A THYMOCYTE RECEPTOR WHICH INTERACTS WITH ERYTHROCYTES AND THE Fc FRAGMENT OF IgG

I. A. Bezvershenko, L. M. Bykova,
and A. L. Sinel'nikova

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The plasma membrane of T-lymphocytes contains receptors which interact with autologous and xenogeneic erythrocytes in the rosette-formation test [1, 2] and also with Fc fragments of immunoglobulins (Ig) [6-8]. A receptor structure with affinity for rabbit IgG and for autologous and xenogeneic erythrocytes was isolated previously from rat thymocytes [3]. Since interaction between this structure and erythrocytes is expressed by agglutination of the cells, and since rabbit IgG can inhibit agglutination by binding with the agglutinating factor, this suggests that affinity for erythrocytes and for IgG may be due to the same substance [4]. This substance has been isolated from thymocytes in a homogeneous state. In its physicochemical properties it is a glycoprotein with mol. wt. of about 12,000 [1].

The object of this investigation was to determine whether the glycoprotein agglutinating erythrocytes belongs to the group of receptors responsible for rosette formation and whether it possesses the properties of an Fc receptor.

EXPERIMENTAL METHOD

The receptor structure was isolated by a method involving incubation of thymocytes with IgG-cellulose, disintegration of the cells in a closely ground homogenizer, washing the sorbent toremove unbound material, and elution of the adsorbed material at pH 2 (a detailed account of the method was published previously [3]). The glycoprotein agglutinating sheep's erythrocytes was isolated from bovine thymocytes by the method described previously. Complete purification was achieved by chromatography on immobilized rabbit IgG [4].

The rosette formation test with autologous erythrocytes was carried out as described by Gluckman et al. [6]. Receptors responsible for rosette formation were removed from the membrane of the thymocytes by the method of Mendes et al. [9] by heating the cell suspension in isotonic medium at 45°C for 1 h followed by washing off the detached receptors. Different quantities (0.25, 0.5, or 1 mg/1 ml suspension) of the receptor structure or purified agglutinating factor were added to the thymocytes ($2.4 \cdot 10^7$ in 2 ml of 0.14 M NaCl in 0.01 M $NaHCO_3$, pH 7.2) after removal of receptors by the method of Mendes et al.[9], the sample was incubated for 1 h at 4°C, washed off three times with buffered 0.14 M NaCl, after which the number of rosette-forming thymocytes was determined by the method of Gluckman et al. [6].

Fab and Fc fragments of rabbit IgG were isolated by Porter's method [10] after treatment of the IgG with papain. Instead of carboxymethylcellulose, carboxymethyl-Sephadex C-50 was used for chromatography.

The agglutination and agglutination inhibition tests were carried out in the usual way.

EXPERIMENTAL RESULTS

Heating rat thymocytes in isotonic medium at 45°C for 1 h prevented them from forming rosettes with autologous erythrocytes in the same way as Mendes et al. [9] observed for human peripheral blood lymphocytes and sheep's erythrocytes. The addition of receptor structure isolated from thymocytes by incubation with immobilized IgG (IgG-cellulose), followed by dis-

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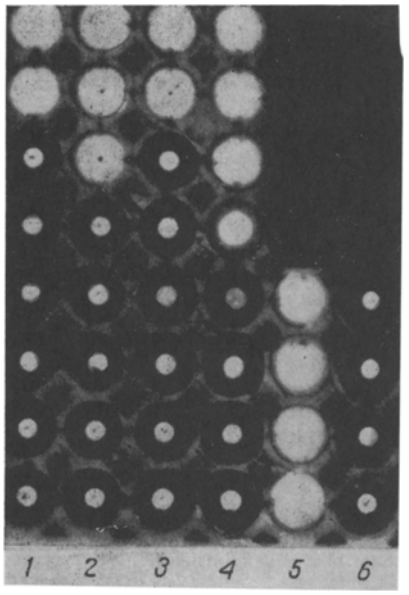

