NEW BIOMEDICAL TECHNOLOGIES

An Enzyme Immunoassay System for Neonatal Screening for Predisposition to Allergy

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 120, № 10, pp. 418-421, October, 1995
Original article submitted April 21, 1995

A test system has been developed for rapid, simple, and reliable measurement of immunoglobulin E in umbilical blood in the range from 0 to 10 IU/ml with a sensitivity of 0.1 IU/ml. The system may be used for the detection of a predisposition to allergy at a very early age, allowing for timely measures to prevent allergic diseases.

Key Words: *umbilical immunoglobulin E; atopy; enzyme immunoassay system*

The wide prevalence and severity of allergic diseases in children today makes neonatal screening a must. This is particularly important for children born to atopic parents in order to detect a predisposition to allergy. The diagnosis of atopic allergy at a very young age helps delay the onset and alleviate the symptoms of future disease.

The clinical atopy syndrome is associated with a capacity to produce immunoglobulin E (IgE) in response to stimulation with an allergen. IgE production starts during the 11th week of human embryo development [7]. IgE antibodies detected in the umbilicus at birth are of fetal origin [5], and their synthesis depends on some hereditary and external factors, including family history of atopy, the child's sex, and the month of birth [3]. The level of IgE in children has been shown to be genetically encoded as early as at birth, that is, in children with a high IgE level in the umbilical blood the level of serum IgE was found to be increased from the age of 18 months to 3 years. Hence, measurement of umbilical IgE, together with investigation of the family history of disease, has been proposed as the most reliable method for neonatal detection of an atopic disorder [4,6]. The concentration of IgE in the umbilical blood is low, and the commercial kits commonly used (e.g. PRIST, Pharmacia, with a minimal measurable concentration of 0.5 IU/ml) are unsuitable for measuring it. A reliable, highly sensitive method is needed for measuring concentrations so low. The purpose of this study was to develop a test system for rapid, simple, and reliable measurement of IgE in concentrations ranging from 0.1 to 10 IU/ml. The use of high-affinity monoclonal antibodies (MAb) [2] to IgE enabled us to develop such a method.

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MATERIALS AND METHODS

Clinical material: umbilical blood serum (20 samples) and neonatal serum (16 samples) together with blood serum (36 samples) of mothers infected with herpesvirus and/or cytomegalovirus were from the Department of Clinical Immunology of the Center for Perinatology, Obstetrics, and Gynecology. Six serum samples of children aged 1 to 3 years were from the Institute of Immunology, Ministry of Health of Russia.

The method is based on two-stage solid-phase enzyme immunoassay with two types of MAb specifically binding various portions of the human IgE molecule. MAb from ascitic fluid were purified by ion-exchange chromatography on DEAE cellulose, and IgE/11-4 MAb were immobilized on solid

0007-4888/95/0010-1050$12.50

phase (polystyrene Nunc-maxisorb plates) from 0.05 M carbonate buffer, pH 9.6, at a concentration of MAb of 10 μg/ml for 16 h at 4°C. Nonspecific binding sites were then blocked with 0.1% bovine serum albumin in buffered saline (BS), pH 7.4, with 0.1% sodium azide. MAb were conjugated with horseradish peroxidase as described previously [1]. IgE standards with concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, 5, and 10 IU/ml were prepared using polyclonal human IgE diluted in equine serum clarified by centrifugation. The standards were calibrated according to the Second International WHO Standard 75/502 for human serum IgE.

IgE measurements were carried out in two stages. Serum (50 μl) was put in plate wells and 50 μl BS were added. The samples, standards, and control serum were incubated for 1 h at 37°C with vigorous stirring. After double washing the second stage of the reaction was carried out: incubation with conjugate (30 min at 37°C). Four-fold washing was followed by the enzymatic reaction with tetramethylbenzidine (20 min at room temperature).

Standard Diafarm kits were used for measuring IgE concentrations in adult sera.

