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INTERACTION OF POLYANION MOLECULES WITH THE PLASMA MEMBRANE OF LYMPHOCYTES WITH DIFFERENT DENSITIES OF CHARGED GROUPS ON THE CELL SURFACE

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The writers showed previously that polyacrylic acid (PAA), a polyanionic mitogen, activates proliferation only of lymphocytes adherent to nylon wadding, but does not affect cells incapable of adhesion [3]. Cells not adherent to nylon wadding (mature T lymphocytes) are known to differ from adherent cells (mainly B lymphocytes, but also macrophages and T suppressor lymphocytes) in their high electrophoretic mobility, due to the larger number of negatively charged groups on the cell surface. These groups consist mainly of terminal N-acetylneuraminic acid and, to a much lesser degree, of terminal N-acetyl-glucosamine and N-acetylgalactosamine. In connection with the facts described above it has been suggested that interaction of the polyanionic polymer with the cell membrane of mature T cells may be impeded or blocked because of the high density of negatively charged groups on the surface of these cells.

To test this hypothesis the effect of PAA was studied on cell subpopulations enriched with B lymphocytes, with mature T cells or immature T lymphocytes, and also on mature T cells, treated beforehand with neuraminidase, in order to remove by enzyme action the terminal N-acetylneuraminic groups present on the cell surface.

EXPERIMENTAL METHOD

In vitro cultures of lymphocytes from mouse (CBA × C57BL)F_1 spleen and thymus were used. The conditions of induction of lymphocyte division with the aid of PAA and the technique of fractionation of the spleen cells based on ability to adhere to nylon wadding were described previously [3, 4]. The fraction of nonadherent cells was described as enriched with mature T cells, the fraction of adherent cells as enriched with B lymphocytes and with a small admixture of T cells [3]. A suspension of thymocytes was used as the source of immature T lymphocytes without any marked glycocalyx, i.e., a high density of charged groups on the cell surface.

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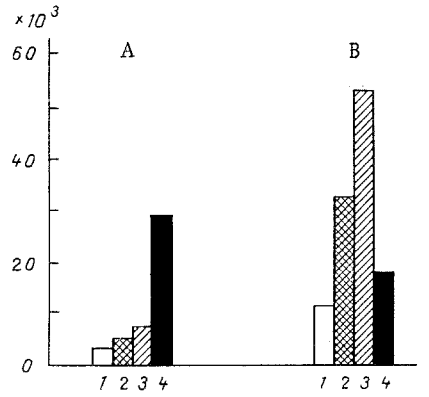

Fig. 1. Proliferative reaction of cells of T and B fractions (A and B) obtained on nylon wadding. Intensity of DNA synthesis shown in cell cultures activated *in vitro*. Horizontal axis: 1) control (without mitogen); 2) PAA (50 μg/ml); 3) lipopolysaccharide from *E. coli* (20 μg/ml); 4) concanavalin A (2 μg/ml). Vertical axis, intensity of incorporation of [^{3}H]-thymidine (in cpm).

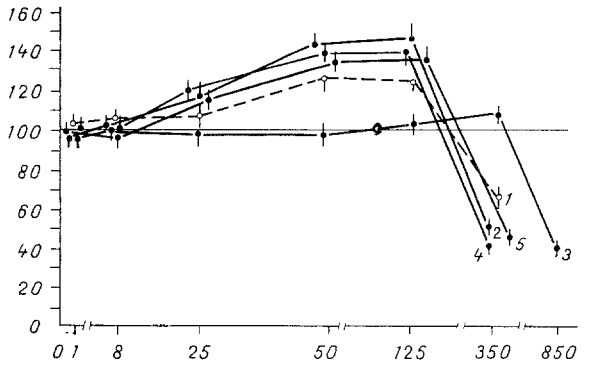

Fig. 2. Effect of PAA on ATPase activity. 1) Spleen cells; 2) splenic B lymphocytes; 3) splenic T lymphocytes; 4) splenic T cells treated beforehand with neuraminidase; 5) thymus lymphocytes. Ordinate, total cellular ATPase (in %); abscissa, final PAA concentration *in vitro* (in μg/ml). Initial level (horizontal line) of ATPase activity of cells, measured immediately before addition of PAA, taken as 100%.

Mature T lymphocytes were treated with neuraminidase (from Calbiochem; 500 U/ml) in 20 mM phosphate buffer, pH 5.8, with a final enzyme concentration of 0.5-1.0 U/ml and with a cell density of 3×10^7 to 5×10^7/ml for 2 h at room temperature.

The effect of PAA on the lymphocytes was assessed relative to an early effect at the cell membrane level, namely activation of membrane (Na^+, K^+)- and Ca^{++}-ATPases. As was shown previously, activation of total cellular ATPase observed under the influence of PAA is due practically completely to activation of these enzymes [1, 2]. The rate of utilization of ATP by the cells (total cellular ATPase) and the rate of production of ATP in the cell, equal to it (under conditions of a stationary ATP level) were estimated from the rate of O_2 consumption by lymphocytes in an airtight polarographic cell with O_2-electrode [1].

EXPERIMENTAL RESULTS

The experimental results showed (Fig. 1) that division of lymphocytes adherent to nylon wadding (fraction of B cells) is activated by PAA *in vitro*, but division of cells not adherent to nylon wadding (fraction of mature T cells) is not activated. The mitogenic action of PAA on thymocytes could not be studied because of rapid death of these cells under conditions of

culture *in vitro*. Under the influence of the same mitogenic doses of PAA considerable activation of total cellular ATPase (up to 130% of the initial level) of unfractionated splenic lymphocytes was observed (Fig. 2). Cells of the T fraction virtually did not react to PAA by activation of ATPases, even when the concentration of the polyanion was increased up to the toxic level. Conversely, total ATPase was activated in cells of the B fraction immediately after treatment with mitogenic doses of PAA. The effect on cells of the B fraction in this case was stronger than on a mixed population of unfractionated spleen cells (up to 148% of the initial level). Addition of mitogenic doses of PAA to the thymocyte suspension led to marked activation of ATPases (total ATPase was increased to 130% of the initial level). It is interesting to note that mature T cells, treated beforehand with neuraminidase, also acquired the ability to react to addition of PAA by activation of total ATPase (up to 136% of the initial level).

These experiments thus showed that mature splenic T lymphocytes do not react to the mitogenic polyanion PAA either by cell proliferation or by activation of membrane ATPases. Conversely, membrane ion-transporting ATPases are activated considerably in splenic B lymphocytes during the first 1-2 min after addition of mitogenic doses of PAA, and later the cells complete a cycle of mitotic division. The reason for the absence of response of mature T cells to PAA is evidently the high density of the negatively charged groups on the surface of these cells. Mature T lymphocytes become sensitive to the activating action of PAA after an artificial decrease in density of the terminal N-acetylneuraminic residues, which account for the greater part of the negatively charged groups on the cell surface. Thymocytes — immature T lymphocytes without a large number of negatively charged groups on the cell surface — also were activated by PAA. Consequently, the high density of negatively charged terminal N-acetylneuraminic groups in the glycocalyx prevents interaction of the negatively charged polymer with the cell membrane.

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