SUBSTANCE REACTING WITH SRBC (SHEEP RED BLOOD CELLS) AND RABBIT IgG: ISOLATION FROM THYMUS AND SPLEEN

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1. Introduction

In recent years the concept that lymphocytes recognize antigens by means of a surface receptor has come to be widely accepted [1–4]. It is known that B lymphocytes carry immunoglobulins (Ig) on their surface membranes, that can be identified and quantitated by suitable technique [5–8]. The receptor function of these immunoglobulin determinants is based on radioautographic observations of specific binding of highly radioactive antigen to B lymphocyte surface membranes, inhibition of such antigen-binding by anti-immunoglobulin sera and on evidence obtained by other methods [9–11].

Evidence for the presence of immunoglobulin receptors on T cells has been more difficult to obtain because they are undetectable by direct techniques. Nevertheless the inhibition of some T cells functions by antibodies directed against immunoglobulins or their subunits was observed and interpreted to indicate that the T cell receptor consists of subunits of immunoglobulin structures [8, 12–14]. However, some data for a non-Ig recognition system in T lymphocytes exist [15,16].

In this paper data on a thymic substance which has affinity to thymus dependent antigens and some evidence for the non-Ig nature of this substance are presented.

2. Materials and methods

2.1. *Thymus fractionation*

Calf thymus was homogenized with a blendor in 0.14 M NaCl and phosphate buffer solution, 10^{-3} M, pH 7 (1:1) and extracted with 1 M H_2SO_4 (final concentration) overnight at 4°C. Then the mixture was neutralized with 25% NH_4OH. Supernatant was obtained by centrifugation of mixture at 3000 rev/min for 20 min.

The concentration of ammonium sulfate in the supernatant was maintained at 50% saturation at pH 7. The preparation was allowed to stand overnight at 4°C. The precipitate was separated by centrifugation and dissolved in a small volume of 0.03 M HCl. Ammonium sulfate was removed by dialysis against a large volume of 0.001 M KH_2PO_4 pH 7.0. After dialysis the preparation was heated to 90°C for 5 min in a water bath. The precipitate was removed by centrifugation. The supernatant (crude preparation) was diluted with an equal volume of 0.28 M sodium chloride and examined in an agglutination test with sheep red blood cells (SRBC). The same procedure was used for the rat thymus, spleen, bone marrow and liver fractionation.

Further purification was performed by affinity chromatography on a rabbit-IgG-cellulose column.

2.2. *Chromatography*

Cellulose LK (Chemapol firm), previously mercerised in 5 N NaOH in a nitrogen atmosphere for 48 h, was activated by cyanogen bromide and conjugated with rabbit IgG as described [18]. The contents of rabbit IgG was 100 μg per 1 ml of the swollen preparation. Rabbit IgG was obtained from sera by chromatography on DEAE-Sephadex A-50 according to Baumstarc [19]. Protein determination was performed by the method of Lowry et al. [20]. The 20 mg of crude preparation in 2 ml of 0.14 M NaCl saline was applied to the cellulose IgG derivative column. The

first elution was performed by 0.14 M NaCl and the second by 0.1 M HCl–glycine buffer pH 2.2 or 0.01 M HCl. The fractions (2 ml) were collected and examined photometrically at 280 and 260 nM. Absorption spectra were obtained in 0.14 M NaCl at pH 7.0 on a Hitachi spectrophotometer over the 220–330 nm range.

Both fractions were examined in agglutination tests with SRBC. The second fraction was neutralized with 0.1 N NaOH before use in agglutination.

The sedimentation constant of the crude preparation was determined in the analytical ultracentrifuge Spinco E-model [21].

