Effect of Long-Term Exposure to Insulin Lispro on the Induction of Antibody Response in Patients With Type 1 or Type 2 Diabetes

S. Edwin Fineberg, md[1]
Jie Huang, phd[2]
Rocco Brunelle, ms[2]

K. S. Gulliya, phd[2]
James H. Anderson, Jr., md[1,2]

OBJECTIVE — To determine the long-term effects of insulin lispro on inducing lispro-specific, insulin-specific, and cross-reactive (reactive with both insulin lispro and human insulin) antibodies.

RESEARCH DESIGN AND METHODS — A multinational, multicenter combination of controlled and noncontrolled, open-label studies of 4.5 years' duration was designed to evaluate the long-term immunologic profile of subcutaneously administered insulin lispro. A total of 1,221 patients (men and women; 12–81 years of age) with type 1 or type 2 diabetes were enrolled. Circulating anti-insulin antibodies were measured using radioimmunoassays.

RESULTS — Insulin-specific and lispro-specific antibody responses were within the background noise levels of the assays. Significant elevations of antibody were confined to a cross-reactive antibody response. Antibody levels resulting from prior exposure to long- and short-acting insulins changed little after transfer to insulin lispro and remained within or near the baseline levels. De novo exposure to insulin lispro resulted in increases in cross-reactive but not insulin- or lispro-specific antibody levels. Cross-reactive insulin antibodies developed more readily in patients with type 1 diabetes than in those with type 2 diabetes. Long-term antibody responses tended to decrease over time and returned to baseline or near-baseline levels by the end of the long-term studies. No evidence of an anamnestic antibody response could be found in individuals treated intermittently with insulin lispro.

CONCLUSIONS — The immunogenic profile of patients treated with insulin lispro was comparable to that of patients treated with recombinant human insulin. Inductions of significant levels of specific or cross-reactive antibodies were not observed in patients who had received insulin previously. No significant antibody-dependent increases in insulin dosage requirements were noted in these patients. The incidence of insulin allergy was not different from that in patients treated with recombinant regular human insulin.

Diabetes Care 26:89–96, 2003

Insulin antibodies have been linked with cutaneous and systemic allergic reactions (1–11) and insulin resistance (12–24). Before highly purified insulin products, allergic responses were seen in up to 30% of patients. Animal insulin preparations were linked to the development of high levels of circulating anti-insulin antibodies in virtually all patients. With the use of highly purified animal and human insulins, insulin antibody levels are lower and clinically observable allergic phenomena are unusual. Although anti-insulin antibodies developed in patients treated with recombinant human insulin and purified animal insulins (25), local allergic skin reactions were seen in <2% of patients and lipoatrophy was seen rarely (26).

In patients with a history of treatment with animal insulins of various degrees of purity, the presence of insulin antibodies has been shown variably to correlate with increased insulin requirements (27). Currently, insulin antibody–mediated insulin resistance resulting in insulin requirements of >1.5 units · kg^{-1} · day^{-1} in adults and >2.5 units · kg^{-1} · day^{-1} in children is an extremely rare complication of therapy (26).

To develop insulin analogs with more desirable pharmacokinetic properties, alterations have been made in the amino acid sequence and/or the chemical makeup of human insulin. Ideally, analogs should neither produce an immune response that results in local or systemic allergic manifestations in excess of those of human insulin preparations nor result in the generation of antibodies that bind to and neutralize exogenous or endogenous insulin. However, insulin analogs may present new epitopes for recognition by the immune system.

Insulin lispro, a rapid-acting analog of human insulin, was created by the reversal of the sequence of amino acids 28 and 29 of the human insulin B-chain (human insulin = Pro B28-Lys B29, insulin lispro = Lys B28-Pro B29) (28).

To assess the long-term immunogenic response to insulin lispro in patients with type 1 or type 2 diabetes, insulin lispro-specific antibodies (LSA), insulin-specific antibodies (ISA), and antibodies reactive with both insulins (i.e., cross-reactive antibodies) were measured. Insulin-mediated allergy and the effect of anti-

Table 1—*Parent and extension insulin lispro studies*

Parent studies (A–H) and insulin treatment history	Study design
Prior insulin treatment	1-year, parallel
Study A	Type 1
Study B	Type 2
Study C	Type 1
Study D	Type 2
Insulin-naïve	1-year, parallel
Study E	Type 1
Study F	Type 2
Prior insulin treatment	6-month, cross-over
Study G	Type 1
Study H	Type 2

Extension studies (K, O, M, N) (enrolled patients) age; gender; origin	Design (completed patients)
Study K (271)	Single arm, up to 4 years (214)
13.4–71.5 years; 53/47% M/F; 86.4% Caucasian	
Study O (167)	Single arm, up to 4 years (121)
13.7–69.4 years; 60/40% M/F; 96.1% Caucasian	
Studies M and N (771)	Single arm, up to 4 years (653)
13.7–80.7 years; 56/44% M/F; 95.4% Caucasian	

insulin antibodies on the dose of insulin were also evaluated.

RESEARCH DESIGN AND METHODS
— The studies were conducted according to the applicable laws of each participating country, the ethical principles of the Declaration of Helsinki, and guidelines for Good Clinical Practice.

Study objective
The primary objective of the combined studies was to assess the safety of insulin lispro with respect to the long-term immunologic response. One-year immunologic effects were previously reported (29).

Study protocol
This composite multinational, multicenter, controlled and noncontrolled, open-label study evaluated 1,221 men and women with type 1 or type 2 diabetes (30) from parent studies (demographics detailed previously) (29,31,32) who continued in extension studies with 988 patients completing ~4 additional years (Table 1). Exclusion criteria included significant renal, hepatic, or cardiac disease, cancer, drug or alcohol abuse, significant insulin allergy, recurrent severe hypoglycemia, anemia or hemoglobinopathy, BMI >35 kg/m^2, pregnancy, lactation, and treatment with oral hypoglycemic agents, systemic glucocorticoids, insulin therapy via pump, or insulin doses >2.0 units $\cdot$ kg^{-1} $\cdot$ day^{-1}.

