PERMEABILITY OF LYSOSOMAL MEMBRANES OF SKIN MACROPHAGES IN HYPERSENSITIVITY OF DELAYED TYPE TO STREPTOCOCCAL ANTIGENS

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Acid phosphatase was determined in lysosomes of skin macrophages of animals with hypersensitivity of delayed type (HDT) to group A streptococcal antigens or tuberculoproteins. Acid phosphatase was determined in skin sections by a histochemical method without preliminary fixation of the tissues. Intradermal injection of the specific antigen in HDT was shown to increase the permeability of the lysosomal membranes of the skin macrophages. These results confirm the hypothesis that lysosomal enzymes of macrophages may act as factors inducing tissue destruction in HDT.

KEY WORDS: *hypersensitivity of delayed type; streptococci; membrane permeability; lysosomes; acid phosphatase.*

Results obtained with various models of infection have shown that two different phenomena arise with the development of hypersensitivity of delayed type (HDT): the harmful action of immune lymphocytes in the presence of specific antigen on target cells; the more intensive destruction of microorganisms by macrophages of the sensitized animals than by macrophages of nonsensitized animals. However, the mechanism of the role of macrophages in the reactions connected with HDT is not yet clear. The suggestion has been made that enzymes liberated from macrophages may take part in tissue destruction during infectious processes [5, 12]. During detection of the lysosomal marker (acid phosphatase) by Gomori's method preliminary fixation of the preparation is known to increase the degree of permeability of the membranes artificially. This enables the substrate used to determine the enzyme to penetrate into the lysosomes. A modification of Gomori's method [6, 8] and determination of acid phosphatase without preliminary fixation enable the degree of permeability of the lysosomal membranes to be revealed. This is because acid phosphatase can be detected in unfixed cells only under the influence of substances increasing membrane permeability, and the abolition of this phenomenon under the influence of substances stabilizing lysosomal membranes [15].

In previous investigations [3-5] using a modified Gomori's method it was shown that the permeability of the lysosomal membranes is increased by the action of the specific antigen in a culture of peritoneal macrophages obtained from sensitized animals. The percentage of cells in which acid phosphatase was detected was reduced when the macrophages of sensitized animals, after incubation with antigen, were treated with dexamethasone, which reduces the permeability of lysosomal membranes. During determination of acid phosphatase by a quantitative method no differences were found in disintegrated material of macrophages obtained both from normal (unsensitized) and sensitized animals. It was postulated on the basis of these findings that increased permeability of the lysosomal membranes of macrophages contributes to the liberation of enzymes from the lysosomes and to tissue damage by those enzymes in HDT [4].

The morphological picture of changes in the skin of animals at the site of injection of the reacting dose of specific antigen in HDT caused by various antigens (including streptococcal antigens) has been described by several workers [1, 10, 11, 13]. In the loose con-

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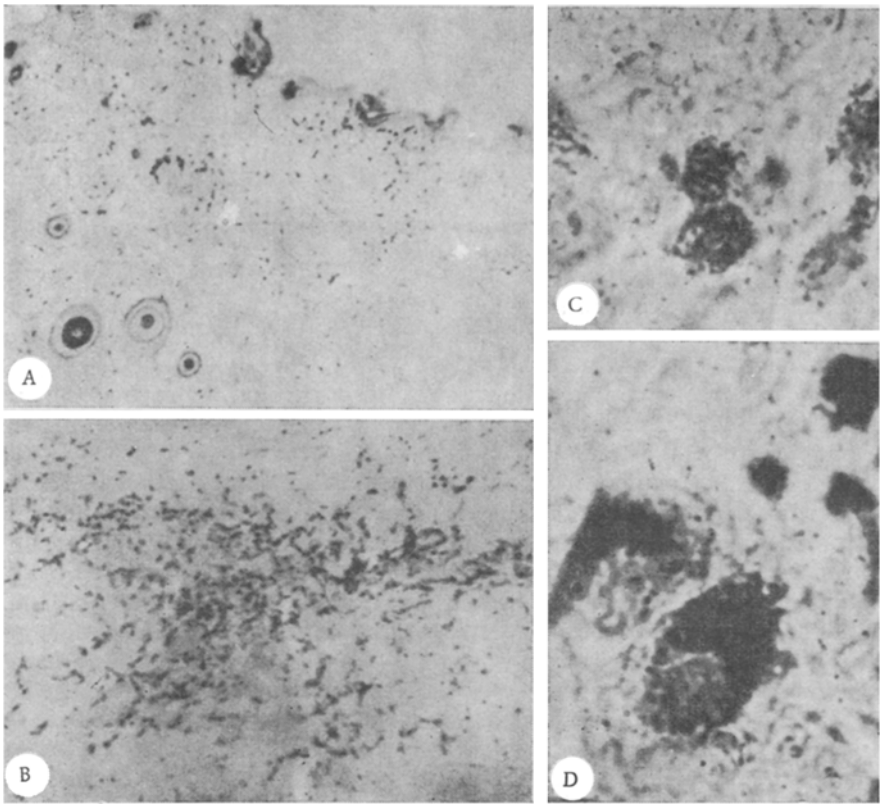

Fig. 1. Determination of acid phosphatase in skin cells of sensitized animals at site of injection of specific antigen (Gomori's method without preliminary fixation). A) In section through dermis, 120×; B) in section through loose connective tissue adjacent to dermis, 120×; C) in macrophages of focus of infiltration in loose connective tissue, 900×, immersion; D) histiocyte of interstitial tissue in muscular layer adjacent to skin, 900×, immersion.

nective tissue of the skin in animals around small blood vessels (veins) and nerve trunks a focal cellular response has been shown to develop, in which the leading role was played by monocytes — lymphocytes and macrophages. Polymorphs are present in the focus of infiltration only as contaminants, or they are present in it only for a short time. In the dense connective tissue of the skin (the dermis proper) hypertrophy of the fixed macrophages (histiocytes) is found.