3. Results

Crude preparations from calf thymus (see Materials and methods) react with sheep red blood cells at relatively low concentrations. 0.2 ml of crude preparation (10–12 μg of protein per ml) was active in agglutination test with 0.1 ml 3% SRBC. This concentration is the lowest for producing agglutination of 0.1 ml 3% SRBC. Rabbit IgG inhibits this agglutination at a concentration of 100 μg per ml (fig.1) in the

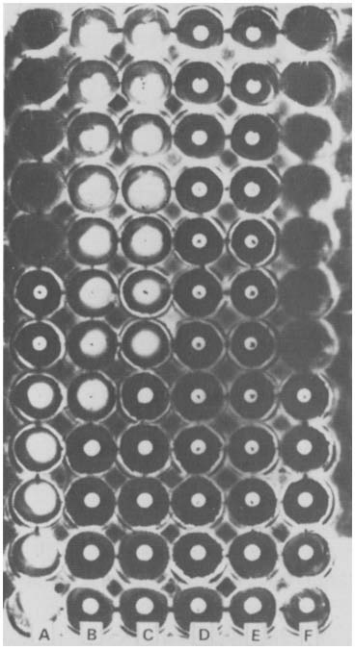

Fig.1. Agglutination of SRBC by crude calf thymus preparation. (b–c). Inhibition of agglutination by rabbit IgG. (d–e). Rabbit globulin alone. (f) SRBC in 0.14 M NaCl solution.

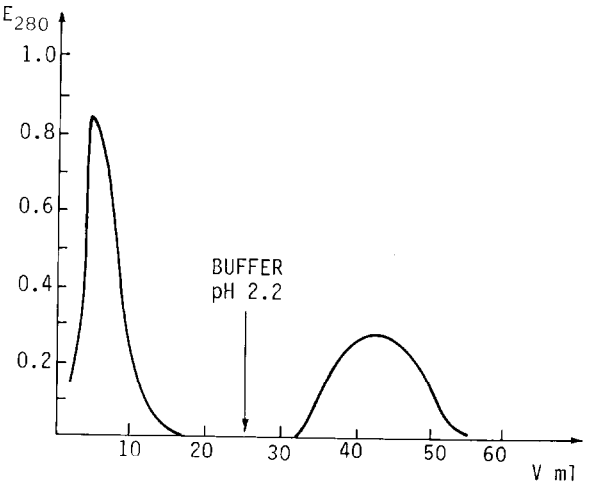

Fig.2. Affinity chromatography of crude preparation on the IgG-cellulose column.

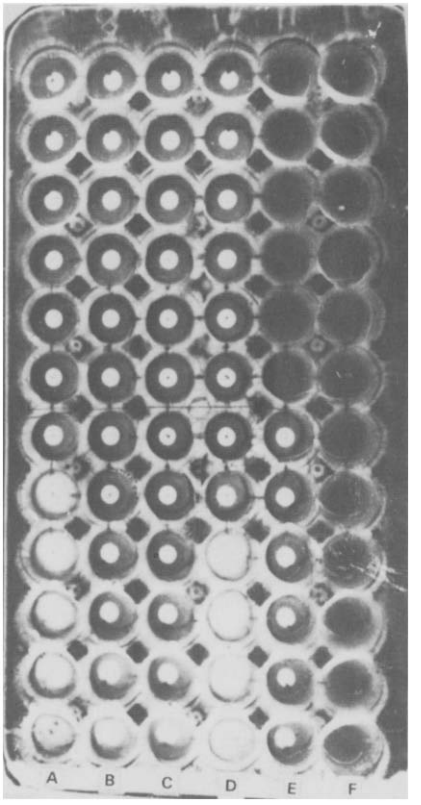

Fig.3. Agglutination of SRBC by preparations obtained from IgG-cellulose column. (a) Crude preparation. (b) Excluded fraction. (c) Normal rabbit serum globulin. (d) The second fraction obtained by elution at pH=2.2. (e) SRBC in 0.14 M NaCl solution.

presence of 20 μg of crude thymus preparation. Crude preparations of the rat thymus are active under the same conditions. On the contrary, preparations obtained from rat liver and from bone marrow were not active in agglutination tests with SRBC even at mg concentrations. The crude preparation obtained from rat spleen was active at high concentrations (above 100 μg per ml). The crude preparation was divided into two fractions by affinity chromatography (fig.2). The first fraction was inactive in the agglutination test with SRBC. The second fraction was obtained from the column by elution with 0.1 M glycine–HCl buffer pH 2. This fraction was active in agglutination test with SRBC at concentration 4 μg per ml (fig.3). Absorption spectra of both fractions in the 220–300 nM range at pH 7 are demonstrated on fig.4. Their shapes are considerably different. The spectrum of the second fraction has no maximum at 280 mM. Fig.5 shows the sedimentation coefficients as a function of concentration. The sedimentation constant is 1.8 s.

