Ultrastructural Changes in Bronchoalveolar Lavage Cells in Sarcoidosis and Comparison With the Tissue Granuloma

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The authors undertook this study to determine whether there were any morphologic changes in bronchoalveolar lavage lymphocytes and macrophages in sarcoidosis and, in particular, to determine whether changes described previously in the mononuclear phagocytes of sarcoid granulomas were also evident in such cells obtained by lavage. Lavage cells from 28 sarcoidosis patients were studied by transmission electron microscopy and compared with lavage cells from 17 control subjects and with lung tissue granulomas from 5 sarcoidosis patients. Interactions between mononuclear phagocytes, especially subplasmalemmal linear densities, and between these cells and lymphocytes were observed in both the tissue granulomas and lavage specimens from sarcoidosis patients. Subplasmalemmal linear densities were never observed in control lavage specimens. Fully developed epithelioid cells were not identified in lavage specimens, but differences were nevertheless found between the lavage cells from sarcoidosis patients and control subjects: in particular, alveolar macrophages in sarcoidosis were larger and showed better developed pseudopodia, more marked polarity, less nuclear heterochromatin, and lysosomes that were larger and more numerous but less electron-dense than normal. Lymphocytes were also enlarged and contained more lysosomes. It is concluded that although there are only a few similarities between the cells of the granuloma and those obtained by bronchoalveolar lavage in sarcoidosis, there are noticeable differences between the lavage cells of sarcoidosis patients and control subjects. In sarcoidosis, a variable proportion (10–70%) of the lavage cells show morphologic features of "activation." (Am J Pathol 1983, 112:7–17)

IN RECENT YEARS there have been several electron-microscopic studies of the sarcoid granuloma, and the ultrastructure of this lesion is now well known.[1–4] At the same time, there have been many reports of alterations in the cellular constituents of bronchoalveolar lavage (BAL) fluid recovered from patients with pulmonary sarcoidosis. Generally, however, BAL studies have concentrated on changes in cell number, an increase in lavage lymphocytes being characteristic of pulmonary sarcoidosis.[5,6] Apart from macrophage alterations in smokers[7–11] and the recognition of Langerhans-type cells in histiocytosis X,[12] information on the ultrastructure of BAL cells is rather sparse.[13–15] Therefore, although the ultrastructure of the granuloma is well described, less is known of the ultrastructure of BAL cells in sarcoidosis. We undertook the present study to determine whether the electron-microscopic examination BAL cells would show any changes helpful either diagnostically or in promoting a better understanding of the pathogenesis of sarcoidosis.

Materials and Methods

Fifty subjects were studied by the use of both light and electron microscopy. BAL cells from 28 sarcoidosis patients were compared with BAL cells from 17 control subjects (Table 1) and also with tissue granulomas from 5 other sarcoidosis patients. Because cigarette smoke induces morphologic alterations in alveolar macrophages,[7–11] smokers and nonsmokers were studied separately. Smokers were defined as having smoked five or more cigarettes a

Accepted for publication February 14, 1983.

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0002-9440/83/0707-0007$01.35

Table 1—Details of Lavage Groups Studied

	Number of Patients				
	Male	Female	Total	Mean age ± SD (years)	Age range (years)
Controls					
Nonsmokers	4	3	7	30 ± 6	21-39
Smokers	6	4	10	39 ± 13	25-63
Total	10	7	17	35 ± 11	21-63
Sarcoidosis					
Nonsmokers	8	10	18	37 ± 11	23-58
Smokers	8	2	10	28 ± 6	20-38
Total	16	12	28	34 ± 11	20-58

day for the last 5 years. In the nonsmoking group, 20 had never smoked and 5 had stopped smoking at least three years previously. The diagnosis of sarcoidosis was established on the basis of compatible clinical and radiologic data,[16,17] together with the finding of noncaseating granulomas in biopsy specimens of bronchus (n = 20), lymph node (n = 5), or skin (n = 3), and in 21 patients, a positive Kveim test. Four of these patients were receiving steroids at the time of the lavage. The tissue granulomas studied by electron microscopy were obtained by open-lung biopsy from 5 other patients with sarcoidosis. The control group included 11 healthy volunteers and 6 patients who underwent bronchoscopy for suspected