Insulin dose and administration procedures
Human insulin analog insulin lispro ([LYS (B28), PRO (B29)], rDNA) (Humalog; Eli Lilly and Company, Indianapolis, IN) was provided in 10-ml vials or 1.5-ml cartridges (100 units/ml [3.5 mg/ml]). Basal insulin study drugs were Humulin N provided in 10-ml vials or 1.5-ml cartridges (100 units/ml [3.5 mg/ml]) and Humulin U provided in 10-ml vials (100 units/ml [3.5 mg/ml]). All insulins were administered subcutaneously. For the extension studies, patients used commercially available intermediate or long-acting formulations of human insulin as their basal insulin, and the investigator adjusted the dosage and time of injection of insulin lispro and basal insulin in ac-cordance with the metabolic needs of the patient.

Organization of data
Data from these trials were segregated into three parts as described below.

Part 1 (Studies A–D and Extension Study K). Patients with type 1 or type 2 diabetes previously treated with human insulin for at least the last 2 years who were randomized to insulin lispro and successfully completed Studies A, B, C, or D (12-month parallel studies) continued treatment with insulin lispro in Extension Study K. Of 271 patients (145 men, 126 women) who were enrolled, 57 discontinued the study. Four patients discontinued the study due to unintended pregnancies and four patients died due to causes unrelated to the study. In addition, 7 patients changed their place of residence, 25 patients had personal conflicts or other personal reasons for discontinuation, 8 patients discontinued because of a protocol violation, 5 patients discontinued because of lack of efficacy (either patient's or physician's perception or both), and 4 patients were lost to follow-up. A total of 214 patients continued throughout the extension study.

Part 2 (Studies E–F and Extension Study O). Patients who were new to insulin therapy who were randomized to insulin lispro and successfully completed Studies E or F (12-month parallel studies) continued treatment with insulin lispro in Extension Study O. Of the 179 patients who entered from the parent studies E or F, 167 had received insulin lispro.

Of the 167 patients who received insulin lispro (39 patients with type 1 diabetes and 128 patients with type 2 diabetes), 46 discontinued the study. One patient developed squamous cell carcinoma, 3 patients died due to causes unrelated to the study, 7 patients were lost to follow-up, 5 patients changed their place of residence, 17 patients discontinued because of a personal conflict or other personal decision, 6 patients discontinued because of a protocol violation, and 7 patients discontinued because of their physician's decision. A total of 121 patients completed the extension study.

Part 3 (Cross-Over Studies G and H and Extension Studies M and N). Patients previously treated with insulin were randomized to insulin lispro or human insulin in a 6-month cross-over design and continued treatment with insulin lis-
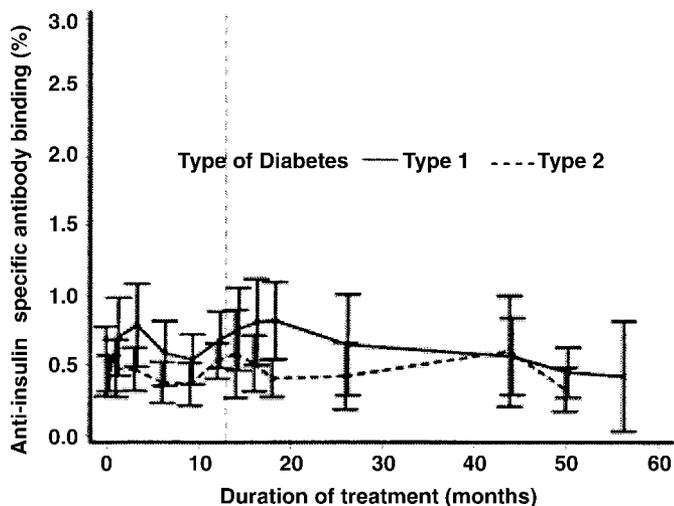
Figure 1—*ISA data for patients with type 1 or type 2 diabetes. Mean and 95% CIs over time are shown. The vertical dotted line separates parent and extension studies.*

pro in the Extension Studies M and N. These studies allowed determination of whether an anamnestic response would occur after intermittent exposure to insulin lispro in patients who were randomized to the insulin lispro first and regular human insulin second followed by lispro in the extension.

A total of 445 men and 326 women with type 1 ($n = 509$) or type 2 ($n = 262$) diabetes continued on insulin lispro during the extension studies. A total of 653 patients completed the studies. Reasons for discontinuation of the study included adverse events (19 patients), death (9 patients), change of place of residence (9 patients), loss to follow-up (12 patients), withdrawal from the study (28 patients), violation of protocol (12 patients), and perceived lack of efficacy (29 patients).

Antibody assay
Blood samples for the measurement of ISA, LSA, and cross-reactive antibodies were collected at various times during each study. Although these measurements were made in two separate laboratories, the assay protocols were identical and extensive cross-referencing of sera was performed. In brief, the antibody assay used was a self-blank assay in which prebound insulin is removed using dextran charcoal, samples are studied in duplicate, and specificity is established by differential displacement of bound, labeled insulin by addition of excess antigens (33). The interassay coefficient of variation of diluted antibody run at dilu-

tions of 1:20,000 and 1:40,000 were 10% (mean binding 48.2%) and 14.9% (mean binding 23.2%), respectively, for cross-reactive antibodies. The reference ranges used (based on healthy subjects not previously exposed to exogenous insulin) for each antibody assayed were 0–2.5% for ISA, 0–3.2% for LSA, and 0–2.5% for cross-reactive antibodies in laboratory 1 and 0–0.80% for ISA, 0–0.85% for LSA, 0–1.57% for cross-reactive antibodies in laboratory 2. In one laboratory, the assays were performed during the parent phase of the trials, and in the other laboratory, the assays were performed during the extension phase. For assessing parent and extension phase cross-reactive antibody

data together, the values from both laboratories were transformed using an empirical cumulative distribution function (CDF) transformation (34).