The object of this investigation was to study the permeability of the lysosomal membranes of cells of the skin and adjacent tissue in vivo in animals sensitized by a culture of streptococci or BCG, at the site of injection of the specific antigen.

EXPERIMENTAL METHOD

Guinea pigs were sensitized by a single injection of 10^{10} group A streptococci, type 10 (strain Dochej NY-5) in incomplete adjuvant into the plantar pads. Freund's complete adjuvant (600 μg BGC) was injected by the same method into the animals of another group. Some animals received no injection and served as the control. The thermostable streptococcal fraction (TST), prepared by the method of Ando and Verzhikovskii [2, 7], and old tuberculin (prepared at the Khar'kov Institute of Vaccines and Sera) were used as the reacting injection. On the 17th to 20th day after sensitization of the animals, different doses of antigens in a volume of 0.1 ml were injected intradermally into the animals: TST 0.1, 0.5, 1.0, and 3.0 μg (as protein content) and tuberculin in dilutions of 1:10, 1:50, 1:100, and 1:200. The animals were decapitated 24 h later. Tissues were taken from parts where the skin reaction was unaccompanied by visible hemorrhages or necrosis. Tissues from the region of injection of TST and tuberculin into animals sensitized by the streptococcal culture or BGC were used. In addition, the skin of unsensitized animals and skin taken from sensitized animals away from the region of the skin test also were studied.

Sections 5-7 μ thick were cut in a cryostat (−20°C) from unfixed frozen tissue. The acid phosphatase content was determined by Gomori's method without preliminary fixation [6, 8]. The reaction was carried out at pH 5.6 and 37°C. The sections were left in the substrate for 25-40 min. The results were read at times when the control sections gave negative or only weakly positive results. The preparations were stained with eosin and mounted in gelatin. Some of the material was fixed, embedded in paraffin wax, and stained with hematoxylin–eosin, toluidine blue, or by Schiff's method.

EXPERIMENTAL RESULTS

Foci of cellular infiltration consisting of lymphocytes and macrophages, with a small admixture of neutrophils, were observed in the skin of the animals sensitized by streptococci at the site of injection of the reacting dose of TST around the small blood vessels and nerve trunks. A similar picture was observed when sections through the skin of guinea pigs sensitized with BCG were studied, at the site of injection of tuberculin. Determination of the acid phosphatase content in the tissue of the control and experimental animals after preliminary fixation of the sections in a mixture of solutions of calcium chloride and formalin revealed the enzyme in the lysosomes of most cells of the dermis. For subsequent investigations sections of tissues were used without preliminary fixation. After unfixed sections of tissues taken from animals sensitized with streptococci had been placed in the substrate, acid phosphatase was detected at the site of injection of TST in the macrophages (histiocytes) of the skin and interstitial tissue of the muscular layer, and in macrophages and polymorphs infiltrating the loose connective tissue (Fig. 1). A similar phenomenon was observed when sections through the skin taken at the site of injection of tuberculin into animals sensitized with BCG were studied. In the connective tissue of the skin of the unsensitized animals only weak cellular infiltration by monocytes was observed at the site of injection of TST or tuberculin. When acid phosphatase was determined in sections from these animals without preliminary fixation, the enzyme was revealed only in granules of individual macrophages in the focus of infiltration. In the histiocytes of the skin (fixed macrophages) and the interstitial tissue of the adjacent muscular layer no phosphatase could be detected. In cases when guinea pigs sensitized with the streptococcal culture received an injection of tuberculin (0.1 ml in a dilution of 1:100) into the skin, acid phosphatase could not be detected in macrophages of the skin and muscular layer (after incubation for 25 min). Injection of streptococcal antigens into animals sensitized with BCG was accompanied by only a weak reaction, possibly on account of the toxic properties of the TST. In macrophages of control regions of the skin of sensitized animals located outside the region of injection of the reacting dose of antigen, no acid phosphatase could be detected under those conditions.

When acid phosphatase was determined by Gomori's method after preliminary fixation, which artificially increases membrane permeability and facilitates penetration of the substrate into the lysosomes, the enzyme could thus be detected in macrophages of all skin sections. When the enzyme was determined by Gomori's method without preliminary fixation acid phosphatase was detected only at the site of injection of the specific antigen in animals with HDT. The results are evidence that injection of the specific antigen leads to increased permeability of the lysosomal membranes of the skin macrophages. As was stated above, similar results were obtained in vitro in cultures of peritoneal macrophages obtained from animals with HDT [3-5]. The results are evidence in support of the view that lysosomal enzymes of macrophages can participate in the destruction of tissues in pathological processes during which HDT develops.

It will be the task of future investigations to determine the effect of increased permeability of lysosomal membranes on the intracellular digestion of microorganisms by macrophages in HDT. Several workers are of the opinion that the factor (or factors) secreted by the macrophages have a regulatory effect on DNA synthesis by lymphocytes, which promotes the depression or, conversely, potentiation of the immune response [9, 14]. Hence the importance of the further study of the phenomenon of an increase in the permeability of the lysosomal membranes of macrophages in HDT under the influence of the specific antigen, for the possibility cannot be ruled out that these factors could be the lysosomal enzymes of macrophages.

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