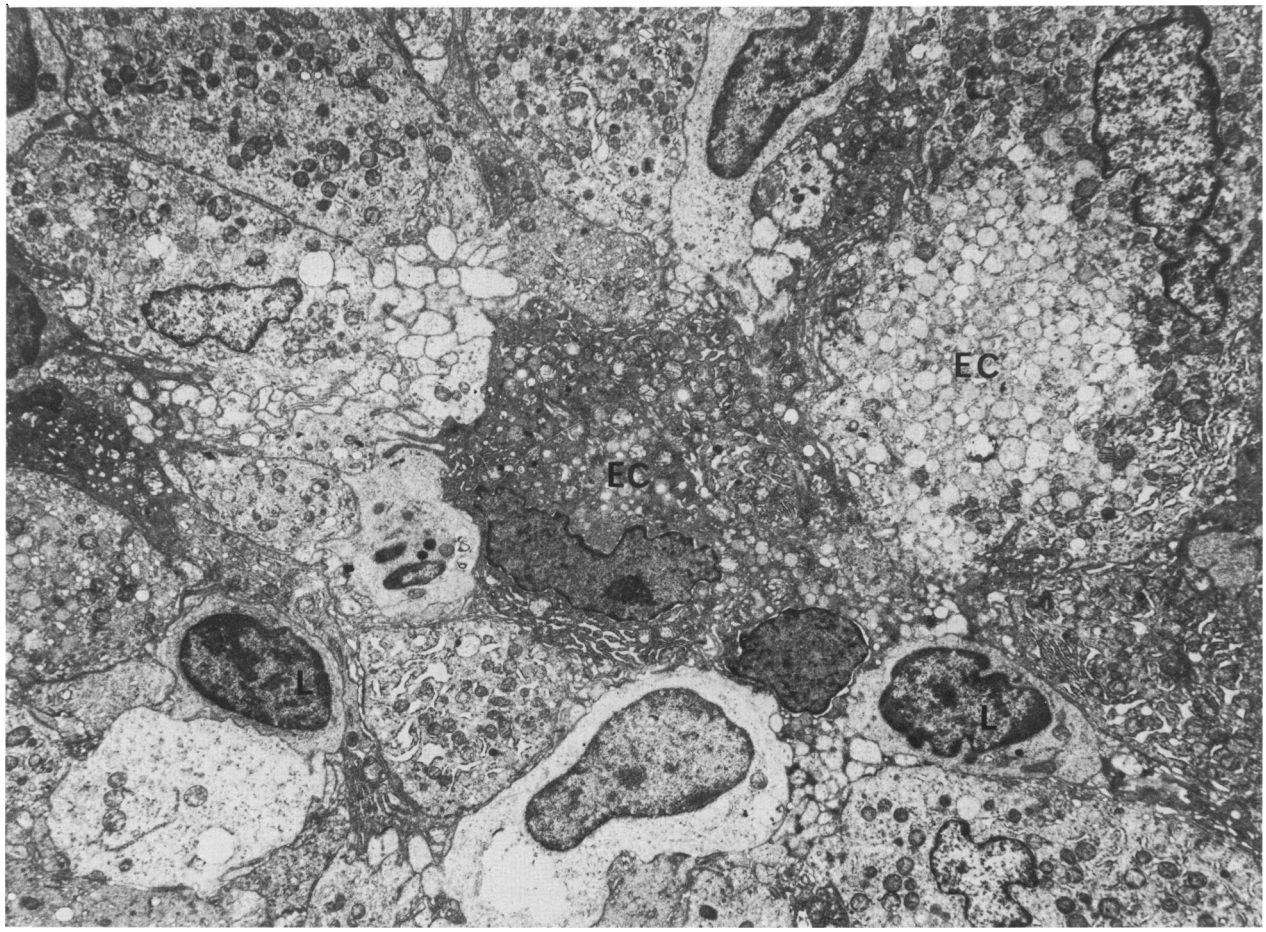


Figure 1—Sarcoid granuloma in lung biopsy showing epithelioid cells (*EC*) with characteristic vesicles and lymphocytes (*L*). (×3500)

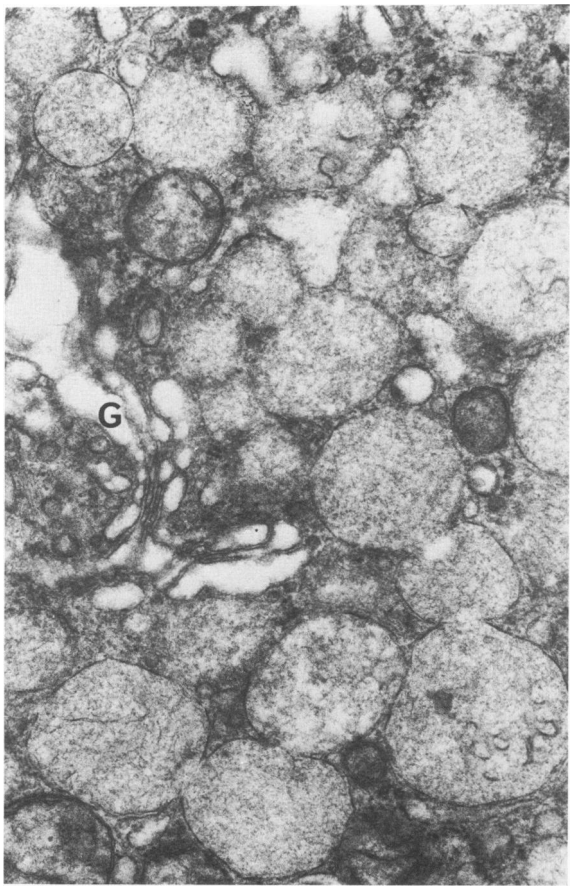


Figure 2—Detail of typical epithelioid cell vesicles and dilated Golgi apparatus (*G*) in a sarcoid granuloma. The vesicles are uniformly filled by finely granular material and lack a clear halo beneath the investing membrane. (×23,500)

bronchial carcinoma, either because of hemoptysis (4 cases) or radiographic opacities in the contralateral lung. In all these cases the lung lavaged was bronchoscopically and radiographically normal. None had clinical or physiologic evidence of chronic bronchitis.

