Development of IgE Antibodies to Human (recombinant DNA), Porcine, and Bovine Insulins in Diabetic Subjects

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Thirty-one previously untreated diabetic individuals received only human insulin (recombinant DNA) for 1 yr with no adverse reactions. The development of serum IgE antibodies to human, porcine, and bovine insulins was assessed by a sepharose radioallergoabsorbent test (RAST). Immunoglobulin (total Ig antibody) binding was assessed by a nonabsorbed species-specific radioimmunoassay. During therapy 2 patients developed IgE antibodies to human insulin as well as increased total Ig binding. The IgE antibodies to human insulin cross-reacted with porcine and bovine insulins, were transient, and were not accompanied by insulin allergy. Ig binding to insulin developed and persisted in 11 of the human insulin-treated diabetics. In comparison, 62 previously untreated diabetic persons received only purified porcine insulin (PPI, < 5 ppm proinsulin, $N = 40$) or a mixed bovine-porcine insulin (proinsulin < 50 ppm, $N = 21$). Increased Ig antibody developed in 16 of 21 patients receiving mixed bovine-porcine insulin and 25 of 41 PPI-treated patients ($P < 0.05$). Seven of 41 PPI-treated patients and 4 of 21 mixed bovine-porcine-treated patients developed anti-insulin IgE antibodies, which were transient in 4 and persisted in 6 diabetic patients. IgE antibody levels did not correlate with total Ig antibody. These data suggest that IgE and total Ig antibodies develop less often after human insulin treatment. Also, the immunoregulation mechanisms responsible for anti-insulin IgE antibody synthesis differ from those regulating other Ig that bind to insulins. Since none of the patients in this study have developed clinical manifestations of insulin allergy or resistance, the clinical relevance of the antibody data must remain speculative. DIABETES CARE 5 (SUPPL. 2): 119–125, 1982.

Many diabetic patients develop antibodies to insulin within weeks to months after initiating therapy with porcine or bovine insulins.[1,2] These immune responses may represent for the diabetic patient a potential complication, especially insulin allergy and/or insulin resistance. The clinical manifestations of insulin allergy vary greatly and range from mild local cutaneous erythema, pruritis, edema, or induration at the insulin injection site to generalized urticaria, angioedema, or systemic anaphylaxis.[3] Insulin-specific IgE antibodies have been found in certain diabetic patients with insulin allergy.[4,5] High levels of anti-insulin antibodies, mostly IgG, are associated with the insulin resistance syndrome, which is defined as a daily insulin requirement of more than 200 U.[6,7] On the other hand, the development of these immune responses to insulin provides the clinical immunologist a unique investigational model to explore the many facets of immunoregulation in the diabetic patient, using insulin as the well-defined antigen probe.

The biosynthesis of human insulin (recombinant DNA), its development as a pharmacologic agent, and the initiation of clinical trials to investigate its clinical efficacy and pharmacology provided us the opportunity to design a prospective study of the immune responses to human insulin. The purpose of this study was to compare the synthesis of specific IgE antibodies to insulins in diabetic patients who were initiating therapy with human insulin with the development of IgE antibodies to insulins in diabetic patients who were beginning therapy with porcine and bovine insulins. The synthesis of total Ig antibodies to human, porcine, and bovine insulins was also measured in these diabetic patients. These studies showed that fewer diabetic patients treated with human in-

TABLE 1
Characteristics of diabetic patients treated with human, porcine, or porcine-bovine insulins

Insulins studied	Diabetics studied	Age	Disease duration	% ideal body weight
Human	31	44 ± 2*	5.2 ± 0.1*	118 ± 3†
Porcine (PPI)	41	45 ± 3	2.4 ± 0.5	117 ± 5
Porcine-bovine (mixed)	21	48 ± 3	5.8 ± 0.7	120 ± 3

*Mean years ± S.E.
†Mean % ± S.E.

sulin developed increased specific IgE or total Ig antibodies to insulins as compared with those diabetic patients treated with porcine and bovine insulins.

