Electron microscopy of giant-cell (temporal) arteritis¹

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Definitive histological studies of giant-cell (temporal) arteritis have been published by several workers (Harrison, 1948; Heptinstall, Porter, and Barkley, 1954; McCormick and Neuberger, 1958). The lesions are fairly uniform and have involved all layers of many different arteries and veins—large and small, intracranial as well as extracranial. The distinctive histological finding in this disease has been a giant-cell reaction with disruption of the internal elastic membrane. Aetiological factors are unknown. Biopsy specimens from a patient with a classical clinical and pathological case of giant-cell (temporal) arteritis have been examined electron microscopically. This report describes the ultrastructural details of this diseased artery.

CASE REPORT

A 64-year-old man was referred to the Saint Louis University Hospital in July 1967 by Dr. James Mulvill, Alton, Illinois, with a main complaint of right fronto-temporal headaches for three months. He also had noted malaise, weight loss, and mild intermittent fever. His past history was remarkable for a partial loss of vision in the right eye for eight years with an attack of gout two years ago. He took digitalis and probenecid regularly and quinidine intermittently. He denied arthralgia and myalgia, and had no symptoms of cerebral dysfunction. He never noted redness or tenderness of the temporal regions.

Examination revealed normal vital signs. The temporal arteries were firm and cord-like with weak pulsations, but were not tender, and there were no signs of inflammation surrounding them. There were no bruits and pulses were good in the extremities. Funduscopic examination showed mild arteriosclerotic changes bilaterally with old perimacular exudates on the right. There was a central scotoma of the right visual field. There was no evidence of recent ocular disease. The cause of his old visual loss was not ascertained. The remainder of the general and neurological examination was normal.

Laboratory studies were normal except for erythrocytic sedimentation rate of 116 mm/hr, a diabetic glucose tolerance test, and serum uric acid 7.0 mg%. There was no eosinophilia, serum protein electrophoresis was

normal, and cerebrospinal fluid was normal. Mercury-203 chlormerodrin brain scan was normal. An electroencephalogram showed mild, diffuse, slow dysrhythmia. Radiographs of chest, skull, and paranasal sinuses were normal except for bilateral maxillary sinusitis.

The left superficial temporal artery was biopsied. The lumen was obliterated in some regions. Histological sections (Fig. 1) demonstrated a marked inflammatory reaction associated with proliferative changes of intima, media, and adventitia. Numerous eosinophils, lymphocytes, and plasma cells were present. The intima had a nodular proliferation. At the junction of the intima and media many giant cells were seen. Some were of foreign-body type, but most were of the Langhans type. No tuberculoid granulomata were noted.

The patient was treated with prednisone 30 mg per day for one month and then the drug was gradually decreased. He has made an excellent recovery from his symptoms of headache and malaise. He has gained weight and has no more febrile episodes.

ELECTRON MICROSCOPIC OBSERVATIONS

Small bits of the temporal artery were immersed in Millonig's OsO₄ fixative for one hour and then dehydrated and embedded in epoxy resin. The sections were stained with lead citrate and uranyl acetate. Phosphotungstic acid (2% in 50% ethanol) was also added to some sections to stain elastica and connective tissue. For control observations, a temporal artery from a 60-year-old man with an internal carotid artery aneurysm was similarly processed and examined.

The adventitia was infiltrated with numerous lymphocytes and some polymorphonuclear leucocytes. The vasa vasorum were prominent in the sections examined and appeared to be normal. Dense bundles of collagen were noted throughout the adventitia and no abnormal connective tissue was seen. The media consisted of parallel arrays of smooth muscle cells surrounded by a framework of collagen and some elastic fibres. Several regions of such normal media were found; however, in other sections this normal pattern was disrupted by the appearance of giant cells especially near the internal elastic membrane, as will be discussed below.

