REPLACEMENT OF IMMUNOGLOBULIN RECEPTORS BY LYMPHOCYTES CONTAINED IN THE MOUSE SPLEEN AT THE PEAK OF THE PRIMARY IMMUNE RESPONSE

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Spleen cells obtained from mice on the fifth day after injection of sheep's red cells (SRBC) irreversibly lose 50% of their surface immunoglobulin receptors during culture in vitro for 4 h. On incubation of spleen cells obtained on the ninth day after injection of the antigen no changes were observed in the total quantity of surface immunoglobulins. Metabolism of the antigen-binding receptors of immune splenic lymphocytes was studied by rosette formation. Culture of spleen cells obtained on the fifth day after injection of SRBC for 20 h showed that 70% of the rosette-forming cells (RFC) were lost. The remaining RFC belonged to θ-positive lymphocytes. The half-replacement time of their antigen-binding receptors was approximately 4 h. Replacement of receptors of RFC in the mouse spleen on the ninth day after antigenic stimulation takes place at the same rate. During culture of spleen cells for 20 h no decrease in the number of RFC was observed. It is postulated that the decrease in the number of RFC obtained at the peak of the primary immune response may be the result of inability of the immune lymphocytes to synthesize new receptors or the result of blocking the newly formed receptors by a soluble factor produced by immune lymphocytes during culture in vitro.

KEY WORDS: B lymphocytes; rosette-forming cells; immunoglobulin receptors; antigen-binding receptors.

Results were obtained previously to show that the antigen-binding receptors of rosette-forming B cells appearing in the spleen of mice at the peak of the primary immune response are aggregated immunoglobulins. Rosette-forming cells (RFC) with receptors of this structure were not found before immunization or 9 days after injection of the antigen (sheep's red cells – SRBC) [1, 2]. Preliminary experiments showed that aggregated immunoglobulins, which perform the function of antigen-binding receptors, are rapidly and irreversibly lost by lymphocytes when cultured in vitro [4]. This could indicate that receptors of this type are not synthesized in culture de novo but are evidently immune complexes adsorbed on the surface of the lymphocytes.

To test this hypothesis, the kinetics of immunoglobulins associated with the surface of lymphocytes and the rate of renewal of antigen-binding receptors of RFC obtained on the fifth and ninth days after immunization with SRBC was studied in experiments in vitro.

EXPERIMENTAL METHOD

The antisera used were: 1) polyvalent rabbit antiserum against mouse immunoglobulins; 2) monospecific rabbit antiserum against mouse IgM, obtained as described previously [1]; 3) donkey antibodies against rabbit γ-globulin, purified on an immunosorbent and labeled with ^{125}I ("Medradiopreparat" Factory, Ministry of Health of the USSR, batch No. 1379171175). The specific radioactivity of the preparation was 1 μCi/μg.

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TABLE 1. Changes in Content of Immunoglobulin on Small Lymphocytes from Spleen of Immune Mice during Culture in vitro (counts of ^{125}I in 2 min $\times 10^{-2}$; M ± m)

Time after immunization, days	Reagent	Duration of culture, h		
		0	4	20
5	Normal rabbit serum	6,9±0,1 (6,3—7,4)	8,9±0,2 (9,1—9,8)	9,6±0,1 (9,2—10,0)
	Anti-Ig serum	135,4±2,3 (126,5—144,2)	66.2±0,4 (64,8—67,6)	93,9±1.9 (88,5—99,3)
	Anti-IgM serum	44,1±0,7 (39,7—48,6)	30,9±0,9 (28,1—33,8)	43,3±2,0 (37,0—49,7)
9	Normal rabbit serum	10,3±0,2 (9,8—10,9)	11,4±0,2 (10,8—11,9)	—
	Anto-Ig serum	50,0±0,4 (48,8—51,2)	49,8±0,3 (48,7—50,8)	—
	Anti-IgM serum	12,8±0,2 (12.2—13,4)	20,5±0,3 (19,6—21,4)	—

Legend. Results of two or three experiments summarized in Table; in each sample $1 \cdot 10^6$ small lymphocytes were labeled with ^{125}I in a dilution of 1 : 500. Level of significance calculated for $P \leq 0.01$.

Mice (CBA) were immunized intravenously with $5 \cdot 10^8$ SRBC. Their fraction of small splenic lymphocytes was obtained by gradient centrifugation in bovine serum albumin (BSA) with a specific gravity of 1.093 (Sigma), by the method of Raidt et al. [5]. The lymphocytes were cultured in vitro under conditions described previously [4]. The content of surface immunoglobulins of the lymphocytes was estimated by indirect radioimmune analysis. The cells were first treated for 45 min in the cold with one of the antiglobulin sera and, after being washed twice with Eagle's medium (with 5% embryonic calf serum), they were incubated under the conditions described above with antibodies against ^{125}I-labeled rabbit immunoglobulins in a dilution of 1:500. The radioactivity of the cells ($1 \cdot 10^6$) was counted on a Gamma spectrometer (Intertechnique). The number of lymphocytes containing θ antigen was determined by the cytotoxic test after incubation of the cells with anti-θ serum and rabbit complement [1]. The number of RFC was estimated by a modified Biozzi's method [3].