MATERIALS AND METHODS

Subjects. In Table 1 are listed the 3 groups of diabetic patients treated with either human, porcine (PPI), or porcine-bovine (mixed) insulins. All of these diabetic patients denied previous therapy with insulin. Even though these diabetics were not preselected or matched for age, disease duration, or percentage of ideal body weight, they were quite similar, with comparable mean ages and ideal body weight. However, the duration of diagnosed illness prior to initiating insulin therapy was somewhat less in those patients selected for therapy with PPI.

Sera. Blood samples were obtained before and at 1- or 2-mo intervals after starting insulin therapy in all study patients. Sera was separated, frozen, and stored at −70°C until studied.

Insulins. The human, PPI, and mixed porcine-bovine insulins were provided for patient use by the Lilly Research Laboratories. The PPI contained less than 5 PPM proinsulin and less than 0.5% bovine insulin; whereas, the mixed porcine-bovine insulin contained less than 50 PPM proinsulin and greater than 80% bovine insulin.

IgE insulin antibodies. The radioallergoabsorbent test (RAST) was utilized to measure specific IgE antibodies to human, porcine, and bovine insulins, as previously described.[8] The species-specific insulins, which were provided by Dr. R. Chance, Lilly Research Laboratories, were individually covalently coupled to cyanogen bromide activated sepharose beads. After incubation of the insulin-sepharose with patient's serum and washing three times, ^{125}I rabbit anti-human IgE antibody (Pharmacia, Uppsala, Sweden) was added. After washing procedures, the radioactivity bound to the antibody-antigen complex on the sepharose particles was counted and the data expressed as percentage counts bound to total counts added (% B/T). Sera from normal subjects who had never received insulin had a mean 3.5% ± 1.7% B/T, and 7% B/T (2 standard deviations above the mean) was considered the upper limits of normal.

Total Ig insulin antibody. Species-specific total Ig antibody (mostly IgG) was measured by a nonabsorbed species-specific radioimmunoassay with ^{125}I insulin as previously described in detail by Fineberg.[9] The antibody activity was expressed as

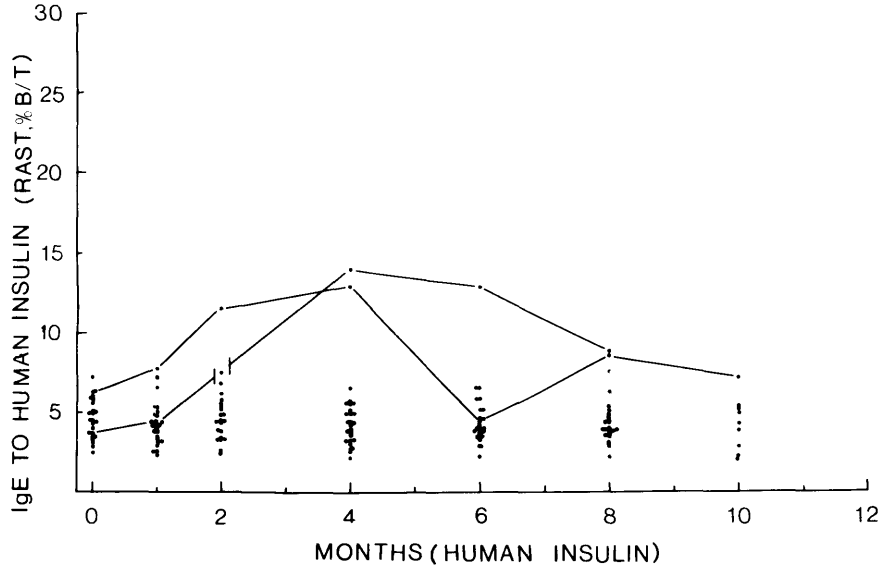

FIG. 1. IgE antibody levels are measured by RAST to human insulin before and during therapy with human insulin. Subjects who developed increased IgE antibody are indicated by the line graphs.