BAL was performed at fiberoptic bronchoscopy, and a cell suspension obtained as described previously.[18,19] Data on the number of cells recovered and the lavage cell differential counts are provided elsewhere[18,19]; briefly, increases in both the percentage and the absolute number of lymphocytes were noted in the patients with sarcoidosis. A few samples were fixed as a centrifuged pellet, but most were fixed in suspension. The latter method was preferred because it avoided differential sedimentation and cellular distortion and facilitated the study of intercellular relationships.[13] Lavage fluid was added to cacodylate-buffered glutaraldehyde (final concentration approximately 2% glutaraldehyde). The cells were postfixed in osmium tetroxide and mixed with a small amount of warm (42 C) agar, and droplets of the agar/cell mixture were allowed to solidify. Incorporation in agar prevented loss of cells at later stages in the embedding process. The cells were finally embedded in either Epon or Araldite. Tissue samples were fixed in glutaraldehyde, followed by osmium tetroxide, and processed to resin without the use of agar. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined by transmission electron microscopy. Cell diameter was measured by light microscopy with the use of a ×100 oil immersion objective and an eyepiece micrometer for examination of 1-μ-thick toluidine-blue–stained plastic sections. In each case the greatest diameter of 50 macrophages and 25 lymphocytes was measured, only nucleated cells being assessed. Macrophages and lymphocytes were generally distinguished with ease; but in the few instances where there was difficulty, the cell was not counted. Statistical analysis of the cell diameters was performed by the Mann–Whitney test for nonparametric data.

Results

Comparison of Granuloma and Lavage Cells in Sarcoidosis

Because the ultrastructural features of sarcoid granulomas have been well described by previous workers,[1-4,20,21] our observations are limited to those aspects useful for comparison with BAL cells: the fine structure of epithelioid cells and Langhans giant cells, and the interrelationships between cells of the mononuclear phagocyte system (MPS) and between these cells and lymphocytes.

Sarcoid granulomas consisted of compact aggregates of epithelioid cells characterized by numerous membrane-bound cytoplasmic vesicles containing granular, relatively electron-lucent material (Figure 1). The vesicles were uniformly filled and lacked a peripheral halo beneath their outer membranes (Figure 2). Lysosomes were poorly represented, and there were no phagolysosomes. The epithelioid cells showed extensive folding of the cytoplasmic membrane, a feature that was also seen in giant cells (Figure 3). In giant cells there was a combination of the cytoplasmic features of epithelioid cells and macrophages, in that they contained both vesicles and phagolysosomes.

Lymphocytes were often closely associated with other cells, the close apposition sometimes being

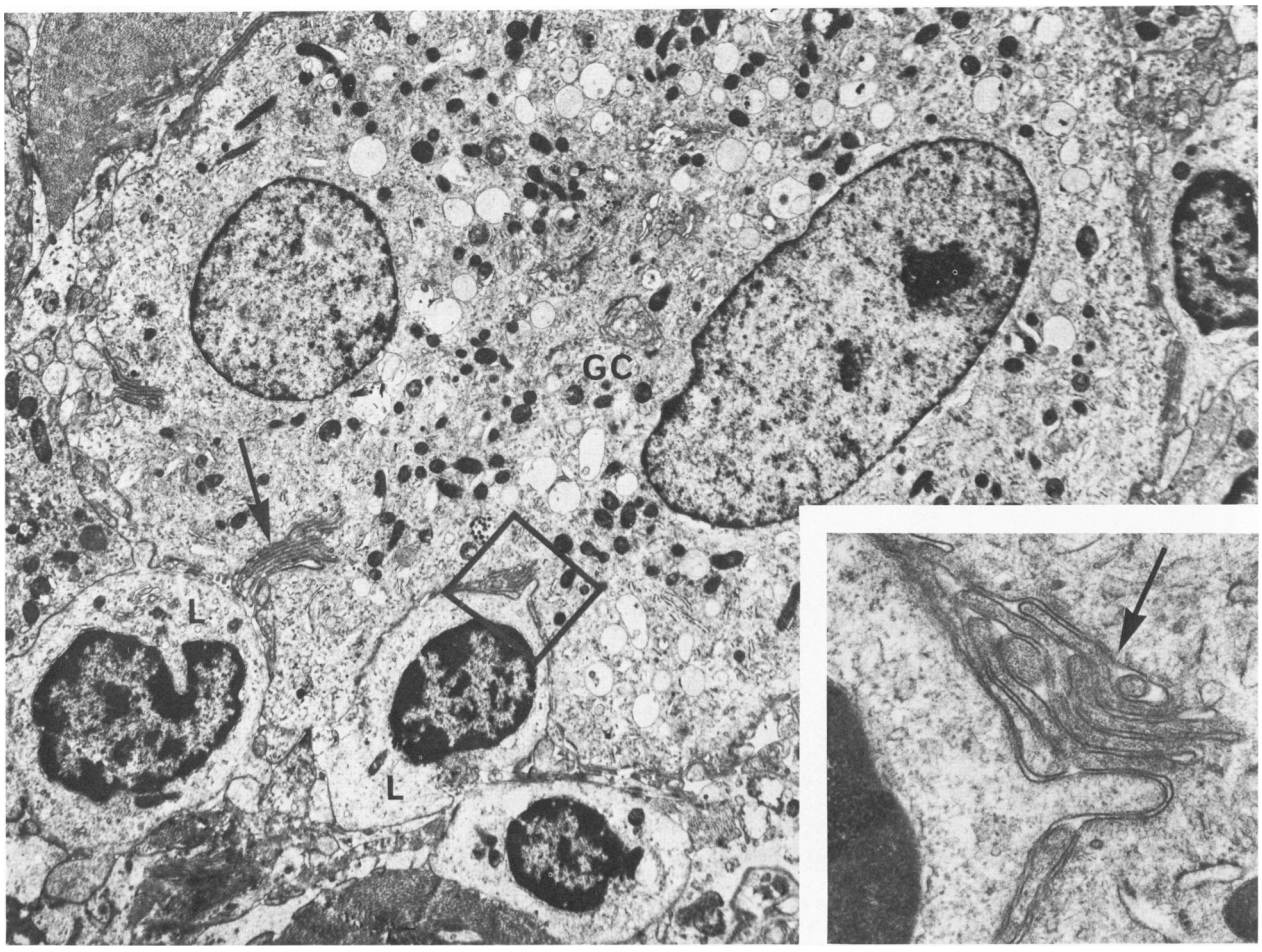
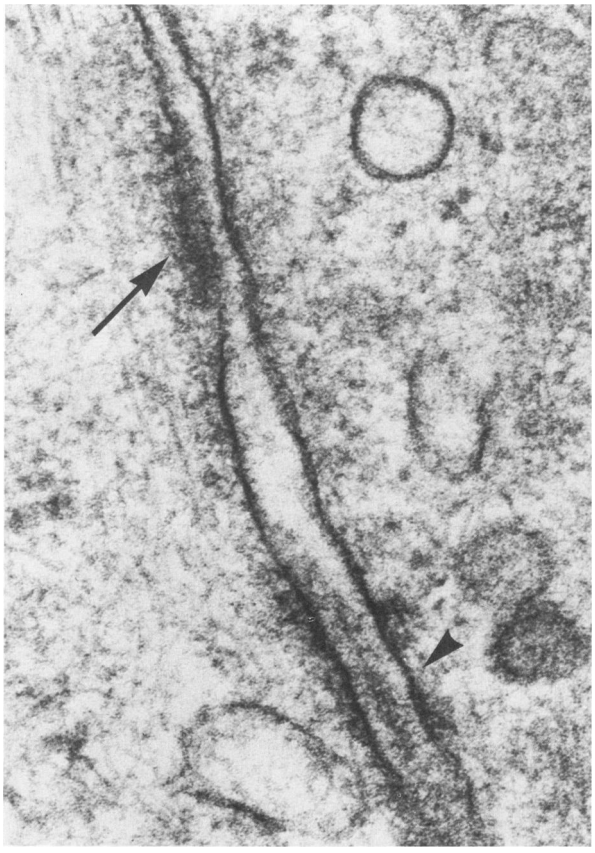


