ULTRASTRUCTURE OF LYMPHOID CELLS OF EXPERIMENTAL TUBERCULOUS GRANULOMAS

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On the basis of their immunologic properties and various other features, lymphocytes are nowadays subdivided into two classes (T and B lymphocytes). No clear morphological differences can be detected between T and B cells by methods of light and transmission electron microscopy, even after their identification on the basis of rosette formation [2, 5, 6, 8, 13, 14]. Nevertheless there are certain histochemical differences between them [2, 5, 8]. More encouraging results have been obtained by the use of scanning electron microscopy (SEM). It can be concluded from a comparison of the results of experiments with the use of immunologic markers and data obtained by SEM that T lymphocytes have a relatively smooth surface, with only a few projections and folds, whereas B lymphocytes have a complex villous surface [13]. However, it is not always possible to investigate T and B cells by SEM, for groups of cells with an intermediate type of surface, probably subpopulations of lymphocytes, also may be observed. Investigations into the subcellular morphology of lymphocytes under conditions of active interaction and, in particular, of pathology, are also of definite interest [3, 4, 10, 11].

Tuberculous inflammation is accompanied by various manifestations of reactions of delayed-type hypersensitivity, involving active participation of lymphocytes [1, 7]. Meanwhile, lymphocyte ultrastructure has been described mainly in studies of BCG vaccination [7]. The object of the present investigation was thus a combined morphological study of lymphoid cells participating in the formation of tuberculous granulomas in the lungs.

EXPERIMENTAL METHOD

Experiments were carried out on 35 guinea pigs weighing 300-350 g, infected subcutaneously with cells of Mycobacterium tuberculosis of the virulent strain $H_{37}R_V$, in a dose of 0.0001 mg. The animals were decapitated at assigned stages of the experiment in the course of 3 months. The lungs were used as the test object. Pieces of granulation tissue obtained from a tuberculous focus, and also pieces of adjacent and remote portions of the lung were fixed in buffered 2.5% glutaraldehyde solution, postfixed in 1% OsO_4 solution in phosphate buffer, and embedded in Epon-Araldite. Ultrathin sections were studied in the YEM-100B electron microscope. Preparations of the lung, lyophilized in a sublimation apparatus of the KS-30 type, and sprayed with carbon and gold, were examined in the scanning electron microscope. Histological sections also were stained with hematoxylin–eosin, for RNA (Brachet), DNA (Feulgen), succinate dehydrogenase (SDH), lactate dehydrogenase, and NAD-diaphorase (Nachlas), and acid and alkaline phosphatase (Gomori; azo-coupling method with naphthol salts).

EXPERIMENTAL RESULTS

Whereas in the early stages of tuberculous inflammation lymphoid cells were found in blood capillaries and in the interstices of the lung tissue, in the granuloma (1.5-3 months after infection) they were found within the structure and at the periphery of the tuberculous foci. Histochemical investigation showed that activity of certain respiratory and hydrolytic enzymes, chiefly SDH and alkaline phosphatase, was increased in the lymphoid cells in the early periods after infection. Plasma cells were distinguished by an intensive reaction for RNA and respiratory enzymes. Progression of tuberculous inflammation with replacement of the specific

