ULTRASTRUCTURAL IMMUNOMORPHOLOGY OF ANTIBODY SYNTHESIS

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An ultrastructural study of antibody formation in the lymphoid cells of the lymph glands was carried out in C3HA mice using horseradish peroxidase as the antigen. The perinuclear space and tubules of the granular endoplasmic reticulum are the primary zones of synthesis and accumulation of antibodies inside the immunocompetent cells. Much less activity of the reaction was observed in the lamellar component of the Golgi apparatus and on the ribosomes.

KEY WORDS: ultrastructure of lymphocytes; intracellular localization of antibody synthesis.

Until recently the electron-microscopic study of antibody synthesis has been carried out chiefly on the basis of indirect evidence of an increase in protein synthesis: an increase in the number of tubules of the granular endoplasmic reticulum (GER) and of free ribosomes, widening of the perinuclear space and the lumen of the tubules of the GER, and hypertrophy of the Golgi apparatus [1-4, 9, 10]. More conclusive proof would be the direct demonstration of immune globulins inside the cells synthesisizing them [5, 8].

In this investigation an attempt was made to study the features distinguishing the ultrastructure of cells of the lymphoid series in mice after reimmunization with peroxidase.

EXPERIMENTAL METHOD

C3HA mice aged 4 months were used. Horseradish peroxidase (Reanal, Budapest) was used as the antigen. For primary immunization 0.5 mg peroxidase was emulsified in 0.3 ml Freund's adjuvant. The emulsion was injected either subcutaneously into the plantar pad of the hind limb or intraperitoneally. Reimmunization was carried out 14-18 days later with a similar dose of peroxidase in 0.9% NaCl solution.

Under ether anesthesia 72-96 h after reimmunization the inguinal or mesenteric lymph glands, respectively were removed from the animals, teased with needles in Hanks's solution at 4°C, filtered through two layers of Kapron, and centrifuged for 3-10 min at 3000 rpm.

Antibodies were identified by the method of Leduc et al. [6]. The residue was fixed for 30 min in 1.25% glutaraldehyde solution in 0.1 M Sorensen's buffer, broken up with a platinum loop, and incubated in a solution of horseradish peroxidase (0.5 mg/ml) for 30 min. The clumps were washed in buffer, postfixed in glutaraldehyde, and incubated in a 0.025% solution of 3,3'-diaminobenzidine with 0.01% hydrogen peroxide for 30 min. The material was washed twice in phosphate buffer, postfixed in 1% osmium fixative, dehydrated in alcohols of increasing strength, and embedded in Epon by Lufts' method [7].

Two series of controls were set up: 1) cells of a suspension of lymphocytes from lymph glands of unimmunized animals were studied without incubation and after incubation with antigen; second, lymphocytes from immunized animals were treated with a 0.025% solution of 3,3'-diaminobenzidine without preliminary incubation with the antigen.

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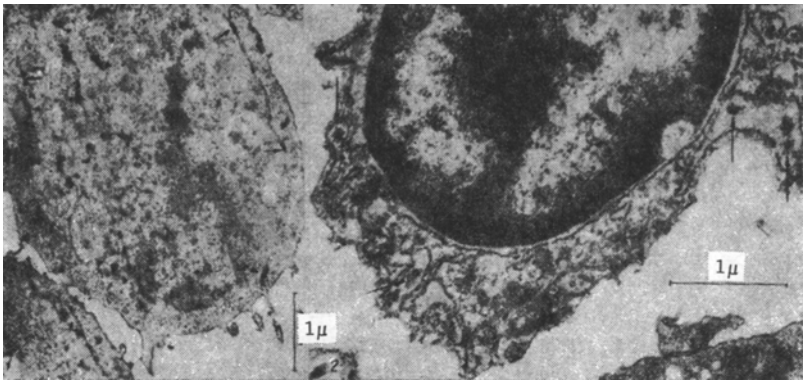

Fig. 1 Fig. 2

Fig. 1. Fragments of two lymphocytes from lymph gland of a mouse 72 h after reimmunization with peroxidase. Reaction for peroxidase activity. Antiperoxidase antibodies detected as discrete loci in the perinuclear space and tubules of the GER (arrows). Uranyl acetate, 18,000×.

