁵I]human insulin compared with [¹²⁵I]porcine insulin (41). In previous studies (3, 42, 43), the greatest affinity of pre-existing insulin antibodies has been noted for beef insulin, which might be of clinical relevance at least in diabetic patients with high insulin antibodies. Interestingly, in one report (41), all patients with insulin antibody-mediated insulin resistance were positive for HLA-DR4, but negative for HLA-DR3, supporting the concept of an immunogenetically transferred anti-insulin immune response in insulin-treated diabetic individuals. Based on the reduced binding of human insulin to IgG antibodies of very high levels, a potential therapeutic advantage of human insulin therapy can be

expected in such infrequent cases of immunological insulin resistance.

Very recently, Davidson et al. (44) evaluated immunological and metabolic responses of patients with a history of antibody-induced beef insulin resistance to treatment with beef, pork, human, and sulfated beef insulin. A randomized, double-blind, sequential, crossover study was performed, and each insulin was administered for 56 days unless dose reached 200 U/day or allergy developed. In total, 26 patients with a history of beef/pork insulin dosage >200 U/day and insulin binding capacities >30 mU/ml of serum were studied. Rechallenge with beef insulin resulted in anamnestic immunological response and deterioration of metabolic control. Human, pork, and sulfated beef insulin had equivalent effects in patients with insulin antibody-mediated immunological resistance (44). Interestingly, long-term therapy with sulfated beef insulin reduced the risk of recurrent immunological resistance on subsequent beef insulin therapy. Naquet et al. (45) demonstrated that therapy with sulfated beef insulin for one year has been associated with a virtual

disappearance of T-cell and antibody responses to beef, pork, and human insulin, parallel with the appearance of insulin-specific CD8⁺ suppressor T-cells.

EFFECT OF PURITY AND SPECIES SPECIFICITY OF INSULIN PREPARATIONS ON IgG INSULIN ANTIBODY FORMATION

It is generally accepted that purity of insulin preparations is more important for immunogenicity and allergenicity than the species specificity. Table 4 shows the frequency and mean levels of IgG insulin antibodies in diabetic patients after a 2-year treatment with insulin preparations of different purity and species specificity. IgG insulin antibody measurement was performed with the Christiansen method (46), as described previously (17). The sensitivity of this method for the detection of IgG-insulin antibodies is 0.05 U/L. IgG-insulin antibodies of very low levels were found in approximately half of the diabetic patients after exclusive treatment with human insulin for 2 years. Frequency and levels of IgG insulin antibodies were not statistically different,

whether biosynthetic or semisynthetic human insulin preparations were used (Table 4). However, relatively high levels of IgG insulin antibodies were observed in most of those patients who had a long history of pretreatment with impure or insufficiently purified insulin preparations (Table 4).

INSULIN ANTIBODIES AND INSULIN PHARMACOKINETICS

It is disputed whether insulin antibodies exert a stabilizing effect on glycemic control, assuming that dissociating insulin-antibody complexes would help mimic basal insulin secretion, or whether they cause a hyperlabile state of glycemic control. Antibody binding, as assessed by Scatchard analysis as either high or low affinity and high or low capacity, is a reversible process that can lead to changes in the availability of plasma free insulin. Thus, the presence of high levels of insulin antibodies could be expected to have clinical consequences. Van Haefen et al. (47,48) have described a slower rise in free insulin levels after injection of short-acting preparations in insulin antibody positive as compared with insulin antibody negative patients. Although some reports cast doubt on the clinical relevance of this observation (49,50), this delay in insulin availability may contribute to postprandial hyperglycemia. Conversely, high levels of insulin antibodies can also cause an increase in the half-life of plasma free insulin with resultant prolongation of postprandial and nighttime hyperinsulinemia and consequent hypoglycemia (51). Very recently, Wredling et al. (52) were not able to demonstrate any relation between insulin antibody levels and severe hypoglycemia in a large group of type 1 diabetic patients.

Certainly, this does not exclude the possibility that insulin antibodies of extremely high levels may be of importance in some diabetic patients with impaired adrenaline response to hypoglycemia (53). Increasing the time period between insulin injection and meal con-

sumption may prevent the clinical problems of immediate postprandial hyperglycemia and nighttime hypoglycemia that result from a high level of insulin antibodies. Recent advances in molecular modeling in combination with rDNA technology have allowed the development of insulin analogues that remain dimeric or even monomeric (54). Whether human insulin analogues are useful candidates for short-acting insulin treatment under this particular condition is unknown. These analogues have to be carefully studied with regard to immunogenicity and other potential clinical side effects. Small therapeutic trials have also been conducted with biosynthetic human proinsulin in patients with diabetes (55,56), and in this setting, human proinsulin was only a weak immunogen (57).

