ELECTROPHORETIC SEPARATION OF LYMPHOCYTES FROM RAT SPLEEN AND THYMUS AND THEIR RESPONSE TO MITOGENS in vitro

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Electrophoretic mobility (EPM) of lymphocytes from the thymus and spleen of Wistar and August rats was investigated by free flow electrophoresis and the ability of lymphocytes with different surface electric charges to undergo mitogenic transformation under the influence of phytohemagglutinin and concanavalin A was compared. An in vitro culture showed that splenic lymphocytes can be divided into two principal groups depending on their surface charge: cells with high and low mobility respectively. Separation of the thymocytes showed them to be a group of cells consisting of several (8 to 10) fractions differing in EPM. Ability of the lymphocytes to be stimulated by mitogens was shown to depend on their surface charge. Stimulation of [^{3}H]thymidine incorporation was observed during exposure of splenic lymphocytes with high mobility in an electric field to mitogens. Lymphocytes with low mobility were not stimulated by mitogens. The subpopulation of thymocytes with low EPM was not stimulated by concanavalin A. Rat thymocytes were shown virtually not to react to phytohemagglutinin irrespective of their EPM.

KEY WORDS: lymphocyte; electrophoretic mobility; mitogens.

Lymphocytes from lymphoid organs and blood have an over-all negative surface charge [1, 2], which is due to exposure of various chemical groups, notably amino, phosphate, and sulfhydryl groups, α-carboxyl groups of N-acetylneuraminic acid, and certain others [2], on their plasma membrane. It has been shown that T and B lymphocytes differ in their surface charge and that they can be separated on the basis of their electrophoretic mobility (EPM) by the method of free flow electrophoresis into two principal populations: cells (T and B respectively) with high and low mobility [3, 7].

Because of the need to study the behavior of cells from rat lymphoid organs during exposure to antigens and, in particular, during organ transplantation, the investigation described below was undertaken in order to study interaction between the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) with various subpopulations of lymphocytes from rat spleen and thymus, separated on the basis of their EPM by means of the Elphor VaP5 apparatus for preparative cell electrophoresis [4, 6].

EXPERIMENTAL METHODS

Male Wistar and August rats aged 6-8 weeks were used in the experiments. To obtain a sufficient number of lymphocytes for separation and the subsequent experiments, cells from lymphoid organs of three animals were pooled. The lymphocytes were washed with medium No. 199 and placed in sorbitol buffer of low ionic strength (pH 7.2). Dead and damaged cells were removed by passage through cotton wool [3]. The cells (initial concentration $2 \cdot 10^7$ cells/ml) were separated on the Elphor VaP5 apparatus for preparative cell electrophoresis (Bender Hobein, West Germany), the working parameters of which were kept constant as follows: voltage 800 V, current 150 mA, rate of flow of buffer in the chamber 500 ml/h, rate at which the cells entered the chamber $1 \cdot 10^8$ cells/h, temperature in the separation chamber 6°C.

The number of lymphocytes obtained in individual fractions after separation was determined with a Picoscale hemocytometer (Hungary). Viability was tested at all stages of the investigations by means of

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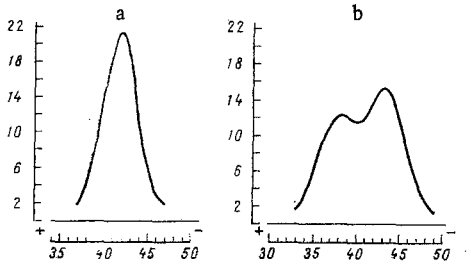

Fig. 1. Electrophoretic separation of lymphocytes from thymus (a) and spleen (b) of Wistar and August rats. Abscissa, fraction No.; ordinate, number of cells in fraction as percentage of total number of cells separated.

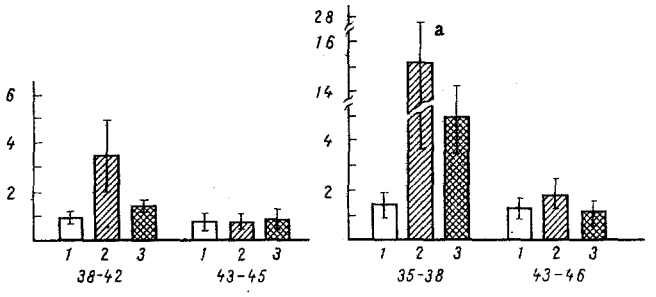

Fig. 2. Incorporation of [^{3}H]thymidine into lymphocytes. 1) Incorporation of [^{3}H]thymidine without mitogen; 2) incorporation of [^{3}H]thymidine after administration of Con A; 3) incorporation of [^{3}H]thymidine after administration of PHA. Abscissa, fraction Nos.; ordinate, incorporation of thymidine (in cpm × 1000). In one experiment (a) increased incorporation of [^{3}H]thymidine was observed in a group of spleen cells with high mobility.

