and SE but did not affect activity of α- and β-interferons. Antisera to human α- and β-interferons neutralized the antiviral action only of standard homologous interferons, and did not affect activity of interferons induced by SE, PHA, and con A. The antiviral properties of the latter were neutralized on treatment with antiserum to human γ-interferon which, in turn, was inactive against standard α- and β-interferons. The results indicate that the SE preparations induce the production of an interferon, by human peripheral blood lymphocytes, whose physicochemical and antigenic properties correspond to those of human γ-interferon.

It can thus be concluded from these investigations that SE possess marked mitogenic and interferon-inducing properties.

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MONOCLONAL ENZYME IMMUNOASSAY TEST SYSTEM FOR TOTAL IMMUNOGLOBULIN E DETERMINATION IN CHILDREN AND ADOLESCENTS

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UDC 616.153.962.4-097-078.73-053.2

KEY WORDS: immunoglobulin E; enzyme immunoassay; monoclonal antibodies

Determination of the total immunoglobulin E (IgE) concentration in the blood serum or plasma of children and adolescents can be informative for the discovery of persons predisposed to allergic diseases [1]. The serum IgE level in children is known to be lower than in adults, and in the normal neonate it is 1-2 IU/ml [5]. With age the IgE concentration rises, to reach the adult level at ~15 years of age [6]. To detect such insignificant concentrations of IgE, a reliable and highly sensitive method of determination of the IgE level is needed. The use of monoclonal antibodies (McAb) with high binding constants, specific for particular epitopes of antigen molecule (in this case the IgE molecule) enables such a method to be developed.

The aim of this investigation was to develop a test system capable of the sufficiently rapid, easy, and reliable measurement of IgE concentrations of between 1 and 100 IU/ml in undiluted blood serum, with a minimal volume of the serum sample and with one-stage conduct of the immunochemical reaction.

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(Presented by Academician of the Academy of Medical Sciences of the USSR, R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 6, pp. 722-724, June, 1989. Original article submitted June 2, 1988.

0007-4888/89/0006-0842$12.50

TABLE 1. Basic Characteristics of Enzyme Immunoassay Test Systems Based on McAb for Quantitative Determination of Total Blood Serum or Plasma IgE Level in Children and Adolescents

Parameter measured	Basic characteristics
Principle of method of analysis	One-stage solid-phase enzyme immunoassay based on a combination of two McAb differing in specificity
Total time of analysis	1.5 h
Sensitivity	1 IU/ml
Range of measurements	1-100 IU/ml
Type of calibration curve	Linear or near-linear
Reproducibility (coefficient of variability)	±5%
Additivity ("uncovering" test)	100 ± 5%
Volume of sample (analysis conducted in duplicate)	15 µl × 2

TABLE 2. Effect of Anticoagulants and Serum Components on Determination of IgE

Substance added	Concentration of substance, mg/ml	Mean o.d. at 492 nm	Percent relative to original serum
Original serum	--	0.782	100
Heparin	1	0.792	101.3
EDTA	7.2	0.782	100.0
Sodium citrate	5	0.748	95.6
Sodium oxalate	3	0.776	99.2
Hemoglobin	12	0.854	109.2
Bilirubin	0.3	0.708	90.5
Phosphatidylethanolamine dipalmitoyl	12.2	0.741	94.8
Lysophosphatidylcholine	12.2	0.932	119.1
Theophylline	0.4	0.721	92.2

EXPERIMENTAL METHOD

Human McAb and IgE described previously [2] were used. Purification of the McAb from ascites fluid was carried out by ion-exchange chromatography on DEAE-cellulose [4]; IgE/11-4 McAb were immobilized on a solid phase (Nune-Immunol polystyrene panels) from carbonate buffer, 0.05 M, pH 9.6, with McAb concentration of 5-10 µg/ml for 16 h at 4°C. Nonspecific binding sites were then blocked with 0.2% gelatin in phosphate-buffered physiological saline, pH 7.4, containing 0.1% sodium azide. The panels thus imprinted were kept at 4°C for several months. The IgE/11-1 McAb were conjugated with the enzyme, horseradish peroxidase, by the periodate oxidation method [7] with some modifications: dialysis after oxidation of the peroxidase was replaced by gel-filtration on Sephadex G-25. An equal volume of glycerol was added to the solution of the conjugate thus obtained and the mixture was kept at −20°C for several months. The conjugate of McAb with horseradish peroxidase, thus prepared, did not require chromatographic purification by gel filtration, for it contained virtually no high-molecular-weight polymers or free peroxidase. The working dilution of the conjugate was chosen so that the maximal standard of 100 IU/ml, under the assigned reaction conditions, gave an optical density (o.d.) of 1.5-1.9 o.d. units. IgE standards were prepared by diluting IgE myeloma protein (I-0) in horse serum, clarified by centrifugation (100,000g,

