CYTOCHEMICAL CHANGES IN CIRCULATING BLOOD LEUKOCYTES DURING EXPERIMENTAL IMMUNOLOGIC STIMULATION

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The acid phosphatase level in the blood lymphocytes of August rats was increased 7 days after injection of Freund's complete adjuvant into the animals, while the dehydrogenase level was lowered. The opposite changes were observed after 14 days. After repeated injection of adjuvant, acid phosphatase activity in the blood granulocytes reached a maximum on the 14th day and remained high until the 45th day.

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It was previously shown that after intraperitoneal injection of Freund's adjuvant with BCG vaccine into rats the animals develop a syndrome resembling in some manifestations human lupus erythematosus (the LE phenomenon, systemic vasculitis, dermatitis, the "wire loop" phenomenon in the kidneys) [5, 7]. It has also been shown that the lymphocytes of sick animals have a marked cytotoxic action on a fibroblast tissue culture [6]. The manifestation of this aggressive behavior of lymphocytes is perhaps accompanied by changes in their metabolic processes. This suggestion is made all the more probable because immunization is accompanied by considerable changes in the level of lysosomal and mitochondrial enzymes [1, 3].

The object of this investigation was to study the activity of the following enzymes in the leukocytes: acid phosphatase, as a marker of lysosomes and an index of catabolic activity, succinate dehydrogenase as a marker of mitochondria and an index of the activity of energy processes in the Krebs cycle, and mitochondrial α-glycerophosphate dehydrogenase, characterizing the connection between glycolysis and respiration and the degree of participation of the mitochondria in oxidation of the hyaloplasmic reduced form of nicotinamide-adenine dinucleotide (NAD).

EXPERIMENTAL METHOD

Experiments were carried out on 25 August rats weighing 150-200 g. Freund's adjuvant was injected once into 5 animals and twice into 15 rats at intervals of 10 days, while 5 animals comprised the control group. Blood films were taken from the caudal vein. The animals were examined before injection of adjuvant, and 7 and 14 days and 1.5 months after injection. Individual data were periodically compared with initial observations for the same animal.

Enzyme activity in the blood lymphocytes was investigated cytochemically: acid phosphatase (3.1.3.2) by the method of Goldberg and Barka [9], succinate dehydrogenase (1.3.99.1) by the method of Quaglino and Hayhoe [13], and α-glycerophosphate dehydrogenase (1.1.99.5) by Nartsissov's method [2]. Acid phosphatase activity was also studied in granulocytes. When assessing activity of the enzyme, 100 cells were counted in suitably stained films and divided into groups depending on the intensity of deposition of reaction products. The cells were divided into two groups: all lymphocytes (or granulocytes) in which enzyme activity was absent or only traces of deposition of reaction products were observed in the cytoplasm were included in one group, and leukocytes whose cytoplasm was completely (high activity) or almost completely (moderate activity) filled with reaction products were included in the second group. This method of counting was fully described previously [1]. The results were expressed as percentages of cells with high and moderately high enzyme activity. The results were analyzed by statistical methods with assessment of the significance of the difference between the arithmetic means of two sets.

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EXPERIMENTAL RESULTS

Considerable changes in enzyme activity in the leukocytes were found in the experimental animals depending on the times of taking blood after injection of the adjuvant. Significant differences were also found in reactions to primary and repeated stimulation. After the first injection of adjuvant (Fig. 1) a decrease in activity of oxidative enzymes with preservation of high activity, or an actual increase in activity of acid phosphatase were observed in the lymphocytes. The decrease in activity of succinate dehydrogenase on the 14th day after injection of adjuvant compared with its initial level is statistically significant (before injection of adjuvant 20 ± 6.8*, on the 14th day 0; $P < 0.05$). The increase in acid phosphatase activity in the lymphocytes on the 7th day (49 ± 1.5) compared with the initial data (37 ± 4.27) is also statistically significant ($P < 0.05$). Changes in α-glycerophosphate dehydrogenase activity on the 14th day compared with the initial values are no more than a tendency ($0.1 > P > 0.05$).

Changes of this type persisted for two weeks. Acid phosphatase activity in the granulocytes changed only slightly at these times, the values remaining close to those observed initially.

On the 7th day after restimulation the high level of acid phosphatase activity in the lymphocytes fell, sometimes to 0 by the 14th day (Fig. 1). Dehydrogenase activity showed changes in the opposite direction. Changes in the activity of these enzymes on the 7th day compared with initially were statistically significant (for acid phosphatase in the lymphocytes $P < 0.02$, for succinate dehydrogenase $P < 0.05$, for α-glycerophosphate dehydrogenase $P < 0.01$).

Acid phosphatase activity in the blood granulocytes differed only slightly from its initial value on the 7th day. On the 14th day, however, its activity was sharply increased, definite granules appearing in the leukocytes, filling the cytoplasm in large numbers. Later (Fig. 1) no definite relationship could be seen between activity of the enzymes in the lymphocytes. Their indices differed only a little from the corresponding values obtained in the control group and also from their initial values. High acid phosphatase activity in the granulocytes was characteristic of these periods.

