The results described in this paper thus indicate that both T and A cells must be present in the productive phase of the immune response; this confirms the earlier suggestion [12] that interaction between individual types of cells not only takes place during induction of the immune response, but continues during the subsequent stages of its development, at the level of mature antibody producers.

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AGGREGATED IMMUNOGLOBULINS AS ANTIGEN-BINDING RECEPTORS OF IMMUNE LYMPHOCYTES

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UDC 612.112.94.017.1

At the peak of the primary immune response to sheep's red cells rosette-forming cells, effectively inactivated by antibodies against aggregated mouse immunoglobulin and by a polyA:polyU complex, appear in the spleen of mice. These rosette-forming cells disappear from the spleen on the ninth day after primary immunization and cannot be detected at the peak of the secondary immune response. During cultivation for 24 h *in vitro* of small lymphocytes taken from the spleen of mice on the fith day after immunization with sheep's red cells all rosette-forming cells inactivated by antibodies against aggregated mouse immunoglubulin are seen to disappear. The results are regarded as evidence of the existence of rosette-forming cells possessing antigen–antibody complexes as antigen-binding receptors, at the peak of the primary immune response.

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

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(Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 6, pp. 702-704, June, 1976. Original article submitted September 22, 1975.

The ability of lymphocytes of bone-marrow origin (B cells) to fix antigen–antibody complexes or nonspecifically aggregated IgG on their surface has been conclusively demonstrated in experiments *in vitro* [4, 5, 7]. These results suggest that B-lymphocytes on whose surface there exist antigen–antibody complexes with the properties of antigen-binding receptors, may appear after immunization. In fact, as the writers showed previously, antibodies interacting specifically with aggregated mouse immunoglobulins and with antigen–antibody complexes block the antigen-binding receptors of rosette-forming B cells (RFCs) which appear in the spleen of mice immunized with sheep's red cells [1].

The object of the present investigation was to continue the study of the structure and properties of antigen-binding receptors of RFCs found in the peripheral lymphoid organs after primary and secondary immunization.

EXPERIMENTAL METHOD

Rabbit antiserum against aggregated mouse immunoglobulins (AAS) and a monospecific antiserum against mouse IgM were obtained by methods described previously [1, 2]. The polynucleotide complex polyA:polyU (C. F. Boehringer und Soehne, GmbH, Mannheim, West Germany) was obtained under the conditions described previously [2].

Mice of strain CBA were immunized intravenously with $5 \cdot 10^8$ sheep's red cells. When immunization was carried out twice, as a first step the mice were given an intravenous injection of 10^6 sheep's red cells, followed by an injection of $5 \cdot 10^8$ cells 40 days later. Spleen cells were obtained in the cold and treated with reagents in optimal doses [1, 2]. To obtain the fraction of small lymphocytes a suspension of spleen cells of immune mice in Eagle's medium with 10% embryonic calf serum (Bethesda, Maryland, USA) was first freed from cell conglomerates and disintegrated cells by layering above whole embryonic calf serum as described by Shortman et al. [9], after which it was fractionated in a gradient of bovine serum albumin (Sigma Co.) by the method of Raidt et al. [8]. The small lymphocytes contained in layer D, after washing three times with Eagle's medium with 10% embryonic calf serum, were cultivated in flasks in the same medium at 37°C for 20 h. Each flask contained 10^7 cells in 2 ml of medium. The cells were treated with the above-mentioned reagents at different time of incubation.

A modified method of Biozzi et al. [3] was used to determine RFCs in the cold. Rosettes formed by lymphocytes with five or more sheep's red cells were counted. Antibody-forming cells were determined by the method of Jerne and Nordin [6].

EXPERIMENTAL RESULTS

Data are given in Fig. 1 on the numbers of RFCs in the spleen inactivated by AAS, by anti-IgM serum, and by polyA:polyU, both before immunization and at different times after injection of $5 \cdot 10^8$ sheep's red cells. Among the spontaneous RFCs there were none which were inactivated by AAS and polyA:poly U; RFCs inactivated by these reagents began to appear 24 h after antigenic stimulation. After 3 days their number reached 70% of the total number of RFCs. By the fifth day after immunization only 60% of RFCs were inactivated by AAS and 50% by polyA:polyU. After 8 days and at subsequent times of observation, RFCs inactivated by these reagents were completely absent from the spleen. AAS and polyA:polyU evidently inactivate the same RFCs. If spleen cells taken on the third day after immunization were treated initially with polyA:polyU and, after removal of that reagent, were incubated with AAS the total number of inactivated RFCs was the same as after treatment with polyA:polyU alone. Similar results were obtained by treatment of the cells with these reagents in the opposite order. Comparison of the data for the kinetics of RFCs inactivated by anti-IgM serum, on the one hand, and by AAS and polyA:polyU on the other hand (Fig. 1) gave results in agreement with those obtained previously [1, 2], namely that RFCs inactivated by AAS and polyA:polyU have antigen-binding receptors predominantly of the IgM type.

The appearance of RFCs inactivated by antibodies against aggregated immunoglobulins only after injection of the antigen is in agreement with the hypothesis that these cells have antigen–antibody complexes on their surface. Inactivation of these cells with polyA:polyU can evidently be attributed to the fact that the double-helical polynucleotide interacts with the immune complex. The largest number of RFCs inactivated by AAS and polyA:polyU was observed during the period of maximal production of 19S hemagglutinins in the primary immune response. The disappearance of RFCs inactivated by AAS and polyA:polyU 8 days after immuniza tion can be explained by elimination of persisting earlier immune complexes by this time.