For evaluating antibody data over time, data from the parent and extension studies were combined. Data were analyzed by type 1 and type 2 diabetes and presented by visit. The baseline measurement from the parent studies was considered Month 0. Visit 1 of the extension studies was conducted at 12 months of therapy (except for Part 3, which was conducted at 6 months of therapy).

Statistical methods
The statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Mean and 95% CIs are presented for each visit (Figs. 1–5). End point was compared with baseline using Wilcoxon's signed-rank test. Repeated cross-reactive antibody measurements over time for patients were analyzed using a quadratic model (PROC MIXED in SAS) in which the exposure time on insulin lispro was the independent variable and the cross-reactive antibody binding was the dependent variable. The intercept and the linear and quadratic coefficients were defined as random effects, because these parameters differ for each patient, and an unstructured covariance term was used.

RESULTS

Part 1
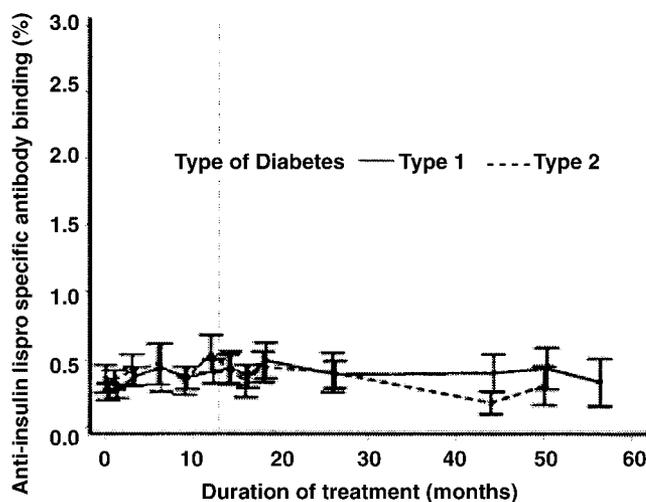In patients who were previously treated with human insulin, data obtained were

Figure 2—*LSA data for patients with type 1 or type 2 diabetes. Mean and 95% CIs over time are shown. The vertical dotted line separates parent and extension studies.*
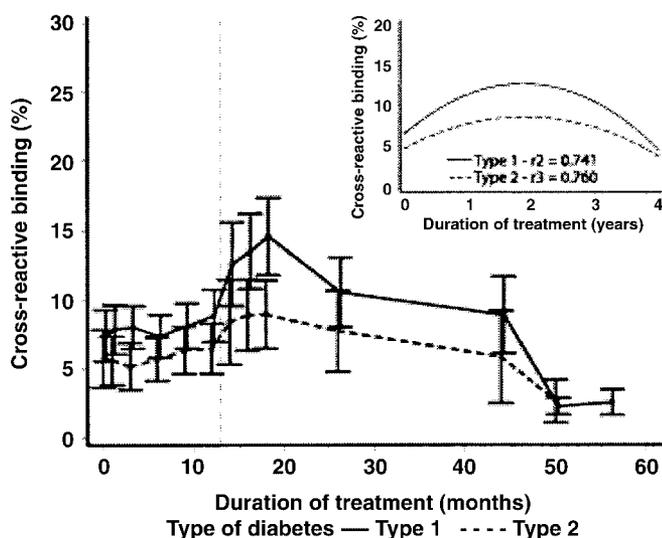
Figure 3—*Percent binding of cross-reactive antibodies from parallel studies in insulin-treated patients with type 1 or type 2 diabetes. Mean and 95% CIs over time are shown. The vertical dotted line separates parent and extension studies. The inset graph is the fitted quadratic random mixed model.*

analyzed with respect to change from baseline to end point. For ISA, the mean values and upper 95% CIs for all visits were within or near the reference range. The ISA response (Fig. 1) in type 1 patients was consistently higher than in type 2 patients until after 40 months of the study. The end points were not statistically different from baseline. For LSA, the mean values and upper 95% CIs for most of the visits were within or near the reference range. There was a virtually complete overlap (Fig. 2) in LSA response between patients with type 1 and type 2 diabetes for a period of more than 25 months. Inconsistent differences between different visits and baseline were detected for both types of patients, but they remained within or near the reference range. The mean end point was statistically higher than baseline in patients with type 1 diabetes ($P = 0.034$), but the median of baseline and end point values for type 1 diabetes were still within the reference range in nonexposed subjects.

Cross-reactive antibody responses for patients with type 1 and type 2 diabetes are shown in Fig. 3. The mean values and 95% CIs for almost all visits were higher than the reference range (for non-insulin–exposed individuals). During most visits, cross-reactive antibody levels were statistically higher than baseline in patients with type 1 diabetes. The trend of cross-reactive antibody levels was downward after the peak near 18 months of therapy

for patients with type 1 diabetes and 17 months of therapy for patients with type 2 diabetes. In both patient groups, cross-reactive antibody levels then decreased to within reference range values by the end of the study.

Individual cross-reactive antibody values (Fig. 3) show good fit of the data ($r^2 = 0.741$ for patients with type 1 diabetes and $r^2 = 0.760$ for patients with type 2 diabetes, between predicted and

original values), indicating that the observed antibody response was consistent with the visit versus time analysis.

Part 2
The antibody data were assessed in patients who were not previously treated with insulin with respect to change from baseline to end point. For ISA, the mean values and upper 95% CIs for all visits were within or near the reference range. The ISA response (data similar to Fig. 1 are not shown) in type 1 diabetes patients was slightly higher than in type 2 patients. The end points were statistically not different from baseline.

