# AGGREGATED IMMUNOGLOBULINS ON THE SURFACE OF RAPIDLY DIVIDING ANTIGEN-BINDING LYMPHOCYTES

A. Ya. Kul'berg, D. R. Kaulen,
V. V. Khorobrykh, and B. L. Yurin

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In the primary immune response of mice to sheep's red cells rosette-forming cells with aggregated immunoglobulins on their surface appear in the fraction of small lymphocytes in the spleen. These cells effectively incorporate thymidine and can be eliminated in vivo by injecting the mice with large doses of the tritiated base. It can be concluded from the results that rapidly dividing lymphocytes, specifically binding antigen, characteristically have aggregated immunoglobulins on their surface.

KEY WORDS: *rosette-forming cells*; *aggregated immunoglobulins*; *antigen-binding receptors*.

Previous work has shown that at the peak of the immune response most of the rosette-forming cells (RFC) contained in the spleen of mice are inactivated by antibodies against aggregated mouse immunoglobulins; consequently, these cells must contain aggregated immunoglobulins on their surface. Since no such RFC can be discovered before immunization or in the late stages after immunization it can be postulated that the presence of immunoglobulin receptors is a feature of rapidly dividing lymphocytes.

The investigation described below was carried out to study this problem.

## EXPERIMENTAL METHOD

Experiments were carried out on the spleen cells of CBA mice, obtained at different times after immunization with sheep's red cells in a dose of $5 \cdot 10^8$ cells. In order to eliminate rapidly dividing cells, the animals were given four intraperitoneal injections of 1 μCi tritiated thymidine ($^3$HT; specific activity 20 Ci/mmole) at intervals of 12 h starting from the third day after immunization. The animals were killed 2 days after injection of $^3$HT or on the fifth day after the beginning of immunization, and the number of RFC and of antibody-forming cells (AFC) in the spleen was determined by methods described previously [2, 4]. To examine the structural organization of the antigen-binding receptors, the effect of antibodies against aggregated mouse immunoglobulins (AAS) on the ability of the lymphocytes to form rosettes was investigated. The antiserum against aggregated mouse immunoglobulins was obtained as described previously [2].

Spleen cells were treated for 45 min in the cold. To determine the number of RFC in the fraction of small lymphocytes, spleen cells from mice of the control and experimental groups were fractionated by the method described earlier [1]. The procedure consisted of removing conglomerates and destroyed cells from the suspension of spleen cells as described by Shortman et al. [8] and then fractionating the spleen cells by centrifugation in a bovine serum albumin density gradient by the method of Raidt et al. [7]. The viability of the lymphocytes was determined relative to the absorption of a 0.1% solution of trypan blue.

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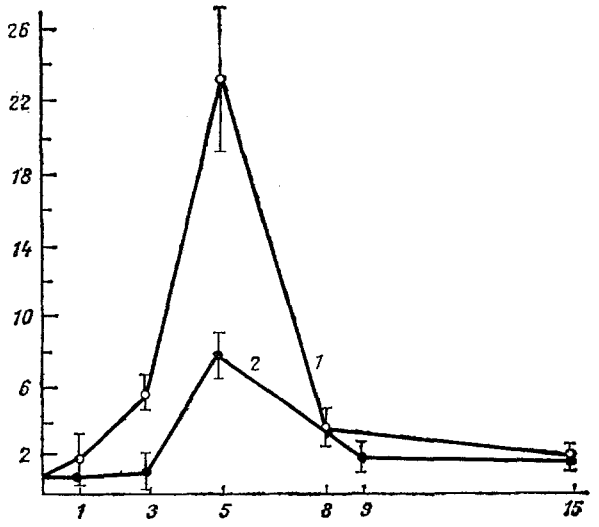


Fig. 1. Kinetics of rosette-forming cells sensitive to AAS in mouse spleen at different times after immunization with sheep's red cells: 1) control (cells treated with normal rabbit serum heated to 56°C); 2) experiments (cells treated with AAS). Abscissa, time of investigation of animals after immunization, days; ordinate, number of RFC per $10^3$ splenic lymphocytes.