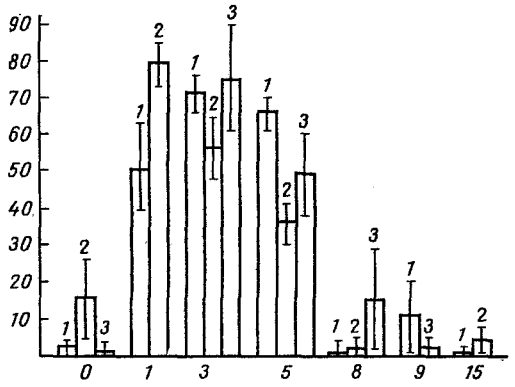

Fig. 1. Inhibition of rosette-forming cells by AAS, anti-IgM serum, and polyA:polyU before and after immunization with sheep's red cells. Ordinate, degree of inhibition of RFCs (in % of control); abscissa, time of investigation of animals before and after immunization (in days). Significance of columns: 1) AAS; 2) anti-IgM serum; 3) polyA:polyU.

TABLE 1. Changes in Number of Rosette-Forming Cells Inactivated by ASS and polyA:polyU during Cultivation of Immune Lymphocytes *in vitro* (number of RFCs per 10^3 small lymphocytes, M ± m)

Reagent	Time of cultivation (in h)			
	0	1	4	20
—	18,9± 1,2	13,3± 0,6	8,4± 0,8	8,9± 0,7
AAS	7,1± 0,5	9,2± 0,3	5,0± 0,6	9,4± 1,1
PolyA : polyU	7,0± 0,6	9,0± 2,0	4,6± 0,6	9,3± 1,0

Legend. Results of two or three experiments summarized in this table.

During the secondary immune response no RFCs inactivated by the reagents used could be detected. This was evidently due to the rapid elimination of antigen—antibody complexes during the secondary immune response.

During cultivation of lymphocytes from the spleen of nonimmune animals *in vitro*, antigen-binding receptors are liberated from the surface of the cells and are also restored as a result of synthesis in the cell [10]. The restoration cannot, however, take place during cultivation of lymphocytes with absorbed antigen—antibody complexes as their receptors *in vitro*. This would result in disappearance of RFCs inactivated by AAS and by polyA:polyU in the course of incubation. This was confirmed by experiments in which lymphocytes obtained from the spleen on the fifth day after immunization were cultivated.

The cell fraction isolated by gradient centrifugation consisted to the extent of 99% of small lymphocytes. The viability of these cells was 96%. The population was found to contain 17 RFCs per 10^3 lymphocytes (Table 1). The number of antibody-forming cells did not exceed 25 per 10^6 lymphocytes. It will be clear from Table 1 that during incubation of the immune lymphocytes for 4 h the number of RFCs detected fell by 50%, and it remained at that level during the next 16 h. The viability of the cells by this time was 83% and, consequently, it was reduced by not more than 15%. The number of RFCs inactivated by AAS and by polyA:polyU 1 h after the beginning of incubation was reduced by half, but toward the end of the period of incubation no RFCs with receptors of this type could be detected. Remembering that complete renewal of intrinsic Ig-receptors as a result of their formation *de novo* is achieved *in vitro* in 20-24 h [10], the results of the present experiments indicate that during the period of incubation some lymphocytes must have irreversibly lost their antigen-binding receptors inactivated by antibodies against aggregated mouse immunoglobulins.

It follows from the results described above that these cells had receptors which were inactivated by antibodies against aggregated mouse immunoglobulins. This may be further evidence

in support of the view that the RFCs appearing at the peak of the immune response and, as was established previously, belonging to the class of B lymphocytes [1], have adsorbed antigen–antibody complexes as their antigen-binding receptors.

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CYTOTOXIC AND COMPLEMENT-FIXING ACTIVITY OF RABBIT ANTISERA AGAINST RAT AND HUMAN BRAIN CORTEX AND WHITE MATTER

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UDC 615.365.82+612.82.017.1

The cytotoxic and complement-fixing activity of antisera against the cortex and white matter of the rat and human brain was investigated on thymus and bone marrow cells of mice and rats. The cytotoxicity test proved to be more sensitive and accurate and showed that cytotoxins against rodent thymocytes were present only in antisera against the cortex of the human brain but they were present in antisera against both the cortex and the white matter of the rat brain, although in significantly larger amounts in the former. Sera against rat cerebral cortex, when exhausted by the homonymous antigen, lost their cytotoxicity but they retained it after exhaustion with white matter.

KEY WORDS: cross-reacting antigens; thymus; brain.

The existence of cross-reacting antigens has not been clearly demonstrated in the brain and thymus of many species of animals [3, 4]. However, there have been few attempts to study with which components of the brain, i.e., with the gray or white matter, these antigens are mainly associated [3, 5].

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

EXPERIMENTAL METHOD

Antisera against homogenates of the cortex and white matter of the rat or human brain were obtained from rabbits by the method described previously [2]. The resulting sera were heated to 56°C for 30 min and absorbed with homogenates of rat or human liver and erythrocytes at room temperature, twice for 1 h each time, in the ratio of 1 ml antiserum to 0.1 ml solid

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 6, pp. 704-706, June, 1976. Original article submitted August 11, 1975.