# Insulin Allergy: Differences in the Binding of Porcine, Bovine, and Human Insulins with Anti-Insulin IgE

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We investigated in vitro binding of insulins of various species sources (bovine, porcine, and human) with anti-insulin reaginic immunoglobulins (IgE) in 10 patients with systemic insulin allergy and in 5 nonallergic but insulin-resistant cases. Anti-insulin IgE had a higher avidity for bovine compared with porcine insulin in both groups. Avidity for bovine insulin was also significantly higher compared with human or desalanine porcine insulin in the insulin-allergic patients. These observations provide a rationale for using porcine insulin in the treatment of systemic insulin allergy. DIABETES CARE 4: 104–107, JANUARY–FEBRUARY 1981.

Reaginic immunoglobins (IgE) are associated with allergic reactions. Insulin-binding IgE is found in the patients with systemic allergy to insulin[1–8] and also in some insulin-treated patients without insulin allergy.[1,6] Patterson et al.[3] investigated the immunoreactivity of anti-insulin IgE to insulins of varied species sources in three patients and observed that IgE bound more avidly to bovine insulin compared with porcine or human insulin. Since the number of patients studied previously has been small, and those tested showed marked variations in immunoreactivities to various insulins, we investigated the species specificity of anti-insulin IgE in a larger group of insulin-treated patients with and without systemic insulin allergy.

## MATERIALS AND METHODS

Sera were collected from the following two groups of diabetic patients.

*Insulin-allergic group.* Ten patients presented with systemic allergic reactions to insulin therapy. The clinical data of these cases are summarized in Table 1. Intradermal tests with commercial bovine and porcine insulins (0.2 U/test) were 4+ in all cases.[9] Nine patients were tested with single-component bovine[10] and single-component porcine insulins (Eli Lilly and Co., Indianapolis, Indiana); eight had 4+ dermal responses to both these insulins, and one patient (#9) showed a 3+ reaction to single-component porcine but a 4+ response to single-component bovine insulin. All patients were given oral diphenhydramine 100–150 mg/day. Five cases received porcine insulin in one-third the daily dose, gradually increasing as described by Mattson et al.[4] One patient (#9) was treated with single-component porcine insulin. This decision was based on the results of the intradermal test.[8,11] However, a subsequent in vitro investigation for reaginic antibodies to proinsulin-like contaminant proteins was negative.[9] Two patients (#2 and #4) required desensitization. These patients had not taken any insulin injections for over 24 h after the systemic allergic reaction. Since these patients had a higher risk of recurrent and profound systemic reaction on resumption of insulin therapy,[4,5] it was necessary to desensitize. The remaining two patients (#1 and #7) were continued on bovine-porcine insulin.

*Nonallergic insulin-resistant group.* Five patients who had no manifestations of insulin allergy but had high titers of insulin-binding antibodies were selected for comparison. Their mean age was 61.8 ± 1.6 yr, and duration of diabetes was 12 ± 5 yr. In the past, all these cases had required high doses of insulin, 240–7200 U/day, and were insulin resistant. Their maintenance insulin dose was 130 ± 50 U/day. Three cases received monospecies bovine, one was on porcine, and one on bovine-porcine mixed insulin.

Blood samples were obtained after an overnight fast before the morning dose of insulin. In the allergic group samples were collected within 24 h of the systemic reaction. Sera were stored at −20°C until used. Laboratory results were not available for the clinical management of patients.

*Radioiodinated insulin.* Bovine insulin (lot No. 795372, Eli Lilly and Co.) was iodinated by the method of Hunter and

TABLE 1
Clinical data on insulin-allergic patients*

| Patient | Age | Sex | Duration of diabetes (yr) | Allergic manifestations | Insulin used in treatment† | Remarks |
|---|---|---|---|---|---|---|
| 1 | 24 | F | 2 | Urticaria | Bovine-porcine mixed | Insulin resistant |
| 2 | 60 | F | 18 | Urticaria | Porcine | Desensitized |
| 3 | 58 | F | 28 | Urticaria | Porcine | |
| 4 | 46 | F | 3 | Urticaria | Bovine-porcine mixed | Desensitized |
| 5 | 52 | F | 10 | Urticaria | Porcine | Oral prednisone was also given |
| 6 | 52 | M | 4 | Urticaria | Porcine | |
| 7 | 26 | F | 6 | Urticaria | Bovine-porcine mixed | Systemic lupus erythematosus; patient was on prednisone 20 mg/day, which was increased to 40 mg/day for 3 wk |
| 8 | 46 | F | 6 | Urticaria | Porcine | |
| 9‡ | 27 | F | 2 | Urticaria | Single-component porcine | |
| 10 | 56 | F | 2 | Urticaria plus angioedema | Porcine | |

* All patients had a history of discontinuous therapy with bovine-porcine mixed insulin. Allergic reactions appeared in 1–4 wk after insulin therapy was resumed.
† Oral diphenhydramine, 100–150 mg/day, was prescribed in each case.
‡ Skin test with single-component porcine insulin produced 3+ response.