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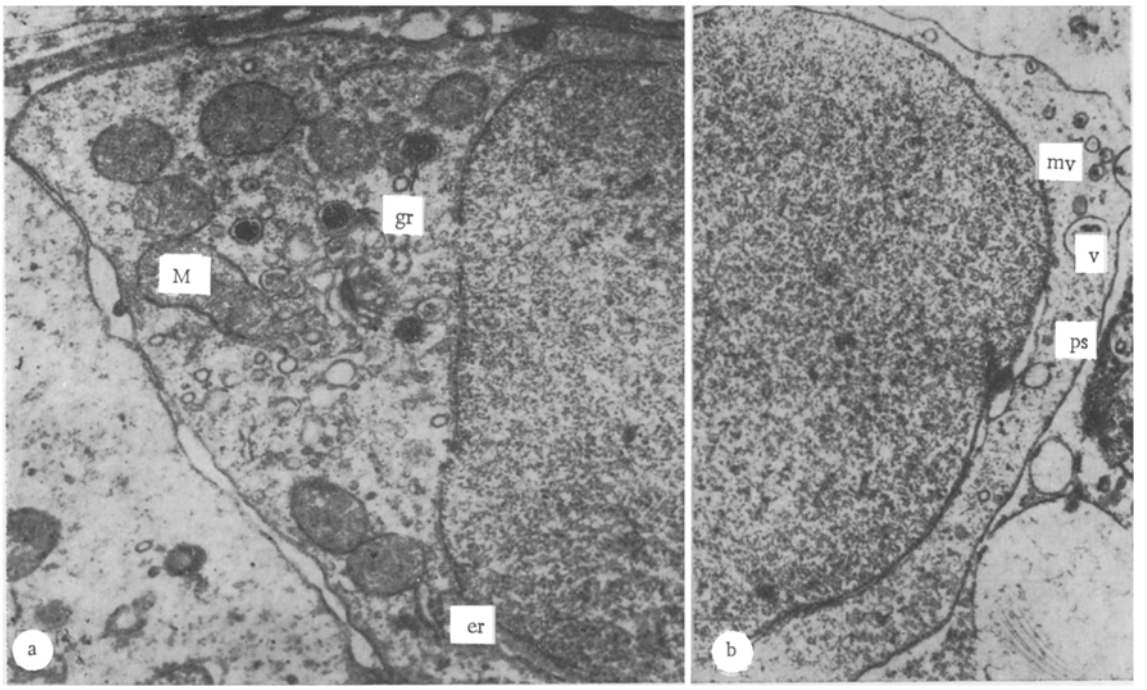

Fig. 1. Ultrastructure of lymphoid cells in tuberculous granuloma: a) group I lymphoid cell. Increased number of mitochondria (M) and of osmiophilic granules (gr) formed in the Golgi zone of structures of endoplasmic reticulum (er) at periphery of a tuberculous focus, 3 months after infection. 32,000×. b) Group II lymphoid cell. Invagination of plasmalemma into cell, multiple micropinocytotic vesicles (mv), small vacuole (v) with osmiophilic contents, Golgi complex, polysomes (ps) at periphery of tuberculous focus 3 months after infection. 16,500×.

granulation tissue by caseous necrosis was accompanied by a reduction of enzyme activity. The change in the intensity of the histochemical reactions reflected the level of metabolic changes in lymphocytes and plasma cells.

The electron-microscopic study showed that lymphoid cells which participate in the development of specific inflammation differed in their ultrastructure in different phases of the inflammatory process. Together with mononuclear cells of monocytic origin, the early or young granuloma contained many lymphocytes whose cytoplasm contained few ultrastructures. As well as the above-mentioned cells with only a few organelles, the mature granuloma also contained activated lymphoid cells, with varied submicroscopic organization, reflecting their functional state. Some lymphocytes (group I) were characterized by a well-developed ultrastructure, evidence of their high functional activity. The number of mitochondria in such activated cells was increased, the rough endoplasmic reticulum was well developed, with hypertrophy of the lamellar complex (Fig. 1a), and lysosomes and lipid inclusions appeared. These changes indicated intensive synthetic and secretory activity of the cells. Furthermore, crystalloid structures, bounded by a single or double membrane, also were found in the cytoplasm of the lymphoid cells. Their appearance was connected with changes in the matrix of the mitochondria (Fig. 2a).

Besides the lymphocytes described above, which were distinguished by the more intensive development of intracellular structures, other lymphoid cells (group II) with high activity of their cytoplasmic membrane, assimilating material adsorbed on it by invagination into the cell, were found in the tuberculous focus. All stages of this process were observed – from contact between the material in the surrounding medium and the cell surface to invagination of the plasmalemma and absorption of the material by the formation of microvesicles or small vacuoles (Fig. 1b). The ability of lymphoid cells to ingest insoluble substances by pinocytosis is due to the functional specialization of cells of this series [9]. Possibly in the course of intracellular interaction they may receive antigenic information [12].

