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ROLE OF DIFFERENT TYPES OF CELLS IN THE EFFECT OF STIMULATION OF IMMUNOGLOBULIN SYNTHESIS IN MIXED CULTURES

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The synthesis of antibodies and nonspecific immunoglobulins in mono- and mixed cultures of immune lymph nodes and intact bone marrow after removal of adherent cells or T cells was studied by a radioisotope method using immunosorbents. Treatment of the lymph node cell population with anti-θ serum depressed antibody synthesis to 30%, whereas removal of the adherent cells depressed it to 70%. In mixed cultures of immune lymph node cells, after removal of adherent cells, and intact bone marrow cells no effect of stimulation of immunoglobulin synthesis was observed, but treatment of immune lymph node cells with anti-θ serum doubled this effect. The possible mechanisms of the effect of stimulation of immunoglobulin synthesis in mixed cultures are discussed and it is concluded that cooperation between individual types of cells is essential in the productive phase of the immune response.

KEY WORDS: immune response; intercellular interaction; T cells; A cells.

Previous investigations have shown that the intensity of antibody synthesis is two to three times greater in a mixed culture of immune lymph node cells and intact bone marrow cells than in a monoculture [3, 4, 11, 12]. This effect has been shown to be connected with the appearance of an additional number of antibody-forming cells in the population of the immune lymph nodes through the participation of a humoral factor secreted by intact bone marrow cells [2, 5, 9]. The view has been substantiated that, besides cellular cooperation observed

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TABLE 1. Immunoglobulin Synthesis in Immune Lymph Node Cells after Removal of Adherent Cells or T Cells from Them

Method of treatment of immune lymph node cells	Number of experiments	Magnitude of immunoglobulin synthesis (in %)	
		antibodies	nonspecific immunoglobulins
Removal of adherent cells	5	68±6,7	51±10,3
Treatment with anti-θ serum and complement	5	26±5,3	36±9,6
Treatment with normal mouse serum and complement	5	49±4,2	54±7,2

TABLE 2. Stimulation of Immunoglobulin Synthesis in Mixed Cultures after Removal of Adherent Cells

Source of cultivated cells	Number of experiments	Coefficient of stimulation	
		antibodies	nonspecific immunoglobulins
Immune lymph nodes + intact bone marrow	6	3,2±1,3	1,57±0,09
Lymph nodes without adherent cells + intact bone marrow	4	3,0±0,01	1,6±0,16
Adherent cells of immune lymph nodes intact bone marrow	3	1,6±0,13	0,9±0,06
Immune lymph nodes + bone marrow without adherent cells	5	1,32±0,03	0,9±0,07
Immune lymph nodes + adherent bone marrow cells	6	1,42±0,25	0,97±0,21

TABLE 3. Stimulation of Immunoglobulin Synthesis in Mixed Cultures after Treatment of Lymph Node Cells with Anti-θ Serum or with Normal Mouse Serum

Source of cultivated cells	Number of experiments	Coefficient of stimulation	
		antibodies	nonspecific immunoglobulins
Immune lymph nodes + intact bone marrow	4	3,48±0,73	1,88±0,10
Immune lymph nodes treated with normal mouse serum + complement + intact bone marrow	4	5,89±1,28	3,26±0,69
Immune lymph nodes treated with anti-θ serum + complement + intact bone marrow	4	9,89±0,36	3,32±1,45

during induction of the immune response, interaction between different types of cells also takes place at the level of mature antibody producers in the productive phase of immunogenesis [12].

In the investigation described below the role of individual types of cells was studied in cooperative processes taking place at the peak of the immune response and the influence of these cells on the stimulation of antibody formation in a mixed culture of immune lymph node cells and intact bone marrow cells was examined. For this purpose, T cells or adherent cells (A cells) were removed from mixed cell populations.

EXPERIMENTAL METHOD

Experiments were carried out on inbred CBA mice (males and females) weighing 18-22 g. The animals were immunized by the method described previously [3]. Lymph nodes were taken from immune mice on the fourth day after reimmunization. Bone marrow cells were obtained from intact animals. The cell suspensions were diluted to a concentration of $3 \cdot 10^7$ nucleated cells to 1 ml and grown on Eagle's medium with 20% bovine serum, antibiotics, and glycine-^{14}C (1 μCi/ml).