The internal elastic membrane was disrupted in

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FIG. 1. This cross-section of the temporal artery demonstrates intimal hyperplasia, subintimal haemorrhage with disruption of the internal elastic membrane, and giant cell response (arrow) and marked inflammatory cell infiltration of the media and adventitia. The outer border of the adventitia is at the left. L = lumen. Paraffin section stained with haematoxylin and eosin. $\times 70$.

many regions both in paraffin sections and in electron microscopic sections. Orcinol staining of paraffin sections revealed no definite differences in staining characteristics of the remaining internal elastic membrane in the patient and in temporal arteries unaffected by arteritis. For electron microscopic study, the internal elastic membranes of control and diseased temporal arteries were examined unstained, with phosphotungstic acid alone, with lead and uranyl stains alone, and with a combination of lead, uranyl, and phosphotungstic acid stains. The intact areas of the internal elastic membrane in this patient did not differ from that of the control subject in

either the stained or unstained state. In unstained sections the matrix of the membrane had approximately the same density as surrounding extracellular substance. The matrix was amorphous and slightly stippled. Within this matrix were numerous fibrils oriented circumferentially to the lumen of the artery. The fibrils measured 25 to 100 m μ in width. After exposure to lead and uranyl salts, the basement membranes adjacent to the elastica interna and the fibrils within the membrane stained fairly densely, as did surrounding cells, leaving the matrix of the membrane relatively electron lucent (Fig. 2). Phosphotungstic acid stained all portions of the membrane

FIG. 2. A portion of internal elastic membrane adjacent to a fenestration is seen in this micrograph. The majority of the internal elastic membrane has vanished, but this remnant has normal structure and staining characteristics with an amorphous, electron-lucent matrix containing numerous fibrillar components. I = intima. M = media. Lead citrate and uranyl acetate. $\times 13,000$.

FIG. 3. This section of internal elastic membrane is only a few microns away from Fig. 2 and has been prepared similarly except for the addition of phosphotungstic acid after staining with lead citrate and uranyl acetate. The matrix of the membrane stains densely. Collagen fibres also stain with the phosphotungstic acid. I = intima. M = media. $\times 13,000$.

extremely densely (Fig. 3). Short exposures to this agent demonstrated uniform darkening of both matrix and fibrils, rather than preferential staining of one element of the membrane. The morphology as well as the staining properties of the intact internal elastic membrane in the diseased artery seemed identical to the control.

The intima of both the diseased and the control artery was greatly thickened by collagen, reduplicated layers of elastica, and myointimal cells. These proliferative changes were more severe in the patient with arteritis, but no apparent qualitative differences within the intima were noted in the two subjects. Figure 4 illustrates the pseudo-replication of layers of elastica within the intima of the diseased artery. Two to five such layers were seen in some sections. Such replications were always considerably thinner, more convoluted, and more fragmented than the true elastic membrane, but had similar staining properties.

Myointimal cells had a broad spectrum of appearances from fairly typical, spindle-shaped, smooth muscle cells with abundant myofibrils and occasional 'attachment devices' to cells similar to fibroblasts, with polygonal shape and containing few fibrils. Well-preserved endothelial cells were rarely seen in electron microscopic sections of the diseased artery. No changes in endothelium were noted by light microscopy.

The most striking finding in the artery of the patient with temporal arteritis was the great number of multinucleated giant-cells located at the junction of the media and intima in areas where the internal elastic membrane was absent. All giant cells examined electron microscopically had similar nuclei and