INSULIN IMMUNOGENICITY IN GRAVIDITY

— Because IgG antibodies are known to cross the placenta, the question of whether insulin antibodies affect fetal metabolism has been raised. Because infants have antibody titers similar to their mothers, insulin antibodies could theoretically lead to fetal hyperglycemia through neutralization of fetal insulin (58,59). Alternatively, fetal and neonatal hypoglycemia may result from inopportune release of insulin from antibody-antigen complexes and may be a factor in the occurrence of this complication in infants of diabetic mothers. In a recent report (60), anti-insulin antibody-bound insulin was found to be transferred from mother to fetus. Further analysis from this study (60) implicated antibodies to animal insulin as a causative factor in the macrosomia seen in infants of diabetic mothers.

Previous studies have demonstrated that switching from beef/pork to purified pork insulin leads to a reduction of insulin antibodies (61), which may be related to improved β -cell function in the newborn (59). To decrease the incidence of maternal and fetal insulin antibodies further, a recent report has rec-

ommended using only human insulin during pregnancy (61). Nonetheless, little direct evidence indicates that insulin antibodies cause harm to the fetus. Very recently, Jovanovic-Peterson et al. (62) designed a randomized study to compare human with animal insulin in pregnant women with pre-existing diabetes. Women receiving human insulin required significantly less insulin and showed less hyperglycemia or hypoglycemia compared with diabetic patients who continued using animal insulins. Interestingly, the infants born to mothers who received human insulin were less prone to macrosomia and had less neonatal hyperinsulinemia. However, measurement of anti-insulin antibody levels did not reveal significant differences between either group.

Recently, insulin allergy and moderate insulin resistance (150 U insulin/day) was demonstrated in a nonobese woman with gestational diabetes who was treated initially and exclusively with human insulin (63). In this patient, serum IgE and IgG binding antibodies to human insulin were inhibited similarly with human insulin, bovine insulin, and porcine insulin (63).

IMMUNOGENICITY OF HUMAN INSULIN VERSUS PORCINE INSULIN

— In 1981, studies were first undertaken to clarify whether human insulin is nonimmunogenic in newly diagnosed type 1 diabetic patients. Surprisingly, only two thirds of newly diagnosed type 1 diabetic patients remained IgG-insulin antibody free, whereas about one third formed low anti-insulin antibodies after short-term treatment with semisynthetic insulin (4). By comparing patients treated either with porcine or human monocomponent insulin preparations over a 12-mo period, the IgG insulin antibody titers were very low in both groups but somewhat higher in patients on porcine monocomponent insulins (4). The main message of this study was, however, that human insulin was immunogenic in a considerable number of di-

abetic patients. This fact has since been confirmed in several double-blind and open studies (5,64–69). The immunogenicity of biosynthetic human insulin preparations was studied by Fineberg et al. (5) in a mixed population of type II and type I diabetic patients. The percentage of insulin bound was identical in patients treated with biosynthetic human insulin or purified pork insulin during the first 3 mo, but was significantly lower during the follow-up in the human insulin-treated group. Furthermore, the percentage of patients with antibody formation was significantly lower in the human insulin-treated group compared with patients on purified pork insulin.

The preliminary report of the randomized, double-blind, Scandinavian, multicenter trial (64) was confirmed; human insulin is indeed immunogenic in diabetic children and mean insulin antibody levels after one year are significantly lower in human insulin compared with porcine insulin-treated patients. The 2-yr follow-up of the Scandinavian study demonstrated (69) that monocomponent human insulin is only slightly less immunogenic than monocomponent porcine insulin. Of the diabetic children treated for 1 or 2 years with human semisynthetic insulin, 72 and 94%, respectively, developed insulin antibodies. In agreement with these findings, we observed a considerably higher frequency of IgG insulin antibodies after treatment with human insulin in diabetic children compared with type 1 diabetic patients older than 20 yr of age (Table 5). Most reports find that both the prevalence and titers of insulin antibodies in patients treated with human insulin are lower than in those treated with bovine/porcine insulin (4,5,64–66,69), although, only slightly less than those observed with purified pork preparations. Table 3 summarizes all the published studies concerning immunogenicity of human insulin versus porcine insulin in newly treated patients. Of the eight studies, six reported small differences between the immunogenicity of highly pu-