There was a virtually complete overlap in LSA response (data similar to Fig. 2 are not shown) between patients with type 1 and type 2 diabetes for a period of 20 months, followed by a sustained divergence of patients with type 1 diabetes, with a slightly higher response. Inconsistent differences at some individual visits and at baseline were detected for patients with both types of diabetes. The end point was not statistically different from baseline. Overall, the levels of both ISA and LSA remained within the reference range.

Baseline cross-reactive antibody levels were relatively low in these insulin-naïve patients. After initiation of insulin therapy, these levels increased during the first 12 months, followed by a gradual decrease that continued for the remainder of
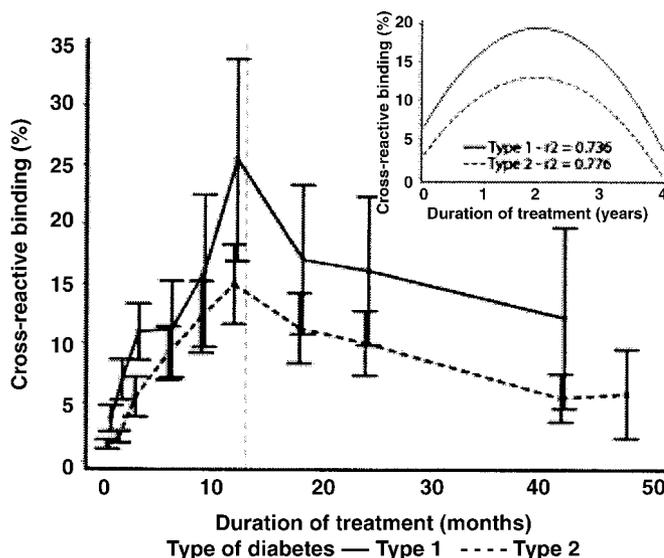

Figure 4—*Percent binding of cross-reactive antibodies from parallel studies in insulin-naive patients with type 1 or type 2 diabetes. Mean and 95% CIs over time are shown. The vertical dotted line separates parent and extension studies. The inset graph is the fitted quadratic random mixed model.*

Figure 5—*Percent binding of cross-reactive antibodies from cross-over studies in insulin-treated patients with type 1 or type 2 diabetes. Mean and 95% CIs over time are shown. The vertical dotted line separates parent and extension studies. The inset graph is the fitted quadratic random mixed model.*
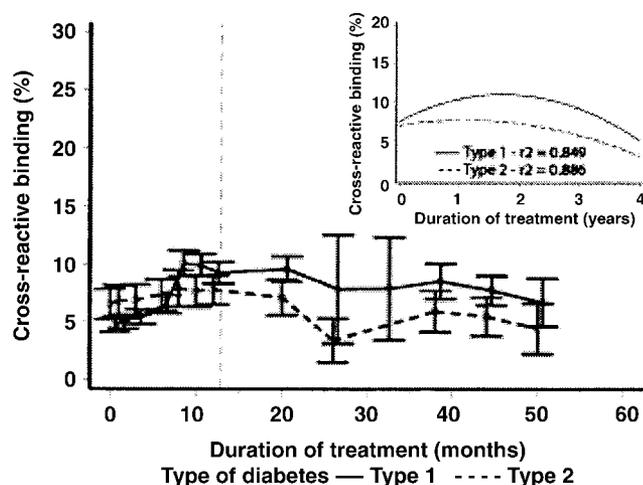
the study. The mean cross-reactive antibody levels in patients with type 2 diabetes were higher than the upper limit of the range for patients who were never exposed to insulin (Fig. 4). However, these levels were within the range for patients who were previously exposed to insulin (0–43.0%). Most patients had very low levels of antibodies with an occasional high value (50 and 75 percentile values were only 2 and 7%, respectively). The end point was statistically different from baseline in patients with type 1 diabetes ($P = 0.001$) and those with type 2 diabetes ($P = 0.001$).

In addition, as previously observed with other insulins, patients with type 1 diabetes and insulin-naïve patients had higher antibody binding levels than the patients with type 2 diabetes. Results of cross-reactive antibody binding (Fig. 4) show a good fit of the data ($r^2 = 0.736$ for type 1 diabetes, and $r^2 = 0.776$ for type 2 diabetes), indicating that the observed antibody response was consistent with the visit versus time analysis.

Part 3
The antibody response in patients who were exposed to insulin intermittently was analyzed with respect to change from baseline to end point. For ISA, the mean values and upper 95% CIs for all visits were within or near the reference range (data very similar to Fig. 1 are not shown). Occasional differences between visits and baseline were also observed. The end

points were statistically different from the baseline in patients with type 1 diabetes ($P = 0.04$) and those with type 2 diabetes ($P = 0.002$).

For LSA, the values for most of the visits were within or near the reference range (data similar to Fig. 2 are not shown). Differences between various visits and baseline were detected occasionally. The end points were not different from baseline.

For cross-reactive antibodies, a comparative analysis between two cross-over sequence groups for each diabetes type was performed. This analysis showed that there was no difference in cross-reactive antibody response between patients treated intermittently and patients treated consecutively with insulin lispro. Data were then analyzed by type of diabetes.

End point was not significantly different from baseline in patients with type 2 diabetes ($P = 0.940$), but for cross-reactive antibodies, the end point was higher than baseline in patients with type 1 diabetes ($P < 0.001$) (Fig. 5). Patients with type 1 diabetes demonstrated a sustained divergence; at most visits, cross-reactive antibody levels were statistically higher than baseline. Cross-reactive antibody binding (Fig. 5) results show a good fit ($r^2 = 0.849$ for type 1 diabetes, and $r^2 = 0.886$ for type 2 diabetes) of the data, indicating that the observed antibody response was consistent with the visit versus time analysis.

Insulin antibodies and insulin dose (parent studies only)
In all studies, these analyses did not show any statistically significant relationship between the change in the total daily insulin dose and the change in percent antibody binding for any of the antibody types (ISA, LSA, cross-reactive antibody).