Figure 3 — Sarcoid granuloma showing a multinucleated giant cell (*GC*) with closely apposed lymphocytes (*L*). (×4700) **Inset** — High magnification of the boxed area. (×23,000) Note the tongue of lymphocyte cytoplasm protruding into the giant cell and the interdigitations of the giant cell plasma membrane (*arrow*).

reinforced by a small tongue of cytoplasm, free of organelles, which protruded into cells of the MPS series, invaginating their cell membranes (Figure 3); but no specialized cell junctions or evidence of cell fusion were seen between lymphocytes and cells of the MPS series. However, junctional complexes were noted linking MPS cells. These junctions were characterized by a thin layer of electron-dense material subjacent to the plasma membrane with an extracellular coating of fibrillary material, corresponding to the paired subplasmalemmal linear densities (SPLDs) described by other authors.[3,20,22] Unpaired SPLDs were also observed at the surface of epithelioid and giant cells (Figure 4).

In the lavage samples true epithelioid cells were not found, but many MPS cells possessed vesiclelike structures. These differed from the vesicles of epithelioid cells in having a range of electron density and generally having a clear peripheral halo, although some of the paler-staining vesicles closely resembled those of the epithelioid cells (Figure 5). Lysosomes and phagolysosomes were also seen in these cells. Giant cells were present in the lavage in only 9 sarcoidosis patients and formed only a small proportion (0.2%) of the lavage cells in these patients. Close associations between lymphocytes and macrophages were seen in lavage specimens from 16 sarcoidosis patients, but the tonguelike evaginations found in the

Figure 5a — A bronchoalveolar macrophage from a nonsmoking sarcoidosis patient. Note the numerous large granules of varying electron density that are clustered centrally and the heterochromatin-deficient nucleus. (×6900) **b** — Detail of macrophage granules. Most have clear peripheral halos, contrasting with the epithelioid cell vesicles shown in Figure 2. A few lighter-stained granules appear to lack a halo and thus resemble epithelioid cell vesicles. (×23,500)