Fig. 2. Plasmablast from lymph gland of a mouse 96 h after reimmunization with peroxidase. Reaction for peroxidase activity. Membranes of GER and perinculear space increased in density because of the presence of antiperoxidase antibodies. Compact zones of condensation of the reaction product can be seen (arrows) in individual cisterns of the GER. Uranyl acetate, 32,000×.

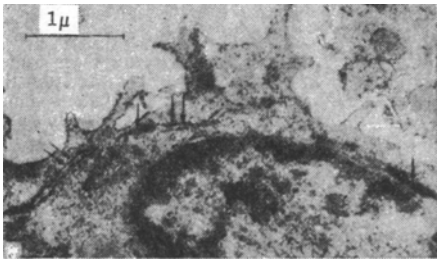

Fig. 3. Blast form of lymphocyte from lymph gland of a mouse 96 h after reimmunization with peroxidase. Reaction for peroxidase activity. Antiperoxidase antibodies distributed as electron-dense masses in individual segements of the GER (single arrows). Outlines of some tubules stained deep black (double arrows). Uranyl acetate, 26,000×.

Sections cut on the LKB-4800 ultramicrotome were stained with uranyl acetate [11] and studied in the IÉM-100V electron microscope with an accelerating voltage of 80 kV.

EXPERIMENTAL RESULTS

No peroxidase activity was detected in the cells of the lymphoid series in any of the control tests.

The electron-microscopic investigations showed that antibodies against peroxidases were contained chiefly in blast forms of lymphocytes (BFL), although adequate condensation of the reaction product could also be observed on structures of the intermediate cell forms between small lymphocytes and BFL, confirming the possibility that immune synthesis starts in small lymphocytes, when it accompanies their transformation into BFL and, later, into plasma cells [8].

Antibodies were discovered as discrete foci in the perinuclear space and in the dilated tubules of the GER 72 h after reimmunization with peroxidase (Fig. 1). Later (after 96 h) the number of blast cell forms increased appreciably, lymphocytes with a wide cytoplasm, resembling plasmablasts with initial features of antibody formation, appeared in the tubules of the abundant ergastoplasm (Fig. 2).

Uneveness of the outline and dilatation of the lumen were frequently observed in tubules of the GER reacting positively for peroxidase activity (positive profiles). In some cells the inner wall of the GER tubule was stained black with dense masses of antibodies.

The concentration of immune globulins, to judge from the density of the reaction product, varied in different parts of the GER considerably. Usually an alternation of weakly and strongly positive segments of the GER was observed, and on this basis it can be postulated that antibodies are synthesized in well-defined areas of the ergastoplasm (Fig. 3).

The tubules of the GER 72-96 h after reimmunization were arranged irregularly in the cell cytoplasm, making excretion of the antibodies through the GER, such as takes place in the phase of intensive immunity [5, 8]. Antiperoxidase antibodies were found in the perinuclear space in many cells, and sometimes this zone was the only place where they occurred. Here, just as in the tubules of the GER, besides widening of the lumen, alternation of segments of high density, corresponding to the antibody concentration, and segments of lower density or even segments free from antibodies was observed (Fig. 1). Antibodies were found in the Golgi apparatus of only very few cells, and then chiefly on its lamellar component. Zones of grouped ribosomes, marked by the presence of specific antibodies, could be seeen in the cytoplasm of individual lymphocytes. The presence of antibodies on the polysomes evidently reflects the earliest phases of synthesis of immune globulins. Some cells in the material studied did not contain antibodies although, in their ultrastructural characteristics, they were analogous to other lymphocytes in which antibodies were clearly detected.

The principal zones of antibody localization within the synthesizing cells 72-96 h after reimmunization were thus clearly defined segments of the perinuclear space and tubules of the GER. Conjecturally the results can also be used as an indirect morphological criterion of the thymus-independent population of lymphocytes.

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