Greenwood.[12] It was purified by column chromatography.[13] The specific activity was 120 mCi/mg.

*Unlabeled insulins.* Human, porcine (lot nos. 21372 and S823036, respectively, Novo Laboratories, Copenhagen, Denmark), desalanine porcine (lot no. 615-1082B-243, free from unmodified porcine insulin, Eli Lilly and Co.), and bovine insulins were dissolved in 0.1 N HCl and diluted to 50 and 250 ng/ml with borate buffer, pH 7.4, containing 1% horse serum for immunoassays. These insulin solutions were used in the competition assay without further dilution. The same preparations of $^{125}$I-insulin and unlabeled insulins were used for all sera.

*Anti-insulin IgE.* Anti-insulin IgE titers were assayed by our previously described paper radioimmunosorbent technique.[1] Fifty microliters of serum was added to a paper disc covalently coupled with anti-human IgE sheep serum (Phadebas IgE, PRIST, Pharmacia Diagnostics, Uppsala, Sweden), and preabsorbed with an excess of human IgG (Miles Laboratories, Inc., Elkhart, Indiana). After an incubation period of 20 h at 4°C, the disc was washed and reincubated with 30 $\mu$l of $^{125}$I-insulin (50 pg) and 20 $\mu$l of buffer. The disc was washed after 20 h and the radioactivity bound was counted in a gamma well counter (Tracor, model 1195). After subtracting the nonimmune counts obtained with normal human serum (<3% of total counts), the anti-insulin IgE titer was expressed as amounts of labeled insulin bound/ml of serum. We have shown that this technique estimates insulin-specific IgE, and intra- and interassay coefficients of variation are 6.2% and 10.1%, respectively. In the competition assays, 20-$\mu$l vol of unlabeled insulin solutions (1–5 ng/test) were mixed with tracer, and percentage reduction in tracer binding due to various insulins was observed. The immunoreactivity of a serum with all unlabeled insulins was studied in a single assay.

## RESULTS

The amounts of $^{125}$I-bovine insulin bound with IgE were 17,393 ± 4342 SEM and 10,868 ± 4838 cpm/ml in the insulin-allergic and nonallergic groups, respectively. Unlabeled insulins reduced

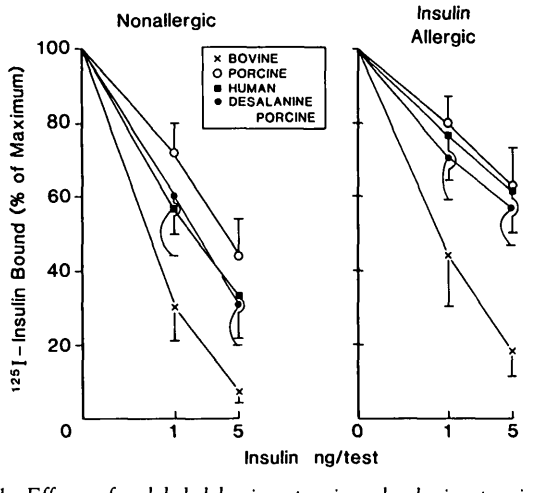


*FIG. 1. Effects of unlabeled bovine, porcine, desalanine porcine, and human insulins on the binding of $^{125}$I-bovine insulin. Data points are group mean ± SEM. Bovine insulin produces more displacement of tracer compared with porcine insulin ($P < 0.05$) in both groups.*

the binding of $^{125}$I-bovine insulin (Figure 1). In the insulin-allergic group unlabeled bovine insulin was more effective ($P < 0.05$) in reducing tracer binding compared with all other insulins (porcine, desalanine porcine, and human). The difference between bovine and porcine insulins was observed in 9 of the 10 insulin-allergic patients. In the nonallergic but insulin-resistant cases the binding of $^{125}$I-bovine insulin was also reduced more by bovine than by porcine insulin ($P < 0.05$). In these cases, however, the reduction in binding caused by bovine insulin was not significantly different when compared with human and desalanine porcine insulins. The competition caused by porcine insulin appeared to be less than that of human insulin, but it was insignificant statistically.

## DISCUSSION

We previously reported[1] that low titers of anti-insulin IgE were found in insulin-treated patients who were nonallergic and nonresistant to insulin. Patients with systemic insulin allergy had high titers of anti-insulin IgE, and their titers did not differ significantly when compared with the titers found in immune-type insulin-resistant patients. The ratios of anti-insulin IgE/anti-insulin IgG were significantly higher in systemic insulin allergy compared with insulin-resistant or nonallergic, nonresistant insulin-treated cases. Those studies[1] suggested that a relative excess of anti-insulin IgE, but not the absolute titers of anti-insulin IgE, were associated with the overt insulin-allergic state.

