Subplasmalemmal Linear Densities in Cells of the Mononuclear Phagocyte System in Lung

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The presence of subplasmalemmal linear densities in cells of the mononuclear phagocyte system was investigated in pulmonary biopsies from 33 patients with fibrotic lung disorders. Subplasmalemmal linear densities, consisting of a thin layer of electron-dense material immediately subjacent to the inner leaflet of the plasma membrane, were found in 30 of the 33 patients, including each of 6 patients with pulmonary sarcoidosis, 18 of 19 patients with idiopathic pulmonary fibrosis, 4 of 5 patients with collagen-vascular diseases, 1 patient with pulmonary lymphangioleiomyomatosis, and 1 patient with marked interstitial pulmonary fibrosis associated with squamous cell carcinoma of the lung. Subplasmalemmal linear densities were found in epithelioid cells, macrophages, and giant cells in granulomas in the 6 patients with sarcoidosis and in alveolar macrophages in 4 of these patients. In patients with other fibrotic lung disorders, subplasmalemmal linear densities were limited in distribution to interstitial and alveolar macrophages. In all patients with sarcoidosis some of the subplasmalemmal linear densities of adjacent mononuclear phagocytes, particularly of those in granulomas, were paired and formed specialized intercellular junctions. Such junctions also were observed in macrophages in 10 of the patients with other fibrotic lung disorders. The junctions formed by subplasmalemmal linear densities differed from other types of junctional structures. Subplasmalemmal linear densities appear to function in 1) the binding of actin filaments to the cytoplasmic surface of the plasma membrane and 2) the formation of intercellular junctions, which may contribute to the immobilization of mononuclear phagocytes in granulomas and alveolar lumens. (Am J Pathol 1980, 100:131-150)

SUBPLASMALEMMAL LINEAR DENSITIES consist of a thin layer of electron-dense material immediately subjacent to the inner leaflet of the plasma membrane. In those areas where subplasmalemmal linear densities are present, the outer leaflet of the plasma membrane is associated with a thin layer of granular or finely fibrillar extracellular material.[1] Although subplasmalemmal linear densities often are present in regions of the cell surface that are not in immediate contact with another cell, in other instances they are paired symmetrically with those of an adjacent cell, thus forming specialized intercellular junctions.[1-9] In most reports of the occurrence of subplasmalemmal linear densities, these structures have been found only in cells of the mononuclear phagocyte system,[1-6] suggesting that the ability to form subplasmalemmal linear densities is a specialized property of this type of cells. Individual case reports have mentioned

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the occurrence of subplasmalemmal linear densities in epithelioid cells in various granulomas, including those in sarcoidosis,[2,3,6–8,12,15] tuberculosis,[2] and fungal infections,[16] as well as in those produced experimentally by intradermal injections of beryllium and zirconium salts.[10] However, the true prevalence of subplasmalemmal linear densities is unknown, because these structures have not been described in numerous other reports of the ultrastructure of different types of granulomas. The presence of numerous cells of the mononuclear phagocyte system in the lungs of patients with sarcoidosis, idiopathic pulmonary fibrosis and other fibrotic lung disorders presents a unique opportunity to study certain characteristics of subplasmalemmal linear densities, including their localization in various types of cells within granulomas, in interstitium, and in alveolar spaces and their contribution to the formation of intercellular junctions.

Materials and Methods

The study group consisted of 33 patients, including 6 with pulmonary sarcoidosis, 19 with idiopathic pulmonary fibrosis, 5 with collagen–vascular disease, 1 with pulmonary lymphangioleiomyomatosis, 1 with chronic eosinophilic pneumonia, and 1 with marked interstitial fibrosis associated with squamous cell carcinoma of the lung. These diseases were diagnosed on the basis of criteria given in detail elsewhere.[17,18] All but one of the patients were considered to be clinically in the mid course of their disease. One patient with idiopathic pulmonary fibrosis had end-stage lung. Pertinent clinical data on the patients are presented in Table 1.

Pulmonary biopsies were obtained from each patient at open thoracotomy and processed for light and electron microscopic studies using methods previously described.[18] The presence of subplasmalemmal linear densities was evaluated qualitatively by transmission electron microscopy in the different types of cells in granulomas, interstitium, and alveolar lumens. The determination of presence or absence of subplasmalemmal linear densitites in cells in interstitium and alveolar lumens was based on study of at least 10 cells in each of these 2 locations. The subplasmalemmal linear densities observed were further classified according to whether they were found either singly or paired with those of adjacent cells, forming intercellular junctions.

Results

Sarcoid granulomas were found in the pulmonary biopsy specimens from each of the 6 patients with pulmonary sarcoidosis. The granulomas contained mainly the following types of cells: monocytes, lymphocytes, macrophages, epithelioid cells, and multinucleated giant cells. Lymphocytes and monocytes tended to be located at the periphery of the granulomas, while macrophages, epithelioid cells, and giant cells tended to collect in central areas, where they established close contact with adjacent cells. Most pulmonary macrophages in patients with sarcoidosis were present in granulomas; few macrophages were found in other areas of lung. Fibrous connective tissue was abundant in some regions of the granulomas but very scarce in others.

Table 1—Occurrence of Subplasmalemmal Linear Densities in Lungs of Patients With Fibrotic Lung Disorders

		Subplasmalemmal linear densities			
		In alveolar lumens		In interstitium	
Age and sex	Clinical diagnosis	Single	Paired	Single	Paired
39 M	Sarcoidosis	+	+	+	+
24 M	Sarcoidosis	0	0	+	+
23 F	Sarcoidosis	–	–	+	+
24 M	Sarcoidosis	+	+	+	+
26 M	Sarcoidosis	+	+	+	+
28 F	Sarcoidosis	+	+	+	+
65 M	IPF	+	+	+	0
48 M	IPF	+	+	0	0
69 M	IPF	0	0	+	0
43 M	IPF	0	0	+	0
68 M	IPF	+	+	+	+
66 M	IPF	+	0	0	0
64 F	IPF	0	0	+	0
41 M	IPF	+	0	0	0
48 M	IPF	0	0	–	–
46 F	IPF	+	0	0	0
55 M	IPF	+	+	0	0
45 F	IPF	0	0	+	0
33 F	IPF	+	0	0	0
54 M	IPF	–	–	+	0
38 M	IPF	+	+	+	+
49 F	IPF	+	0	+	+
26 F	IPF	0	0	+	0
68 F	IPF	0	0	+	0
44 F	IPF	+	+	0	0
57 M	CVD	0	0	0	0
27 F	CVD	–	–	+	0
53 F	CVD	–	–	+	0
43 F	CVD	0	0	+	0
25 M	CVD	+	+	+	+
55 F	Lymphangio-leiomyomatosis	+	+	+	+
60 M	Chronic eosinophilic pneumonia	0	0	0	0
45 F	Squamous cell carcinoma	0	+	0	0

+ = present; 0 = absent; – = data not available; CVD = collagen–vascular disease; IPF = idiopathic pulmonary fibrosis.