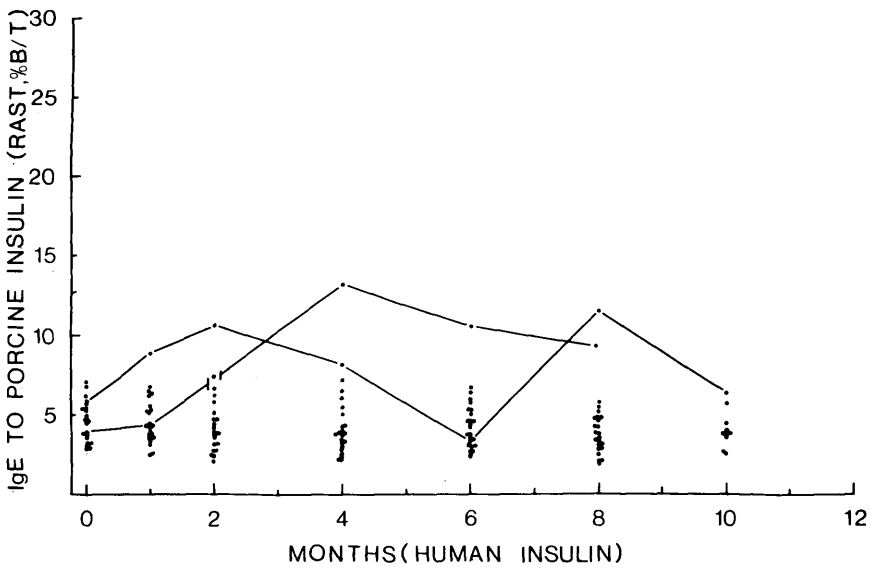

FIG. 2. IgE antibody levels as measured by RAST to porcine insulin before and during therapy with human insulin. Subjects who developed increased IgE antibody are indicated by the line graphs.

percentage bound/total counts (% B/T) minus control serum binding. The upper limits of normal were 4% B/T.

RESULTS

The development of IgE antibodies to human insulin in the 31 patients treated with human insulin is shown in Figure 1. None of these patients had elevated IgE antibodies prior to beginning insulin therapy. Two of the 31 diabetic patients developed increased IgE insulin antibody (greater than 7% B/T), which peaked 2–6 mo after onset of human insulin treatment and returned towards normal by 10 mo of therapy. These 2 patients did not develop any signs or symptoms of insulin allergy or adverse reactions to the human insulin; nor did any of the other 29 subjects treated with human insulin.

As shown in Figures 2 and 3, these same 2 diabetic patients' sera had comparable and transient increases in IgE antibodies to porcine and bovine insulins. These data indicate that the IgE antibodies to human insulin that developed during human insulin therapy cross-reacted with porcine and bovine insulins.

The development of total Ig antibodies to human insulin is shown in Figure 4. Increased Ig antibodies were found in 10 of the 31 patients who were treated with human insulin, including the 2 patients who had increased IgE antibody. The Ig antibodies to human insulin gradually increased during the initial 6 mo of the study and did not appear to correlate with development of species-specific anti-insulin IgE antibodies.