RESULTS

We thus developed a solid-phase enzyme immunoassay system for measuring the concentration of total IgE in human umbilical blood after the sandwich principle in the range from 0.1 to 10 IU/ml with a sensitivity of 0.1 IU/ml. An analysis takes 2.5 h. This test system may be used for neonatal diagnosis and in model systems, for example, to follow up the time course of IgE synthesis in cell cultures.

The new test system is based on a kit we developed previously for measuring total IgE in concentrations of 1 to 100 IU/ml [1]. Our task now was

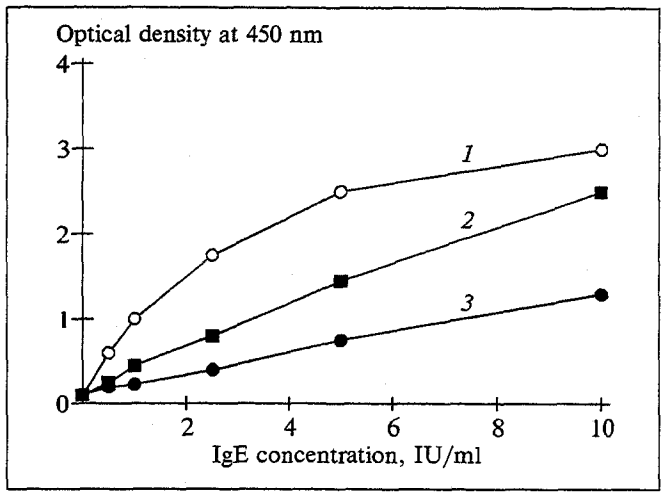

Fig. 1. Calibration curves at various dilutions of calibration standards with BS. *1*) no dilution; *2*) double dilution; *3*) 5-fold dilution.

to improve the sensitivity of the kit with the least detriment to other characteristics. For this purpose we proposed performing the enzyme immunoassay in two stages: 1) incubation with peripheral or umbilical serum and 2) incubation with conjugate.

The optimal concentrations of the lower antibodies were selected with standards of 0, 0.1, 1, and 10 IU/ml without dilution of the standards and with double dilution with BS (Table 1). The concentration of 10 μg/ml antibodies of the IgE/11-4 series was selected as the optimal concentration of the lower antibodies.

Serum dilution is advisable in the event of neonatal measurements, for the quantity of blood which can be taken from a newborn is very small. The results of diluting the standards 2- and 5-fold and of using them undiluted are presented in Fig. 1. Hence, double dilution with BS was found to be optimal for a two-stage test. However, we showed that the volume of a sample may be reduced to 20

