ROLE OF MACROPHAGES IN THE MECHANISM OF SPECIFIC RETENTION OF SENSITIZED LYMPHOCYTES IN LYMPH GLANDS CONTAINING ANTIGEN

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The role of macrophages in the phenomenon of specific retention of antigen-activated lymphocytes in lymph glands containing the same antigen was investigated. Injection of peritoneal macrophages, phagocytosing sheep's red cells (as well as of the red cells alone) was found to cause specific retention of lymphocytes in the regional lymph glands. Preliminary treatment of the antigen-loaded macrophages with antierythrocytic serum reduced the retention of lymphocytes. It is postulated that lymphocytes are fixed by means of receptors on their surface to the membrane of macrophages, into which antigen molecules are incorporated.
KEY WORDS: *macrophages; antigen; specific retention of lymphocytes.*

It has now been shown that interaction between T- and B-lymphocytes and macrophages is necessary for the biosynthesis of antibodies against many antigens. The suggestion has been made that *in vivo* and in tissue culture close contact is brought about between these cells by means of antigen and receptors on the surface of the interacting cells [10-13].

Specific retention of antigen-sensitized lymphocytes in the lymph glands and spleen, containing the same antigen, has been investigated [5, 6]. However, this work has not solved the problem of the role of specific receptors of lymphocytes in the retention phenomenon. The role of the T- and B-lymphocytes in this phenomenon has not been discovered. Finally, it is not yet clear whether antigen captured by the macrophages of lymphoid tissue participates in fixation of the cells. An indirect answer to the first question was obtained by the writers [1] in experiments in which antired-cell antibodies completely abolished the specific retention of "irradiated" lymphocytes in lymph glands containing sheep's erythrocytes.

In experiments in which "irradiated" thymocytes (taken from the spleen of lethally irradiated mice previously given injections of thymocytes together with sheep's red cells) were injected into recipients it was found that T-lymphocytes participate in the specific retention phenomenon [2].

The present investigation had two objects. The first was to discover whether specific retention of sensitized lymphocytes can take place in lymph glands into which macrophages loaded with antigen have been introduced. It was also hoped to discover how the hypothetical participation of macrophages depends on their functional state. This problem had to be studied because other workers [4, 7, 8] and the present writers [3] have previously described a sharp decrease in the immunogenicity of peritoneal macrophages loaded with sheep's red cells if obtained from mice receiving antierythrocytic serum.

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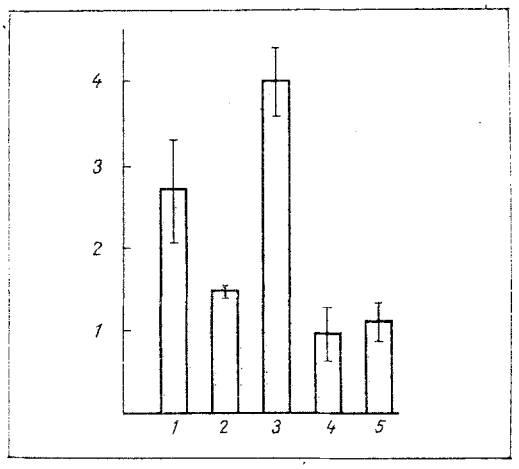

Fig. 1. Specific retention of "trained" lymphocytes when antigen is introduced by different methods into lymph glands. Abscissa, no. of experiment; ordinate, index of asymmetry (explanation in text).

EXPERIMENTAL METHOD

In the experiments of group 1, $2 \cdot 10^8$ sheep's red cells in 0.02 ml physiological saline was injected into the footpad of the right forelimb of 35 CBA mice and the same volume of rat's red cells was injected into the footpad of the left forelimb. An intravenous injection of $5 \cdot 10^7$ syngeneic spleen cells, labeled for 1 h with thymidine-H^3 *in vitro* (10μCi per 10^8 cells in 1 ml Eagle's medium) was given 2 h later. The donors were given an injection of 0.5 ml of a 5% suspension of sheep's red cells 3 days before the experiment. The recipients were decapitated 18 h after the injection of labeled cells and their axillary lymph glands were removed and weighed. The lymph glands were hydrolyzed in concentrated formic acid (2 mg tissue to 0.1 ml acid) and the radioactivity of the digest was estimated on a Packard Tri-Carb scintillation counter. The difference between the retention of labeled cells in the experimental and control lymph glands was expressed as the ratio beyween the radioactivity of the tissue of the experimental glands and the radioactivity of the contralateral lymph glands (the index of asymmetry).

In the experiments of groups 2, 24 CBA mice received an injection, not of sheep's red cells, but of $3.5 \cdot 10^6$ syngeneic peritoneal macrophages loaded with sheep's red cells ($7 \cdot 10^6$ according to counts in films), into the footpad. The peritoneal macrophages were obtained 3 days after intraperitoneal injection of 1.5 ml 10% peptone and 1 h after intraperitoneal injection of 10^9 sheep's red cells. To remove unphagocytosed red cells from the suspension of macrophages, the cell suspension was subjected to hypotonic shock and then washed 3 times [9].