Lung tissue from the patients with idiopathic pulmonary fibrosis, collagen–vascular disease, lymphangioleiomyomatosis, chronic eosinophilic pneumonia, and pulmonary fibrosis associated with squamous cell carcinoma of the lung showed variable degrees of interstitial fibrosis and of cuboidalization of the alveolar epithelial cells. Granulomas were not found in any of the biopsies from these patients. The majority of the biopsies from patients with idiopathic pulmonary fibrosis contained large numbers of macrophages, singly and in clusters, in alveolar lumens and in fibrotic

interstitium; however, there was no correlation between the degree of fibrosis and the number of macrophages. Biopsies from the patients with collagen–vascular disease and the patient with lymphangioleiomyomatosis had only small numbers of macrophages in the alveolar spaces. Only a few macrophages were present in alveolar spaces and interstitium of the patient with chronic eosinophilic pneumonia. A large number of macrophages were found in alveolar lumens and fibrotic interstitium of the patient with squamous cell carcinoma of the lung. This patient had marked fibrosis and cuboidal alveolar epithelial cells adjacent to nodules of neoplastic cells.

Incidence of Subplasmalemmal Linear Densities

Paired or unpaired subplasmalemmal linear densities (Figures 1–7) were found in a total of 30 of the 33 patients in the study group (Table 1). Paired and unpaired subplasmalemmal linear densities were found in macrophages, epithelioid cells, and giant cells in interstitial granulomas in each of the 6 patients with pulmonary sarcoidosis (Figures 1 and 2). Alveolar macrophages in 4 of these patients also had both paired and unpaired subplasmalemmal linear densities; macrophages in alveolar lumens in 1 patient did not have either paired or unpaired subplasmalemmal linear densities, and no macrophages were found by ultrastructural examination in alveolar lumens of the remaining patient with sarcoidosis.

Subplasmalemmal linear densities were found in 23 of the 26 patients with other fibrotic lung disorders, including 18 of 19 patients with idiopathic pulmonary fibrosis, 4 of the 5 patients with collagen–vascular diseases, 1 patient with lymphangioleiomyomatosis, and 1 patient with marked interstitial fibrosis and squamous cell carcinoma of the lung; they were not present in 1 patient with idiopathic pulmonary fibrosis, 1 patient with collagen–vascular disease, and 1 patient with chronic eosinophilic pneumonia. In each of the 23 patients just cited, subplasmalemmal linear densities were limited in distribution to macrophages, including alveolar macrophages in 8 patients, interstitial macrophages in 10 patients, and both alveolar and interstitial macrophages in 5 patients. The subplasmalemmal linear densities were paired in 1 patient, unpaired in 14 patients, and both paired and unpaired in 9 patients. Thus, paired subplasmalemmal linear densities forming intercellular junctions between mononuclear phagocytes were found in each of the 6 patients with sarcoidosis and in 10 of the 23 patients with other fibrotic lung disorders.

The subplasmalemmal linear densities occurring in macrophages, epithelioid cells, and multinucleated giant cells were structurally similar and did not show any significant differences related to cell type. In macro-

phages, subplasmalemmal linear densities were found mostly in cells that formed clusters, especially in the interstitial granulomas of patients with sarcoidosis and in alveolar lumens of patients with idiopathic pulmonary fibrosis. The subplasmalemmal linear densities in macrophages did not show any differences related to the location of the cells in interstitium or alveolar lumens.

Morphology of Subplasmalemmal Linear Densities

The subplasmalemmal linear densities that occurred singly ranged from 0.28 to 1.7 μ in length and from 220 to 380 Å in thickness (Figures 5 and 6). The subplasmalemmal linear densities were composed of electron-dense material that, at high magnification, appeared very finely granular and without any organized substructure. This material was immediately subjacent to the inner leaflet of the plasma membrane and was not separated from it by an electron-lucent layer. The subplasmalemmal linear densities were not associated with a plaque-like condensation of material such as that observed subjacent to the plasma membrane in the desmosomes and hemidesmosomes of epithelial cells. The regions of the cell surfaces in which subplasmalemmal linear densities were present usually appeared smooth in contour. The majority of the subplasmalemmal linear densities were not located in areas of formation of filopodia. Two types of cytoplasmic filaments were present in cells (macrophages, epithelioid cells, and multinucleated giant cells) containing subplasmalemmal linear densitites: microfilaments of 50–60 Å in thickness and larger filaments of about 100 Å in thickness. The thin filaments often were selectively distributed in subplasmalemmal areas, whereas the 100 Å filaments were more widely distributed throughout the cytoplasm. Some of the subplasmalemmal linear densities were intimately associated with both types of filaments. Other subplasmalemmal linear densities were not selectively associated with either of these filaments, although because of their peripheral location they were in the vicinity of the 50 Å filaments. The external surface of the plasma membrane overlying areas of formation of subplasmalemmal linear densities consistently had a coating layer that measured about 500 Å in overall thickness. Two components were evident in this external coating layer: a more lucent inner zone, which measured from 100 to 250 Å in thickness and was immediately adjacent to the plasma membrane, and a denser outer zone, which measured about 380 Å in thickness. Because of the two-layered substructure just described, this cell coating resembled a basal lamina; however, it was limited almost exclusively to the areas of formation of subplasmalemmal linear densities. In contrast to these areas, other regions of the surfaces of cells bearing sub-

plasmalemmal linear densities were not associated with a coating or with a true basal lamina.

Subplasmalemmal linear densities that occurred in pairs (Figure 7), forming junctional structures between two adjacent cells, ranged from 0.4 to 2.6 μ in length and from 220 to 380 Å in thickness. In regions in which paired subplasmalemmal linear densities were present the plasma membranes of the adjacent cells were separated by a space that varied from 500 to 800 Å in width. This space was filled by finely filamentous material similar to that forming discrete coatings over unpaired subplasmalemmal linear densities. This space was bisected by a denser layer, about 100 Å in width. Fine strands extended from this central dense layer toward each of the two plasma membranes. The paired subplasmalemmal linear densities were symmetrical and in close parallelism, although coated vesicles occasionally were seen in association with one of the two components of a paired subplasmalemmal linear density. The material of which paired subplasmalemmal linear densities were composed appeared morphologically similar to that in unpaired subplasmalemmal linear densities, as did the types of filaments with which the subplasmalemmal linear densities were associated. As in the case of unpaired subplasmalemmal linear densities, the dense material in paired subplasmalemmal linear densities was not separated from the inner aspect of the plasma membrane, and it did not form a cytoplasmic dense plaque such as that seen in the desmosomes of epithelial cells. Paired subplasmalemmal linear densities usually were present in relatively smooth areas of the cell surfaces rather than in areas of extensive formation of filopodia or of interdigitations between adjacent cells.

Discussion

The present study demonstrates that subplasmalemmal linear densitites are common in macrophages in interstitium and alveolar spaces and in epithelioid cells, macrophages, and multinucleated giant cells in granulomas in the lungs of patients with a variety of pulmonary disorders. Subplasmalemmal linear densities were found in 30 of the 33 patients. They occurred singly in 29 of these patients, and they were paired, forming specialized intercellular junctions, in 16 patients.