It is interesting to compare the results obtained with those of the study of the cytotoxic effect of lymphocytes from animals with experimental autoimmune diseases in tissue culture. For instance, the cytotoxic effect of lymphocytes from animals with "adjuvant" nephritis appeared on the 8th-11th day after injection of the nephritogenic mixture [11]. Transfer of the pathological process is possible at these times, e.g., "adjuvant" arthritis can be transferred by means of lymphocytes of the sick animal to recipients of the same line [16]. Lymphocytes taken from donors on the 14th day after injection of adjuvant cannot transfer the pathological process, and no cytotoxic effect was observed after the 11th day. At the same time, as the present observations show, acid phosphatase activity of the lymphocytes reached a maximum by the 7th day; by the 14th day after injection of adjuvant it was down to a minimum even if a further injection of adjuvant had been given (on the 10th day after the first injection). Changes in succinate dehydrogenase activity were in the opposite direction: with an increase in acid phosphatase activity, succinate dehydrogenase activity fell. These relationships between changes in the biological properties of lymphocytes and changes in indices characterizing the state of their lysosomes and mitochondria are probably not fortuitous.

Considerable attention is being paid at the present time to the role of lysosomal structures in the pathogenesis of allergic conditions. In particular, during lysis of granules in the neutrophils which constitute a special type of lysosomes, the Arthüs and Schwartzmann phenomena take place; a marked increase in acid phosphatase activity is found in allergic diseases [3, 15], after immunization followed by various complications [3], and after sensitization of animals with oxazalone and tuberculin [10]. At the same time, administration of antihistamine preparations and glucocorticoids is known to stabilize lysosomal membranes and to reduce the number of these organelles in cells [17].

The results described above show that the development of various types of hypersensitivity in the organism is accompanied by an increase in acid phosphatase activity both in lymphocytes and in other cells. If it is remembered that injection of Freund's complete adjuvant simulates a state of allergy of the delayed type as well as stimulating immunogenesis, in the present investigations changes could naturally be expected in the acid phosphatase activity in the lymphocytes. These changes were found to occur in phases, an increase in acid phosphatase activity being combined with a decrease in activity of the oxidative enzymes of energy metabolism. It is considered that this relationship can be regarded as a

*Values of the mean sampling error are shown.

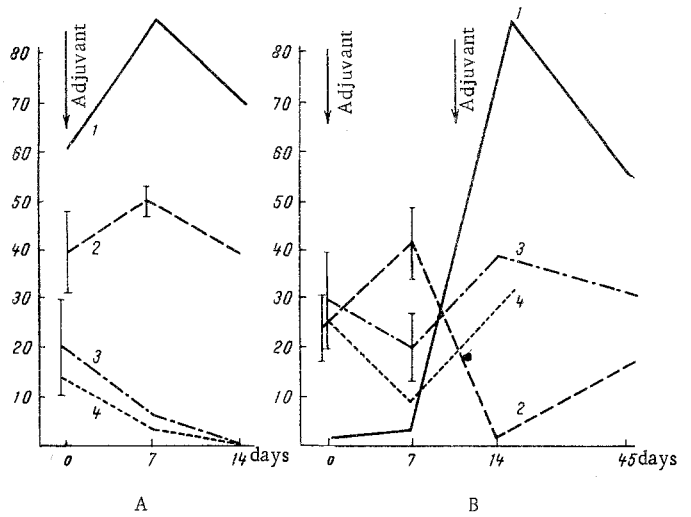

Fig. 1. Changes in acid phosphatase and dehydrogenase activity in rats under the influence of a single (A) and two (B) injections of Freund's complete adjuvant (mean data and standard deviations – $M \pm \sigma$). 1) Acid phosphatase activity in granulocytes; 2) acid phosphatase activity in lymphocytes; 3) succinate dehydrogenase activity in lymphocytes; 4) α-glycerophosphate dehydrogenase activity in lymphocytes. Abscissa, times of investigation (in days); ordinate, percentage of lymphocytes (granulocytes) with high and moderate enzyme activity.

cytochemical criterion of hypersensitivity of the delayed type in the period of its formation, and judging from comparison of the biological properties of the lymphocytes, it reflects this hypersensitivity to the highest degree.

An increase in acid phosphatase activity in the circulating blood lymphocytes was observed by the writers in children after injection of DPT vaccine, when the period of immunization was uncomplicated [1]. Reactions of the same type were also observed during blast-transformation of lymphocytes in culture on the addition of phytohemagglutinin [14]. In both cases, however, the increase in acid phosphatase activity was accompanied by a considerable, or actually a greater increase in activity of the oxidative enzymes. Combinations of this type evidently reflect physiological activation of lymphocytes and are not characteristic of delayed hypersensitivity. The reasons for the opposite character of changes in acid phosphatase and dehydrogenase activity are not clear. The increase in acid phosphatase activity evidently corresponds to an increase in the number of lysosomes in the cells accompanying differentiation of the lymphocytes. The same phenomenon has previously been observed after injection of brucella vaccine, prodigiosan, and Vi-antigen in mouse macrophages [4]. The possibility of an increase in enzyme activity because of increased synthesis is not ruled out. It is impossible at this stage to explain the decrease in activity of the oxidative enzymes.

The decrease in succinate dehydrogenase activity discovered experimentally is evidence of a significant fall in energy metabolism in the mitochondria. Low activity of mitochondrial α-glycerophosphate dehydrogenase indirectly suggests a disturbance of the integration of glycolysis and respiration. Active migration of ions and molecules through membrane barriers is known to be strictly coordinated with the utilization and re-creation of energy [10]. A decrease in ATP formation in the cell, for instance, is accompanied by a sharp increase in liberation of proteins from it [12].

The decrease in activity of dehydrogenases participating in energy metabolism demonstrated in these experiments suggests that production of high-energy compounds in the lymphocytes is reduced. Such changes must be accompanied by a disturbance of permeability and integrity of the cell membrane and membranes of the organelles, especially lysosomes. Under those conditions the lysosomal enzymes (having lost their isolation) and the cells themselves, especially lymphocytes, can evidently become passive chemical aggressors.

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