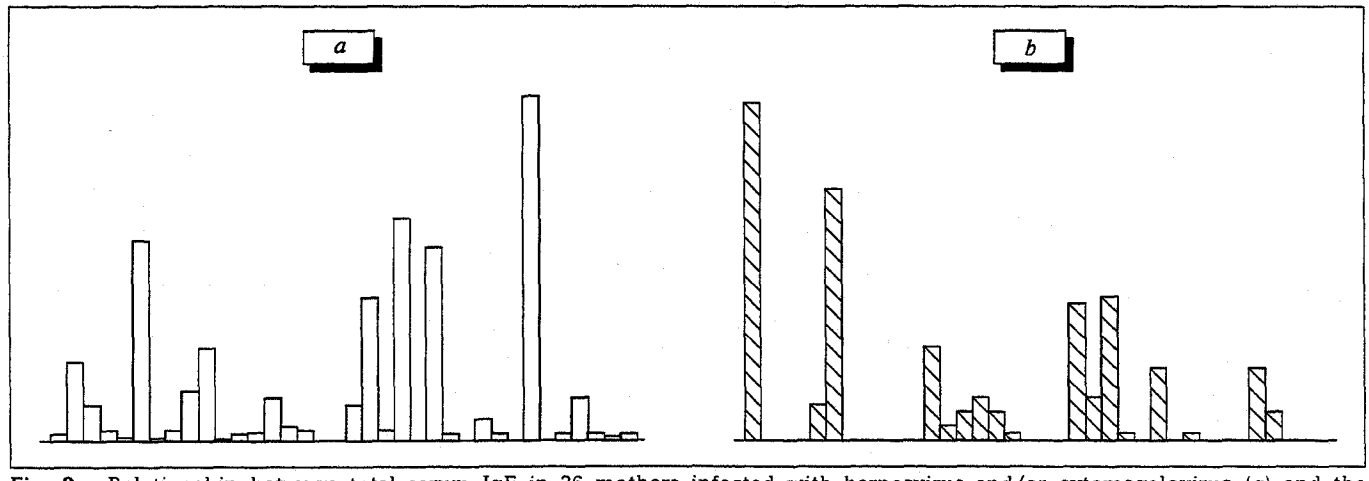

Fig. 2. Relationship between total serum IgE in 36 mothers infected with herpesvirus and/or cytomegalovirus (*a*) and the corresponding umbilical and peripheral sera of newborns (*b*).

TABLE 1. Optical Density (at 450 nm) Corresponding to IgE Concentrations Undiluted and Diluted Twofold at Different Concentrations of the Lower Antibodies

IgE concentration, IU/ml	Concentration of lower antibodies, μg/ml		
	20	10	5
0, undiluted	0.118	0.097	0.098
0.1, undiluted	0.256	0.196	0.151
0.1, double diluted	0.203	0.155	0.128
1.0, undiluted	0.935	0.655	0.585
1.0, double diluted	0.619	0.456	0.384
10, undiluted	3.070	2.860	2.515
10, double diluted	2.95	2.420	2.120

TABLE 2. Test for Linearity of Dilutions for the IgE Measurement Kit (at Various Serum IgE Concentrations)

IgE concentration in serum, IU/ml	Extent of dilution	IgE concentration in a sample, IU/ml		Actual/ theoretical×100%
		theoretical	actual	
7.21	2	3.6	3.8	105.5
	4	1.8	1.95	108.3
	8	0.9	0.79	91.3
	16	0.45	0.43	94.3
13.96	2	7.0	7.3	104.2
	4	3.5	3.8	108.6
	8	1.7	1.9	105.9
	16	0.87	0.99	109.7

μl if necessary, but the sensitivity will then be somewhat lower.

The effect of high concentrations (hook effect) is absent in this test system. Serum samples containing IgE in high (up to 1000 IU/ml) concentrations were tested. All the resultant values surpassed the maximum on the optical density scale.

The results of testing the linearity of dilutions for this kit are presented in Table 2. Deviations in the concentrations of samples obtained by diluting the sera with IgE concentrations of 7.21 and 13.94 IU/ml with serum without IgE were no more than 10% in comparison with the theoretically estimated concentrations.

Using the described methods, we measured IgE concentrations in 36 serum samples from newborns, in 6 samples of children aged 1 to 3 years, and in 36 adult sera. IgE concentrations for umbilical blood sera ranged from 0 to 5.3 IU/ml and for neonatal sera from 0 to 8.7 IU/ml. The concentrations in sera of children aged 1 to 3 years were higher than 10 IU/ml, and therefore the calibration standards for the test system had to be changed: a 20 IU/ml standard was added and the age of children in whom the test system may be used had to be limited to one year.

We failed to detect individual correlations for mother-child pairs (Fig. 2). A low concentration of IgE, 11 IU/ml, was observed in the mother of a newborn with a maximal IgE level (4.5 IU/ml). On the other hand, umbilical IgE was not detected in the newborns of many mothers with elevated IgE (more than 120 IU/ml). The lacke of correlation may be attributed to effects of other hereditary factors on the umbilical IgE, for example, atopy in the father, or environmental factors. On the other hand, this confirms the hypothesis that IgE production is an immunological characteristic of the fetus itself, not a result of passive transport across the placenta, and is genetically determined. The developed test system may be used for screening very young infants for predisposition to allergy, which will enable timely measures to be taken to prevent the development of an allergic disease.

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