rified human and highly purified porcine insulin, whereas two studies reported no such observation. The authors of the Swiss-Italian study (68) did not observe a difference in the prevalence or titers of insulin antibodies in 52 diabetic children treated with either human monocomponent or porcine monocomponent insulin. Because the prevalence of HLA-DR3 was higher in the diabetic children on porcine insulin than in the group treated with human insulin, the authors themselves questioned whether this difference may have had some influence on insulin antibody formation. In the Australian report (67), very heterogeneous type II diabetic patients were studied. Follow-up study was performed in only 10 patients from each group. It should be noted that insulin antibody titers were very low in all eight studies and that differences in insulin antibodies between human insulin- and porcine insulin-treated patients were relatively modest. Although controlled comparative immunological studies with biosynthetic or semisynthetic human insulin were not undertaken, antibody measurements in our laboratory do not indicate relevant differences in frequency or mean titers of insulin antibodies after treatment with human insulin of different origin (Table 4).

The clinical relevance of the slightly lower immunogenicity of human insulin compared with highly purified porcine insulin is questionable. Earlier studies suggested an adverse effect of even low levels of insulin antibodies on β -cell function (70,71). In the large, randomized, controlled Scandinavian study, however, the differences in insulin antibody levels were insufficient for inducing differences in β -cell function between human monocomponent and porcine monocomponent insulin-treated patients (69).

The most important message of all these studies is that human insulin is immunogenic in diabetic individuals. Although the insulin antibody levels seem to be lower in patients on human insulin, the proportion of patients with or with-

out insulin antibody formation is not very different between patients treated exclusively with human or highly purified porcine insulin. Why is human insulin immunogenic in humans? One important contributing factor could be the route of insulin administration. Preliminary investigations have shown that intravenously administered insulin is more or less nonimmunogenic. Deamidation of insulin, as well as the repository insulin formulations, may also have some influence. Finally, the inherit immunogenicity of the body's own proteins, including insulin could also be involved.

TRANSFER STUDIES FROM PORCINE INSULIN TO HUMAN INSULIN

— Transfer from highly purified porcine insulin to human insulin decreased the insulin binding to IgG in already sensitized patients in some (72,73) but not all studies (74–76). The glycemic control, as assessed by HbA_{1c} levels, tended to deteriorate in the human insulin group during the first 3 months of the trial and returned to the baseline level in only one study (76). By contrast, Virgil et al. (73), who reported a reduction in insulin antibodies 42 mo after transfer from porcine to human monocomponent insulins, did not obtain a significant modification of metabolic control, insulin requirement, nor hypoglycemic episodes. Higher fasting blood glucose levels were found in patients on biosynthetic human insulin compared with animal-insulin treatment in several short-term, double-blind, crossover trials (77). The observed differences cannot be explained by immunological mechanism, but may be attributable to the differences in the pharmacokinetics of the two insulins (78). The slightly accelerated absorption of human compared with porcine insulin is possibly associated with a shorter duration of insulin action during the night.

INDICATION FOR THE USE OF HUMAN INSULIN — A number of possible benefits (altered absorption

Table 5—Comparative studies of the immunogenicity of human versus porcine insulin

Author	Year	Type of diabetes	Patients (n)	Study design	Differences (≠) or identity (=)
Scherthaner et al.	1983	Type I	102	Open study	≠
Fineberg et al.	1983	Type I/II	221	Open study	≠
Heding et al.	1984	Type I	135	Double-blind study	≠
Iavicoli et al.	1984	Type I	43	Open study	≠
Luyckx et al.	1986	Type I	33	Open study	≠
Larkins et al.	1986	Type II	20	Double-blind study	=
Zuppingner et al.	1987	Type I	52	Double-blind study	=
Marshall et al.	1988	Type I	138	Double-blind study	≠

characteristics, increased potency, altered distribution of metabolic effects, and protection of endogenous insulin secretion) of human insulin preparations have been discussed during the last 10 years. However, a great number of comparative studies concerning the potency of human and porcine insulins reached near universal agreement on the equivalence of potency of human and porcine insulin (77,79). Thus, the only advantage of human insulin preparations for treatment is the very low immunogenicity and allergenicity. Human insulin should be used in patients with insulin allergy, immunological insulin resistance, or lipotrophy. Human insulin also should preferably be given in cases with intermittent insulin therapy, gestational diabetes, and in all patients with newly diagnosed type I diabetes. There are no obvious circumstances in which human insulin would appear to be contraindicated. On the other hand, there are no clear indications for switching long-standing well-controlled diabetic patients to human insulin, except in the presence of immunological controlled complications of insulin therapy, as noted above.

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