The synthesis of antibodies and nonspecific immunoglobulins was determined from incorporation of the radioactive label into these proteins after their removal from the medium with the aid of specific immunosorbents [1]. The magnitude of the effect of stimulation of immunoglobulin synthesis was estimated as a coefficient expressing the ratio between the intensity

of antibody and nonspecific immunoglobulin synthesis in the mixed culture to the intensity of synthesis of these proteins in the corresponding individual cultures.

To separate the adherent cells from nonadherent the suspensions of lymph node or bone marrow cells were incubated at 37°C in glass flasks for 30-40 min with a density of $2.8 \cdot 10^5$ cells/cm^2. To remove the T cells the suspension of lymph node cells was treated with anti-θ serum and complement [8]. In parallel experiments the cells were treated with normal isologous mouse serum and complement.

To obtain anti-θ serum, mice of strain AKR were immunized 6 times intraperitoneally with thymus cells from CBA mice ($1 \cdot 10^7$-$2 \cdot 10^7$ cells per mouse) at weekly intervals. The serum was tested on thymus, lymph node, and bone marrow cells. Most of the sera obtained were active in a dilution of 1:8.

EXPERIMENTAL RESULTS

In the experiments of series I antibody and nonspecific immunoglobulin synthesis was studied in the initial lymph node cell population and after removal of A or T cells from them. The magnitude of synthesis of these proteins by immune lymph node cells after the various treatments is shown in Table 1 as percentages of synthesis in the original population.

It will be clear from Table 1 that removal of both the adherent and the T cells from the population of immune lymph node cells led to a decrease in the intensity of antibody synthesis. Consequently, the presence of other helper cells (A cells, T cells), which do not themselves synthesize antibodies, is essential for the normal activity of mature antibody-forming cells at the peak of the immune response. These cells evidently play an important role in the regulation of antibody formation. Work showing that different subpopulations of cells possess the functions of helper cells and suppressor cells has recently been published [6, 7, 13]. According to Gorczynski [7],for instance, T cells with θ antigen on their surface stimulate antibody formation, whereas T cells with no θ antigen are suppressors. Treatment of immune lymph node cells with anti-θ serum possibly disturbs the equilibrium between these subpopulations, as a result of which the effect of inhibitors liberated by the suppressor cells is intensified.

Treatment of the population of lymph node cells with normal mouse serum and complement also reduced immunoglobulin synthesis to 50%. The number of living cells was not reduced under these circumstances compared with the control. Inhibition of the immune response under the influence of normal mouse serum has also been observed by other workers [10, 14, 15]. The thought that the effects of treatment with anti-θ serum and with normal mouse serum in these experiments were in the same direction suggests that normal mouse serum in some way acts on cells carrying θ antigen.

In the next series of experiments the effect of removal of the adherent cells or T cells from the population of immune lymph node or intact bone marrow cells on the stimulation of immunoglobulin synthesis in mixed cultures was studied. The results of these experiments are given in Tables 2 and 3.

The results in Table 2 show that removal of the adherent cells from the immune lymph node population did not abolish the effect of stimulation of immunoglobulin synthesis, whereas in the cell population of the intact bone marrow, in order to obtain a complete effect both adherent and nonadherent cells must be present. In the immune lymph node population there is evidently a certain number of "silent" antibody producers, which embark upon synthesis without the participation of adherent cells under the influence of a factor secreted by the bone marrow cells.

When cells of immune lymph nodes were treated with anti-θ serum and complement and cultivated together with bone marrow, the effect of stimulation of antibody formation was approximately doubled compared with that of the mixed culture in which the cells were not treated in any way. Normal mouse serum also increased the stimulation effect, but this increase was much smaller than on treatment with anti-θ serum (Table 3).

The increase in the effect of stimulation of immunoglobulin synthesis in the mixed culture after treatment of the population of lymph node cells with anti-θ serum is evidence that T cells participate in the cooperative processes resulting in increased antibody formation. Stimulation of the immune response by bone marrow cells could perhaps be due to their effect on suppressor T cells. However, the mechanism of this phenomenon is not yet completely clear and requires further study.

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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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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