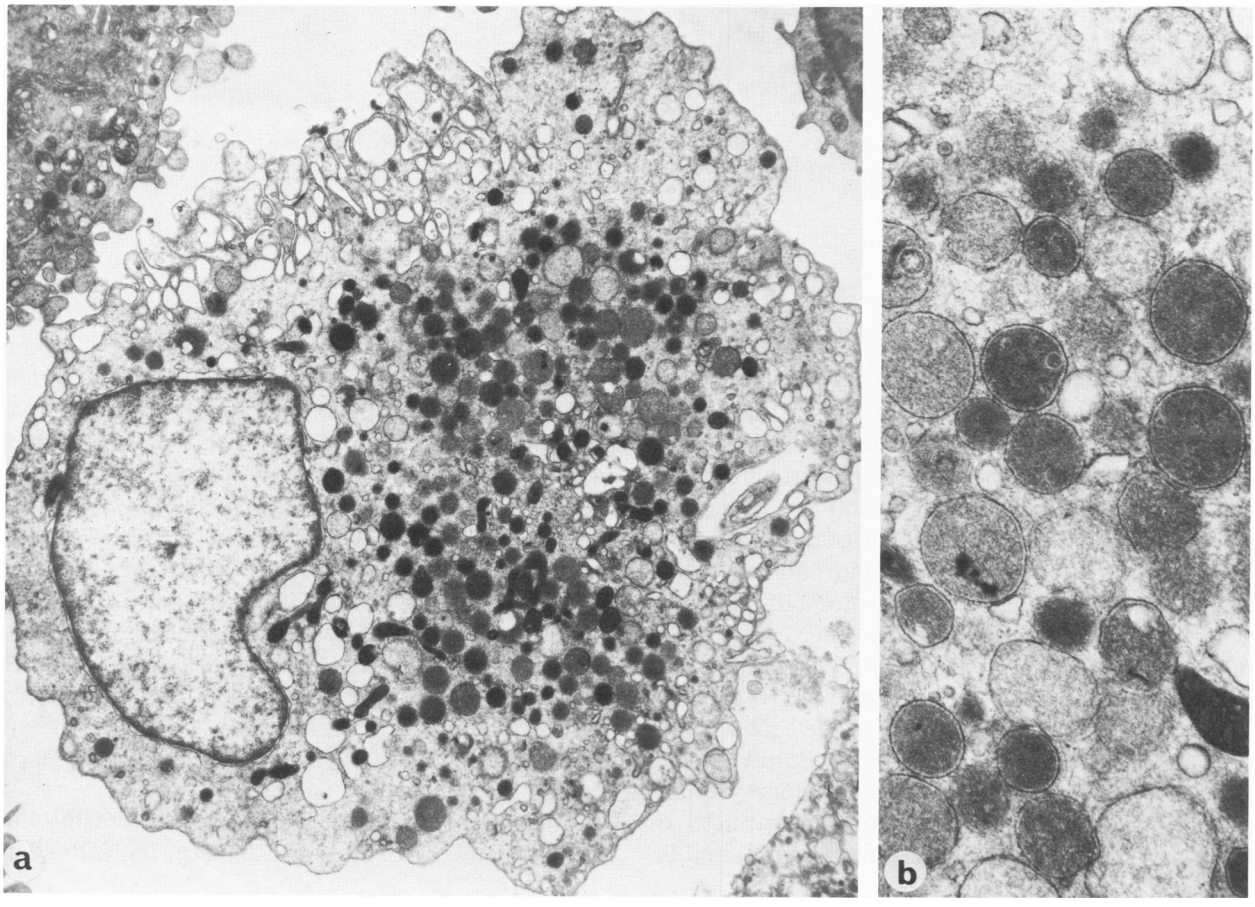
granulomas were never observed. Paired SPLDs between macrophages were found in 14 lavage samples from patients with sarcoidosis (Figure 6).

Comparison Between Sarcoidosis and Control Lavage Cells

Light-microscopic analysis of 1-μ-thick plastic sections showed that both macrophages and lymphocytes were larger in sarcoidosis than in controls (Figures 7 and 8), the difference being highly significant for both smokers and nonsmokers, and in the case of lymphocytes, there being little overlap. For macrophages this difference in size was due not to a subpopulation of very large cells, but to a general increase in cell size, as shown in the frequency distribution graph (Figure 9). Smoking alone did not significantly influence macrophage diameter.

By electron microscopy, monocytes or small macrophages constituted about 60% of the MPS cells in the control group, correlating well with the mor-

Figure 4 — Paired (*arrowhead*) and unpaired (*arrow*) subplasmalemmal linear densities connecting two epithelioid cells in a granuloma. (× 100,000)