In the present study, we report the reactivities of anti-insulin IgE with various insulins. Since serum samples with high titers of anti-insulin IgE were required for these investigations, we selected sera from (1) systemic insulin-allergic patients and (2) immune-type insulin-resistant cases. Anti-insulin IgE bound bovine insulin more avidly compared with porcine, desalanine porcine, or human insulins. The high avidity for bovine insulin was a common phenomenon as seen in 9 of our 10 insulin-allergic cases, in all 5 nonallergic insulin-resistant cases, and in three insulin-allergic cases reported by Patterson et al.[3] In this respect, the insulin-specific IgE resemble other insulin-binding immunoglobulins, anti-insulin IgG, which are generally found to have higher avidity for bovine than for porcine insulin.[14,15]

Intradermal tests performed with 0.2 U (equivalent to 8 $\mu$g) of insulin failed to differentiate bovine and porcine insulins in our allergic patients. There was, therefore, a discrepancy between in vivo and in vitro tests. This may be due to greater versatility of in vitro techniques in which we were able (1) to compare the binding affinities of anti-insulin IgE for various insulins at very low concentrations (1–5 ng/test) and (2) to study a direct competition between bovine and other insulins. Perhaps intradermal tests performed with decreased insulin dose may have shown differences between bovine and porcine insulins.

In insulin-allergic patients, skin tests have dual purposes: (1) to confirm the diagnosis of insulin-allergy and (2) to help in the selection of better tolerated insulin. Although we were unable to demonstrate any differences between porcine and bovine insulins in skin tests, Kahn and Rosenthal[8] state in a recent review on insulin allergy that porcine insulin is better tolerated in almost all cases. In view of these remarks, as well as our present in vitro observations that anti-insulin IgE have lower avidity for porcine than for bovine insulin, it is reasonable to recommend the use of porcine insulin in the treatment of systemic insulin allergy, with the exception of a rare case, where a skin test shows a better tolerance to nonporcine insulin.

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## REFERENCES

[1] Kumar, D.: Anti-insulin IgE in diabetics. J. Clin. Endocrinol. Metab. *45*: 1159–64, 1977.

[2] Lieberman, P., Patterson, R., Metz, R., and Lucena, G.: Allergic reactions to insulin. JAMA *215*: 1106–12, 1971.

[3] Patterson, R., Mellies, C. J., and Roberts, M.: Immunologic reactions against insulin. II. IgE anti-insulin, insulin allergy, and combined IgE and IgG immunologic insulin resistance. J. Immunol. *110*: 1135–45, 1973.

[4] Mattson, J. E., Patterson, R., and Roberts, M.: Insulin therapy in patients with systemic allergy. Arch. Intern. Med. *135*: 818–21, 1975.

[5] Lamkin, N., Lieberman, P., Hashimoto, K., Morohashi, M., and Sullivan, P.: Allergic reactions to insulin. J. Allergy Clin. Immunol. 58: 213–33, 1976.

[6] Velcovsky, H. G., Jonatha, E. M., Schmidt, G., and Federlin, K.: Quantitative measurement of insulin specific IgE antibodies in diabetics with and without insulin allergy. Diabetologia *13*: 437 (abstract 325), 1977.

[7] Nakagawa, S., Saito, N., Nakagawa, H., Sasaki, T., Watanabe, T., and Aoki, S.: Detection of IgE insulin antibodies with radioallergosorbent test. Diabetologia *14*: 33–38, 1978.

[8] Kahn, R. C., and Rosenthal, A. S.: Immunologic reactions to insulin: insulin allergy, insulin resistance, and the autoimmune insulin syndrome. Diabetes Care *2*: 283–95, 1979.

[9] Kumar, D., Rosenquist, R. J., and Parameswaran, V.: Insulin allergy, reaginic antibodies to insulin and proinsulin. J. Clin. Endocrinol. Metab. *49*: 252–54, 1979.

[10] Chance, R. E., Root, M. A., and Galloway, J. A.: The im-

munogenicity of insulin preparations. Acta Endocrinol. (Copenhagen) 83(Suppl. 205): 185–96, 1976.

[11] Galloway, J. A., and Bressler, R.: Insulin treatment in diabetes. Med. Clin. North Am. 62: 663–80, 1978.

[12] Hunter, W. M., and Greenwood, F. C.: Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature 194: 495–96, 1962.

[13] Kumar, D., and Miller, L. V.: Proinsulin specific antibodies in human sera. Diabetes 22: 361–66, 1973.

[14] Berson, S. A., and Yalow, R. S.: Species-specificity of human anti-beef, pork insulin serum. J. Clin. Invest. 38: 2017–25, 1959.

[15] Kurtz, A. B., Matthews, J. A., and Nabarro, J.D.N.: Insulin binding antibody: reaction differences with bovine and porcine insulins. Diabetologia 15: 19–22, 1978.