Isolation of Normal Immunoglobulin E by Means of an Immunoabsorbent

By S. CARREL, LORE THEILKÄS, A. MORELL, F. SKVARIL AND S. BARANDUN
Schweizerische Zentrale für klinische Tumorforschung, Tiefenau, 3004 Berne, Switzerland

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An improved method for the isolation of normal immunoglobulin E is described. The method is based on the use of an immunoabsorbent formed by the mechanical entrapment of antibodies against a myeloma immunoglobulin E into a lattice of a highly cross-linked macroporous polyacrylamide gel. Normal immunoglobulin E was isolated from an immunoglobulin E-rich serum pool as well as from an individual healthy donor. The isolated immunoglobulin E was contaminated with about 20% of immunoglobulin G. An antiserum against normal immunoglobulin E was prepared by immunizing rabbits with the isolated immunoglobulin E.

IgE* is now well established as a new immunoglobulin class. It was first detected in an 8S myeloma component by Johansson & Bennich (1967) and later in a second case of multiple myeloma by Ogawa, Kochwa, Smith, Ishizaka & McIntyre (1969). By the use of appropriate sensitive methods Johansson, Bennich & Wide (1968) also detected IgE in normal serum. A direct proof for IgE as a carrier of reaginic activity was obtained after purification of the protein from reaginic sera by Ishizaka & Ishizaka (1970).

Our aim was to isolate IgE from a normal individual serum as well as from a serum pool with a relatively high IgE concentration by using polyacrylamide gel containing specific antibody as immunoabsorbent. The immunoabsorbent was based on the mechanical entrapment of antibodies against IgE into a lattice of a highly cross-linked macroporous synthetic polyacrylamide gel, as reported by Carrel, Gerber & Barandun (1969) and Carrel & Barandun (1971).

MATERIALS AND METHODS

Sera. A serum pool from six donors was provided by the Blood Transfusion Service, University College Hospital, Ibadan, Nigeria. (This pool was designated as 'African high IgE plasma pool 1' and kindly given by Dr Rowe from World Health Organization International Reference Centre for Immunoglobulins, Lausanne, Switzerland.) The IgE concentration of this pool and in the serum of a healthy individual European blood donor (A.M.) were determined by a radio-immunoabsorbent assay as described by Wide & Porath (1966) by the modification given by Mann, Granger & Fahey

(1969). The immunoabsorbent was prepared by coupling the immunoglobulin fraction of an anti-IgE serum to bromoacetyl-cellulose. The binding of ^{125}I -labelled IgE myeloma protein to this immunoabsorbent was inhibited quantitatively by isolated IgE and IgE present in serum samples. With this method it was possible to detect $0.03\mu\text{g}$ of IgE/ml. The IgE concentration in the African serum pool was found to be $27\mu\text{g/ml}$, whereas the concentration in the serum of the European donor was about $2\mu\text{g/ml}$. Small quantities of other sera with relatively high IgE concentrations were provided by the World Health Organization Reference Centre for Immunoglobulins.

Myeloma IgE (PS). This was isolated from serum by ion-exchange chromatography on a DEAE-Sephadex A-50 column ($29\text{cm} \times 3.2\text{cm}$) equilibrated with 0.1M -tris-HCl buffer, pH 8.0, at room temperature by using a stepwise elution from 0.1 to 0.2M -tris-HCl buffer, pH 8.0. The serum (10 ml) was equilibrated by dialysis against the starting buffer before addition to the column and 4.5 ml fractions were collected. The eluted fractions (89–133) were pooled, concentrated by vacuum dialysis to 24mg/ml and applied to a Sephadex G-200 column ($145\text{cm} \times 3\text{cm}$) equilibrated with 0.1M -tris-HCl– 0.5M -NaCl buffer, pH 8; 5 ml fractions were collected. The eluted fractions (70–110) were concentrated and examined for contamination with other serum proteins by agar diffusion precipitin analysis (Ouchterlony, 1949) against specific anti-IgA, anti-IgE, anti-IgD and anti-IgM sera. The results revealed that the purified IgE (PS) was contaminated by IgG only. The amount of IgG was estimated to be 2% by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965).

Normal IgG. This was prepared by block electrophoresis as originally described by Kunkel & Slater (1952) with Pevikon-C 870 (Fosfatbolaget, Stockholm, Sweden), a copolymer of polyvinyl chloride and polyvinyl acetate as supporting media in barbital buffer at pH 8.6 and 1.005 . Cohn fraction II isolated from pooled normal human plasma (provided by the Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross, Berne Switzerland) was used as starting material, as all four

* Abbreviations: IgA, IgE, IgG and IgM, immunoglobulins A, E, G and M (macroglobulin) respectively.