Subplasmalemmal linear densities have not been previously studied systematically in lung. Our observations on tissues from 6 patients with pulmonary sarcoidosis show that subplasmalemmal linear densities are a consistent finding in sarcoid granulomas. However, these structures are specific neither for sarcoidosis nor for granulomas, since they also occur in

alveolar and interstitial macrophages in patients with other pulmonary disorders. Subplasmalemmal linear densities were not found in monocytes, which suggests that these structures develop as macrophages, epithelioid cells, and giant cells differentiate from monocytes. However, subplasmalemmal linear densities do not seem to be an obligatory feature of such a differentiation, because they are not present in all macrophages. Our findings confirm and extend previous observations concerning the presence of subplasmalemmal linear densities in other cells of the mononuclear phagoctye system (Table 2), including microglia in brain in multiple sclerosis,[14] phagocytic cells in lymphoma,[11] macrophages in toruloma and blastomycoma,[16] epithelioid cells in sarcoidosis,[2-4,6-8] tuberculosis,[2] and cat-scratch fever,[1] globoid cells in naturally occurring and experimentally induced globoid cell leukodystrophy,[1] and histiocytes in giant cell arteritis,[13] in skin in multicentric reticulohistiocytosis,[5] and in infantile papular self-healing histiocytosis of the head.[9] We observed subplasmalemmal linear densities to be most prominent and numerous in granulomas, where they most frequently formed intercellular junctions. The formation of these junctions may be related to the immobilization of cells of the mononuclear phagocyte system in granulomas.

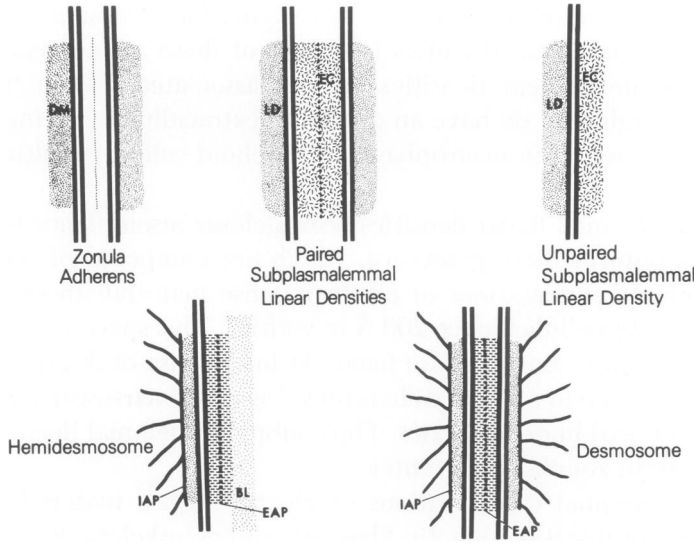
Mononuclear phagocytes usually are not considered to be capable of forming junctional structures, although some studies have shown that under certain circumstances these cells have the ability to develop paired subplasmalemmal linear densities [1-4,5-10] as well as gap junctions.[19] The junctions described in this report differ morphologically from other types of junctional structures, including gap junctions, desmosomes, hemidesmosomes, zonulae adherentes, and the myofilament insertion sites of cardiac and smooth muscle. These differences are illustrated in Text-figure 1.

Electrotonic coupling, attributable to the presence of gap junctions, has been demonstrated in cultured macrophages.[20] More recently, gap junctions have been observed, using freeze-fracture techniques, in macrophages derived from canine bone marrow.[19] These junctions were smaller than usual and could not be visualized in sections of material fixed with glutaraldehyde and osmium tetroxide, but in material treated with lanthanum they revealed the hexagonal array of particles typical of gap junctions. Porvaznik and MacVittie [19] suggested that gap junction interaction between macrophages is directed by chemotactic factors. The structure of the subplasmalemmal linear densities in our material differed clearly from that of gap junctions, in which the intercellular space is reduced to 20–30 Å in width. The presence of the latter junctions could not

Table 2—Reports of the Occurrence of Subplasmalemmal Linear Densities (SPLD)

Authors	Species	Tissue	Cell type	SPLD		Condition
				Single	Paired	
Mikata et al [2-4]	Human	Lung, lymph node	EC	+	+	Sarcoidosis, tuberculosis
Bernaudin et al [7]	Human	Lung	EC	0	+	Sarcoidosis
Judd et al [8]	Human	Lung	EC	0	+	Sarcoidosis
Carr [6]	Human	—	EC	0	+	Sarcoidosis
Morgenroth and Fasske [12]	Human	Lymph node	EC	+	0	Sarcoidosis
Trombley et al [15]	Human	Brain	EC, giant cell	—	—	Sarcoidosis
Elias and Epstein [10]	Human	Skin	EC	+	0	Be-, Zi-induced granulomas
Ebner and Gerhart [5]	Human	Skin	Histiocyte	0	+	Multicentric reticulohistiocytosis
Caputo and Gianotti [9]	Human	Skin	Histiocyte	0	+	"Papular self-healing histiocytosis of the head"
Parker et al [13]	Human	Artery	EC, histiocyte	+	0	Giant cell arteritis
Prineas and Raine [14]	Human	Brain	Microglial cell	+	0	Multiple sclerosis
Hirano et al [11]	Human	Brain	Phagocytic cell	+	0	Malignant lymphoma
Yajima et al [1]	Human	Brain	Globoid cell	+	+	Globoid leukodystrophy
	Dog	Brain	Globoid cell	+	+	Globoid leukodystrophy
	Rat	Brain	Globoid cell	+	+	Galactocerebroside injection
	Human	Lymph node	EC	+	+	Cat-scratch fever
Mirra et al [16]	Human	Brain	Macrophage	—	—	Toruloma, blastomycoma

0 = absent; + = present; — = information not available; EC = epithelioid cell.



TEXT-FIGURE 1—Diagram of the structure of a zonula adherens, paired and unpaired subplasmalemmal linear densities, a hemidesmosome, and a desmosome. The zonula adherens is composed of two accumulations of electron-dense material (*DM*) closely apposed to the cytoplasmic surfaces of the two plasma membranes; a central line may be present in the intercellular space in the junctional area. The subplasmalemmal linear densities (*LD*) are associated with an external coating (*EC*); when the subplasmalemmal linear densities are paired, a dense central line is found in the intercellular space in the junctional area. A hemidesmosome has an intracellular attachment plaque (*IAP*) associated with 100 Å filaments and an extracellular attachment plaque (EAP); from the latter fine filaments extend to the plasma membrane and to the basal lamina (*BL*). A desmosome is composed of two paired hemidesmosomes; however, a basal lamina is not present in the intercellular space in the area of desmosome formation.

be evaluated in our material, which was not studied using freeze-fracture techniques.

Desmosomes and hemidesmosomes differ from subplasmalemmal linear densities in several important respects. Desmosomes have an attachment plaque along the inner aspect of the plasma membrane, as well as cytoskeletal filaments, 100 Å in diameter, which insert into the attachment plaque.[21] This attachment plaque is not evident in subplasmalemmal linear densities. Paired subplasmalemmal linear densities and desmosomes have a central dense zone in the intercellular space; however, the width of the space between the two outer leaflets of the apposed plasma membranes is considerably less (240 Å) in desmosomes than in paired subplasmalemmal linear densities (500–800 Å). Hemidesmosomes are characterized by an attachment plaque, similar to that of desmosomes, and by an extracellular plaque that is connected by fine filaments to the outer leaflet

of the plasma membrane and to the overlying basal lamina.[21] Unpaired subplasmalemmal linear densities lack both of these attachment plaques. Subplasmalemmal linear densities are not associated with a true basal lamina, although they do have an overlying extracellular coating. A basal lamina is not present in macrophages, epithelioid cells, or multinucleated giant cells.