TABLE 1. Presence of Aggregated Immunoglobulins on Surface of Rapidly Dividing Lymphocytes from Immune Mouse Spleen (M ± m and 99% confidence limits)

| Experiment No. | Spleen cells taken 5 days after immunization of mice with sheep's red cells | Number of cells in spleen, millions | No. of viable spleen cells (lymphocytes), % | Number of RFC per $10^3$ spleen cells (lymphocytes) | | No. of AFC per $10^6$ spleen cells (lymphocytes) |
|---|---|---|---|---|---|---|
| | | | | untreated | treated with AAS | |
| 1 | Without gradient centrifugation | 120,6±1,7 | 84,5±1,0 | 23,8±1,5 (17,2÷29,7) | — | 182,2±40,7 (20,4÷345,2) |
| | Fourfold introduction of $^3$HT | 66,6±2,4 | 73,3±2,4 | 7,2±0,4 (5,5÷8,9) | — | 11,0±1,1 (10,5÷11,5) |
| 2 | After gradient centrifugation | — | 91 | 23,2±1,5 (17,2÷29,2) | 8,2±0,2 (7,4÷9,0) | — |
| | Fourfold introduction of $^3$HT | — | 90—91 | 4,0±0,6 (1,4÷6,6) | 3,5±0,8 (0,4÷6,6) | — |

## EXPERIMENTAL RESULTS

Data for the kinetics of RFC in the spleen of mice immunized with $5 \cdot 10^8$ sheep's red cells are given in Fig. 1. The number of RFC inactivated by AAS also is shown. The absolute number of cells sensitive to AAS evidently reaches a maximum on the fifth day after the beginning of immunization, but the relative effectiveness of inactivation was about equal on the third and fifth days. The absolute increase in the number of cells inactivated by AAS, despite the constant ratio between the numbers of resistant and sensitive RFC, could indicate that between the third and fifth days there was intensive proliferation of an RFC population characterized by the possession of aggregated immunoglobulins on their cytoplasmic membrane.

To test this hypothesis, lymphocytes with a high rate of division were eliminated in vivo by the radioactive "suicide" method [9]. For this purpose, high doses of $^3$HT were injected into the animals on the third day after immunization in accordance with the scheme described above. As a result of the injection of labeled $^3$HT the total number of nucleated cells in the spleen was reduced by half in the course of 2 days (i.e., on the fifth day after immunization) compared with their number in the immune animals not treated with tritiated thymidine (Table 1, experiment 1). The number of RFC in the mice receiving $^3$HT remained the

same as when the radioactive agent was injected. It was also found that no AFC were present in the spleen of the mice receiving $^3$HT. Considering that the life span of AFC is 48 h [3] and also that most of the precursors of AFC are rapidly dividing cells [5, 6] it can be concluded that the scheme of injection of $^3$HT as used is capable of effectively eliminating cells with a high rate of division.

To make a reliable comparative assessment of the RFC population in animals receiving $^3$HT and in the control animals, it was deemed necessary to determine RFC in purified fractions of small splenic lymphocytes. The reasons for this were as follows: a) the evident difference in the cell composition of the spleens of the control and experimental groups; b) the presence of a large number of nonviable cells in the spleen of the animals receiving $^3$HT which could interfere with the detection of true RFC.

Figures for the number of RFC and their sensitivity to AAS in the fraction of small splenic lymphocytes from mice receiving $^3$HT for 2 days and from mice not receiving this treatment, are given in Table 1 (experiment 2). The number of RFC per $10^6$ lymphocytes by the fifth day after immunization was clearly approximately 8 times greater in the mice not receiving tritiated thymidine. About 70% of RFC in the mice not receiving $^3$HT were inactivated by AAS and, consequently, contained aggregated immunoglobulins on their surface. Conversely, animals receiving $^3$HT did not possess RFC carrying aggregated immunoglobulins, as could be judged from their resistance to AAS. It was also important to note that the absolute number of RFC not inactivated by AAS was less only by half in the animals receiving $^3$HT than in mice not treated in this way.

It can be concluded from these results that immune RFC carrying aggregated immunoglobulins on their surface belong to the population of radiosensitive, rapidly dividing lymphocytes. At the same time, RFC not containing aggregated immunoglobulins on their cytoplasmic membrane evidently have a substantially slower rate of division. Bearing in mind earlier observations that RFC carrying aggregated immunoglobulins belong to the B lymphocyte class [2], it can be assumed that the appearance of aggregated immunoglobulins on the cytoplasmic membranes of these cells is connected with their activation by the antigen or by another mitogen.

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