The new type of "dendritic" lymphoid cells, found in the organs of mice and described in the literature, confirms differences in their functional specialization [15]. The study of a purified population of these cells has shown that they possess adhesive properties, they have thin projecting processes of cytoplasm, and are capable of active endocytosis. Lymphoid cells with an active ingestive function, participating in the formation of the tuberculous granuloma, contained fewer organelles than the group I lymphocytes.

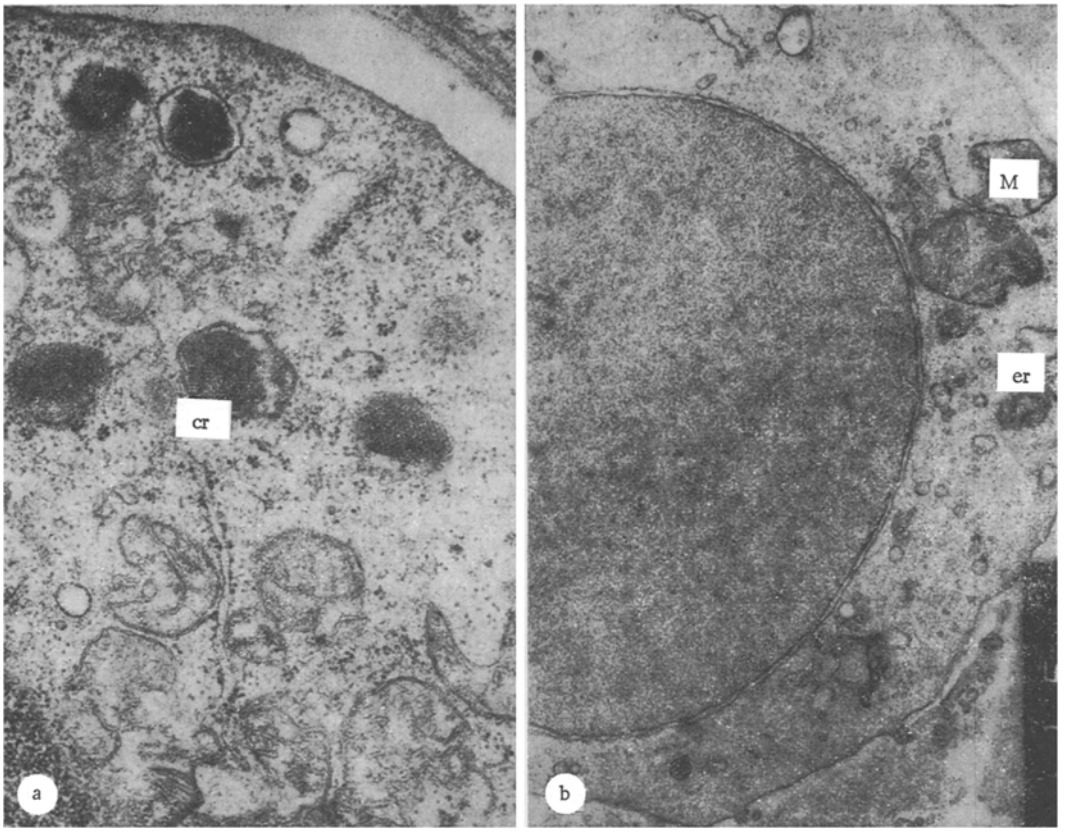

Fig. 2. Ultrastructural changes in lymphoid cell at periphery of tuberculous focus: a) crystalloid structures (cr) in cytoplasm of lymphoid cell. 33,600×. b) Lymphoid cell with degenerative and destructive changes in cytoplasm. Structures of rough endoplasmic reticulum (er) are vacuolated and disintegrating; periphery of tuberculous focus 3 months after infection. 30,500×.