Subplasmalemmal linear densities bear a closer resemblance to zonulae adherentes (intermediate junctions), which are composed of paired subplasmalemmal condensations of electron-dense material; these are separated by an intercellular space 200 Å in width.[21] This space is occasionally bisected by a thin, central dense band. As in the case of desmosomes, the intercellular space in zonulae adherentes [21] is much narrower than that in subplasmalemmal linear densities. Thus, subplasmalemmal linear densities also differ from zonulae adherentes.

Subplasmalemmal condensations of electron-dense material that also serve as sites of insertion of actin filaments and cytoskeletal filaments (100 Å in diameter) are also present in cardiac muscle cells,[22] smooth muscle cells,[23–26] and myofibroblasts.[27] In cardiac muscle cells [22] these condensations become paired and form part of intercellular junctions (myofibrillar insertion sites of the intercalated disks, which are separated by a space of 200 Å), whereas in smooth muscle cells [23–26] and myofibroblasts [27] they are unpaired and do not form junctions.

It is evident from the preceding that subplasmalemmal linear densities represent only one of the various types of structures resulting from condensations of cytoplasmic protein material associated with the inner leaflet of the plasma membrane. Other examples of these structures include not only the desmosomes, zonulae adherentes, and myofilament attachment sites described above but also bristle-coated vesicles.[28,29] The latter are characterized by the distinctive substructure of their subplasmalemmal layer, which differs clearly from that of subplasmalemmal linear densities. The bristles in coated vesicles are thought to be composed of clathrin.[29] Coated vesicles are of importance in mediating the uptake of macromolecules, such as low-density lipoproteins [30] and α_2 macroglobulin,[31] which become bound to specific cell surface receptors.[32] The cytoplasmic protein forming subplasmalemmal condensations in a variety of cells, including the peripherally located dense zones in smooth muscle cells, the myofibrillar insertion sites and Z bands in cardiac muscle cells, and the desmosomes of various cell types has been identified as α-actinin.[26,33] An α-actininlike protein has been isolated from a variety of nonmuscle cells, where it has been found to be attached to the cytoplasmic surface of the plasma membrane in association with a network of micro-

filaments.[26,33] Alpha-actinin also has been detected in the peripheral regions of spreading rat embryo cells, where it forms a regular network that precedes the formation of the straight actin filament bundles that are seen in the cells that are fully spread out.[34] Actin also has been found to be associated with α-actinin in the membranes of secretory vesicles of adrenal chromaffin cells.[35] This again suggests that α-actinin in nonmuscle cells serves to anchor microfilaments to the inner surfaces of the plasma membranes. The role of α-actinin in anchoring actin filaments in the sarcomeres of striated muscle is well known.[33,36]

Resting macrophages have a poorly organized subplasmalemmal network of actin filaments, which become organized into oriented bundles as these cells are activated to phagocytose particles.[37] The actin filaments mediate the cell surface changes involved in phagocytosis by macrophages.[38,39] The tissue culture studies of Sutton and Weiss have shown that both the cytoskeletal (100 Å) and the actin filaments in monocytes and macrophages increase in number as these cells differentiate into epithelioid cells and that the greatest concentration of filaments occurs in aged giant cells.[40] Macrophages, epithelioid cells, and multinucleated giant cells in granulomas in lung contain variable numbers of actinlike filaments in peripheral regions of their cytoplasm. These variations probably reflect differences in the functional states of these cells. Such filaments usually appear poorly organized, and their association with subplasmalemmal linear densities is not always consistent; however, it is reasonable to believe that these cells can develop focal condensations of proteins capable of anchoring the filaments. Although the evidence derived from other cell types suggests that α-actinin is the protein anchoring cytoplasmic filaments in pulmonary macrophages and epithelioid cells, this remains uncertain because biochemical or immunofluorescence studies have not been made to demonstrate the presence of α-actinin in pulmonary cells. Actin (which constitutes 10–12% of the total protein of pulmonary macrophages) and myosin have been isolated from pulmonary macrophages, together with large amounts of a high-molecular-weight protein known as actin-binding protein.[38,39,41] This protein, which is similar in several respects to the filamin isolated from other cell types, binds to actin, producing a gelation reaction. Such a reaction is thought to be of importance in mediating changes in cytoplasmic consistency, presumably by forming crosslinked complexes of laterally aggregated actin filaments.[38,39,41,42] The subcellular localization of the protein has not been studied by immunofluorescence techniques.

Subplasmalemmal linear densities in pulmonary macrophages, epithelioid cells, and multinucleated giant cells are capable of participating in

two important structural functions: 1) the binding of subplasmalemmal actin-like filaments to the cytoplasmic surface of the plasma membrane and 2) cell-to-cell interactions leading to specialized attachments between adjacent cells. It is uncertain whether the material in subplasmalemmal linear densities is α-actinin, actin-binding protein, or another, unidentified component.

References

1. Yajima K, Fletcher TF, Suzuki K: Sub-plasmalemmal linear density: A common structure in globoid cells and mesenchymal cells. Acta Neuropathol (Berl) 1977, 39:195–200
2. Mikata A: Utilization of electron microscopy for a routine diagnosis of lymph node biopsies. Jpn J Clin Pathol 1972 20:719–724
3. Mikata A: [Electron microscopic observation of sarcoid granuloma of the lymph nodes.] Jpn J Clin Med 1968, 26:1320–1323
4. Mikata A, Kano Y, Okui S, Hamano S, Okamoto R, Furuya Y, Watanabe S, Kikuchi K, Terunuma K, Ishi K, Yanagisawa M, Iwasaki M, Koizumi T: [Electron microscopic and enzyme-histochemical observation of sarcoidosis granuloma.] Iryo 1968, 22:637–644
5. Ebner H, Gebhart W: Zur Ultrastruktur der multizentrischen Reticulohistiocytose. Arch Dermatol Forsch 1971, 240:259–270
6. Carr I: The Macrophage: A review of Ultrastructure and Function. London, New York, Academic Press, 1973, pp 77
7. Bernaudin J-F, Soler P, Basset F, Chrétien J: La cellule épithélioïde: Données ultrastructurales au cours de diverses entités pathologiques humaines. Pathol Biol (Paris) 1975, 23:494–498
8. Judd PA, Finnegan P, Curran RC: Pulmonary sarcoidosis: A clinicopathological study. J Pathol 1975, 115:191–198
9. Caputo R, Gianotti F: Junctions between histiocytes: Role of coated vesicles. J Ultrastruct Res 1979, 68:256–264
10. Elias PM, Epstein WL: Ultrastructural observations on experimentally induced foreign-body and organized epithelioid-cell granulomas in man. Am J Pathol 1968, 52:1207–1223
11. Hirano A, Ghatak NR, Becker NH, Zimmerman HM: A comparison of the fine structure of small blood vessels in intracranial and retroperitoneal malignant lymphomas. Acta Neuropathol (Berl) 1974, 27:93–104
12. Morgenroth K, Fasske E: Examination of mediastinal lymph node sarcoidosis by electron microscope. Beitr Pathol 1974, 153:51–64
13. Parker F, Healey LA, Wilske KR, Odland GF: Light and electron microscopic studies on human temporal arteries with special reference to alterations related to senescence, atherosclerosis and giant cell arteritis. Am J Pathol 1975, 79:57–80
14. Prineas JW, Raine CS: Electron microscopy and immunoperoxidase studies of early multiple sclerosis lesions. (Abstr) Neurology 1976, 26:29–32
15. Trombley IK, Mirra SS, Miles ML: An electron microscopic (EM) study of central nervous system (CNS) sarcoidosis (Abstr). VIIIth International Congress, American Association of Neuropathologists, September 24–29, 1978, Washington, DC, pp 702
16. Mirra SS, Trombley IK, Miles ML: Toruloma and blastomycoma of the central nervous system (CNS): A comparative electron microscopic (EM) study (Abstr). 55th Annual Meeting, American Association of Neuropathologists, June 7–10, 1979, Kansas City, pp 333
17. Crystal RG, Fulmer JD, Roberts WC, Moss ML, Line BR, Reynolds HY: Idiopathic