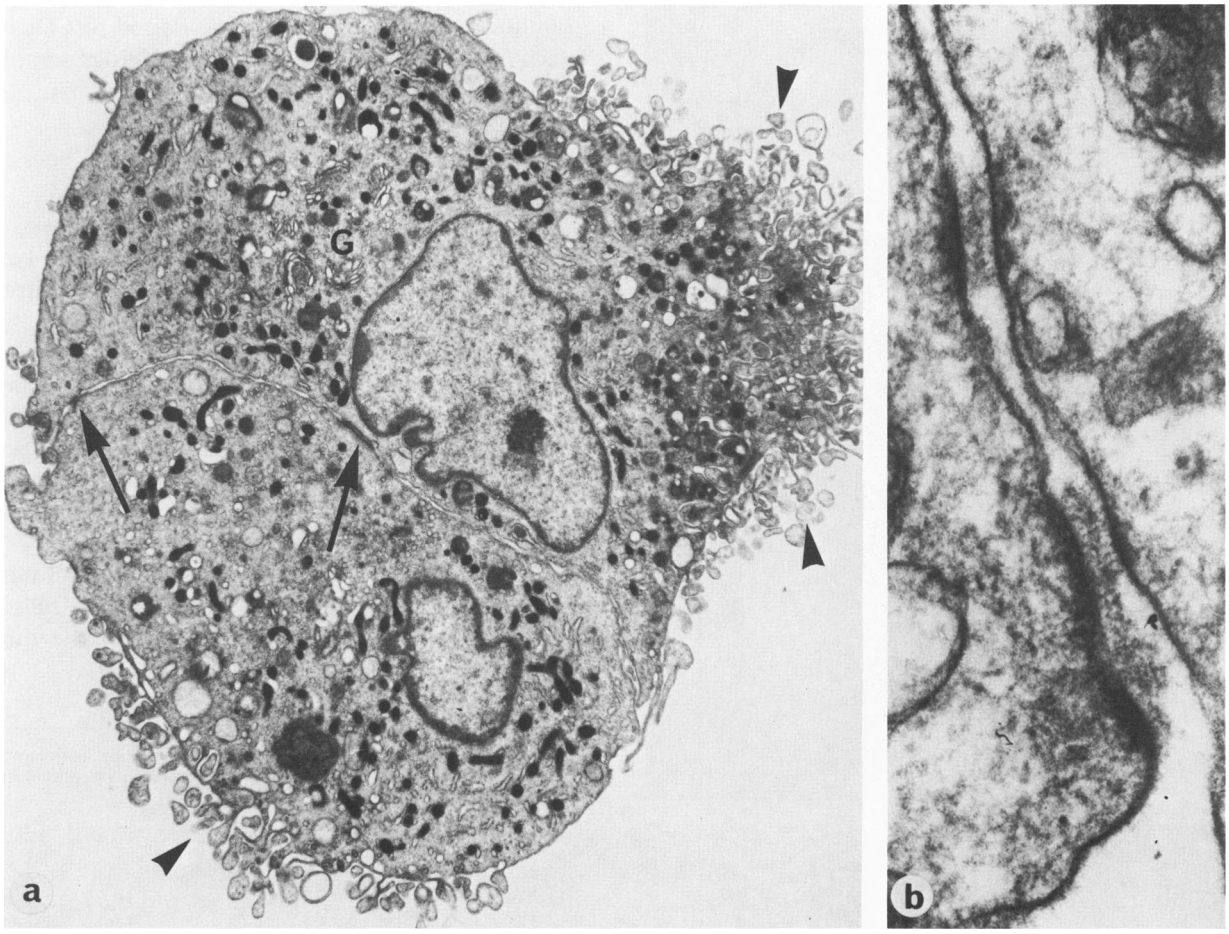


Figure 6a — Bronchoalveolar macrophages from a sarcoidosis patient. The two cells are connected by subplasmalemmal linear density-type junctions (*arrow*). Note the polarization of the cytoplasmic membranes (*arrowhead*), the abundant dilated Golgi apparatus (*G*), and the euchromatin-rich nuclei. (×6000) **b** — Detail of subplasmalemmal linear densities between two macrophages in a sarcoidosis lavage specimen (compare with Figure 4). (×100,000)

phometric findings (Figure 9). These cells were rounded, with small pseudopodia all around the circumference of the cell, many small dense lysosomes distributed throughout the cytoplasm, a few phagolysosomes, and small flat Golgi profiles (Figure 10). The nucleus had peripheral patchy heterochromatin, and a nucleolus was usually seen. In the sarcoid group such cells formed a smaller proportion of the MPS, again correlating with the morphometric data (Figure 9). A very variable proportion (10–70%, but generally about 60%) were larger macrophages containing numerous large granules, which were less electron-dense than those in controls (Figure 5). These granules tended to cluster in the center of the cells around distended Golgi profiles. Empty vesicles were also observed, mainly at the periphery of the cell. Mitochondria were numerous. The nuclei of these cells showed more dispersed chromatin, rendering the nucleolus more prominent. Many of these cells had well-developed pseudopodia, often concentrated toward one pole of the cell (Figure 6). This polarization was also seen in control samples but was significantly less common ($P < 0.05$). Many coated vesicles were evident, generally confined to the side of the cell with pseudopodia. MPS cells were sometimes linked by paired SPLD structures (Figure 6), a feature never observed in the control group. Giant cells were infrequently found in both groups. The ultrastructural differences observed between macrophages of sarcoidosis patients and control subjects were less clearly seen in smokers because of the greater heterogeneity of lysosomal inclusions. Langerhans-type cells, as seen in histiocytosis X, were never observed in this study.

Lymphocytes in control lavages were scanty and usually small, with a smooth surface, few mitochon-

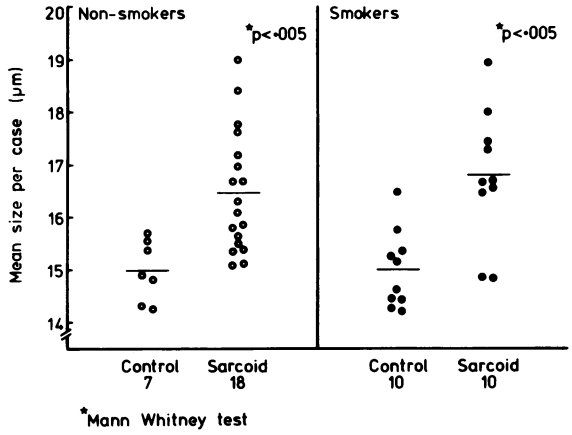


Figure 7 – Mean maximum diameter of macrophages in lung lavage fluids from sarcoidosis cases, compared with that of control subjects.

dria, and a large nucleus rich in heterochromatin (Figure 11a). In sarcoidosis lavages, lymphocytes were more numerous and larger and possessed increased cytoplasm and more mitochondria and dense granules (Figure 11b). Endoplasmic reticulum was not a feature of these cells. Sometimes the cytoplasmic membrane showed thin projections. Close apposition between lymphocytes and macrophages was noted more frequently in sarcoidosis patients than in control subjects, but these cell contacts did not involve any modification of the cell membranes or the formation of cytoplasmic protrusions (Figures 11b and c). The mean percentage of macrophages in close contact with lymphocytes was 6.4 in sarcoidosis patients, compared with 1.6 in control subjects, the ratio being higher in smokers than in nonsmokers.

Figure 8 – Mean maximum diameter of lymphocytes in lung lavage fluid from sarcoidosis cases, compared with controls.

