Insulin-Specific IgG and IgE Antibody Response in Type I Diabetic Subjects Exclusively Treated with Human Insulin (recombinant DNA)

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Bovine and porcine insulins elicit specific antibody response in diabetic subjects after a few months of treatment. In seven type I diabetic individuals who were exclusively treated with human insulin (recombinant DNA) each month, sera were examined for the development of insulin-specific IgG and IgE antibodies. In all patients (except one), IgG antibodies occurred after 2 mo and tended to further increase in concentration after 5–6 mo. IgE antibodies could be detected after 1 mo with a further increase after 2–3 mo and a marked decline thereafter. No patient exhibited allergic symptoms. The results indicate that physicochemical properties of insulin preparations used for treatment and the route of administration are of more importance for immunogenicity than species differences of insulin. DIABETES CARE 5 (SUPPL. 2): 126–128, 1982.

Since the introduction of insulin into the treatment of diabetes mellitus in 1921, patients have suffered in varying degree from such immunologic side effects as insulin allergy, resistance, or lipodystrophy. Although various purification techniques of the formerly crude pancreatic extract and the development of single- or monocomponent insulins led to a worldwide reduction of those immunologic side effects, up to the present they still can be observed in a significant group of patients. Whether the species differences in amino acid composition or other factors such as the route of administration and the aggregated forms of depot insulins are of greater importance remained unknown. The introduction of human insulin (recombinant DNA) into the treatment of diabetes offered a unique chance to answer this question.

MATERIAL AND METHODS

Seven freshly diagnosed type I diabetic subjects aged 14–42 yr received NPH human insulin (HUMULIN, Eli Lilly and Company, Indianapolis, Indiana) in doses between 2 and 36 U/day over a period of 7 mo. Blood samples were taken in a fasting state before and at monthly intervals during the treatment, always 12 h after the last insulin injection. After spontaneous clotting the sera was kept in deep freeze until examination.

Determination of insulin-specific IgG antibodies. IgG antibody concentration was measured using a radioimmunoelectrophoresis technique according to Christiansen.[1] Serum samples are incubated with ^{125}I-A14-labeled insulin (specific activity 350–360 μCi/ml) of human, bovine, or porcine origin (kindly provided by the Eli Lilly Company) in a barbital buffer 0.02 M, pH 8.6 at 4° for 12 h. Specimens of 5 μl are put into small holes of 1% agarose containing rabbit anti-human IgG (Difco) (5 ml ab/50 ml agarose). The agarose plates are exposed to electrophoresis for 16 h with 2–3 volt/cm at 4°. Precipitates are excised and measured for radioactivity in a gamma counter. The content of insulin-specific IgG of a serum sample is given as mU/ml. While in sera of normal control persons and non-insulin-treated diabetic subjects insulin-binding immunoglobins are not detectable, each measurable concentration of insulin binding IgG expresses an immune response to insulin. Concentrations below 0.1 mU/ml are of no clinical relevance while values above 0.3 mU/ml mostly are accompanied by an increased daily insulin requirement.

Determination of insulin-specific IgE antibodies. Measurement of insulin-specific IgE antibodies was performed using our own techniques (Federlin and Velcovsky,[2] and Velcovsky et al.[3]). In this method insulin is fixed to a solid carrier (sepharose B or cellulose paper platelets 0.6 mm diameter) which are activated by cyanbromide. Insulin is coupled to the carrier in a 0.1 M $NaHCO_3$ solution; the coupling process is stopped by ethanol amine. The platelets, which are preferentially used for various advantages compared to sepharose, are dried and lyophilized. Patients' sera are incubated with

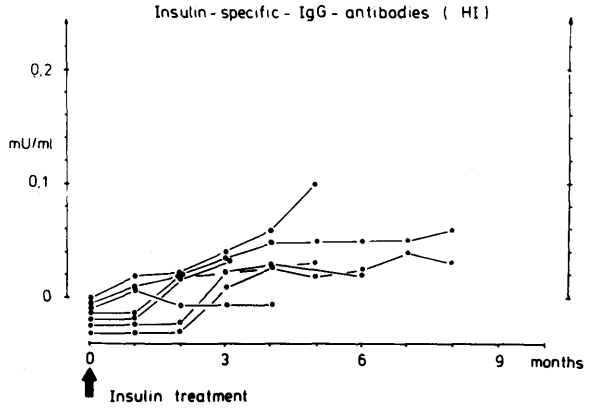

FIG. 1. Development of insulin-specific IgG antibodies in sera of type I diabetic individuals exclusively treated with HI (human insulin) *determined by radioimmunoelectrophoresis using ^{125}I-human insulin as antigen.*

