

SOME IMMUNOCHEMICAL PROPERTIES OF NATURAL ANTIGLOBULIN FACTORS (HOMOREACTANTS)

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UDC 612.017.1-064

A naturally occurring antiglobulin factor against the Fab-fragment of homologous IgG, contained in the rabbit and human blood serum and γ -globulin preparations, was shown by gel-filtration on Sephadex G-200 to have a molecular weight of about 250,000 daltons. In man this factor does not pass through the placenta in normal pregnancy. Despite differences in the physicochemical and effector properties of 7S IgG and protein with homoreactant activity, the latter has the specific antigenic determinants of IgG. These observations suggest that a complex of IgG with another protein or nonprotein compound possesses homoreactant properties.

KEY WORDS: homoreactants; pepsin Fab'-fragments; IgG; placenta.

Homoreactants (or agglutinators) are naturally occurring antiglobulin factors which interact selectively with the Fab-fragments of homologous IgG obtained by the aid of various proteolytic enzymes or cyanogen bromide [3, 7, 9, 12, 14, 16, 17, 20]. These factors may be found in the composition of fractions of serum IgG from most healthy persons [7, 9, 14, 17], rabbits [12, 16, 20], and primates [3]. It has been suggested that homoreactants are autoantibodies against internal antigenic determinants of isologous IgG, which are demasked in the process of its catabolism [6, 7, 12]. This hypothesis has not so far received direct experimental proof [1]. Serious doubts about whether the homoreactants are in fact antibodies arose after it was shown that purified rabbit IgG antibodies against a simple determinant group possess homoreactant activity and retain the ability to interact with pepsin Fab-fragment after blocking of the active centers of the antibodies by haptens [10].

Some immunochemical properties of homoreactants were studied in this investigation.

EXPERIMENTAL METHOD

The sources of the homoreactant were normal rabbit serum and also preparations of rabbit (Serva) and human (donors' antimeasles γ -globulin, batch 36-1, Moscow Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR) γ -globulins. Blood serum from mothers after a normal pregnancy and neonatal cord blood also were used for determination of the homoreactant.

Purified IgG from hyperimmune rabbit serum against sheep's red cells and its pepsin Fab'-fragment were obtained as described earlier [10]. To obtain the F(ab')₂-fragment from human serum against Rh(D)-factor, the globulin fraction was first isolated by precipitation with ammonium sulfate (40% saturation), the protein was hydrolyzed with pepsin, and the digest was then fractionated by the method described in [4].

Rabbit and human homoreactants against pepsin Fab' [or F(ab')₂]-fragments were determined by the passive hemagglutination test. Sheep's red cells sensitized with Fab-fragment from rabbit antibodies against this antigen [10] and Rhesus-positive human red cells sensitized with F(ab')₂-fragment of antibodies from human blood serum against Rh(D)-factor [17] were used. Preparations for determination of the homoreactant were first adsorbed by nonsensitized red cells.

Laboratory of Immunochemistry, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Department of Clinical Pediatrics, N. I. Pirogov Second Moscow Medical Institute. Debreczen Medical Institute, Hungary. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 80, No. 7, pp. 72-75, July, 1975. Original article submitted July 29, 1974.

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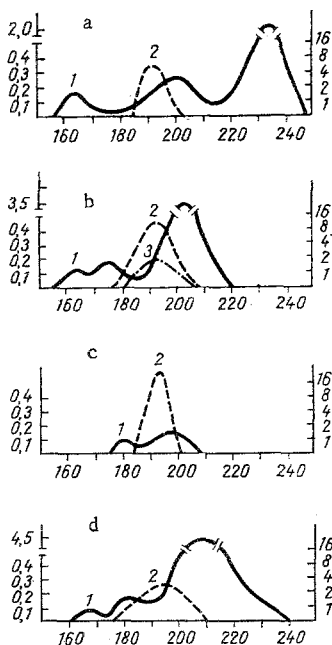


Fig. 1. Chromatography of rabbit serum and preparations of rabbit and human γ -globulin on Sephadex G-200 and distribution of homoreactant activity in fractions. Abscissa, volume of eluate (in ml); ordinate: left - optical density at 280 nm; right - titer of homoreactant per milligram protein in passive hemagglutination test. a) Rabbit serum; b) rabbit γ -globulin; c) rechromatography of fraction from preparation of rabbit γ -globulin with homoreactant activity; d) human γ -globulin. 1) Optical density at 280 nm; 2) homoreactant activity; 3) bovine catalase (optical density at 280 nm).

the immunosorbent, and at the same time, all the homoreactant contained in the sample was bound. The specificity of adsorption of the homoreactant was assessed by passing the preparation of human IgG through the above-mentioned immunosorbent. No perceptible adsorption of protein or loss of homoreactant activity took place.