EXPERIMENTAL RESULTS

All the experiments were carried out on small lymphocytes from mouse spleen obtained by centrifugation in a BSA gradient. The preparations contained not more than 2% of other spleen cells. The ratio between T and B lymphocytes in the fraction used was 55:45. Data for the change in content of immunoglobulins on mouse spleen lymphocytes during culture in vitro are given in Table 1. When lymphocytes obtained from the spleen on the fifth day after immunization with SRBC were used, the quantity of immunoglobulins bound with the cell surface fell by approximately 50% during 4 h of cultivation. Meanwhile, during cultivation of lymphocytes obtained on the ninth day after immunization with SRBC under the same conditions, no changes took place in the quantity of immunoglobulins bound with the cell. The viability of the cells remained practically unchanged during culture in vitro for 4 h. The corresponding control experiments demonstrated the high specificity of the method used to detect immunoglobulins. It can accordingly be concluded that a characteristic feature of lymphocytes obtained at the peak of the primary immune response was the rapid loss of a considerable proportion of the immunoglobulins bound with the cell surface in vitro. Similar experiments using monospecific anti-IgM serum also revealed a decrease in the total quantity of IgM bound with the surface of the lymphocytes during in vitro culture of cells obtained on the fifth day after immunization.

To investigate the kinetics of the antigen-binding receptors of the immune lymphocytes the number of RFC was determined during culture in vitro in the presence or absence of added cycloheximide, an inhibitor of protein synthesis (Fig. 1).

As Fig. 1 shows, in the absence of cycloheximide the number of cells detectable in the lymphocyte culture fell by about one half during the first 4 h, after which it remained constant during the next 16 h of incubation. If the medium contained cycloheximide (50 μg/ml), the number of RFC fell during the first hour of incubation by the same degree as after culture in the absence of the reagent. Later during culture with cycloheximide the decrease in the number of RFC was much greater than in the control, so that after 20 h only single RFC could be found.

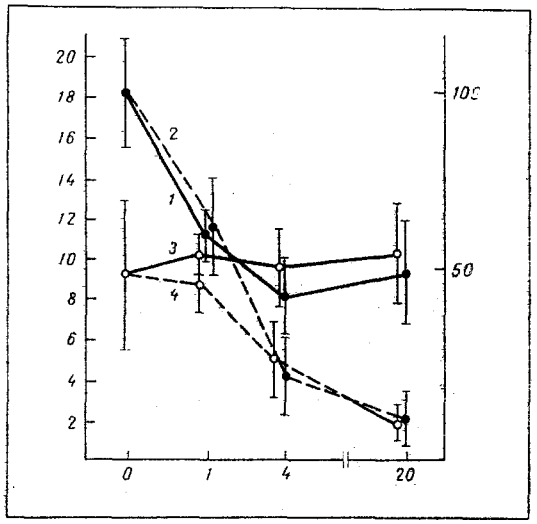

Fig. 1. Analysis in vitro of replacement of antigen-binding receptors of RFC contained in mouse spleen on fifth and ninth days after immunization with SRBC. Abscissa, duration of culture of small lymphocytes in vitro (in h); ordinate, left, number of RFC per 10^3 small lymphocytes on fifth day after antigenic stimulation without cycloheximide (1), in presence of cycloheximide (2), on ninth day after antigenic stimulation without cycloheximide (3), and in presence of cycloheximide (4); on right, percentage of RFC after culture of lymphocytes in vitro for various times (cells obtained on fifth day after immunization).

When lymphocytes obtained on the ninth day after immunization with SRBC were cultured for 20 h no significant decrease was found in the number of RFC. In this case also, cycloheximide effectively depressed the biosynthesis of immunoglobulin receptors (Fig. 1).

The results of these experiments indicate that at the peak of the primary immune response (fifth day after immunization) two subpopulations of RFC, differing essentially in the character of metabolism of their antigen-binding receptors, are present in the mouse spleen. As the experiments with cycloheximide showed, about half of all the RFC during this period have the property of regenerating their antigen-binding receptors in vitro. The half-replacement time was about 4 h, in good agreement with data on replacement of immunoglobulin receptors of lymphocytes from normal mouse spleen [6, 7]. The remainder of the RFC on the fifth day after immunization irreversibly lost its ability to bind antigen specifically in the course of culture in vitro. This may have arisen because the receptors of these cells lose contact with the cell surface and no formation of new receptors takes place. This hypothesis is in agreement on the whole with observations according to which an overall decrease in the quantity of immunoglobulins fixed to cells is observed during in vitro culture of lymphocytes obtained on the fifth day after immunization.

In the course of culture of lymphocytes in vitro no change was observed in the number of θ-positive cells. The number of RFC containing θ antigen also was determined before and after 4 h of culture. Whereas before culture the population contained approximately equal numbers of RFC which could be classed as T and B lymphocytes, after culture for 4 h, besides a general decrease in the number of RFC (by approximately four fifths) there was an increase in the relative number of RFC containing θ antigen. This could mean that RFC which irreversibly lose their antigen-binding receptors belong to the class of B lymphocytes.

It was shown previously that a characteristic feature distinguishing rosette-forming B cells appearing at the peak of the primary immune response is the presence of antigen-binding receptors which can be blocked by antibodies against aggregated mouse immunoglobulins [1, 2]. It could accordingly be concluded that the receptors mentioned above lie on the surface of the cell in an aggregated form. Receptors with this type of structural organization were not found on RFC present in the spleen in the later stages after immunization. As was demonstrated previously [4], a decrease in the number of RFC during culture of lymphocytes obtained at the peak of the primary immune response takes place on account of RFC reacting with antibodies against aggregated mouse immunoglobulins. It can be concluded from these results that the differences described in

this paper in the replacement of antigen-binding receptors of immune RFC may be the result of differences in the supramolecular organization of these receptors.

The question of the causes of the appearance of aggregated immunoglobulin receptors at the peak of the primary immune response and factors leading to the irreversible loss of these receptors during culture of lymphocytes in vitro is of considerable interest. One likely explanation, already mentioned previously [1], is that aggregated antigen-binding receptors are immune complexes fixed on the surface of B lymphocytes. This and other hypotheses are currently being tested.

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