SESSION III

Chairman: Dr H. L. Israel, M.D.

The fine structure of sarcoid and tuberculous granulomas

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Summary

The granulomas of sarcoidosis and non-caseating tuberculosis show similar cell-types: two forms of epithelioid cells, A and B, giant cells, lymphocytes and 'activated' mononuclear cells. The morphology of epithelioid cells suggests that they are primarily biosynthetic rather than phagocytic.

The relationship of these various cells to each other is discussed and the following sequence of granuloma development is suggested: circulating lymphocyte → activated mononuclears → A → B epithelioid cell whose secretory product stimulates transformation of other circulating lymphocytes.

Introduction

The fascination of the search for the causation of sarcoidosis continues and embraces ever widening techniques. The sarcoid granuloma, on light microscopy, consists of closely apposed epithelioid cells, intermingled giant cells, often of the Langhans, tuberculous type and with admixed or poorly delineated numbers of peripheral lymphocytes. The granulomas differ from those commonly found in tuberculosis, by the absence of caseous necrosis, but occasionally show minimal 'fibrinoid' necrosis. The sarcoid granulomas are considered to be morphologically and histochemically identical with those in the Kveim test, non-caseating tuberculosis, chronic beryllium disease, Crohn's disease and farmer's lung (Jones Williams, 1967). The epithelioid cells in all the above conditions are rich in residual bodies, end products of lysosomal activity (Jones Williams & Williams, 1967). They all show high acid phosphatases and moderate pentose cycle and mitochondrial enzyme activity (Williams, Jones Williams & Williams, 1969). On light microscopy, though no causative agent has been detected the epithelioid cells

are obviously metabolically active cells, with properties suggesting both phagocytosis and biosynthesis.

The published accounts of the fine structure of sarcoid granulomas have also not demonstrated any causative agent but most investigators agree that epithelioid cells are active cells though there is no unanimity as to their exact function (Bassett *et al.*, 1967; Gusek & Behrend, 1969; Hirsch, Fedorko & Dwyer, 1967; Kalifat, Bouteille & Delarue, 1967; Kelemen, Soltesz & Mandi, 1969; Wanstrup & Christensen, 1966).

We shall present and discuss our initial fine structural findings and compare the features of the sarcoid granulomas with that of non-caseating tuberculosis.

Materials and methods

The material examined was obtained from one sarcoid spleen and two bacteriologically proven tuberculous lymph nodes. On light microscopy the granulomas examined, in both diseases, showed identical epithelioid and scanty giant cells without caseation.

One millimetre cubes of tissue, for electron-microscope study, were fixed in 3% glutaraldehyde (4 hr), followed by 0.1M phosphate buffer, pH 7.4 (18 hr), post-fixed in Millonig's phosphate-buffered osmium tetroxide (1 hr), all at 4°C. The blocks were then dehydrated with alcohol and embedded in Araldite. Sections were cut with an LKB III ultramicrotome. Multiple photographs, × 4500, were taken of single granulomas and montages constructed to study cell types and their distribution. Thick Araldite sections, (0.5μ), stained with toluidine blue, were examined under the light microscope, and ultrathin sections were stained for electronmicroscopy, with uranyl acetate and lead citrate.

FIG. 1. 'A' type epithelioid cell. Note extensive rough endoplasmic reticulum (ER) and mitochondria (M).

Results

The epithelioid cells, approximately 60μ in diameter, consisted of two main types, A and B, though indistinguishable on light microscopy. 'A' cells appear heavily stained and 'B' lightly stained. Some epithelioid cells with features of both 'A' and 'B' were designated as transitional cells. Giant cells were scanty. Lymphocytes were scanty and located mainly at the outer limit of the granulomas. Occasional other lymphocyte-like cells were present and were termed 'activated mononuclears'. All these cell-types were present and showed similar features in both the sarcoid and tuberculous granulomas. Their distribution however was different as from a study of montages, B cells predominate in sarcoidosis and A cells in tuberculosis.

Both types of epithelioid cells show similar nuclei containing nucleoli and peripherally arranged chromatin. They show numerous mitochondria, varying amounts of endoplasmic reticulum, Golgi complexes and vesicles. Neither type shows identifiable phagocytosed material and evidence of pinocytosis was practically absent. The cell membranes show many fingerlike processes interdigitating with those of adjacent cells. A few however show broad club-like processes. The 'A' type (Fig. 1) is distinguished by the presence of abundant lamellar rough endo-

plasmic reticulum. 'B' cells (Fig. 2) show very prominent Golgi complexes and numerous associated variably shaped vesicles ranging in size from 0.5 – 0.75μ dia. Some vesicles in type B contain lightly stained finely granular material; others, in the absence of attached ribosomes, probably represent smooth endoplasmic reticulum. Occasional very lightly stained cells with sparse organelles are seen, and appear to be degenerate B cells. The transitional type cells show abundant but localized rough endoplasmic reticulum with the remaining cytoplasm showing similar features to the B cell.

