5. T. Kosaka, T. Asahina, and Kobayashi, J. Immunol., 40, 597 (1980).
6. W. Strober, R. Krakauer, and H. L. Klaeveman, N. Engl. J. Med., 294, 351 (1976).
7. T. B. Tomasi, The Immune System of Secretions, Englewood Cliffs, New Jersey (1976).

PRODUCTION OF MONOSPECIFIC ANTISERA TO IgA OF LABORATORY ANIMALS BY CASCADE IMMUNIZATION WITHOUT PRELIMINARY ANTIGEN ISOLATION

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Mice, rats, and guinea pigs are widely used in laboratory practice to study the principles governing the formation of the immune response and, in particular, to evaluate vaccine preparations. In infections of the mucous membranes a definite role for local immunity has been demonstrated, and its study requires determination of serum and secretory IgA and of antibodies bound with them. This raises the problem of obtaining monospecific antisera to the IgA of these laboratory animals.

The traditional method of obtaining antisera, based on isolation and purification of a protein antigen, is unsuitable for the present task because of the small volume of material, especially when the serum IgA level is low (guinea pigs), and the absence of an affinity adsorbent which is sufficiently specific for IgA. Isolation of IgA from secretions in which it is the dominant component is difficult because of the difficulty of obtaining secretions in sufficient amounts for fractionation. Various methods not requiring purification of the antigen, and based on the use of immune precipitates [1, 4, 6, 7, 11] or of other insoluble immune complexes [3, 9, 10], which include the required antigen, have been suggested. These methods, however, can yield monospecific antisera only in the early stages of immunization [4], for during long-term immunization with the same precipitate, obtained with a polyspecific antiserum, antibodies to impurities appear.

The method of cascade immunization suggested in this paper does not require isolation and purification of the antigen, but envisages the use of immune preparation including IgA. The specificity of the antisera is achieved by successive 2-3-stage (cascade) immunization with exchange of the antiserum-producing animals and source of IgA by means of which the precipitate for immunization is obtained.

To obtain the first-order antiserum to mouse IgA, a serum fraction obtained on DEAE-cellulose by elution with 0.01 M phosphate buffer, pH 8.0, was used. This fraction, according to the results of immunochemical testing, contained only IgG, but the serum obtained by immunization with this fraction gave a line not only of IgG, but also of IgA (Fig. 1a). To prepare immune precipitates, a mouse coprofiltrate was used: this was obtained by washing segments of the small intestine with a solution containing protease inhibitors [5], followed by tenfold concentration by precipitation with ammonium sulfate at 50% saturation.

To obtain antiserum to rat IgA two secretions were used: bile, obtained through a cannula inserted into the bile duct of the anesthetized animal, and saliva, stimulated by subcutaneous injection of 1 mg pilocarpine.

To obtain antiserum to guinea pig IgA we used tear fluid, secretion of which was stimulated by application of a piece of red pepper to the conjunctiva of the eye, and colostrum, clarified by centrifugation at 10,000 rpm.

To remove antibodies to IgG present as impurities in antisera to IgA, adsorbents prepared from the IgG fractions of mouse, rat, and guinea pig sera were used. The fractions were obtained on DEAE-cellulose, as described above for mouse serum.

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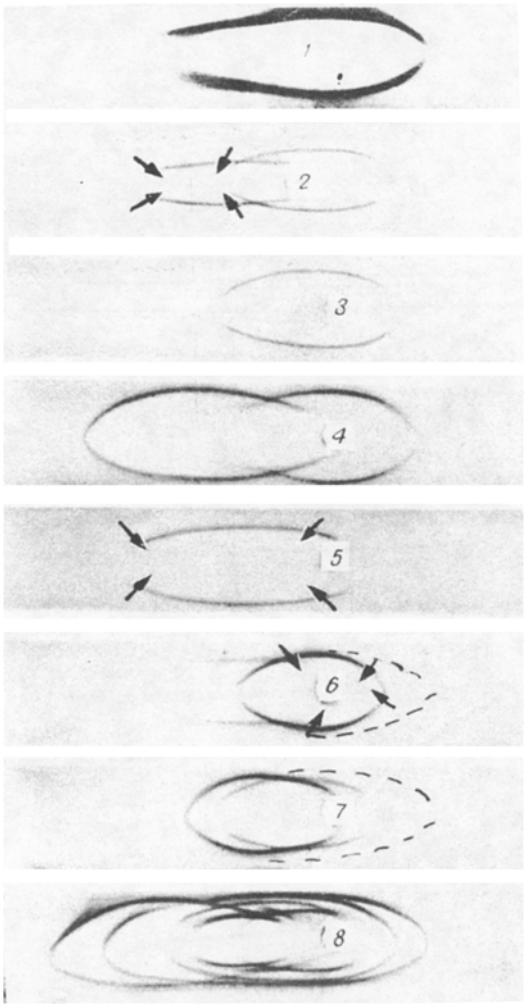