Figure 5 depicts the development of IgE antibodies to por-

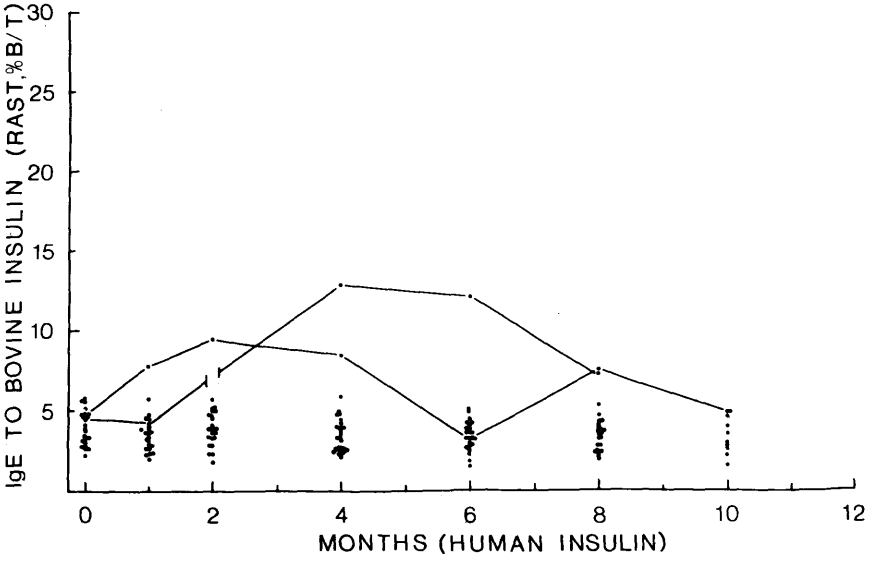

FIG. 3. IgE antibody levels as measured by RAST to bovine insulin before and during therapy with human insulin. Subjects who developed increased IgE antibody are indicated by the line graphs.

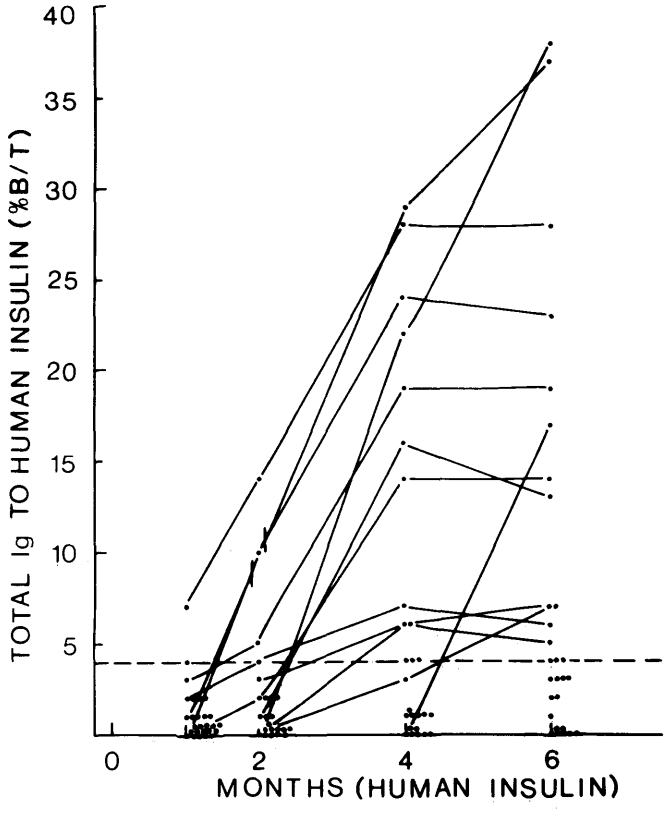

FIG. 4. Total Ig antibody levels as measured to human insulin during therapy with human insulin. Subjects who developed increased Ig antibody are indicated by the line graphs. The dotted line indicates the upper limits of normal (4% B/T).

cine insulin in those patients treated with PPI. Unlike the human-insulin-treated patients, 3 of these diabetic patients had increased IgE antibodies to porcine insulin before starting insulin therapy even though these patients denied previous insulin treatment. After starting PPI therapy, 1 diabetic continued to synthesize increased IgE antibodies to porcine insulin but in the other 2 patients serum IgE antibody to insulin decreased after initiating the insulin therapy. Several patterns of increased IgE antibody to porcine insulin were recognized. Thirty-four of the 41 diabetic patients showed no rise in IgE antibodies. Two patients developed a transient increase of IgE antibodies at 2–4 mo and then returned to normal values after 6–8 mo of insulin therapy, but 3 patients sustained elevated IgE antibody during the 12-mo study. One diabetic gradually developed increased IgE antibodies during the 12 mo of insulin treatment. In spite of increased IgE antibodies, none of these subjects have manifested any clinical allergic or adverse reactions to insulin. Figure 6 shows the 6 patients with increased IgE antibodies to bovine insulin after therapy with PPI. Five of these 6 diabetics also had increased IgE antibodies to bovine insulin, documenting that these IgE antibodies cross-react with both porcine and bovine insulins. However, one patient developed IgE antibodies to only porcine insulin and another had IgE antibodies to only bovine insulin.