pulmonary fibrosis: Clinical, histologic, radiographic, physiologic, scintigraphic, cytologic, and biochemical aspects. Ann Intern Med 1976, 85:769–788
18. Kawanami O, Ferrans VJ, Roberts WC, Crystal RG, Fulmer JD: Anchoring fibrils. A new connective tissue structure in fibrotic lung disease. Am J Pathol 1978, 92:389–410
19. Porvaznik M, MacVittie TJ: Detection of gap junctions between the progeny of a canine macrophage colony-forming cell *in vitro*. J Cell Biol 1979, 82:555–564
20. Levy JA, Weiss RM, Dirksen ER, Rosen MR: Possible communication between murine macrophages oriented in linear chains in tissue culture. Exp Cell Res 1976, 103:375–385
21. Farquhar MG, Palade GE: Junctional complexes in various epithelia. J Cell Biol 1963, 17:375–412
22. Sommer JR, Johnson EA: Ultrastructure of cardiac muscle, Handbook of Physiology. Section 2, The Cardiovascular System. Vol 1, The Heart. Edited by RM Berne, N Sperelakis. Baltimore, Md, Williams & Wilkins, 1979, pp 113–186
23. Cooke PH, Fay FS: Correlation between fiber length, ultrastructure and the length-tension relationship of mammalian smooth muscle. J Cell Biol 1972, 52:105–116
24. Uehara Y, Campbell GR, Burnstock G: Cytoplasmic filaments in developing and adult vertebrate smooth muscle. J Cell Biol 1971, 50:484–497
25. Gimbrone MA Jr, Cotran RS: Human vascular smooth muscle in culture: Growth and ultrastructure. Lab Invest 1975, 33:16–27
26. Schollmeyer JE, Furcht LT, Goll DE, Robson RM, Stromer MH: Localization of contractile proteins in smooth muscle cells and in normal and transformed fibroblasts, Cell Motility. Book A, Motility, Muscle and Nonmuscle Cells. Cold Spring Harbor Conferences on Cell Proliferation. Vol 3. Edited by R Goldman, T Pollard, J Rosenbaum. Cold Spring Harbor Laboratory, 1976, pp 361–388
27. Kapanci Y, Assimacopoulos A, Irle C, Zwahlen A, Gabbiani G: "Contractile interstitial cells" in pulmonary alveolar septa: A possible regulator of ventilation/perfusion ratio? J Cell Biol 1974, 60:375–392
28. Friend DS, Farquhar MG: Functions of coated vesicles during protein absorption in the rat vas deferens. J Cell Biol 1967, 35:357–376
29. Pearse BMF: Clathrin: A Unique protein associated with intracellular transfer of membrane by coated vesicles. Proc Natl Acad Sci USA 1976, 73:1255–1259
30. Anderson RGW, Brown MS, Goldstein JL: Role of the coated endocytic vesicle in the uptake of receptor—bound low density lipoprotein in human fibroblasts. Cell 1977, 10:351–364
31. Willingham MC, Maxfield FR, Pastan IH: α_2 Macroglobulin binding to the plasma membrane of cultured fibroblasts. Diffuse binding followed by clustering in coated regions. J Cell Biol 1979, 82:614–625
32. Goldstein JL, Anderson RGW, Brown MS: Coated pits, coated vesicles, and receptor—mediated endocytosis. Nature 1979, 279:679–685
33. Schollmeyer JV, Goll DE, Tilney L, Mooseker M, Robson R, Stromer M: Localization of α-actinin in nonmuscle material. J Cell Biol 1974, 63:304a
34. Lazarides E: Actin, α-actinin, and tropomyosin interaction in the structural organization of actin filaments in nonmuscle cells. J Cell Biol 1976, 68:202–219
35. Jockusch BM, Burger MM, DaPrada M, Richards JG, Chaponnier C, Gabbiani G: α-Actinin attached to membranes of secretory vesicles. Nature 1977, 270:628–629
36. Korn ED: Biochemistry of actomyosin-dependent cell motility: A review. Proc Natl Acad Sci USA 1978, 75:588–599
37. Reaven EP, Axline SG: Subplasmalemmal microfilaments and microtubules in resting and phagocytizing cultivated macrophages. J Cell Biol 1973, 59:12–27
38. Stossel TP, Hartwig JH: Interactions between actin, myosin, and an actin-binding

protein from rabbit alveolar macrophages: Alveolar macrophage myosin Mg^{2+}-adenosine triphosphatase requires a cofactor for activation by actin. J Biol Chem 1975, 250:5706–5712
39. Stossel TP, Hartwig JH: Phagocytosis and the contractile proteins of pulmonary macrophages, Cell Motility. Book B, Actin, Myosin, and Associated Proteins. Cold Spring Harbor Conferences on Cell Proliferation. Vol 3. Edited by R Goldman, T Pollard, J Rosenbaum. Cold Spring Harbor Laboratory, 1976, pp 529–544
40. Sutton JS, Weiss L: Transformation of monocytes in tissue culture into macrophages, epithelioid cells, and multinucleated giant cells: An electron microscope study. J Cell Biol 1966, 28:303–332
41. Hartwig JH, Stossel TP: Isolation and properties of actin, myosin, and a new actin-binding protein in rabbit alveolar macrophages. J Biol Chem 1975, 250:5696–5705
42. Schloss JA, Goldman RD: Isolation of a high molecular weight actin-binding protein from baby hamster kidney (BHK-21) cells. Proc Natl Acad Sci USA 1979, 76:4484–4488