Fig. 1. Characteristics of polyspecific first-order antisera containing antibodies to IgA. Grooves contain: antisera to IgG-fraction of mouse serum (a), to rat salivary proteins (b), to guinea pig lacrimal proteins (c). Wells contain: mouse serum (1) and coprofiltrate (2); rat serum (3), saliva (4), and bile (5); guinea pig colostrum (6), serum (7), and tear fluid (8). Anode on the left. Regions of IgA-precipitates used for immunization are marked. Broken line indicates weak precipitation lines corresponding to guinea pig IgG.

To obtain antisera to secretions and serum fractions, the antigen was injected subcutaneously in a dose of 0.5-1.0 mg together with Freund's complete adjuvant into rabbits. Reimmunization began one month later and continued with an interval of two weeks with Freund's incomplete adjuvant.

Immune precipitates for immunization were prepared by immunoelectrophoresis (IEP) [12]. Agarose gel (usually 1%) in Veronal–Medinal buffer, pH 8.6 (0.06 M) was used. Electrophoresis of the serum or secretions was carried out for 45 min with a voltage of 5 V/cm, after which the corresponding antiserum was poured into the grooves and the plates were incubated at room temperature until next day. Precipitation lines cut out of the gel were frozen, thawed, and vigorously washed with physiological saline. The washed off precipitates were dissolved in 6M KI solution, emulsified in Freund's complete adjuvant, and injected subcutaneously into rabbits with an interval of two weeks [8]. For one injection 20 precipitation lines were used. The immunization cycle consisted of 2 to 4 injections.

Preparation of Antiserum to Mouse IgA. In the first stage antiserum to the IgG-fraction of the serum isolated on DEAE-cellulose was obtained. This antiserum always contained significant amounts of antibodies to IgA as impurity and gave an IgA-precipitate with the mouse coprofiltrate (Fig. 1a). In the next stage another rabbit was immunized with the same

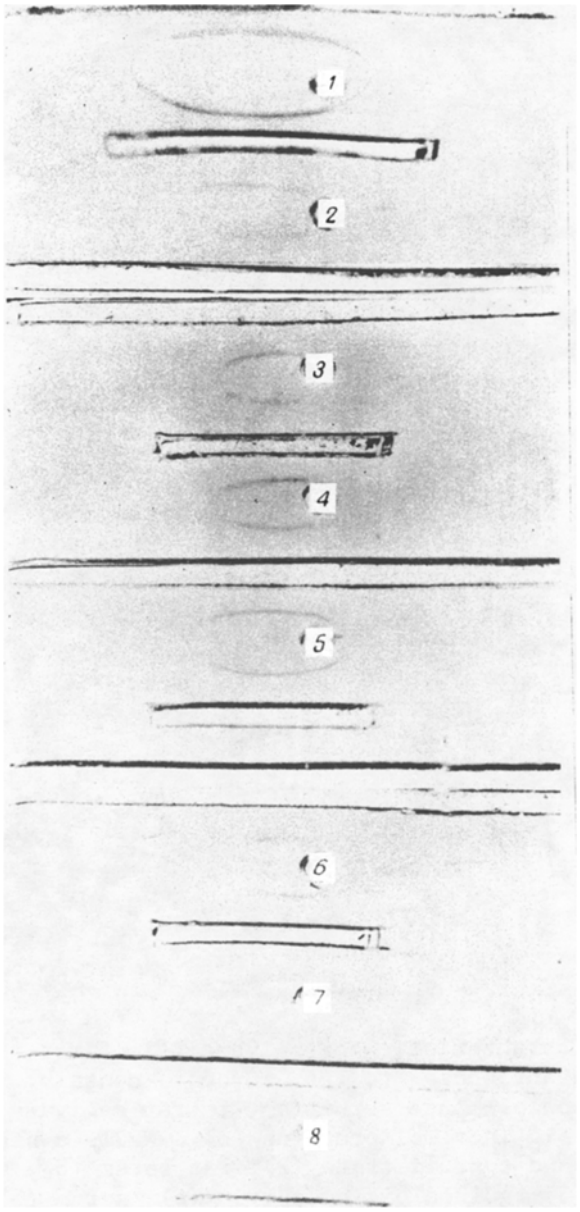

Fig. 2. Characteristics of monospecific antisera to IgA. Grooves contain: monospecific antisera to mouse (a), rat (b), and guinea pig IgA (c); long grooves contain antisera to IgA obtained by the cascade method of immunization, short grooves contain commercial antisera (from Miles, USA), respectively. Wells contain: mouse serum (1) and coprofiltrate (2); rat serum (3), saliva (4), and bile (5); guinea pig tear fluid (6), serum (7), and colostrum (8). Anode on the left.