Figures 7 and 8 depict the development of IgE antibodies to porcine and bovine insulin in the 21 diabetics treated with the mixed porcine-bovine insulin. Two patients had increased IgE antibodies to porcine insulin but not to bovine insulin before starting insulin, and both patients denied prior insulin therapy. One subject sustained the increased IgE antibody to porcine insulin during insulin therapy, but unlike

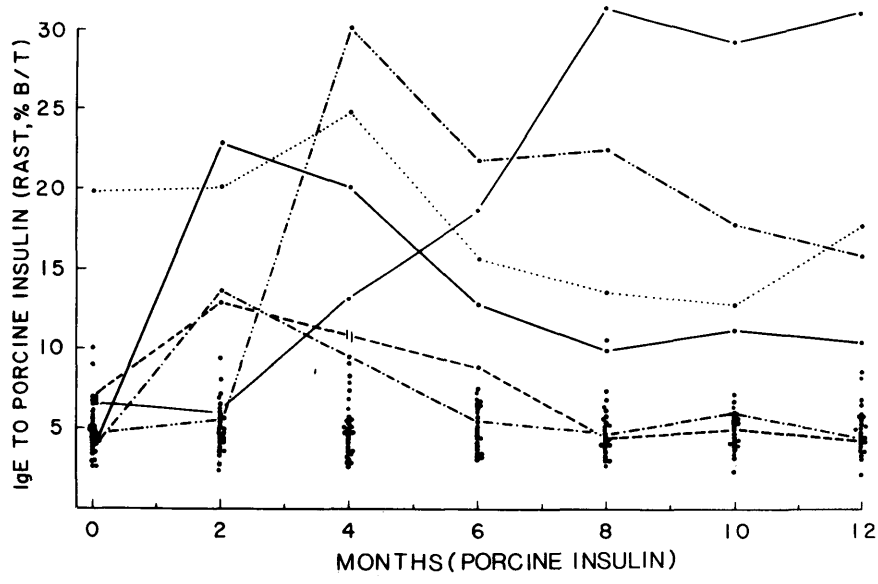

FIG. 5. IgE antibody levels as measured by RAST to porcine insulin before and during therapy with porcine (PPI) insulin. Subjects who developed increased IgE antibody are indicated by the line graphs using the several symbols.