IgG subclasses could be demonstrated in this preparation as described by Skvaril & Morell (1970). In the isolated IgG fraction all IgG subclasses could also be detected by means of specific IgG-subclass antisera.

Antisera. Antibodies to myeloma IgE (PS) were raised in rabbits, sheep and pigs by immunization with purified IgE. Antigen (1 ml containing 0.5–1 mg) mixed with 1 ml of Freund's complete adjuvant was injected intramuscularly and this was repeated after 21 days. The animals were bled 10 days after the last injection. The antisera were tested for specificity by immunoelectrophoresis against normal human serum and by agar-diffusion analysis against purified IgA, IgG, IgM and Bence-Jones proteins (L chains) at different concentrations. All antisera, except one, revealed determinants of IgG heavy chains in gel diffusion and also produced a faint precipitin line in the γ -region when tested by immunoelectrophoresis against normal sera. Absorption with 0.05 ml of pooled normal serum or 0.05 mg IgG/ml of antiserum rendered the antisera specific for IgE.

Antisera to normal IgG were prepared by immunizing rabbits with two injections of 2 mg of antigen in Freund's complete adjuvant in the two hind-foot pads. Each antiserum was adsorbed with light chains isolated from reduced alkylated normal IgG.

Preparation of immunoadsorbent. An immunoadsorbent was prepared by polymerizing an aqueous solution of acrylamide and *NN'*-methylenebisacrylamide as cross-linking agent together with the IgE antibodies to be mechanically trapped. In our experiments 7 ml of a monomer solution (T:C = 8–25; see below) containing 6 g of acrylamide and 2 g of *NN'*-methylenebisacrylamide/100 ml of water was mixed with 1 ml of the immunoglobulin fraction from a specific rabbit anti-IgE serum containing 50 mg of antibody; light-activated polymerization was carried out with ammonium peroxydisulphate and riboflavin as catalysts for 1 h at 27°C. The gels (T:C) are described by the notation of Hjerten (1962), in which the first numeral (T) denotes the total weight (in g) of monomer (acrylamide plus bisacrylamide)/100 ml of solvent and the second numeral (C) the percentage of bisacrylamide expressed as the percentage (w/w) of the total amount of monomer. Since O_2 inhibited polymerization, N_2 was first passed through the monomer solution for 10 min. The block of polymerized protein gel was mechanically dispersed by forcing it through a needle with an inner diameter of 0.8 mm. The gel particles were repeatedly washed with 0.1 M-tris-HCl–0.1 M-NaCl buffer, pH 7.5, until no more protein was detected in the supernatant by measurement of the extinction at 280 nm. About 90% of the added immunoglobulin was trapped in the gel.

Isolation of IgE. A chromatographic column (20 cm $\times$ 1.5 cm) was packed with the antibody-containing gel particles and connected to a u.v. absorptiometer and the extinction of the effluent was recorded at 280 nm. After application of the serum on top of the column, the column was washed with 250 ml of 0.1 M-tris-HCl–0.1 M-NaCl buffer, pH 7.5, until an extinction of less than 0.01 was recorded in the effluent. The flow rate was kept at 5 ml/h for 3 h and then adjusted to 30 ml/h for a very intensive washing for 8 h. The antigen bound to the immunoadsorbent was eluted with 20 ml of 3 M-NaCNS, pH 6.0, or with 15 ml of 0.2 M-glycine-HCl buffer, pH 2.3. After elution of the antigen, the columns were re-equili-

brated with 0.1 M-tris-HCl–0.1 M-NaCl buffer, pH 7.5, and used again.

Identification of isolated IgE. The pH of the eluted antigen was adjusted to neutrality by exchanging the glycine buffer or the NaCNS buffer against 0.1 M-tris-HCl–0.1 M-NaCl buffer, pH 7.5, on a Sephadex G-25 column (38 cm $\times$ 3 cm), and the eluted protein was then concentrated by pressure dialysis to a final volume of 1 ml. As rabbit antibodies to IgE were used for the isolation of IgE, the specificity of the eluted IgE was tested in gel-diffusion analysis against sheep and pig antisera specific for determinants on the heavy chain of IgE. All anti-IgE sera from rabbit, sheep and pig showed a reaction of identity in gel-diffusion analysis against isolated IgE.