precipitates and antiserum to IgA, containing antibodies to IgG as impurities, which were easily adsorbed, was obtained (Fig. 2a).

Preparation of Antiserum to Rat IgA. Basically the same scheme was used as in the previous case. In the first stage antiserum to salivary proteins was obtained. This antiserum gave three precipitation lines on IEP with saliva, one of which corresponded to IgA. However, the antiserum to salivary proteins gave only one precipitation line with serum and bile, corresponding to IgA (Fig. 1b). Since rat bile contained virtually no other immunoglobulins than IgA (data of IEP), we used the precipitate obtained from bile with antiserum to salivary proteins to immunize the next rabbit, and under these circumstances we obtained a monospecific antiserum to rat IgA (Fig. 2b).

Preparation of Antiserum to Guinea Pig IgA. The first step was to obtain a polyspecific antiserum to proteins of tear fluid. This antiserum gave many precipitation lines on IEP with tear fluid, among which it was difficult to distinguish a line corresponding to IgA. A weak IgG line and two anodal lines were obtained with the serum. This same antiserum gave three lines with colostrum, the strongest being the IgA line. The IgA precipitate was cut out and used to immunize the next rabbit (Fig. 1c). The antiserum obtained by this immunization revealed only IgA and IgG in the serum and secretions of the guinea pig. Antibodies to IgG were easily removed by adsorption (Fig. 2c).

Thus as a rule it was possible to obtain monospecific antisera to IgA at the second stage of immunization. If the second-order antisera contained contaminating antibodies to proteins other than IgA and IgG, they were used to obtain new IgA-precipitates and to immunize the next rabbit.

The specificity of the antisera was verified not only by IEP, but also by Ouchterlony's double immunodiffusion method. Completely identical IgA precipitation lines were found with the resulting antisera and also with commercial antisera to IgA (Miles, USA) and also with antisera to IgA obtained previously by traditional methods [2]. Ouchterlony's method also revealed complete identity of the lines of the serum and secretory IgA of the animals, above all in mice and rats, on account of differences in the electrophoretic mobility of these proteins (Figs. 1 and 2). Later, the antisera were used to determine the quantity of IgA in the sera and secretions by radial immunodiffusion in gel and to determine serum and secretory IgA-antibodies in microbial lipopolysaccharides in vaccinated animals by enzyme immunoassay. The specificity of the antisera was adequate for their use in this highly sensitive test.

The principle of the cascade immunization technique can be used to obtain monospecific antisera to other serum and secretory proteins.

LITERATURE CITED

1. V. A. Aleshkin, "Production of monospecific antisera to mouse immunoglobulins and some aspects of their use," Author's Abstract of Dissertation for the Degree of Candidate of Medical Sciences, Moscow (1983).
2. M. M. Lyubinskaya and V. A. Aleshkin, Immunologiya, No. 2, 15 (1984).
3. L. B. Khazenson, Immunologiya, No. 1, 34 (1980).
4. A. R. Bradwell et al., Clin. Chim. Acta, 71, 501 (1976).
5. C. O. Elson, W. Ealding, and J. Lefkowitz, J. Immunol. Methods, 67, 101 (1984).
6. M. Harboe and O. Closs, Scand. J. Immunol., 17, Suppl. 10, 353 (1984).
7. J. Kroll and M. M. Andersen, J. Immunol. Methods, 13, 125 (1976).
8. J. Kroll, Methods Enzymol., 73, 52 (1981).
9. F. Milgrom, T. Lusrehynski, and S. Dubiski, Nature, 177, 329 (1956).
10. M. R. Mordineg and H. J. Müller-Eberhard, J. Immunol., 94, 877 (1965).
11. D. R. Nash, J. P. Vaerman, H. Berin, et al., J. Immunol., 103, 145 (1969).
12. I. I. Scheidegger, Int. Arch. Allergy Appl. Immunol., 7, 103 (1955).