4. Discussion

In the present study a substance which reacts with IgG and SRBC was obtained from the thymus. The same isolation procedure gave low yields from rat

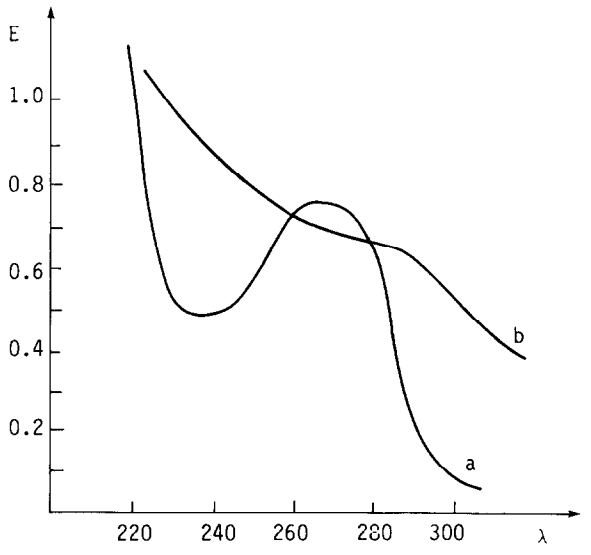

Fig.4. Absorption spectra of fractions obtained by affinity chromatography. (a) Excluded fraction. (b) Fraction obtained by elution at pH 2.2.

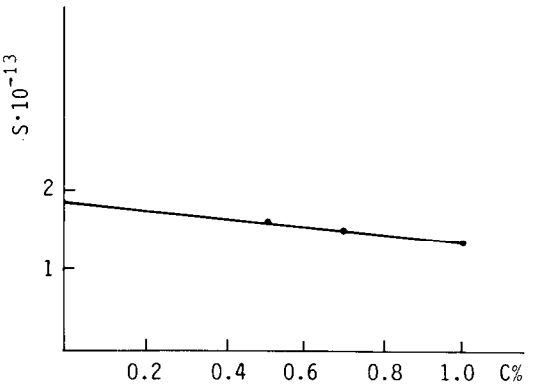

Fig.5. Sedimentation coefficient (calf thymus preparation) as a function of concentration.

spleen. In the liver and bone marrow the substance was not detected. It is likely that the substance is a product of thymus-derived lymphocytes because it was found according to known distribution of T lymphocytes in the thymus and spleen [22]. It is known that only negligible amount of B cells are present in the thymus. Mitchell and Miller showed that thymus-derived lymphocytes had to react first with the specific antigen before interaction with bone marrow cells [2].

It has been suggested that membrane receptors of at least some thymus-derived cells have no restricted specificity since they reacted with a number of thymus-dependent antigens [17]. T lymphocytes of intact animals might react with many kinds of cells including the self-cells [23]. In the present study chemical affinity of a substance isolated from calf or rat thymus to SRBC and rabbit IgG was demonstrated by the agglutination test, inhibition of agglutination, and by the method of affinity chromatography. The affinity of the isolated substance to rabbit IgG is of some interest. For example, radioimmunochemical determination of Ig on the surface of T lymphocytes may be due to the reaction between labelled allogenic Ig and surface receptor of T lymphocytes. The substance now isolated from thymus is not F (ab) fragment of Ig or any of its chains because its sedimentation constant is too low.

The supposition that the substance belongs to the recognizing structure of T cells is purely speculative and it is of interest to decide this question by further work.

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