Substantial differences in the properties of IgG and the protein with homoreactant activity were found by a study of the property of human homoreactant to pass through the placenta. The results showed that human IgG of all subclasses except IgG₂ normally pass through the placenta [4, 19]. However, in the case of normal pregnancy, no homoreactant could be found in any of the newborn infants studied, despite the presence of this factor in all the mothers. These differences were observed despite close agreement between the IgA levels in the mothers and the presence of only trace amounts of IgM.

The results described above indicate that homoreactants have antigenic markers belonging to IgG, but they differ from it in their higher molecular weight and their inability to pass through the placenta (in the case of human homoreactant). Two hypotheses may be put forward regarding the nature of the protein possessing homoreactant activity. First, IgGs with an unusual ratio between their polypeptide chains, aris-

The molecular weight of the factor with homoreactant properties was determined by gel-filtration on Sephadex G-200 (2.2 \times 130 cm), equilibrated with 0.15 M NaCl solution.

To obtain the immunosorbent goat antibodies against rabbit γ -globulin (Calbiochem) were fixed on Sepharose 2B activated by cyanogen bromide (Pharmacia). Serologic analysis showed that the antibodies were directed against the antigenic determinants of the Fc-site of rabbit IgG.

The concentrations of IgG, IgM, and IgA in human blood serum were determined by radial immunodiffusion [5].

EXPERIMENTAL RESULTS

Determination of homoreactant against pepsin Fab-fragment in fractions of rabbit serum proteins obtained by gel-filtration on Sephadex G-200 showed that the factor agglutinating red cells sensitized with Fab'-fragment appears in the second peak of proteins; fractions in the ascending portion of this peak had the highest activity (Fig. 1a). On chromatography of preparations of human and rabbit IgG on Sephadex G-200, besides the basic 7S-component, a heavier material was found. Fractional analysis of the homoreactant content showed that the peak of its activity did not coincide with the 7S IgG peak but corresponded to material eluted from the column before the peak of isologous IgG (Fig. 1b, d). By using bovine catalase as marker, peaks of the fragment and homoreactant were found to coincide almost completely (Fig. 1b) and, consequently, the molecular weight of the protein with homoreactant activity was about 250,000 daltons. To purify this protein more completely from 7S IgG, the homoreactant-containing fractions from the preparation of rabbit IgG were concentrated by ultrafiltration through a Diaflow PM-10 (Amicon) membrane and again passed through the Sephadex G-200 column. As a result of rechromatography the specific activity of the homoreactant was increased substantially (Fig. 1c), a result that could be attributed mainly to the more complete liberation of the factor from 7S IgG. Meanwhile no protein peak corresponding to the peak of homoreactant activity could be found, probably because of the very low concentration of homoreactant in the blood serum [20].

Despite the fact that the protein with homoreactant activity differed in its molecular weight from 7S IgG, it had the characteristic antigenic features of IgG. The use of immunosorbent with antibodies against the Fc-site of the rabbit IgG molecule showed that about 95% of the protein contained in the rabbit IgG preparation was bound with

ing de novo in the process of assembly of the IgG molecule in the plasma cells, may possess homoreactant properties; second, the possibility cannot be ruled out that IgG dimers or complexes of IgG with other proteins, arising in the bloodstream, are characterized by homoreactant activity. The property of IgG to form dimers spontaneously on account of noncovalent interactions has been clearly proved experimentally [11].

Further investigation of the structure of homoreactants and the mechanism of their reaction with the Fab-fragment is of considerable interest, because of the accumulation of increasing evidence of the role of these antiglobulin factors in local and generalized inflammatory reactions and in the nonspecific regulation of the immunologic reactivity of the organism [1, 2, 11, 13, 15].

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