METHOD OF DETERMINATION OF REAGIN ANTIBODIES

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The method of passive sensitization of the isolated intestine of a healthy monkey was used to detect reagin antibodies in the blood serum of patients with pollinosis.

The sensitivity of this method is 3-6 orders higher than that of the known method of passive transfer of increased sensitivity, as described by Prausnitz and Küstner, with respect to reagin detection.

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The development of sensitive methods of determination of reagins in the blood serum of allergic patients is an important problem in contemporary allergology [1].

It is now about 50 years since Prausnitz and Küstner [10] described the passive transfer of increased sensitivity to the skin of healthy persons by means of serum from allergic patients.

Many methods are now available for determination of skin-sensitizing antibodies, or reagins (scarification tests, intradermal allergic tests, application tests, the test of degranulation of the blood basophils, Boyden's passive hemagglutination reaction, the antiglobulin test, and so on). More recently, the method of passive sensitization of the monkey's intestine has attracted the attention of investigators as a means of detection of reagin antibodies [6, 7].

In the present investigation reagins were determined in the blood serum of patients with pollinosis by studying passive sensitization of the intestine of a Macaca rhesus monkey in relation to the incubation time of a segment of intestine with the test antibodies and the concentration of antibodies and allergen. The method was also compared with the Prausnitz – Küstner reaction of passive transfer of increased sensitivity.

EXPERIMENTAL METHOD

Experiments were carried out with the sera of 20 patients sensitive to meadow grass pollen (timothy-grass, wild oats, cocksfoot). The sera were kept at -20° before use. Sera of 7 nonallergic patients acted as controls. The specific allergen consisted of a 7.5% saline extract of timothy-grass pollen (without phenol), prepared by the usual method in the Allergologic Research Institute, Academy of Medical Sciences of the USSR [4].

The ileum of a healthy Macaca rhesus monkey was used for detection of the reagins. The monkey was sacrificed by exsanguination under nembutal anesthesia. A piece of the ileum about 20 cm in length, taken from the region of the ileocecal angle, was perfused with warm Krebs' solution and then cut up into segments 1.5-2 cm long which were kept at 4° until required. As a rule the ileum remained sensitive to histamine for 48 h. Sensitized segments of ileum were placed in an ordinary Schultz – Dale apparatus [3]. Isometric contractions of the ileum were recorded on a medical plethysmograph [2].

To determine the sensitivity of the ileum, various dilutions of histamine were first tested ($5 \cdot 10^{-8}$, 10^{-7}, $2 \cdot 10^{-7}$). The ileum was then treated with specific allergen and its sensitivity to histamine again tested. In this way the allergic contracture could be expressed in histamine equivalents. Passive sensitization of the ileum was also carried out with different concentrations of serum, diluted to the required value with isotonic sucrose solution in a volume of 3 ml, with a continuous oxygen supply and at 37°C.

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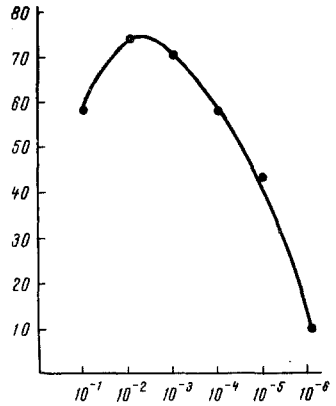

Fig. 1. Strength of allergic contraction of passively sensitized monkey ileum as a function of allergen concentration. Time of passive sensitization 10 min. Abscissa, dilutions of allergen; ordinate, strength of allergic contraction expressed in histamine equivalents (ng).

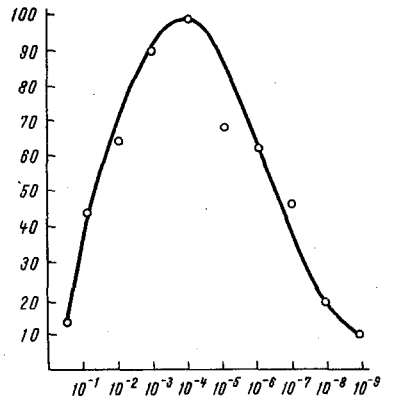

Fig. 2. Passive sensitization of monkey ileum as a function of antibody concentration. Time of passive sensitization 10 min. Abscissa, dilutions of serum; ordinate, strength of allergic contraction expressed in histamine equivalents (ng).