Giant cells (150μ dia.) are most frequent in the tuberculosis cases with cytoplasm showing the feature of type B epithelioid cells. They are very rich in mitochondria, Golgi complexes and vesicular bodies with mainly vesicular RER. As in the epithelioid cells there are no recognizable tubercle bacilli.

The lymphocytes, 7 – 10μ dia., are round or oval in section, show no interdigitations and contain scanty organelles usually confined to one pole. Some lymphocyte-like cells—activated mononuclears (Fig. 3), contained more abundant widely distributed organelles and in particular showed small amounts of lamellar rough endoplasmic reticulum.

In sarcoid and tuberculous granulomas interstitial

FIG. 2. 'B' type epithelioid cell. Very numerous Golgi complexes (G) and secretory vesicles (SB).

tissue is prominent and shows both 50Å and 350Å dia. fibres, some of which showed 650Å collagen-banding.

Discussion

The above results show that epithelioid and other cells in the granuloma of sarcoidosis and tuberculosis are similar. We found two types of epithelioid cells, A and B, which agrees with the results of Wanstrup & Christensen (1966) and Gusek & Behrend (1969) in sarcoidosis and with Gusek (1965) in tuberculosis. Our findings raise many problems, about epithelioid cells, in particular (a) whether they

are primarily phagocytic or biosynthetic, (b) the relative distribution of Types A and B and (c) the relationship of cells A and B to one another and to other cells in the granulomas.

In view of our light microscopy findings (Jones Williams & Williams, 1967 and Williams *et al.*, 1969), we expected epithelioid cells to show features of phagocytosis—pinocytosis with numerous dense bodies (lysosomes) and complexed phago-lysosomes (residual bodies), together with features of biosynthesis. Evidence of phagocytosis in these cells, however, even in tuberculous granulomas, was practically absent. It is important to note that, in

FIG. 3. Activated mononuclear cell. Lymphocyte-like but with increased number of organelles and presence of lamellar rough endoplasmic reticulum. Labelling as in Fig. 1.

this and previous studies by others, special techniques for the identification of lysosomal enzymes at electron microscopy were not done.

Evidence of biosynthetic activity in both types of epithelioid cells was plentiful. The morphology of Type A is reminiscent of plasma cells and is therefore consistent with a protein, possibly immunoglobulin, producing cell. It is of interest that the level of immunoglobulins is often raised in sarcoidosis (Norberg, 1967) but reports do not show any consistent pattern, further, that by immunofluorescent techniques Wanstrup & Elling (1968) showed that epithelioid cells contain both IgM and IgA im-

munoglobulins. The B-type epithelioid cell with its numerous Golgi complexes and associated vesicles is also a biosynthetic cell and may be producing lipo- and muco-proteins. It is also possible that, as intracellular end products of lysosomal digestion were very scanty, the B cells are producing lysosomal enzymes for 'export'. This conjecture, with others, just await the results of our lysosomal enzyme studies.

As a result of our present investigations it appears, therefore, that the majority of epithelioid cells are more concerned with biosynthesis than with phagocytosis.

The study of the montages showed an interesting difference in the distribution of the two types of epithelioid cells. B cells predominate in sarcoidosis and A cells in tuberculosis. This difference may reflect the age of the granuloma, as in the sarcoid patient the disease had been present for about three years while the lymph nodes had been enlarged in the patient with tuberculosis for only a few months. It may reflect a functional difference but it is impossible to exclude sampling error.

The relationship of A to B epithelioid cells and of epithelioid to other cells in the granuloma is summarized in Fig. 4. The morphological features of the

FIG. 4. Circulating mononuclear (M), stimulated mononuclear (SM), epithelioid cells (A) and (B).

transitional cell, intermediate between A and B cells suggests these cells are related. It is likely that A is an early form of B. This is supported by the fact that giant cells show similar features to B cells and experimentally, giant cells are a late development from epithelioid type cells (Sutton & Weiss, 1966). Further, dying cells show the structure of B rather than A cells which also suggests that B is a terminal stage. Our schema is an attempt to explain the relationship of the cell types found and the persistence of granulomas in sarcoid and also in some forms of tuberculosis with very scanty organisms. The primary inciting agent acts on a circulatory

mononuclear cell (M), possibly a lymphocyte, which then develops into the activated mononuclear cell (SM), into A and then B epithelioid cell and sometimes into giant cells. We have shown that epithelioid cells are biosynthetic and they may produce a secondary inciting agent which in turn stimulates another circulating mononuclear cell and thus perpetuates the granulomas.

Further work is thus required to identify the possible secondary inciting agent which may well be the active fraction of the Kveim test in sarcoidosis.

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