In the experiments of group 3, a mixture of macrophages ($3.5 \cdot 10^6$) and sheep's red cells ($2 \cdot 10^8$) prepared in the cold (4°C) immediately before injection, was injected into the right footpad of the recipients.

In the experiments of group 4 sheep's red cells were added in the cold to a suspension of peritoneal macrophages not loaded with antigen (the ratio of macrophages to red cells was 1:100). This was followed by hypotonic shock and washing of the cells. An injection of $3.5 \cdot 10^6$ macrophages was then given into the footpad of four recipients. These experiments were carried out in order to rule out the possibility of incomplete destruction of the red cells by hypotonic shock and also the possibility of passive adsorption of erythrocytic antigens on to the surface of the macrophages.

In the experiments of group 5 the same number of peritoneal macrophages, loaded with sheep's red cells and then treated *in vitro* for 1 h at 37°C with mouse antierythrocytic serum (hemagglutinin titer 1:1024), was injected into the footpad of six CBA mice.

EXPERIMENTAL RESULTS

The results of the five groups of experiments are shown in Fig. 1. Clearly marked retention of labeled lymphocytes took place in the lymph glands in the experiments in which sheep's red cells were injected into the footpad of the mice. The value of the index of asymmetry was unaffected by injection of the control antigen (rat red cells) into the opposite footpad. A particularly clear effect was obtained when a freshly prepared mixture of peritoneal macrophages with sheep's red cells was injected (Fig. 1, 3). In that case, four times more sensitized lymphocytes were fixed in the regional lymph gland than in the contralateral gland. A less marked, but statistically significant retention was observed in the experiments in which peritoneal macrophages phagocytosing sheep's red cells *in vitro*

were injected (Fig. 1, 2). After injection of macrophages not phagocytosing the antigen, retention of sensitized labeled lymphocytes in the regional lymph gland was absent (Fig. 1, 4). After treatment of antigen-containing macrophages with antierythrocytic serum a tendency was observed for retention of the sensitized labeled lymphocytes in the regional lymph glands to diminish (Fig. 1, 5).

The results of these experiments show that macrophages phagocytosing antigen can bring about not only nonspecific, but also specific fixation of labeled lymphocytes activated by the same antigen in a lymph gland. It can be postulated that the lymphocytes interact with antigens of erythrocytic origin incorporated into the membrane of the macrophage. The possibility of comparatively long exposure of antigen molecules on the surface of the plasma membrane of peritoneal macrophages has been stated by Unanue et al. [14].

The phenomenon of retention in experiments with injection of peritoneal macrophages previously loaded with antigen *in vitro* (Figs. 1, 2) was possibly weaker in intensity than in the experiments in which red cells alone were injected (Fig. 1) because the dose of red cells phagocytosed by the macrophages was an order of magnitude lower. However, it is evident that the quantity of antigen was not the only factor. Addition of the corresponding number of red cells ($2 \cdot 10^8$) to the macrophages did not equalized the indices of asymmetry, but caused a twofold increase in the level of retention of the sensitized lymphocytes (compared with experiments in which sheep's red cells only were injected).

Antibodies reduced the ability of the macrophages to interact with the "trained" lymphocytes to some extent, but this problem requires further investigation. Such a possibility was reported by Mosier [11], who worked with another model. A second possibility, retention of labeled erythrocytes as a result of the "secretion" of erythrocytic antigens by macrophages, must be borne in mind, having regard to results obtained by Askonas and Jaroskova [4].

LITERATURE CITED

1. V. P. Zakharova, Byull. Éksperim. Biol. i Med., No. 10, 74 (1973).
2. V. P. Zakharova, and V. V. Malaitsev, in: Immunoreactivity of the Organism [in Russian], Kaliningrad and Tallin (1973), pp. 7-8.
3. V. V. Malaitsev, Zh. Mikrobiol., No. 11, 59 (1973).
4. B. A. Askonas and L. Jaroskova, in: Developmental Aspects of Antibody Formation and Structure (Proceedings of a Symposium), New York (1970), p. 531.
5. E. E. Emeson and D. R. Thursh, J. Immunol., 106, 635 (1971).
6. G. Griscelli, P. Vassalli, and R. T. McClusky, J. Exp. Med., 130, 1427 (1969).
7. G. Haughton and D. O. Adams, J. Reticuloend. Soc., 5, 565 (1968).
8. G. Haughton and D. O. Adams, J. Reticuloend. Soc., 7, 500 (1970).
9. T. Morita and E. R. Perkins, J. Reticuloend. Soc., 2, 406 (1965).
10. D. E. Mosier, Science, 158, 1573 (1967).
11. D. E. Mosier, J. Exp. Med., 129, 351 (1969).
12. D. E. Mosier and L. W. Coppleson, Proc. Nat. Acad. Sci. (Washington), 61, 542 (1968).
13. E. R. Unanue, Advances Immunol., 15, 95 (1972).
14. E. R. Unanue, I. C. Cerottini, and M. Bedford, Nature, 222, 1193 (1969).