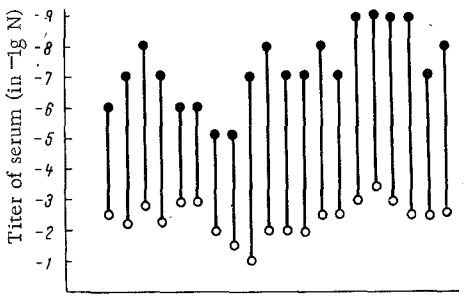

Fig. 3. Comparison of sensitivity estimated by passive sensitization of monkey's ileum and by Prausnitz–Küstner reaction. Black circles show titers of sera determined on passively sensitized ileum; white circles denote titers of sera determined by Prausnitz–Küstner method.

The Prausnitz–Küstner passive transfer test was carried out in the usual manner [5].

EXPERIMENTAL RESULTS

To rule out nonspecific action the allergen was first titrated on unsensitized segments of ileum. The sensitized segment of ileum was treated with dilutions of allergen which did not produce nonspecific contraction of the normal ileum. In the present investigations a nonspecific effect was given only by undiluted 7.5% extract of timothy-grass pollen.

Preliminary experiments were carried out to study the relationship between passive sensitization and the incubation time of ileum with serum. Sera were used in dilutions of 1:100 and 1:1000 and allergen in a dilution of 1:100. Incubation times of 5, 10, 15, 20, 30, 60, and 120 min were tested and the optimum sensitization period was found to be 10 min.

To study the character of the relationship between passive sensitization and allergen concentration in a constant optimum dilution of serum (10^{-3}, 10^{-4}), several dilutions (from 10^{-1} to 10^{-5}–10^{-6}) were used. Dilutions of allergen producing maximum contraction of the ileum were 10^{-2}–10^{-3}. The last dilutions of allergen still capable of detecting the state of sensitization of the ileum were 10^{-4}–10^{-6}. In the presence of an excess or deficiency of specific allergen, the response of the ileum was reduced (Fig. 1). Allergen was titrated in these same dilutions by the Prausnitz–Küstner method. The optimum dilutions of allergen were 1:10 and 1:100 and the titration values 10^{-3}–10^{-5}.

The character of the relationship between passive sensitization and antibody concentration was also investigated and the two methods of detecting reagins were compared. The ileum was sensitized by gradually decreasing concentrations of each serum (from 10^{-1} to 10^{-7}–10^{-10}). The dose of allergen remained constant (1:100). Dilutions of sera producing maximum sensitization of the ileum were 10^{-3}–10^{-4}. The last dilutions of sera still capable of sensitizing the ileum were 10^{-5}–10^{-9}.

For the Prausnitz–Küstner reaction sera were used in dilutions of 1:10, 1:30, and so on to 1:2430. The dose of allergen for each serum was constant (1:10–1:100). The last dilutions of serum still capable of sensitizing human skin varied from 1:10 to 1:2430.

These results confirm the phenomenon of inhibition of sensitization described in the immunologic literature [7, 8, 9] in the presence of an excess or deficiency of antibodies (Fig. 2).

The results thus showed that the sensitivity of the monkey's ileum with respect to detection of reagins in the blood serum of allergic patients is several orders (10^{-3}–10^{-6}) higher than the sensitivity of the Prausnitz–Küstner reaction (Fig. 3). It must also be remembered that one of the main disadvantages of allergic tests in vivo is the need to use healthy human recipients, whereas for tests in vitro this is unnecessary.

On the basis of this investigation it is possible to recommend the method of passive sensitization of the isolated ileum of a healthy monkey instead of Prausnitz–Küstner reaction for detection of reagins in the blood serum of patients with pollinosis. This method can also be suggested for testing allergen used in desensitizing treatment of patients with pollinoses for their biological activity.

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