trypan blue. After fractionation, three or four neighboring fractions of the lymphocytes were pooled and their reaction to mitogens was determined during culture in vitro. Cells in a concentration of $1.5 \cdot 10^6$/ml were suspended in medium 199 containing 5% inactivated human serum (group IV) and antibiotics. The optimal mitogenic concentrations of Con A (Boehringer Mannheim) and PHA (Difco) were determined in preliminary experiments. [^{3}H]Thymidine in a dose of 1 μCi was added to the culture after 48 h and incorporation of [^{3}H]-thymidine (in cpm) into the DNA fraction insoluble in TCA was determined 24 h later by means of a Mark II scintillation counter. All experiments were repeated 5 times in three parallel cultures. Electrophoretic separation of the lymphocytes was carried out under sterile conditions, after appropriate preparation of the Elphor apparatus for work under those conditions.

EXPERIMENTAL RESULTS

Profiles of electrophoretic separation of negatively charged thymus and spleen cells of Wistar and August rats are illustrated in Fig. 1. Splenic lymphocytes from the rats on fractionation formed two principal cell populations differing considerably in EPM, located between fractions 33 and 49 and characterized by two peaks on the distribution curve. Erythrocytes present in the splenic suspension served as the control of EPM and in these experiments were located in the region of fraction 31. The profile of electrophoretic distribution of thymocytes showed one peak in the region of fractions 37-47. The thymocytes closely resembled in their EPM the population of spleen cells with low mobility. Virtually no erythrocytes were present in the thymus. Fractionation profiles of lymphocytes from the spleen and thymus of Wistar and August rats in an electric field did not differ significantly.

The population of spleen cells with high mobility (fractions 35-38) was shown to respond to PHA and Con A, as reflected in a significant increase in [^{3}H]thymidine incorporation. Cells of low mobility (fractions

43-46) did not respond to these mitogens (Fig. 2). On analysis of the thymus cells separated on the basis of EPM it was discovered that there is a subpopulation of thymocytes (fractions 43-45; Fig. 1) with comparatively lower EPM than the majority of thymus cells and no responding to stimulation by Con A and PHA (Fig. 2). Thymocytes distributed over fractions 38 to 42 inclusive (with higher mobility) reacted to Con A but not to PHA.

The results of these experiments to determine EPM of lymphocytes from the rat spleen and thymus agree with those obtained by other workers [8]. Meanwhile different fractions of the thymus and spleen were found to react differently to mitogens. For instance, fractions 38 to 42 of thymocytes could increase their [^{3}H]thymidine incorporation in the presence of Con A, whereas thymocytes of fractions 43 to 46 did not possess this property; this difference may reflect the existence of thymocytes with different functions in the thymus. Unfortunately the results of the present experiments could not be compared with those of other workers for no reference could be found in the periodical literature to studies aimed at determining the response of fractions differing in EPM to mitogens in vitro after electrophoretic fractionation of rat lymphocytes. A paper by Shortman et al. [5], who studied the reaction of lymphocytes from the thymus and spleen of CBA mice, separated in a density gradient, to T-mitogens (PHA and Con A), is interesting. A parallel study of the electrophoretic fractionation profile of the lymphocytes and a search for θ antigen in them were carried out on animals of this strain. These workers found that thymus cells with a low content of θ antigen possessed higher EPM than thymocytes with a high concentration of the antigen. Meanwhile cells with low EPM, unlike thymocytes with high mobility, did not respond to mitogens. These findings are in agreement with the results of the present experiments and they probably reflect the existence of common processes of differentiation and maturation of lymphocytes in the thymus of animals of different species.

LITERATURE CITED

1. K. Hannig and K. Zeiller, Hoppe-Seylers Z. Physiol. Chem., 350, 467 (1969).
2. J. Mehrishi and K. Zeiller, Brit. Med. J., 1, 360 (1974).
3. S. Nordling, L. Anderson, and P. Hayry, Eur. J. Immunol., 2, 405 (1972).
4. K. Shortman, Ann. Rev. Biophys. Bioeng., 1, 93 (1972).
5. K. Shortman, H. von Boehmer, J. Lipp, et al., Transplant. Rev., 25, 163 (1972).
6. H. von Boehmer, K. Shortman, and G. Nossal, J. Cell. Physiol., 84, 231 (1974).
7. K. Zeiller, E. Holzberg, J. Pascher, et al., Hoppe-Seylers Z. Physiol. Chem., 353, 105 (1972).
8. K. Zeiller and K. Hannig, Hoppe-Seylers Z. Physiol. Chem., 352, 1162 (1971).