INVESTIGATION OF IgE BY THERMISTOGRAPHY

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UDC 616-056.43-07:616.153.96-097.5-073:621.316.8

KEY WORDS: thermistography; IgE.

Antibodies of the IgE class are known to play a decisive role in allergic reactions of reagin type. Healthy human blood serum contains this protein in small quantities (20-220 i.u./ml)*, whereas in many diseases with atopic manifestations the IgE level may be 3-4 times higher [1, 3, 9].

Since the IgE concentration in the blood serum is low and since its isolation and purification are very difficult, its structure, functions, and physicochemical properties have not been adequately studied. The most widely used methods of IgE determination are currently radioimmunologic [8, 10], but despite certain advantages, these are very laborious and expensive. The use of new methods and techniques for the investigation of IgE is therefore an extremely promising development. The method of thermistography [4] has been used to determine the serum immunoglobulin levels. By the use of this method it is possible to detect a low IgA level (of the order of nanogram/millileter) in patients with ataxia-telangiectasia, whereas by the usual Mancini immunodiffusion method, IgA was not found in these patients.

The object of this investigation was to study the possibility of using thermistography for qualitative determination of IgE.

EXPERIMENTAL METHOD

The following standards were used: standard human blood serum with a known IgE concentration (10^4 i.u./ml), monospecific antiserum against IgE (from Behringwerke, West Germany), and for the control tests, human blood serum with an assigned IgE concentration (10^3 i.u./ml, from Pharmacia, Sweden).

A highly sensitive semiconductor microthermistor of the MT-54 type, enabling changes in the thermophysical properties of the medium as a result of the specific binding (precipitation) of antigen (AG) and antibody (AB) to be assessed, was used for thermistography. To ensure the high sensitivity of the method and to reduce side effects to the minimum, the dispersive power on the microthermistor should not exceed 3 MW. The intensity of the precipitation test was assessed from the change in intrinsic resistance of the thermistor r in time t ($\delta r/\delta t$), due to a decrease in the thermal conductivity of the medium during the formation of precipitating aggregates.

EXPERIMENTAL RESULTS

To determine the optimal ratio between the dilutions of the reagents (zones of equivalents) in the precipitation reactions and to detect the limits of sensitivity of this method, antiserum against IgE was titrated by the α-method (the method of Dean and Webb) [5]. The precipitation test was carried out with an assigned dilution of antiserum and with varying dilutions of antigen. Antiserum and antigen were used in dilutions of 1:1 to $1:10^6$.

The precipitation reaction for IgE was observed with dilutions of AG and AB to $1:10^6$. For all antiserum concentrations tested zones of equivalents were found, corresponding to maximal values of ($\delta r/\delta t$); the optimal ratio of dilutions of AG and AB was 1. Consequently, IgE, like immunoglobins of the other classes, precipitate when there is a perfectly definite quantitative ratio between AG and AB, but at a different ratio than, for instance, IgA (AG:AB = 1:10) [4].

The effect of the pH of the medium on the precipitation test with antiserum and IgE was studied. Solutions of reagents with pH 2.2-8.4 were made up in a citrate-phosphate buffer with molarity M = 0.15. A logarithmic graph of the change in intrinsic resistance of the thermistor with time ($\delta r/\delta t$) as a function of pH of the precipitating reagents is illustrated in Fig. 1. Maximal binding of AG and AB macromolecules for IgE was observed in the region pH 5.7-7.5, the same as for other classes of immunoglobulins [2, 7]. Later antisera with pH 6 ± 0.2 were used.

The precipitation reaction of denatured serum containing IgE and the corresponding monospecific anti-IgE antiserum also was studied. Heat inactivation of the serum was carried out in a type UT-15 ultrathermostat at 56°C for 15 min. On

*1 i.u./ml = 2.42 ng/ml.

N. I. Pirogov Second Moscow Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 11, pp. 636-637, November, 1981. Original article submitted February 28, 1981.

0007-4888/81/9211-1600$07.50

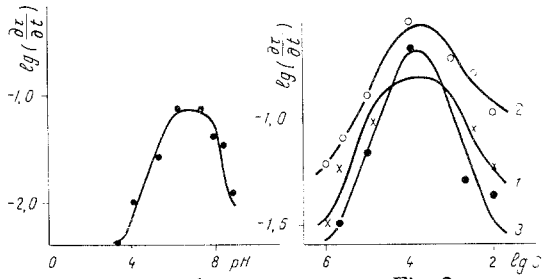

Fig. 1 Fig. 2

Fig. 1. Logarithmic graph of change in intrinsic resistance of thermistor with time as a function of pH of medium for precipitation test with IgE and antiserum against IgE. Dilution of AG and AB $1:10^4$.

Fig. 2. Logarithmic graph of changes in intrinsic resistance of thermistor with time as a function of concentration of precipitating reagents on addition of 0.02 mg (1), 0.06 mg (2), and 0.10 mg (3) of antigen. C) Degree of dilution of reagents.

the addition of specific antiserum to the inactivated serum the resistance of the thermistor remained virtually unchanged, indicating absence of a reaction and confirming the thermolability of the IgE by contrast with immunoglobulins of other classes [3, 8].

A graph of log ($\delta r/\delta t$) as a function of log C of antigen at equivalent dilutions of AB and AG, in the case of addition of different quantities of AG, is illustrated in Fig. 2. All the curves are extremal in character. With an increase in the quantity of added antigen the values initially were shifted into the region of higher values of $\delta r/\delta t$ (the precipitation test was intensified), but later, in the region of lower values of $\delta r/\delta t$ partial lysis of the precipitate was observed. The extremal values under these circumstances corresponded to the same dilutions of antigen and antiserum ($1:10^4$), evidence of the existence of an optimal ratio between concentrations of antiserum and antigen. These data are also evidence of the high sensitivity and specificity of thermistography.

The serum IgE levels in this investigation was determined in healthy subjects and patients. For this purpose, a graduated curve of the relationship between concentration C and log ($\delta r/\delta t$) was plotted for standard reagents in the precipitation test between monospecific antiserum against IgE and the corresponding serum with a known IgE concentration. The graduation curve was used to determine the IgE concentration in the control serum. The calculated value of IgE concentration obtained by thermistography in the control serum was 1060 i.u./ml, which differed by 6% from the assigned known value of 1000 i.u./ml.

Using the graduation curve the IgE level was determined in four healthy subjects and eight patients with psoriasis and bronchial asthma. The IgE level in four healthy subjects was 180, 87, 200, and 120 i.u./ml respectively, i.e., within the range 87-200 i.u./ml, whereas in the three patients with psoriasis it was 250, 230, and 280 i.u./ml, i.e., it was either normal or a little above normal, in agreement with data in the literature [3, 6]. In four patients with bronchial asthma the IgE level was considerably above normal, namely 750, 1500, 1000, and 600 i.u./ml, also in agreement with data in the literature [3, 9].

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