DETERMINATION OF ACID PHOSPHATASE IN MACROPHAGES IN HYPERSENSITIVITY OF THE DELAYED TYPE

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During incubation of macrophages from animals sensitized with group A streptococci with specific antigen, the percentage of cells is increased in which acid phosphatase can be demonstrated histochemically without preliminary fixation of the cells. This indicates increased permeability of the lysosome membranes, which evidently facilitates the liberation of enzymes from the lysosomes.

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An increase in the percentage of lymphocytes and in the number of granules in the cells in which acid phosphatase (APase) can be detected histochemically is regarded as evidence of activation of lysosomes during hypersensitivity of the delayed type (HDT) [8, 9, 11]. An increase in the content of APase in lymphocytes following injection of a BCG suspension into animals has been demonstrated biochemically [7, 13]. The results of determination of APase by histochemical methods depend on two factors: the quantity of enzyme in the lysosomes and the permeability of the membranes, the latter influencing penetration of substrate into the lysosomes [5]. The importance of these factors for activation of lysosomes in the case of HDT has not yet been explained. Nor has it been shown whether activation of lysosomes in general is characteristic of this phenomenon or whether it is determined by the action of a specific antigen on the lymphocytes of the previously sensitized animals.

The objects of this investigation were: 1) to determine the character of changes in the lysosomes of lymphocytes in HDT by parallel determination of APase by different methods, revealing the content of this enzyme and permeability of the membranes of the lysosome system of lymphocytes to be estimated; 2) to elucidate the cause of changes in the lysosomes in HDT: preceding sensitization or the action of specific antigen on the lymphocytes of the previously sensitized animal.

EXPERIMENTAL METHOD

An injection of 5 million cells of a living culture of group A streptococci was given into the soft tissues of the paw of guinea pigs. The specific antigen for intradermal tests and for treatment of the lymphocytes (thermostable fraction) was obtained by repeated precipitation with glacial acetic acid from fluid decantered from a broth culture of the same strain and heated to 100° for 30 min [1, 6]. Absence of O- and S- streptolysins in the nonspecific antigen was verified with a suspension of sheep's erythrocytes [15]. Lymphocytes of the peritoneal exudate (LPE) were obtained without preliminary stimulation by irrigation of the peritoneal cavity with Hanks's solution containing bovine serum and heparin [19] and were attached to cover slips [3]. The macrophages attached to the cover slips were incubated for 2 h in Hanks's solution with serum, carefully washed off, and again incubated for 24 h in the same medium or in medium with 50 μg/ml of specific antigen, or in medium with 50 μg/ml purified tuberculin. APase was determined by Gomori's method (application of substrate Na-β-glycerophosphate to cells preliminarily fixed with 10% Ca-formol solution for 20 min, to increase the permeability of the membranes,) or by the same method in the modification [12] without preliminary fixation. In both cases incubation with substrate continued for 30 min at 18-20°. The content of APase was determined in the disintegrated cells [2, 10]. Macrophages were removed from the cover slips with versene and frozen and thawed six times. An increase in the

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TABLE 1. Use of Histochemical Method without Preliminary Fixation of Cells to Determine APase in Lymphocytes of Peritoneal Exudate of Control and Sensitized Animals in Relation to Action of Specific Antigen, Tuberculin, KCl, or Dexamethazone on Cells

Percent of cells in which APase found	Cells of peritoneal exudate of						
	control animals		animals sensitized with streptococci				
				treated with			
	not treated with SA (17)	treated with SA (17)	not treated with SA (23)	SA (23)	tuberculin (6)	KCl solution (13)	SA and dexametha-zone (13)
< 20	17	15	21	4	6	2	9
20-35	–	1	1	6	–	5	4
36-70	–	1	1	13	–	6	–
Mean	6.7±0.6	10.1±0.7	11.5±0.7	35.8±1.0	9.1±1.2	37.5±1.2	16.5±1.0

Note. Number of animals given in parentheses; SA, specific antigen.

permeability of the membranes in the control experiments was produced by treating the cells with 10^{-6} M KCl. To stabilize the membranes the cells were treated with 20 μg/ml dexamethazone. Incubation in the presence of KCl or dexamethazone took place for 30 min at 37°.

EXPERIMENTAL RESULTS

Marked reactions of the delayed type were observed in all animals receiving intradermal injections of 1 μg specific antigen 2.5-3 weeks after sensitization with streptococci; these reactions were absent in the control animals. Determination of APase in the LPE was carried out in both sensitized and control animals without preliminary intradermal injection of the specific antigen. During investigation of APase by Gomori's method, granules containing the enzyme were found in practically all LPE of the control and sensitized animals. During determination of APase by the histochemical method without preliminary fixation of the cells, in most of the control animals, regardless of treatment of the macrophages with specific antigen, APase was detected in fewer than 10% of the cells (Table 1). The mean percentage of cells containing APase was only 6.7-10.1.

The mean percentage of cells in which APase was detected among the macrophages of sensitized animals untreated with specific antigen was 11.5±0.7. During incubation of LPE of sensitized animals in the presence of specific antigen, APase was discovered in more than 20% of cells in 19 of 23 animals, and in 35-70% in 13 cases. The mean percentage of cells containing APase was 35.8±1.0 (APase was determined in LPE of the same animals during the action of specific antigen and in its absence). Incubation of macrophages from animals sensitized with streptococci in the presence of tuberculin did not increase the percentage of cells with APase. When lymphocytes from sensitized animals untreated with specific antigen were treated with KCl solution, APase was found in most animals in more than 20% of the cells (on the average in 37.5%). After treatment of macrophages of sensitized animals with specific antigen and subsequent incubation with dexamethazone, no appreciable increase in the number of cells containing APase took place compared with the control (Table 1). Considerable variations in the APase content were found (from 3.6 to 9.7 μg P/10^6 cells) in disintegrated cells of the control and sensitized animals, but no difference was found between these groups. Treatment of macrophages with specific antigen had no effect on the amount of enzyme detected in the disintegrated cells. These investigations were carried out on the same animals as those in which an increase in the percentage of cells containing APase was discovered histochemically.

The increase in the number of cells containing APase after treatment of lymphocytes with specific antigen for 24 h, observed in HDT, was evidently due to an increase in permeability of the lysosome membranes (in cases when preliminary fixation of the cells was not carried out). Proof of this is given by: 1) the absence of an increase in enzyme activity in the disintegrated cells; 2) the discovery of APase, by Gomori's method with preliminary fixation of the cells, increasing the permeability of the membranes, in almost 100% of LPE of normal or sensitized animals untreated with specific antigen; 3) the obtaining of a

similar effect following an increase in the permeability of the membranes through the action of KCl and of specific antigen on LPE of the sensitized animals; and 4) the absence of the described effect following treatment of the cells with specific antigen and subsequent treatment with dexamethazone, which stabilizes the lysosome membranes.

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