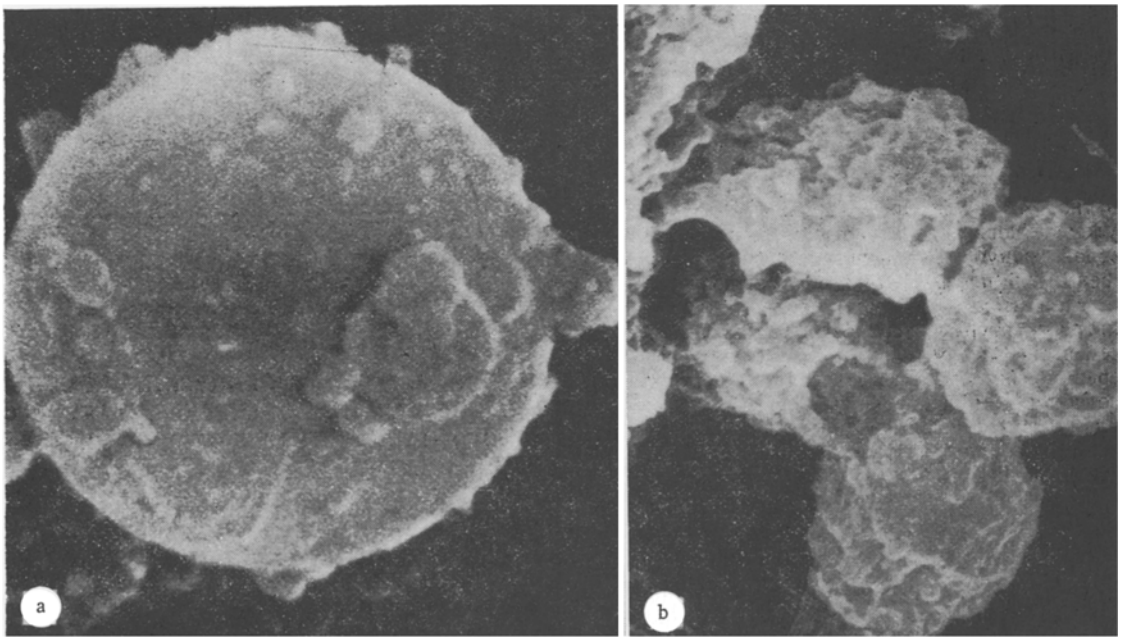

Fig. 3. Lymphocyte in field of vision of scanning microscope: a) lymphocyte with smooth surface, giving off single short processes and wide projections, tuberculous granuloma, 1.5 months after infection. SEM, 18,200×. b) Lymphoid cells with irregular, nodular surface, combined into groups by means of cytoplasmic processes; periphery of tuberculous focus 3 months after infection. SEM, 7200×.

Progressive tuberculous inflammation led to degenerative and destructive changes in the lymphoid cells: swelling of the mitochondria and disturbance of their integrity, vacuolation and disintegration of elements of the rough endoplasmic reticulum, and the development of perinuclear edema (Fig. 2b).

During SEM the lymphocytes appeared as small (diameter 5-6 μ), round formations with a smooth or villous surface. Most lymphoid cells in the early (1.5 months) granuloma had a smooth surface with single short processes or wide projections (Fig. 3a). In the late granuloma (3 months), on the other hand, lymphoid cells with an irregular and nodular surface, combined into groups by means of cytoplasmic processes, were predominant (Fig. 3b). Sometimes crater-like depressions of unknown origin, possibly due to injury, were identified on the surface of the cell.

CONCLUSION

Activated lymphocytes were found in the lymphoid population participating in the formation of the tuberculous granuloma, and two groups of cells characterized by differences both in their ultrastructure and in the manner of manifestation of functional activity could be distinguished among them. Whereas the development of the ultrastructure in the group I lymphoid cells development of the ultrastructure is accompanied by intensive synthetic and secretory activity, the predominant feature of the group II cells is their ingestive function, effected by active pinocytosis. Heterogeneity of the subcellular organization of the lymphoid cells in tuberculous infection reflects differences in their functional specialization. The surface of the cell is a good indicator of its functional state and of the intensity of processes taking place in it. Probably lymphoid cells in the young granuloma are T cells, whereas in the mature granuloma lymphocytes with an irregular surface, resembling B cells, become more numerous. Forms of lymphoid cells with intermediate type of ultrastructure also are encountered. Consequently, without taking into account the degree of differentiation, of intercellular interaction, and density of the population of lymphoid cells in a granuloma it is difficult to identify whether they belong to the T or B class. Tests with special immunologic markers are necessary for this purpose.

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