Allergic reactions
The incidence of treatment-emergent adverse events potentially related to allergy was compiled from the database of all patients treated with insulin lispro, human insulin, or both in Studies A through H (Table 2). With respect to the general terms of allergic reaction, rash, and pruritus, the reported incidence was <3% and comparable between the treatment groups. When events of rash and pruritus were examined individually, 78 rash events and 17 pruritus events could be reasonably associated with an insulin-unrelated event (such as insect bite, sunburn, etc.). In the remaining 83 rash events (40 for human regular insulin and 43 for insulin lispro) and 26 pruritus events (11 for regular human insulin and 15 for insulin lispro), there were no statistical differences between treatment groups. For all other event categories, the incidence was <0.5% and there were no statistical differences between treatment groups. The incidence of allergic reactions for patients with the highest 1% of cross-reactive antibody or LSA binding was not different from the group as a whole.

CONCLUSIONS — Even with the use of human insulin, low-level antibody responses and allergic responses still occur, albeit with much less intensity and frequency. One can speculate as to whether these events are triggered by excipients in commercial insulin, such as protamine or preservatives, small amounts of insulin fragments, or dimers (35,36), or by the fact that therapeutic insulin is usually injected into the subcutaneous tissue. Several structural and pharmacological features of insulin lispro suggested that the immunogenic potential would be low. First, the reversal of the amino acids at B28 and B29 is in a relatively nonimmunogenic area of the molecule, and no additional or foreign amino acids were added to the structure. Secondly, the Lys B28-Pro B29 sequence duplicates the corresponding sequence in

Table 2—*Overall incidence of treatment-emergent adverse events potentially related to manifestations of insulin allergy*

Event classification	Humulin R (N = 2,265)	Insulin lispro (N = 2,247)	Total (N = 1,512)
Patients with ≥1 treatment-emergent adverse events	165 (7.3)	178 (7.9)	343 (7.6)
Patients with no treatment-emergent adverse events	2,100 (92.7)	2,069 (92.1)	4,169 (92.4)
Rash	63 (2.8)	64 (2.9)	127 (2.8)
Allergic reaction	52 (2.3)	60 (2.7)	112 (2.5)
Pruritus	15 (0.7)	26 (1.2)	41 (0.9)
Vasodilatation	10 (0.4)	12 (0.5)	22 (0.5)
Urticaria	4 (0.2)	10 (0.4)	14 (0.3)
Application site reaction	9 (0.4)	4 (0.2)	13 (0.3)
Hypotension	5 (0.2)	5 (0.2)	10 (0.2)
Face edema	5 (0.2)	4 (0.2)	9 (0.2)
Maculopapular rash	3 (0.1)	6 (0.3)	9 (0.2)
Injection site reaction	1 (0.0)	4 (0.2)	5 (0.1)
Generalized edema	3 (0.1)	1 (0.0)	4 (0.1)
Reaction unevaluable	1 (0.0)	3 (0.1)	4 (0.1)
Injection site pain	1 (0.0)	2 (0.1)	3 (0.1)
Injection site atrophy	0	2 (0.1)	2 (0.0)
Shock	1 (0.0)	1 (0.0)	2 (0.0)
Injection site inflammation	1 (0.0)	0	1 (0.0)
Injection site mass	0	1 (0.0)	1 (0.0)
Petechial rash	1 (0.0)	0	1 (0.0)

Data are *n* (%). *N* = total number of patients; *n* = number of patients (Studies A–H) who reported at least one event in the category.

the structurally similar IGF-1 molecule, so the body normally "sees" this amino acid sequence. Thirdly, insulin lispro is very rapidly absorbed from the subcutaneous tissue, and prior insulin studies have suggested that the immunogenic potential of an insulin formulation is correlated with the duration of residence in the subcutaneous tissue. Insulins in suspension form have been shown to be more immunogenic than soluble preparations (25). This view is further supported by the findings of increased concentration of anti-insulin antibodies during continuous subcutaneous insulin infusion and multiple-injection therapy (37).

Previous short-term studies have demonstrated that the immunogenicity of insulin lispro was not different from regular human insulin in patients with type 1 or type 2 diabetes treated for 12 months (29). The present analyses of these combined studies provide the first opportunity to define the immunologic profile of insulin lispro over an extended treatment of period of more than 4 years. For both patients with and without prior insulin therapy, the values of ISA and LSA antibodies generally remained within the nonexposed reference ranges for the duration of the study. Most antibodies generated in these clinical trials were cross-reactive rather than specific (29). In both patients with type 1 or type 2 diabetes previously treated with insulin, there were small increases in cross-reactive antibody levels from baseline to peaks at 14–18 months during insulin lispro therapy. Subsequent to these responses, the cross-reactive antibody levels gradually declined to the baseline levels by 36–48 months of treatment. Although it has been reported that in patients started on either highly purified porcine or recombinant human insulin antibodies develop by 6 months followed by a decline thereafter, earlier work has suggested that less immunogenic insulins are associated with a later peak of smaller magnitude (25,38,39). Patients not previously treated with insulin also exhibited predominantly cross-reactive antibody responses with peak responses by 12 months and a declining over the remaining years of the study. In both treatment groups, patients with type 1 diabetes developed insulin antibodies more readily than patients with type 2 diabetes (29).

In patients who received intermittent therapy with insulin lispro in the crossover followed by extension studies, there was no anamnestic response in either the cross-reactive or the lispro-specific antibodies. In most studies, as previously reported (39), there were no relationships between changes in cross-reactive antibodies and changes in insulin dosage over time. The few statistically significant changes occurred only in patients with type 1 diabetes, and these changes were inconsistent with dosages moving in opposite directions with slight increases in antibody. The incidence of nonspecific allergic response was slightly >2% and occurred with similar frequency in treatment with insulin lispro and human insulin. Therefore, the allergenic profile of insulin lispro is low and similar to that of human insulin (26,29).