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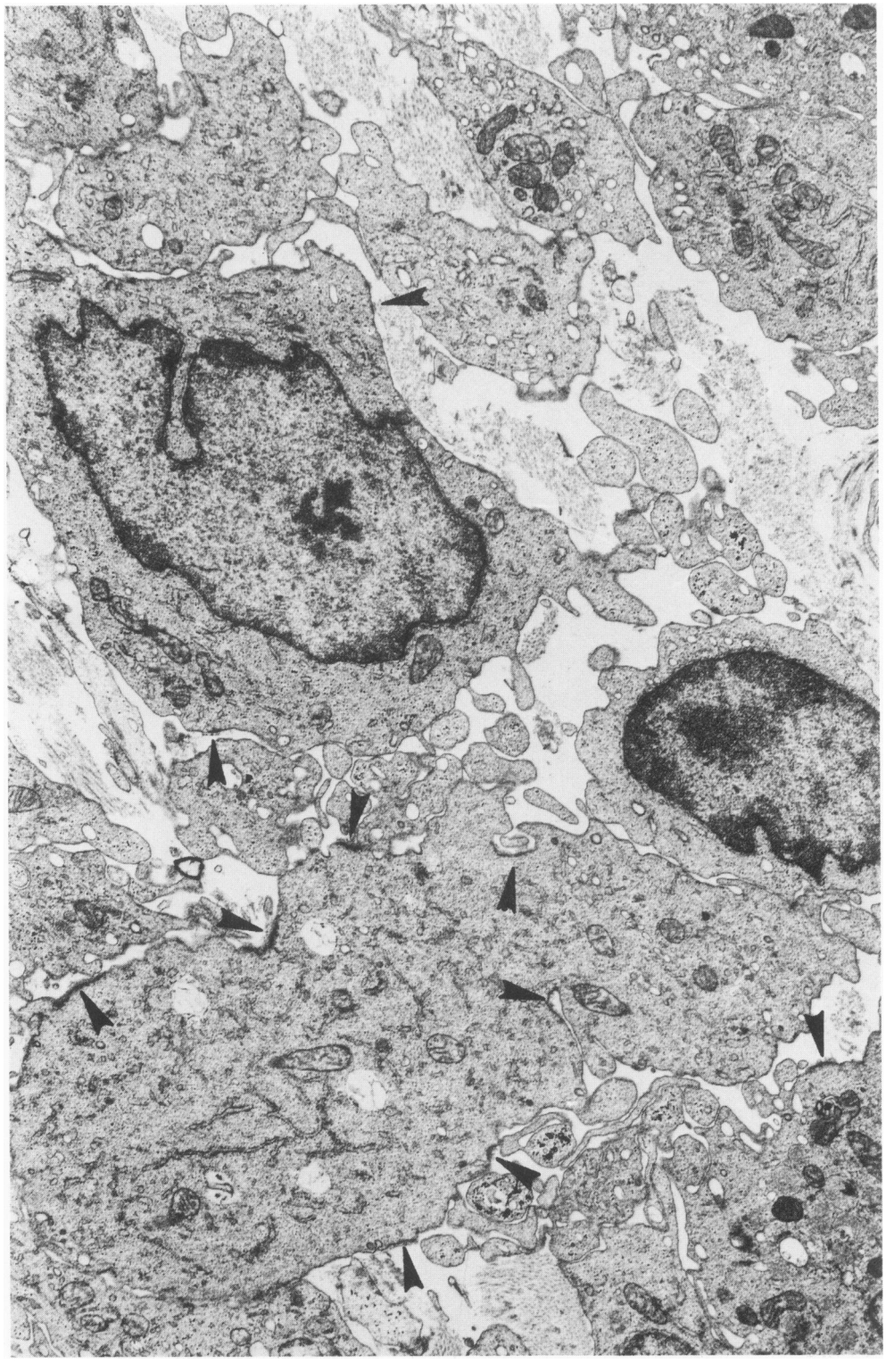


Figure 1—Portion of granuloma in lung of patient with sarcoidosis, showing subplasmalemmal linear densities (*arrowheads*) in epithelioid cells adjacent to a lymphocyte. (×13,300)

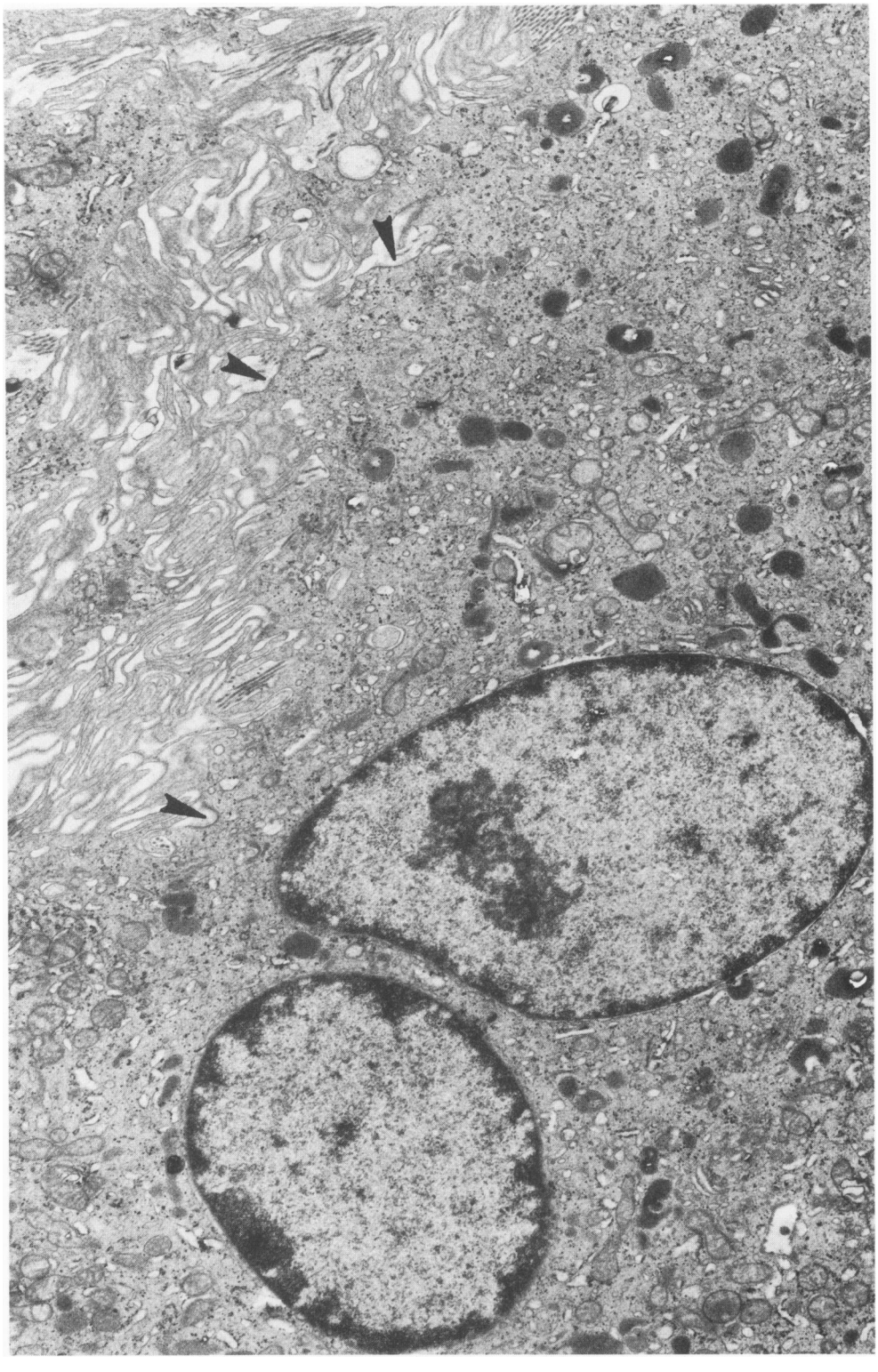


Figure 2—Several subplasmalemmal linear densities (*arrowheads*) are located along the surface of a multinucleated giant cell in a sarcoid granuloma. (×11,500)