Fig. 1

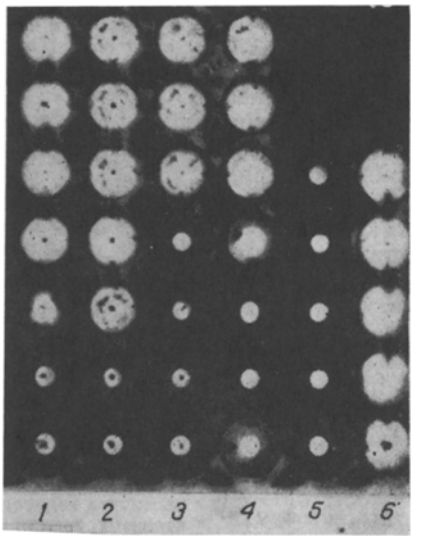

Fig. 2

Fig. 1. Agglutination of sheep's erythrocytes by agglutinating structure of thymocytes from Wistar rats. 1, 2) Inhibition of agglutination by Fab fragments of IgG; 3) inhibition of agglutination by Fc fragment of IgG; 4) inhibition of agglutination by rabbit IgG; 5) agglutination of erythrocytes by thymocyte receptor structure; 6) sheep's erythrocytes in 0.14 M NaCl. A quantity of 1.6 mg (dry weight) of agglutinating structure in 0.1 ml 0.14 M NaCl was incubated with Fab and Fc fragments (initial concentration 0.04 mg in 0.2 ml 0.14 M NaCl, dilution 1:2) for 30 min; 0.2 ml of 3% erythrocyte suspension was added for agglutination.

Fig. 2. Agglutination of sheep's erythrocytes by purified agglutinating substance. 1, 2) Inhibition of agglutination by Fab fragments of IgG; 3) inhibition of agglutination by Fc fragment of IgG; 4) inhibition of agglutination of rabbit IgG; 5) sheep's erythrocytes in 0.14 M NaCl; 6) agglutination of erythrocytes by purified agglutinating substance. A quantity of 0.08 mg of the purified agglutinating substance in 0.1 ml 0.14 M NaCl was incubated with 0.64 mg Fab or Fc fragments in 0.2 ml 0.14 M NaCl (dilution 1:2); 0.2 ml of a 3% suspension of sheep's erythrocytes was added for agglutination.

TABLE 1. Restoration of Number of Thymocytes (in %) Forming Rosettes with Autologous Erythrocytes after Incubation with Receptor Structure

Expt. No.	Intact thymocytes	The same thymocytes, heated to 45°C for 1 h	Heated thymocytes
1	2,3	0,3	2,8
2	3,6	2,1	2,7
3	2,8	1,8	2,8
4	5,7	1,6	2,8
5	2,2	0,6	2,7
6	3,3	0,3	1,3
7	1,1	0,5	1,7

Note. Here and in Table 2 all data subjected to statistical analysis by the method of direct differences ($P < 0.02$).

TABLE 2. Restoration of Number of Thymocytes (in %) Forming Rosettes with Autologous Erythrocytes after Incubation with Purified Agglutinating Factor

Expt. No.	Intact thymocytes	The same thymocytes, heated to 45°C for h	Heated thymocytes after incubation with purified agglutinating factor (0.5 mg/ml suspension)
1	2,3	0,3	2,6
2	3,6	2,1	3,5
3	2,2	0,6	2,5
4	3,3	0,3	1,3
5	1,1	0,3	0,8

integration of the cells, washing to remove unadsorbed material, and elution at pH 2 [3, 4], to the heated thymocytes restored their ability to form rosettes (Table 1).

Agglutination of sheep's erythrocytes induced by this structure was suppressed if the agglutinating structure was incubated with Fab and Fc fragments of IgG (Fig. 1). Agglutination induced by purified agglutinating factor was inhibited by Fc fragment present in a concentration only one-quarter that of the Fab fragment (Fig. 2) or IgG.

Addition of purified factor (glycoprotein) to the heated and washed thymocytes also restored their ability to form rosettes with autologous erythrocytes (Table 2).

Restoration of rosette formation after addition of receptor structure, adsorbed by immobilized IgG, to the heated and washed thymocytes is evidence that IgG can bind with receptors having affinity for erythrocytes. It was shown previously that this applies to both autologous and xenogeneic erythrocytes. It can be tentatively suggested that the structure adsorbed by theIgG contains various receptors, noncovalently bound with one another. However, restoration of rosette formation by purified glycoprotein and inhibition of agglutination which it causes by Fc fragment of IgG are evidence that it is one and the same compound which possesses affinity for IgG and for erythrocytes. Thus, at least one of the receptors possessing properties of an Fc receptor is capable of binding with erythrocytes. This receptor has been found in thymocyte membranes [2] and also in the composition of receptors responsible for rosette formation and separated from thymocytes on heating [2]. Separation of the receptors from T-lymphocytes on heating to 45°C for 1 h was not accompanied by appreciable damage to the cells, for they suffered no loss of viability, as shown by rejection of trypan blue or erythrosin. The existence of such a receptor is indirectly confirmed by the observations of De Cock et al. [5], who observed inhibition of rosette formation by T-lymphocytes bound with aggregated IgG.

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