Insulin lispro has been successfully used to manage patients with cutaneous insulin allergy or immunologic resistance (9,24,40–46). Case reports indicate that insulin lispro seems to have lower immunogenic potency (9,10,24,40). In intradermal tests, the initial wheal-flare response generated by insulin lispro was 50% less intense than human insulin, which progressively declined and vanished by week 25 (9). This reduced immunogenic response may be attributable to the rapid monomeric state of insulin lispro after injection (10). Additionally, a reduction in insulin autoantibodies has been observed after treatment with insulin lispro (41).

Long-term treatment with insulin lispro, similar to recombinant human insulin, elicits a low and clinically inconsequential immunogenic response. As with other insulins, treatment with insulin lispro is associated with slightly greater antibody responses in patients with type 1 diabetes than in those with type 2 diabetes. Intermittent treatment does not exaggerate specific or cross-reactive antibody response. Patients treated with insulin lispro do not develop increased insulin dosage requirements, nor do they experience an increase in events related to insulin allergy.

the following investigators and their site personnel who participated in these multicenter insulin lispro studies: A. de Leiva, Barcelona, Spain; A. Estour, Saint-Etienne, France; A. Jara, P. Sanchez, Madrid, Spain; A. Karasik, Tel-Hashomer, Israel; A. Scheen, Liege, Belgium; A. Tiengo, Padova, Italy; A.A. Motala, Durban, South Africa; A.H. Barnett, Green East, U.K.; A.J. Garber, Houston, Texas; A.J. Nankervis, Parkville, Australia; A.J. Spijker, Roermond, the Netherlands; A.P. Roberts, Adelaide, Australia; A.R. Daniels, Auckland, New Zealand; A.V. Greco, Roma, Italy; B. Charbonnel, Nantes, France; B. Goldberg, Miami, FL; B. Schulze-Schleppinghoff, Essen, Germany; B.O. Boehm, Ulm, Germany; B.R. Zimmerman, Rochester, Minnesota; C. Coscelli, Parma, Italy; C. Rosak, Frankfurt, Germany; C.D. Agardh, Lund, Switzerland; C.M. Kesson, Glasgow, U.K.; D. Andreani, Rome, Italy; D. Maingay, Blaricum, the Netherlands; D. Fedele, Padova, Italy; D.H. Clarke, Salt Lake City, Utah; D.K. Yue, Camperdown, Australia; D.P. Cameron, Woolloongabba, Australia; D.S. Schade, Albuquerque, New Mexico; D.W. Erkelens, Utrecht, the Netherlands; E.J. Bastyr, III, Galveston, Texas; E.L. Toth, Edmonton, Canada; EL Vilardell, Barcelona, Spain; E.N. Janssens, Dordecht, the Netherlands; F. Fery, Brussels, Belgium; F. Stockmann, Goettingen, Germany; F.A. Gries, Auf'm Hennekamp, Germany; F.B. Bonnici, Cape Town, South Africa; F.J. Seif, Tügbingen, Germany; G. Bompiani, Palermo, Italy; G. Cathelineau, Paris, France; G. Crepaldi, Padova, Italy; G. Delluc, Perigueux, France; G. Pagano, Torino, Italy; G. Pozza, Milano, Italy; G. Schernthaner, Vienna, Austria; G. Somers, Brussels, Belgium; G. Menzinger, Rome, Italy; G.A. Hitman, London, U.K.; G.C. Ellis, Somerset West, South Africa; G.E. Storms, Bilthoven, the Netherlands; H.J. Hazenberg, Deventer, the Netherlands; H. Kaplan, Cape Town, South Africa; H. Schatz, Bochum, Germany; H..J. Ruessmann, Dinslaken, Germany; H.P. Chase, Denver, Colorado; H.U. Jastram, Kaiserslautern, Germany; I. Folling, Trondheim, North Dakota; I. Koop, Marburg, Germany; I.H. De Leeuw, Edegem, Belgium; I.M. Fettes, Toronto, Canada; J. Beyer, Mainz, Germany; J. Cassar, Isleworth, U.K.; J. Huisman, Enschede, the Netherlands; J. Mahoudeau, Caen, France; J. Viikari, Turku, Finland; J.A. Davidson, Dallas, Texas; J.A. Vazquez, I. Goikolea, Barakaldo, Spain; J.J. Altman, Paris, France; J.K. Wales, Leeds, U.K.; J.L. Chiasson, Montreal, Canada; J.L. Rosenzweig, Boston, Massachusetts; J.L. Selam, Paris, France; J.P. Lauvaux, Brussels, Belgium; J.P. O'Hare, Bath, U.K.; J.P. Riou, Lyon, France; J.R. Attali, Bondy, France; J.R. Patsch, Innsbruck, Austria; J.S. Soeldner, Sacramento, California; K. Federlin, Giessen, Germany; K. Huth, Frankfurt, Germany; K.E. Schroder, Duisburg, Germany; K.G. Petersen, Freiburg, Germany; K.M. Shaw, Cosham, U.K.; L. Blonde, New Orleans, Louisiana; L. Sacca, Napoli, Italy; L. Van Gaal, Edegem, Belgium; L.G. van Doorn, Tilburg, the Netherlands; L.I. Robertson, Durban, South Africa; M.C. Mengel, Orlando, Florida; M. Cohen, Caulfield, Australia; M. Frank, Hamburg/S, Germany; M. Haslbeck, Munich, Germany; M. Mancini, Napoli, Italy; M. Muggeo, Verona, Italy; M. Dreyer, Hamburg, Germany; M.A. Omar, Durban, South Africa; M.H. Borkenstein, Graz, Austria; M.H. Tan, Halifax, Canada; M.J. Prince, Indianapolis, Indiana; M.L. Reeves, Chattanooga, Tennessee; M.L. Spencer, Minneapolis, Minnesota; O. Lnenicka, Orebro, Switzerland; P. Diem, Bern, Switzerland; P. Drouin, Dommartinlestoul, France; P. Fossati, Lille, France; P. Salmela, Oulu, Finland; P.A. Boyce, Indianapolis, Indiana; P.B. Vialettes, Marseilles, France; P.F. Bougneres, Paris, France; P.J. Guillausseau, Paris, France; R. Astorga, Seville, Spain; R. Bouillon, Leuven, Belgium; R. Giorgino, Via Ennio, Italy; R. Landgraf, Munich, Germany; R. Lauro, Rome, Italy; R. Marechaud, Poitiers, France; R. Moore, Durban Natal, South Africa; R. Moore, Durban, South Africa; R. Vigneri, Catania, Italy; R.A. Guthrie, Wichita, Kansas; R.K. Mayfield, Charleston, West Virginia; R.M. Bergenstall, Minneapolis, Minnesota; S. Vaaler, Oslo, Norway; S. Weitzman, Beersheba, Israel; S.A. Arslanian, Pittsburgh, Pennsylvania; S.R. Heller, Sheffield, U.K.; T. Halmos, Budapest, Hungary; T.A. Welborn, Perth, Australia; V.A. Koivisto, Helsinki, Finland; W. Lourens, Pretoria, South Africa; W. Sturmer, Wuerzburg, Germany; W.D. Alexander, Sidcup, U.K.; W.F. Mollentze, Bloemfontein, South Africa; W.K. Waldhausl, Lasarettgasse, Austria.