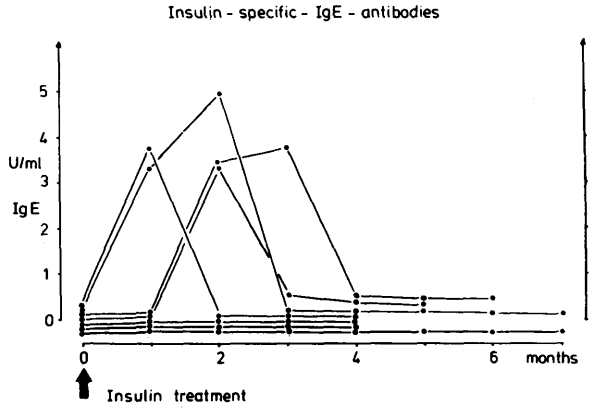

FIG. 3. Insulin-specific IgE antibodies in the sera of type I diabetic individuals (same patients as in Figure 1). RAST (platelet) technique, ^{125}I-bovine insulin as antigen.

the antigen-containing carrier for 16 h and after three washing procedures ^{125}I-rabbit anti-human IgE is added. After a further washing the samples are counted in a gamma counter. The insulin-specific IgE concentrations are given in units per milliliter.

RESULTS

IgG antibodies. As shown in Figure 1, after 1 mo three of seven patients produced small amounts of antibodies, and after 3 mo (with one exception) all patients demonstrated antibodies in the range between 0.01 and 0.1 mU/ml. There was no correlation between the amount of antibodies in serum and the daily insulin dosage.

For comparison, the results of determination of antibodies in six type I diabetic individuals who were treated exclusively with purified porcine insulin (PPI) are shown in Figure 2. The rise of antibody concentrations occurred at the same time—after 1 mo of treatment—however, to much higher values.

IgE antibodies. In contrast to the pattern of IgG antibodies, patients after the initiation of treatment with human insulin developed the highest concentrations of IgE antibodies after 2 mo followed by a marked decline after 4 mo of treatment to values slightly above normal (Figure 3). Whether further treatment will be accompanied by another rise in antibody concentration has to be awaited. None of the patients exhibited allergic symptoms to insulin.

Binding of insulin as antigen. The sera showed different behavior of immunoglobulin binding for the various types of insulin. Immunoglobulins of the IgG type bound human insulin, bovine insulin, and porcine insulin in nearly the same amount. IgE antibodies, however, in the concentrations measured at this early period of treatment, were only detectable using bovine insulin as the antigen (Figure 3).

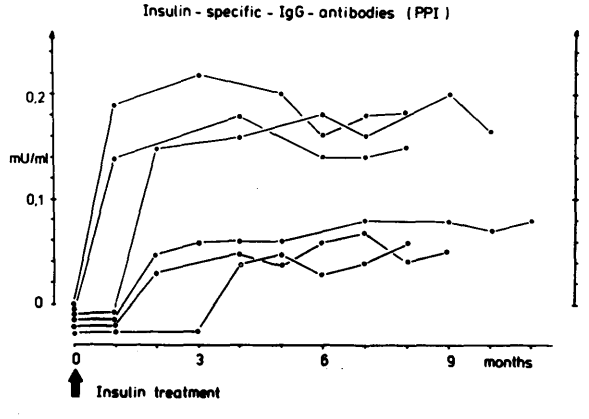

FIG. 2. Development of insulin-specific IgG antibodies in sera of type I diabetic individuals exclusively treated with PPI determined by radioimmunoelectrophoresis using ^{125}I-porcine insulin as antigen.

DISCUSSION

The results confirm recent data that human insulin is—possibly only slightly—immunogenic. Schernthaner et al.[4] reported for the first time increased binding capacity of serum for insulin in 30% of patients treated with human insulin of the semisynthetic type. With regard to human insulin, Fineberg[5] described the first cases of anti-human antibodies using PEG precipitation and the relation of bound to free insulin in a given amount of serum.

The present study describes the specific binding of insulin to immunoglobulins using radioimmunoelectrophoresis in patients treated solely with human insulin. With this method it could be demonstrated that antibody production starts very early and that small concentrations, with one exception, were found in all patients. Insulin-specific IgE antibodies were neglected for a long time in insulin immunology. However in former studies we demonstrated that this type of

immunoglobulin in insulin-treated diabetic subjects may serve as a very sensitive parameter for the immunogenicity of insulin preparations[2,3] except in patients with insulin allergy.[6–8]

For patients treated with human insulin, the results demonstrate the reliability of measuring specific IgE as a parameter for immunogenicity. Interestingly the rapid rise of insulin-specific IgE antibodies after the initiation of human insulin treatment was followed by a sudden decline after 4 mo of treatment. A similar observation was made by Hamilton et al.[9] in patients treated with bovine and porcine insulins. The detection of insulin-specific IgE antibodies after treatment with human insulin by using bovine insulin as the antigen in vitro confirms the observations of Kumar,[10] who described a higher avidity of anti-insulin IgE for bovine compared with porcine and human insulin in patients treated with porcine and/or bovine insulin. This underlines not only the usefulness of porcine or human insulin for the treatment of insulin allergy but also suitability of bovine insulin in the search of immunogenicity of various insulins.[11]

In conclusion it was also shown that human insulin is immunogenic in man. However it is still open how far this might be of clinical relevance. The physicochemical nature of the insulin used for treatment and the subcutaneous route of administration seem to play a greater immunologic role than the species-defined sequence of amino acids.

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