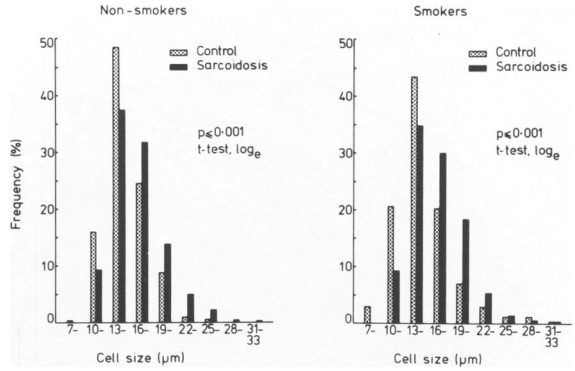


Figure 9 – Frequency distribution of macrophage sizes (maximum diameter) in sarcoidosis patients and control subjects.

Discussion

With regard to sarcoid granulomas, the present study corroborates the findings of others concerning the cell types and their morphological appearance.[1,4,16,17,20,21] The presence of lymphocytes and MPS cells is well recognized in sarcoid granulomas, but the close association we found between them has been reported only infrequently.[1,3,23] This type of close apposition has been emphasized in experimentally induced epithelioid cell granulomas[24] and in cultures of monocytes derived from sarcoidosis patients.[25-26] The mechanism of this physical adherence is not clear but may represent an antigen-triggered cell interaction similar to that described in experimental studies.[27-30] The passage of immunologic information is mediated by cell-surface structures and is promoted by close cellular apposition but without direct cytoplasmic communication.[30,31] The same type of physical interaction has been described in T-cell-mediated target cell lysis.[32,33] Close cellular interactions are believed to be responsible for both T-cell proliferation and macrophage activation.[31,34] Furthermore, activated T cells release soluble mediators capable of attracting monocytes and inhibiting their migration[35] and may have a role in maintaining granulomas.[36] It has also been shown that transformation of macrophages into epithelioid cells is inhibited by the suppression of cell-mediated reactions,[37] suggesting that adjacent lymphocytes take part in the process.

Turning from the granuloma to BAL specimens, the increased apposition between lymphocytes and macrophages we have observed in sarcoidosis has been described previously.[14,38] It may, however, merely reflect the higher proportion of lymphocytes in sarcoidosis lavage fluid, because in those cases where there was a normal proportion of lymphocytes

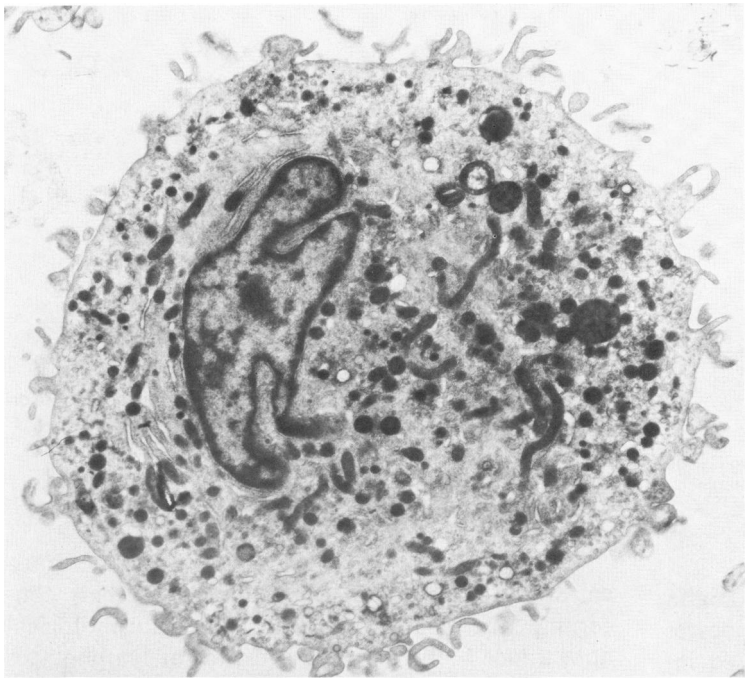


Figure 10—Typical macrophage from a nonsmoking control subject with dispersed small lysosomes and a nucleus rich in heterochromatin. Compare with Figure 5a. (×6900)

we did not find a significant increase in lymphocyte–macrophage apposition. On the other hand, the morphologic appearance of BAL lymphocytes in the sarcoidosis group was markedly different from the control group. The increase in size and our ultrastructural findings of more abundant cytoplasm and increased numbers of mitochondria and dense granules compare favorably with the appearance of activated T-lymphocytes in experimental studies.[34,39] In addition, previous functional studies on BAL cells in sarcoidosis have shown that activated T-lymphocytes are present.[40]

The specialized membrane structures (SPLDs) we observed between MPS cells in both the granuloma and the BAL specimens have been reported previously, mainly in sarcoid granulomas[3,20,22] but also in other diseases.[22,41] SPLDs are found between epithelioid cells, giant cells, and macrophages; but monocyte involvement has not been described. Our study is the first to describe SPLDs in bronchoalveolar lavage specimens. We found them only in our sarcoidosis patients, and their absence from control lavage specimens suggests that these cell junctions are only formed under pathologic conditions or that they are confined to a special class of macrophage which is better represented in sarcoidosis.