References

1. deShazo RD, Levinson AI, Boehm T, Evans R, Ward G: Severe persistent biphasic local (immediate and late) skin reactions to insulin. *J Allergy Clin Immunol* 59:161–164, 1977
2. Velcovsky HG, Beringhoff B, Federlin K: [Immediate type allergy to insulin (author's translation)]. [German] *Immunitat und Infektion* 6:146–152, 1978
3. Deleeuw I, Delvigne C, Beckaert J: Insulin allergy treated with human insulin (recombinant DNA). *Diabetes Care* 5 (Suppl. 2):168–170, 1982
4. Carveth-Johnson AO, Mylvaganam K, Child DF: Generalised allergic reaction with synthetic human insulin (Letter). *Lancet* 2:1287, 1982
5. Blandford RL, Sewell H, Sharp P, Hearnshaw JR: Generalized allergic reaction with synthetic human insulin (Comment – Letter). *Lancet* 2:1468, 1982
6. Altman JJ, Pehuet M, Slama G, Tchaopoutsky C: Three cases of allergic reaction to human insulin (Letter). *Lancet* 2:524, 1983
7. Garcia-Ortega P, Knobel H, Miranda A: Sensitization to human insulin (Letter). *BMJ* 288:1271, 1984
8. Gossain VV, Rouner DR, Homah K: Systemic allergy to human (recombinant DNA) insulin. *Ann Allergy* 55:116–118, 1985
9. Kumar D: Lispro analog for treatment of generalized allergy to human insulin. *Diabetes Care* 20:1357–1359, 1997
10. Frigerio C, Aubry M, Gomez F: Desensitization-resistant insulin allergy. *Allergy* 52:238–239, 1997
11. Hanauer L, Batson JM: Anaphylactic shock following insulin injection. *Diabetes* 10:105–109, 1961
12. Andersen OO: Insulin antibody formation II: the influence of species difference and method of administration. *Acta Endocrinol* 72:33–45, 1973
13. Witters LA, Ohman JL, Weir GC, Raymond LW, Lowell FC: Insulin antibodies in the pathogenesis of insulin allergy and resistance. *Am J Med* 63:703–709, 1977
14. Davidson JK, DeBra DW: Immunologic insulin resistance. *Diabetes* 27:307–318, 1978
15. Van Haeften TW, Bolli GB, Dimitriadis GD, Gottesman IS, Horwitz DL, Gerich JE: Effect of insulin antibodies and their kinetic characteristics on plasma free insulin dynamics in patients with diabetes mellitus. *Metabolism* 35:649–656, 1986
16. Grammer LC, Roberts M, Buchanan TA, Fitzsimons R, Metzger BE, Patterson: Specificity of immunoglobulin E and immunoglobulin G against human (recombinant DNA) insulin in human insulin allergy and resistance. *J Lab Clin Med* 109:141–146, 1987
17. Ganz MA, Unterman T, Roberts M, Uy R, Sahgal S, Samter M, Grammer LC: Resistance and allergy to recombinant human insulin. *J Allergy Clin Immunol* 86:45–52, 1990
18. Semple JS, Delovitch TL: Altered processing of human insulin by B lymphocytes from an immunologically insulin-resistant type 1 diabetic patient. *J Autoimmun* 4:277–289, 1991
19. Micic' D, Brkic' S, Kendereški A, Popovic V, Zoric S, Nikolic JA, Igrutinovic L, Ivanoska D, Manojlovic D, Micic J: Immunological resistance to human biosynthetic insulin: effects of immunosuppression and plasmapheresis. *Diabetes Res Clin Pract* 19:83–89, 1993
20. Uriuhara A, Morita S, Kuroda S, Fumoto M, Araki S, Aoyama N, Amano K, Hayashi Y, Yokono K, Kasuga M: Severe insulin-resistant diabetes mellitus associated with hypereosinophilic syndrome. *Intern Med* 33:632–636, 1994
21. Olsen CL, Chan E, Turner DS, Iravani M, Nagy M, Selam JL, Wong ND, Waxman K, Charles MA: Insulin antibody responses

after long-term intraperitoneal insulin administration via implantable programmable insulin delivery systems. *Diabetes Care* 17:169–176, 1994