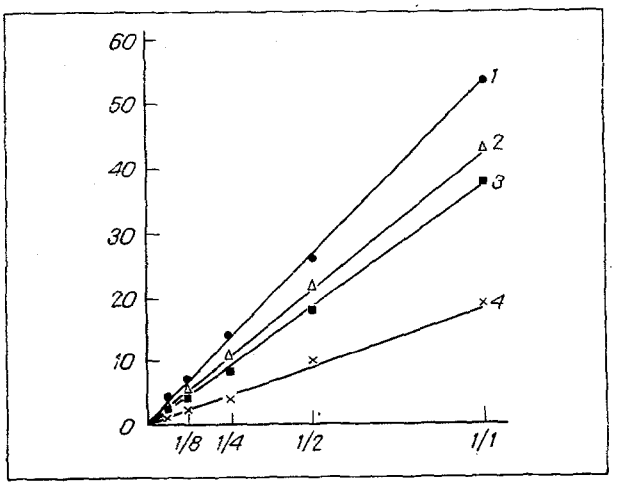

Fig. 1. Dependence of IgE concentration on dilution of serum. Abscissa, dilution of serum; ordinate, IgE concentration (in IU/ml). 1-4) Different samples of sera.

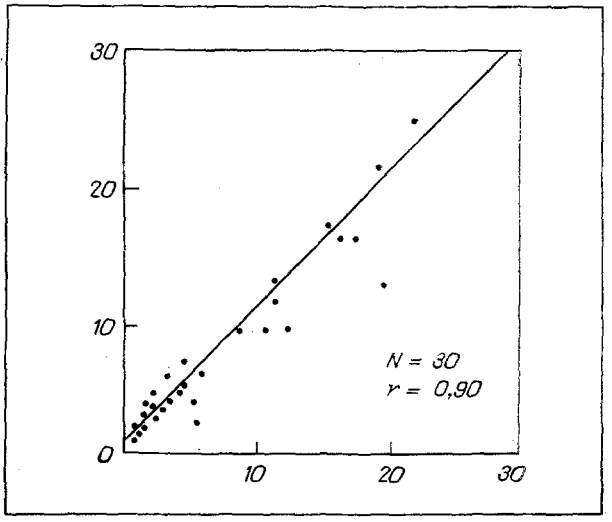

Fig. 2. Correlation of developed test system with commercial kit from "Boehringer." Abscissa, values of IgE concentrations (IU/ml) obtained by developed test systems; ordinate, values of IgE concentrations obtained with kit from "Boehringer." N) Number of measurements, r) coefficient of correlation.

1 h). The standards were calibrated against the second international standard WHO No. 75/502 for human serum IgE [3]. As the zero control, horse serum was used. Enzyme immunoassay of IgE was carried out as described above. The volume of the serum sample was 15 µl and the samples and conjugate of McAb with peroxidase were incubated simultaneously in a panel (for 1 h at 37°C). The enzyme reaction proceeded for 10 min at room temperature, the concentration of o-phenylene diamine being 0.6 mg/ml and of hydrogen peroxide 0.02%. Parallel immunoassay of IgE was carried out with a kit from "Boehringer" (West Germany) in accordance with the instructions provided. Correlation between the results was calculated by the "Statgraph" program on an IBM-PC computer.

EXPERIMENTAL RESULTS

A solid-phase enzyme immunoassay test system was obtained as a result of the procedures described above, by means of which the total IgE concentration in blood serum and plasma could be determined in accordance with the "sandwich" principle, using two McAb with different

specificity. The basic analytical characteristics of the test system and the results of the tests are given in Table 1.

No effect of high concentrations (hook effect) was found to be present in this test system. Samples containing high concentrations of IgE (I-O), up to 160 μg/ml (or 67,000 IU/ml), were measured. All the values obtained exceeded the maximum of the optical density scale; consequently, it was impossible to obtain any spuriously low values of the IgE concentration, i.e., no hook effect was exhibited.

The linearity of the dilutions also was tested. Several samples of sera with a high IgE concentration were diluted 2, 4, 8, and 16 times with the zero control. The dependence of values obtained for IgE concentrations on dilution was close to linear for the samples of sera analyzed (Fig. 1). Thus, sera with an IgE concentration of more than 100 IU/ml can be measured with dilutions of several times without any substantial error.

The effect of anticoagulants and serum components on the results of the measurements also were studied. Anticoagulants, hemoglobin, bilirubin, lipids, and theophylline were added to serum with a measured concentration of IgE. It follows from Table 2 that these substances had no significant effect on the results, so that it was possible to measure IgE not only in the patient's blood plasma as well as his serum, in lipemic, hemolytic, and icteric sera, and also in the serum of patients receiving therapeutic doses of theophylline.

The work was completed by the study of correlation between the results of measurement of the IgE concentration by the test system described above and by the kit obtained from "Boehringer." Parallel measurements were made of the IgE concentration in series of samples of children's sera with the aid of this test system and with the enzyme immunoassay kit from "Boehringer." The coefficient of correlation was 0.90 (Fig. 2).

By means of the test system developed above it is therefore possible to measure the IgE concentration effectively in the blood serum and plasma of children and adolescents. The method is distinguished by its high sensitivity, reproducibility, analytical reliability, rapidity of conduct of the analysis, and its simplicity to perform. The results obtained by the use of a commercial enzyme immunoassay kit from "Boehringer." The test system can be used under clinical conditions for serial measurements of IgE concentrations in pediatric allergology.

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