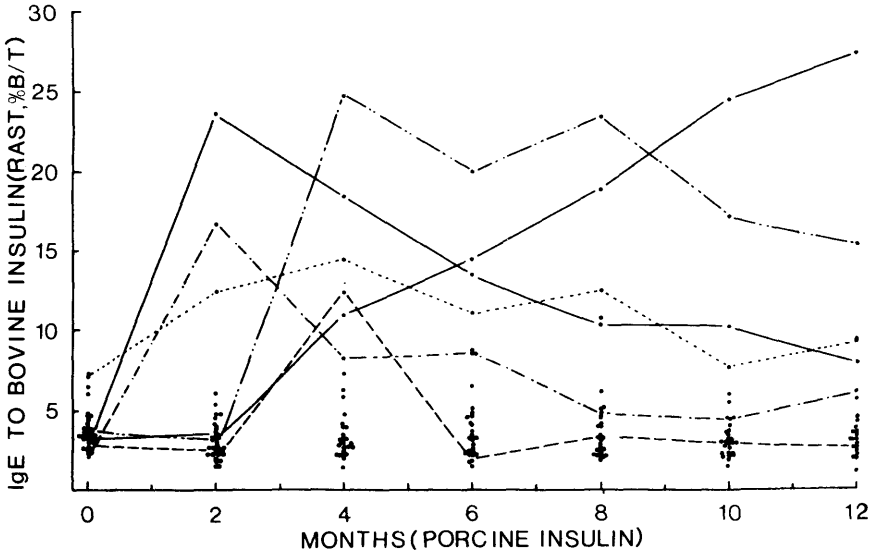

FIG. 6. IgE antibody levels as measured by RAST to bovine insulin before and during therapy with porcine (PPI) insulin. Subjects who developed increased IgE antibody are indicated by the line graphs using the same symbols used in Figure 5.

the other subjects, this patient's porcine IgE antibody did not cross-react with bovine insulin. The transient nature of the increase in IgE antibody at 2–4 mo of insulin therapy was again noted.

Table 2 summarizes the development of IgE and total Ig antibodies to human, porcine, and bovine insulins in patients treated with human, PPI, and porcine-bovine insulins. Whereas 6% of patients treated with human insulin developed increased IgE antibodies, 17% and 19% of those treated with PPI or mixed insulins, respectively, had increased IgE antibodies to insulin. A greater percentage of patients treated with PPI or the mixed porcine-bovine insulins (61% and 76%, respectively) developed total Ig (mostly IgG) antibodies to the several insulins than those patients (35%) who synthesized IgE antibodies to insulin after therapy with human insulin.

DISCUSSION

Since fewer diabetic subjects treated with human insulin developed increased IgE or total Ig antibodies to insulin as compared to patients treated with PPI or mixed porcine-bovine insulins, it appears that human insulin was less immunogenic than porcine or bovine insulin. This observation was not unexpected since human insulin is identical chemically to natural human insulin, and the porcine and bovine insulins have amino acid differences.[10] If human insulin (recombinant DNA) is identical to naturally occurring human insulin, then theoretically it should be considered "self," and there should be little if any antibody synthesis to a native antigen. Whether this expression of antibody synthesis to human insulin in these diabetic patients represents autoimmunity or another phenomenon

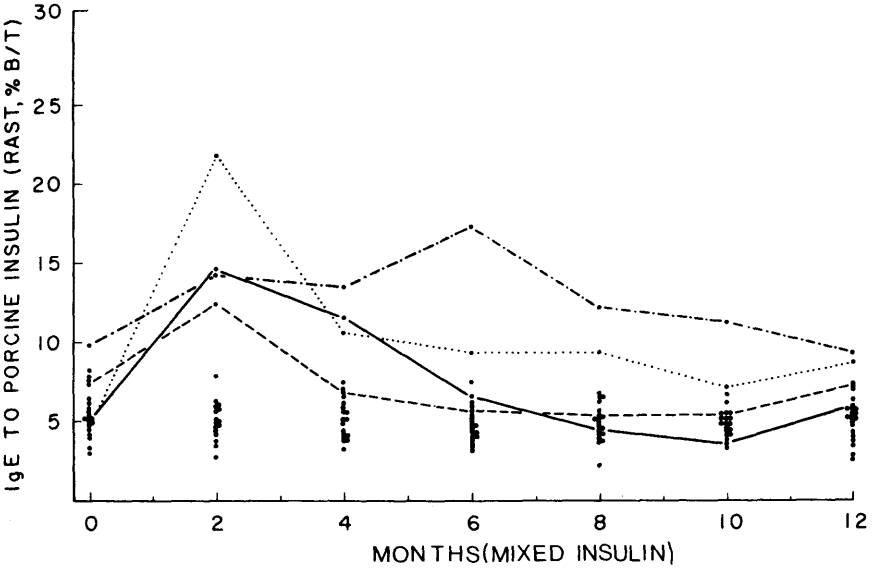

FIG. 7. IgE antibody levels as measured by RAST to porcine insulin before and during therapy with mixed porcine and bovine insulin. Subjects who developed increased IgE antibody are indicated by the line graphs utilizing the several symbols.