22. Wichterle D, Stolba P, Bendlova B, Krnakova A: [Anti-insulin antibodies and insulin resistance]. *Vnitrni Lekarstvi* 41:146–150, 1995

23. Henrichs HR, Unger H, Trautmann ME, Pfützner A: Severe insulin resistance treated with insulin lispro (Letter). *Lancet* 348:1248, 1996

24. Lahtela JT, Knip M, Paul R, Antonen J, Salmi J: Severe antibody-mediated human insulin resistance: successful treatment with the insulin analog lispro: a case report. *Diabetes Care* 20:71–73, 1997

25. Fineberg SE, Galloway JA, Fineberg NS, Goldman J: Effects of species of origin, purification levels, and formulation on insulin immunogenicity. *Diabetes* 32:592–599, 1983

26. Fineberg SE: Insulin allergy and insulin resistance. In *Therapy for Diabetes Mellitus and Related Disorders.* Lebovitz HE, Ed. Alexandria, Virginia, American Diabetes Association, 1994, p. 178–184

27. Walford S, Allison SP, Reeves WG: The effect of insulin antibodies on insulin dose and diabetic control. *Diabetologia* 22:106–110, 1982

28. Howey DC, Bowsher RR, Brunelle RL, Woodworth JR: [Lys(B28)Pro(B29)]-human insulin: a rapidly absorbed analogue of human insulin. *Diabetes* 43:396–402, 1994

29. Fineberg SE, Fineberg SE, Anderson JH, Birkett MA, Gibson RG, Hufferd S: Immunologic effects of insulin lispro [Lys (b28), Pro (B29)human insulin] in IDDM and NIDDM patients previously treated with insulin. *Diabetes* 45:1750–1754, 1996

30. Pickup JC, Williams G: The WHO classification of diabetes. In *The Textbook of Diabetes.* Vol. 1. Oxford, Blackwell Science, 1991, p. 39

31. Anderson JH, Brunelle R, Koivisto VA, Pfützner A, Trautmann ME, Vignati L, DiMarchi R, the Multicenter Insulin Lispro Study Group: Reduction of postprandial hyperglycemia and frequency of hypoglycemia in IDDM patients during insulin analog treatment. *Diabetes* 46:265–270, 1997

32. Anderson JH, Brunelle R, Keohane P, Koivisto VA, Trautmann ME, Vignati L, DiMarchi R: Mealtime treatment with insulin analogue improves postprandial hyperglycemia and hypoglycemia in NIDDM patients. *Arch Intern Med* 157:1249–1255, 1997

33. Fineberg SE, Rathbun MJ, Hufferd S, Fineberg NS, Spradlin CT, Galloway JA, Frank B: Immunologic aspects of human proinsulin therapy. *Diabetes* 37:276–280, 1988

34. Huang J, Brunelle R: A nonparametric method for combining multilaboratory data. *Drug Inf J* 36:395–406, 2002

35. Robbins DC, Shoelson SE, Tager HS, Mead PM, Gaynor DH: Products of therapeutic insulins in the blood of insulin-dependent (type 1) diabetic patients. *Diabetes* 34:510–519, 1985

36. Ratner RE, Phillips TM, Steiner M: Persistent cutaneous insulin allergy resulting from high-molecular-weight insulin aggregates. *Diabetes* 39:728–733, 1990

37. Dahl-Jorgensen K, Torjensen P, Hansssen KF, Sandvick L, Aagenaes O: Increase in insulin antibodies during continuous subcutaneous insulin infusion and multiple-injection therapy in contrast to conventional treatment. *Diabetes* 36:1–5, 1987

38. Braimon J, O'Brien M, Kaulbach H, Moses A: The insulin analog lispro effective treats anti-insulin antibody mediated insulin resistance (Abstract). *Diabetes* 45 (Suppl. 2):185A, 1996

39. Roach P, Varshavsky JA, Gantner K, Anderson JH: Insulin antibody-formation during treatment with human insulin or insulin lispro does not affect insulin dose requirements. *Diabetes* 45 (Suppl. 2):958, 1996

40. Hermoso F, Vazquez M, Chaves G, Reviriego J, Andion R: Generalized allergy to human insulin treated with insulin lispro (Abstract). *Diabetologia* 40 (Suppl. 1): A349, 1997

41. Lahtela JT, Salmi J, Antonen J, Knip M, Paul R: Severe antibody-mediated resistance to insulin reduced by plasmapheresis and by switching to human insulin analog (Abstract). *Eur J Endocrinol* 132 (Suppl. 1):15, 1995

42. Lahtela JT, Salmi J, Antonen J, Knip M, Paul R: Severe human insulin antibody-mediated resistance treated with insulin analogue lispro (B28,29) (Abstract). *Diabetologia* 38 (Suppl. 1):A191, 1995

43. Pfützner A, Kustner E, Forst T, Schulze-Schleppinghoff B, Trautmann ME, Haslbeck M, Schatz H, Beyer J: Intensive insulin therapy with insulin lispro in patients with type 1 diabetes reduces the frequency of hypoglycemia episodes. *Exp Clin Endocrinol Diabetes* 104:25–30, 1996

44. Di Mario U, Perfetti R, Anastasi E, Contreas G, Crisa L, Tiberti C, Amendolea MA, Masala C: Auto antibodies to insulin do appear in non-diabetic patients with autoimmune disorders: comparison with anti-immunoglobulin antibodies and other autoimmune phenomena. *Acta Endocrinol (Copenh)* 1222:3030–3038, 1990

45. Eapen SS, Connor EL, Gern JE: Insulin desensitization with insulin lispro and an insulin pump in a 5-year-old child. *Ann Allergy Asthma Immunol* 85:395–397, 2000

46. Raine CH 3rd, Krzyston MJ, Amr M, Hydrick L: Improvement in severe insulin resistance with frequent injections of lispro insulin. *J Natl Med Assoc* 91:410–413, 1999