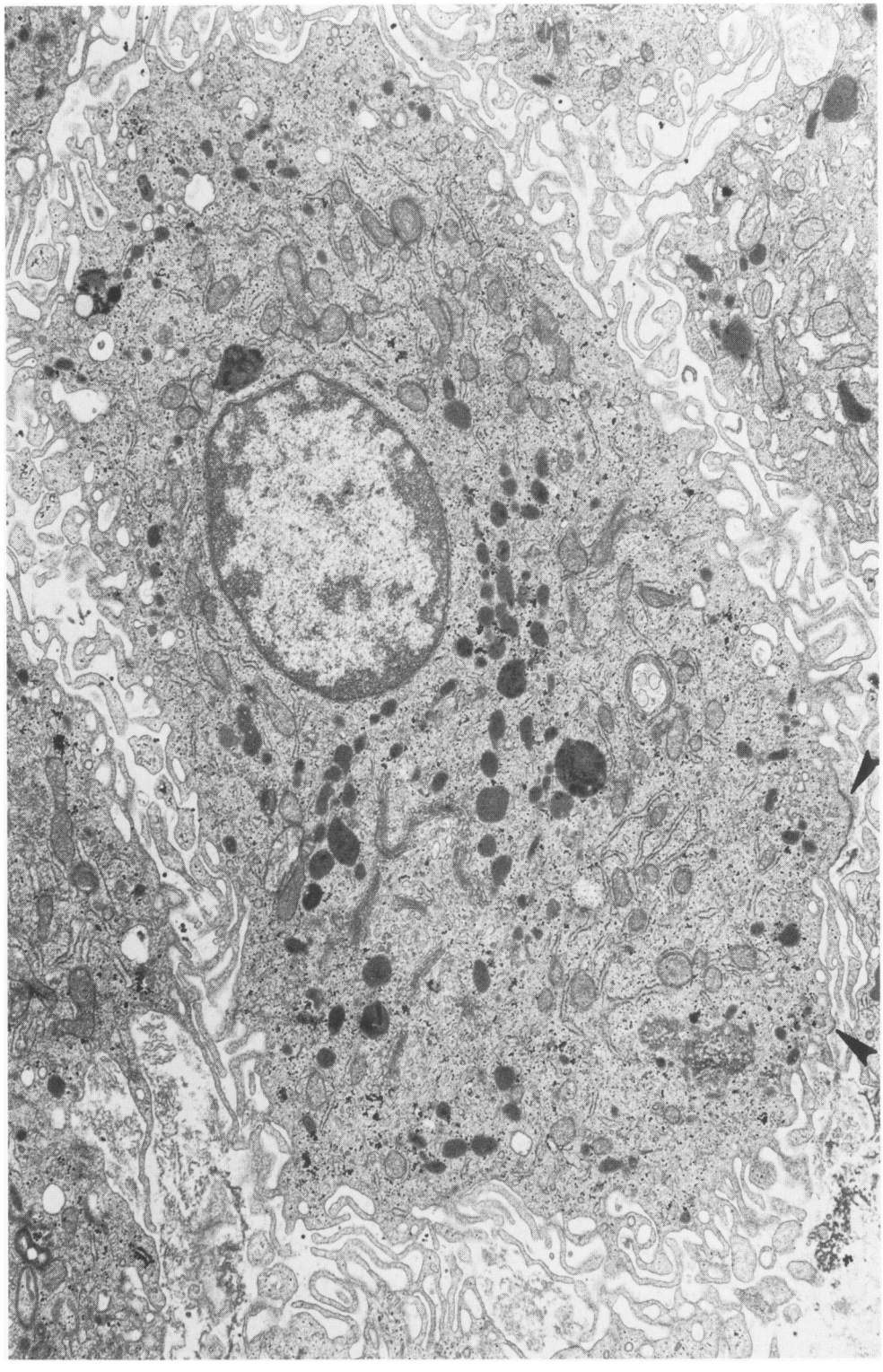


Figure 3—Alveolar macrophage from patient with idiopathic pulmonary fibrosis contains two small, unpaired subplasmalemmal linear densities (*arrowheads*). (×13,300)

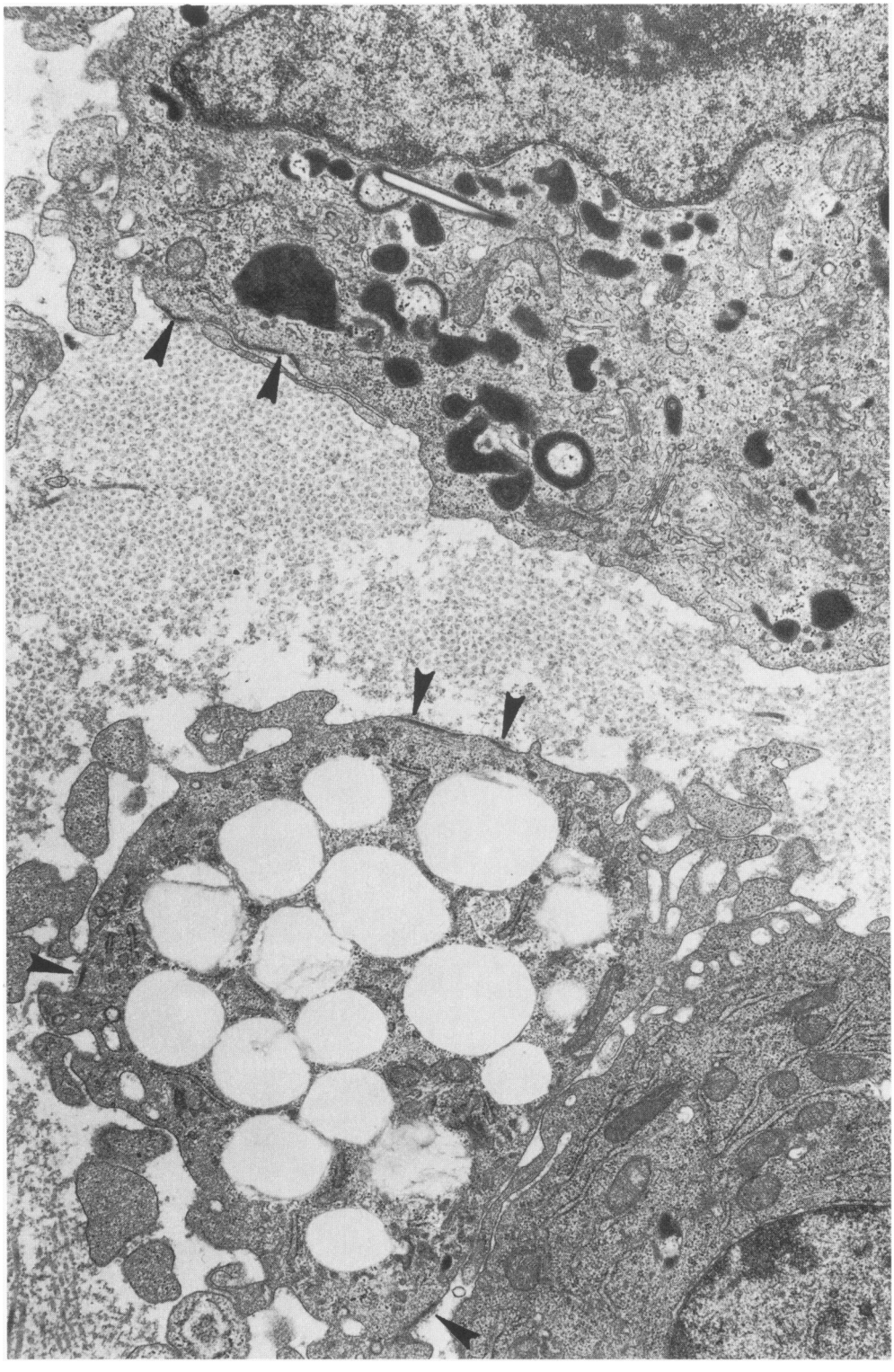
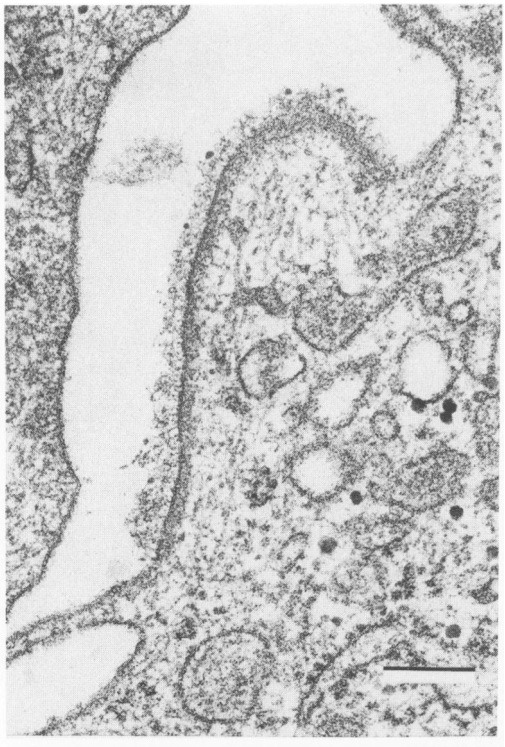