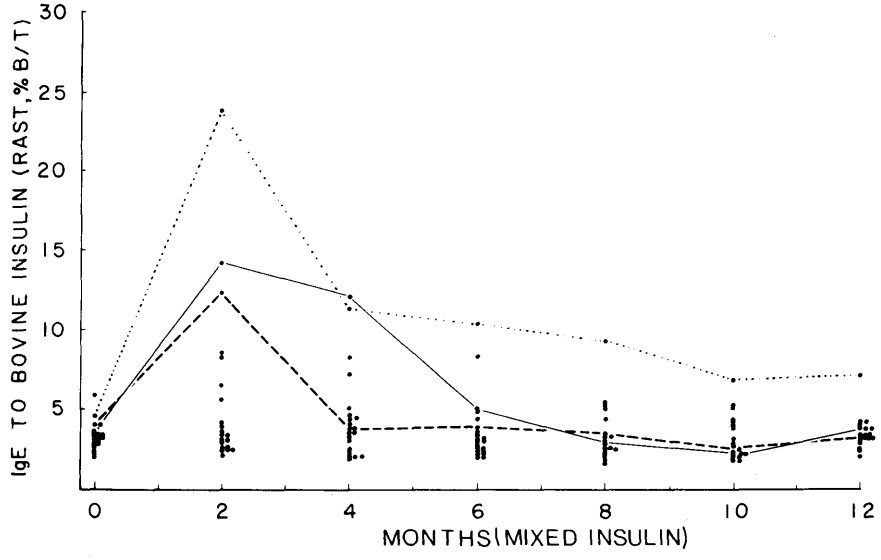

FIG. 8. IgE antibody levels as measured by RAST to bovine insulin before and during therapy with mixed porcine and bovine insulin. Subjects who developed increased IgE antibody are indicated by the line graphs using the same symbols used in Figure 7.

related to the subcutaneous route of insulin administration or some other unidentified mechanism must remain speculative at this time. There may be other possible explanations for these results. Since diabetic patients with a personal or family history of allergic diseases such as allergic rhinitis or asthma were not excluded from the study, it is possible that patients prone to increased IgE synthesis were inadvertently included in the study group. This does not appear to be the case since total serum IgE levels were not elevated in any of those diabetics who developed increased IgE antibodies to insulin. It is also possible that the patients who developed increased IgE antibodies to insulin may have a genetic predisposition to synthesize IgE antibodies. It has been suggested that specific IgE antibody responses to ragweed pollen are influenced by immune response genes, and the IgE antibody response to ragweed pollen was linked to the histocompatibility (HLA) locus.[11] HLA typing will be performed on our study patients, but these studies have not been completed.

It should be emphasized that less than 20% of the diabetic subjects developed IgE antibodies during therapy with human, porcine, or bovine insulins, yet 61–76% of the patients treated with porcine or bovine insulin developed total Ig (mostly IgG) insulin antibodies. In those patients treated with human insulin, 6% developed IgE antibodies, whereas 35% developed total Ig insulin antibodies. Thus, IgE antibody synthesis did not correlate with total Ig antibody synthesis and indicated that the immunoregulation mechanism responsible for anti-insulin IgE antibody differs from those regulating other Ig that can bind to insulin.

Since none of the patients in the three study groups have developed clinical manifestations of insulin allergy, resistance, or other potential immune-mediated complications of diabetes, the clinical relevance of the observed increased antibody levels must remain speculative. It is our anticipation that these prospective studies will be continued and the clinical data will be forthcoming in the not too distant future.

TABLE 2
Development of antibodies to insulins

Insulin studied	Diabetic patients with increased antibodies to insulin	
	IgE*	Total Ig†
Human	2 (6%)‡	11 (35%)
Porcine (PPI)	7 (17%)	25 (61%)
Porcine-bovine (mixed)	4 (19%)	16 (76%)

*After 12 mo of insulin therapy.
†After 6-mo insulin therapy.
‡Percentage of patients studied.

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