At present it is debatable whether BAL cells represent accurately the cells in the lung tissue.[42–44] The absence of true epithelioid cells in BAL specimens indicates that BAL is not completely representative of the disease in the lung. Although true epithelioid cells were not found in BAL, morphologic changes in BAL macrophages were nevertheless found in our sarcoidosis patients. Macrophage size was significantly increased, a feature not identified in previous studies involving a smaller number of patients.[14,15,38] Increased polarization of macrophage pseudopodia was notable in our sarcoidosis patients, but the possibility that this may be artifactual has to be considered. Membrane changes have been ascribed to lidocaine (lignocaine BP),[45,46] the anesthetic used in our studies, but it should be noted that we used the same anesthetic for both patients and control subjects. It has also been suggested that the polarization may be due to centrifugation,[15] but we observed polarization in samples that had not been centrifuged before fixation. We therefore regard membrane polarization as a genuine morphologic change. We also noted a predominance of euchromatin and an increased number and size of lysosomes and Golgi profiles in BAL macrophages in sarcoidosis, features that have been regarded as signs of the high metabolic activity of the cell.[47–50]

We acknowledge that the term "activated cell" can be very ambiguous[51,52] and must be used with care, but consider that the morphologic changes we have described in sarcoidosis lavage macrophages and lymphocytes do indeed represent cellular ac-

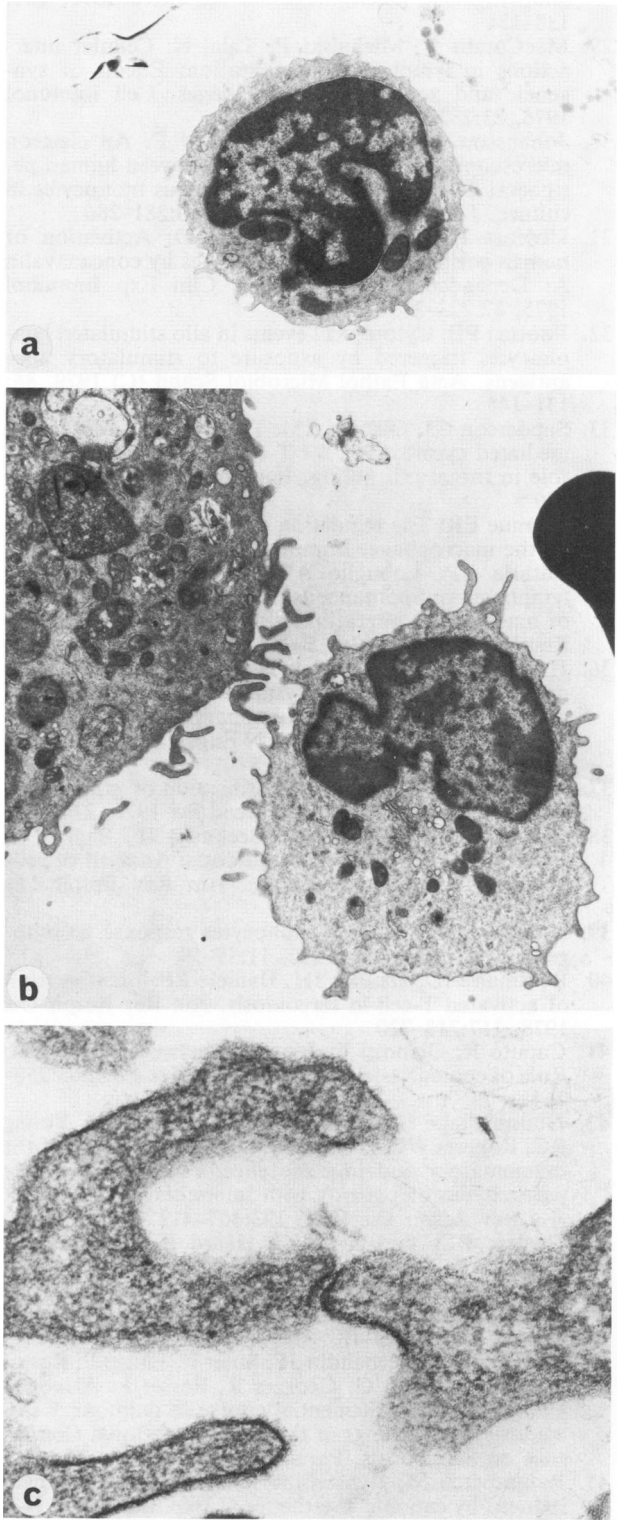


Figure 11a — A lavage lymphocyte from a control subject. (×6750) **b** — A lavage lymphocyte and a macrophage from a sarcoidosis patient. Compare with Figure 11a. (×6750) **c** — Detail of the lymphocyte-macrophage association in Figure 11b. (×69,000)

tivation. Both the cytoplasmic and nuclear changes compare favorably with previous descriptions of activated macrophages[53-55] and activated T-lymphocytes[34,46,47,55-57] and could provide the morphologic basis for the recent description of augmented antigen presentation by alveolar macrophages to T-lymphocytes observed in BAL from sarcoidosis patients.[58]

Pulmonary macrophages and T-lymphocytes are both known to be functionally activated in sarcoidosis,[49,55,59] and the changes we have described provide a structural basis for proposed hypotheses[59,60] that macrophage–lymphocyte interactions play a key role in the pathogenesis of sarcoidosis. T-cell activation may therefore be involved in the increase of activated macrophages in the alveoli and the presence of epithelioid cells within granuloma.[36]

In conclusion, we have found that although the cells in sarcoidosis granulomas show few similarities to those obtained by bronchoalveolar lavage, there are noticeable differences between the lavage cells of sarcoidosis patients and those of control subjects. In sarcoidosis many of the lavage cells appear to be "activated." A clear understanding of the role of these cells cannot be gained from a purely morphologic study, but our results are compatible with the view that immunologic factors are important in the pathogenesis of pulmonary sarcoidosis.

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