Figure 4—Several subplasmalemmal linear densities (*arrowheads*) are present along the surfaces of two interstitial macrophages in lung of a patient with collagen–vascular disease. (×17,000)



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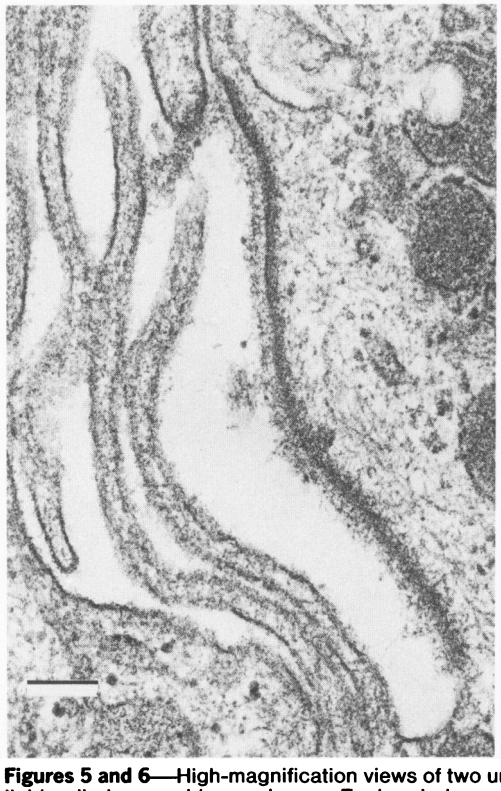

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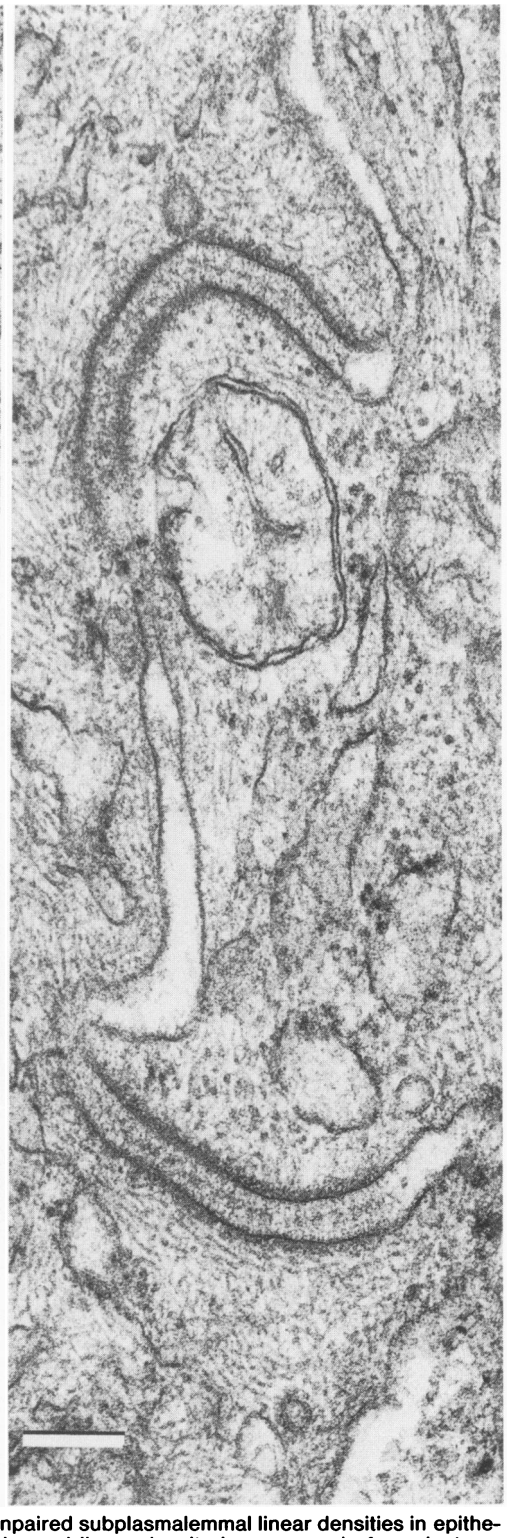

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Figures 5 and 6—High-magnification views of two unpaired subplasmalemmal linear densities in epithelioid cells in sarcoid granulomas. Each subplasmalemmal linear density is composed of an electron-dense zone subjacent to the plasma membrane, associated with a sharply circumscribed extracellular coating of finely fibrillar material. Note the cytoplasmic filaments adjacent to the subplasmalemmal linear densities. Bar = 0.2μ. (×59,000 and ×47,000, respectively) **Figure 7**—Two paired subplasmalemmal linear densities form intercellular junctions connecting two epithelioid cells in a sarcoid granuloma. Note the arrangement of the extracellular coating material in the regions of junction formation. Bar = 0.2μ. (×64,000)

[*End of Article*]