RESULTS

Isolation of IgE from the African (Nigeria) serum pool. A 10 ml portion of serum containing about 270 μ g of IgE was applied to the immunoadsorbent column. The bound IgE was eluted with 3 M-sodium thiocyanate, pH 6, or with 0.2 M-glycine-HCl buffer, pH 2.3. The specificity of the isolated IgE was tested with the Ouchterlony (1949) technique by using a monospecific anti-IgE serum (Fig. 1). With both eluting buffers 230–260 μ g of IgE could be recovered, as determined by the radio-immunoadsorbent assay. The extent of isolation was also tested in double-diffusion analysis (Fig. 1) by collecting all the non-bound ballast proteins (washes) and reconcentrating them up to twice the original protein concentration. No IgE could be detected in the ballast proteins, as shown in Fig. 1 by the absence of a precipitin line, whereas the starting serum pool as well as the isolated IgE reacted strongly with all antisera against myeloma IgE (Fig. 1). Further investigations revealed that isolated IgE was contaminated with some IgG

Fig. 1. Double-diffusion analysis of normal IgE isolated from an IgE-rich serum pool (Nigeria), by using a specific sheep antiserum to myeloma IgE (PS) (centre well) absorbed with 0.02 ml of normal serum/ml of antiserum. Outer wells: (1) serum pool Nigeria; (2) isolated IgE; (3) non-bound ballast proteins.

Fig. 2. Double-diffusion analysis of normal IgE isolated from an IgE-rich serum pool (centre well) against serial dilutions of anti-IgG serum. Outer wells: (1) undiluted; (2) diluted 1:2; (3) diluted 1:4; (4) diluted 1:8; (5) diluted 1:16; (6) diluted 1:32.

Fig. 3. Double-diffusion analysis of normal IgE isolated from an IgE-rich serum pool (1) and of normal IgE isolated from an individual blood donor (A.M.) (2), by using a specific pig antiserum to myeloma IgE (PS) (centre well) adsorbed with 0.03ml of normal serum/ml of anti-serum.

(Fig. 2), estimated to be about 20% by a radio-immunoabsorbent assay specific for IgG.

Isolation of IgE from the serum of a normal European (A.M.). Since the concentration of IgE in this serum was only about 2 µg/ml, no detectable precipitin line formed in gel-diffusion tests with antiserum to IgE. For each experiment 40ml of serum A.M., containing about 80 µg of IgE, was applied to the top of the immunoabsorbent column. The bound protein that was subsequently eluted from the column was concentrated to 1ml and identified as IgE by double diffusion (Fig. 3) against a monospecific anti-IgE serum. The recovery was 70–75 µg of IgE, as determined by radio-immunoabsorbent assay, but the isolated IgE was also contaminated with about 20% of IgG.

Fig. 4. Double-diffusion analysis of rabbit antiserum against isolated normal IgE (centre well) after adsorption with 0.05ml of normal serum/ml of antiserum. Outer wells: (1) serum pool Nigeria; (2) isolated myeloma IgE (PS); (3) normal human serum; (4) serum pool (Gambia); (5) IgE-rich serum (Holland).

Preparation of antisera to normal IgE. Rabbits immunized with 200 µg of our isolated IgE in Freund's complete adjuvant produced antisera reacting with IgE and normal human serum when tested by the Ouchterlony (1949) technique. After appropriate adsorption with normal human serum (0.05ml/ml of antiserum) all antisera were rendered monospecific for IgE, as shown by Fig. 4. The precipitin lines of all systems tested showed complete identity (no spur formation) (Fig. 4).

DISCUSSION

As IgE has been identified as a carrier of human reaginic activity, the investigation of the biological and physicochemical properties of these reaginic antibodies requires isolation of IgE from the sera of normal individuals and of patients suffering from various allergies. Low serum concentrations, however, were a major obstacle for the enrichment or isolation of non-myeloma IgE by routine immunochemical methods. The isolation procedure described here uses a polyacrylamide gel containing antibodies to IgE as an immunoabsorbent. The method is based on the mechanical enclosure of antibodies to IgE in the lattice of the macroporous polyacrylamide gel. Preparation of the immunoabsorbent and isolation of IgE take place under mild conditions, causing no significant denaturation of the enclosed protein. Advantages of this method are therefore its high yield and its simplicity.

IgE isolated from sera was identified in gel-diffusion tests by using an antiserum to a myeloma IgE. About 90% of the IgE present in the serum could be isolated, as demonstrated by quantitative determination with a radioimmunoassay. All IgE preparations, however, were contaminated with

about 20% of IgG. The reason for this is not clear, especially since the anti-myeloma IgE serum was adsorbed with normal IgG that had been purified by Pevikon-block electrophoresis and that contained all four IgG subclasses. Non-precipitating antibodies to IgG were also taken into consideration and removed by IgG-containing immunoadsorbents. Thus far the mixture of IgE and IgG could not be separated. It is possible that complexes are formed between IgG and IgE either *in vivo* or during the isolation procedure.

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