cytoplasm. The nuclei were convoluted and usually concentrated near the periphery. The cytoplasm was distinctive because of the great abundance of mitochondria, lysosomes, and Golgi membranes (Figs. 5 and 6). Highly convoluted, almost microvillous, borders were another characteristic feature of giant cells. Mononuclear cells of similar cytoplasmic content were seen adjacent to giant cells and gave the appearance of coalescing to form giant cells. In the extracellular spaces at the periphery of giant cells, masses of fibrillar material were often present. This material often was surrounded partially by convoluted cytoplasmic processes of giant cells, as if undergoing phagocytosis, but was never actually seen intracellularly. Occasional polymorphonuclear leucocytes were also seen adjacent to giant cells and the fibrillar material (Fig. 7). The fibrillar extracellular substance, so prominent around giant cells, stained fairly densely with lead and uranyl salts (Fig. 7), but was even more densely stained by phosphotungstic acid (Figs. 5 and 8). This material was unlike normal elastica, collagen, and basement membrane in its structure and staining characteristics. Its nature is uncertain, but presumably it corresponds to the 'fibrinoid' or mucinous degeneration or fragmented elastica with altered characteristics noted by light microscopy. It had many of the features of fibrin.

DISCUSSION

The gross description of temporal arteritis was first made by Jonathan Hutchinson (1890). The first microscopic description was by Horton, Magath, and Brown (1932). Definitive surveys of the patho-

FIG. 4.

FIG. 4. Proliferation of collagen and elastica are prominent within the intima in this micrograph. The densely stained lamina parallels the internal elastic membrane and seems to be an abortive duplication of it. This has been described in atherosclerotic arteries (Balis, Haust, and More, 1964) as well as within doubly ligated segments of arteries experimentally (Buck, 1961). Lead citrate, uranyl acetate, and phosphotungstic acid. $\times 3,000$.

FIG. 5.

FIG. 5. Approximately one-quarter of the cross-sectioned area of a multinucleated giant cell is seen in this micrograph. The convoluted nuclei, cytoplasm packed with mitochondria and lysosomes, and darkly stained fibrillar material in the extracellular spaces surrounding the cell are characteristic of all giant-cells seen in this study. Lead citrate, uranyl acetate, and phosphotungstic acid. $\times 4,500$.

FIG. 6.

FIG. 6. This micrograph shows the cytoplasm of a giant cell in more detail. Mitochondria, lysosomes, and Golgi membranes are prominent. Lead citrate and uranyl acetate. $\times 24,000$.

logical findings at biopsy and necropsy have been published by several workers (Harrison, 1948; Heptinstall *et al.*, 1954; McCormick and Neuberger, 1958). The gross features of the disease are not characteristic. The vessels are enlarged and nodular, and have little or no lumen. Histologically, the lesions are distinctive, but may vary from one region to another, so that the lesion is more intense and involves more of the circumference in some sections than in ones taken a few millimetres distant. Thrombosis is the exception and plays little part in the development of the lesion. The intima is always thickened, thus narrowing the lumen.

The adventitia is usually invaded by mononuclear and occasionally, polymorphonuclear inflammatory cells, often cuffing the vasa vasorum. Giant cells are rarely seen in this layer. Fibrous proliferation is frequent in the adventitia. Nerves are sometimes seen to be involved in this fibrosis, which probably accounts for the pain, and its relief by biopsy of the artery. The medial changes are dominated by the presence of giant cells, often located near areas of breakdown of the internal elastic membrane, but occasionally remote from such areas. They are of

the foreign-body type and vary from small cells with two or three nuclei up to masses of 100μ with many nuclei. There is also an invasion of the media by mononuclear cells resembling histiocytes or macrophages, which seemingly are the precursors of the giant-cells. Giant-cells are occasionally absent, probably because of unfortunate planes of section or due to their disappearance in old lesions. Loss of muscle fibres is often noted. Fibrinoid necrosis is infrequently found. When present, it may indicate a very early lesion or it may represent an overlap of this disease with polyarteritis nodosa.

The internal elastic membrane is usually severely degenerated, but this may occur in a segmental or partially circumferential distribution. It often is unduly refractile and loses its affinity for elastic stains. The intimal proliferation can often be divided into two layers. The inner thicker one consists of mucoid, oedematous material with a fibrocytic matrix. The outer zone (nearer the elastic membrane) has much more inflammatory response, with occasional eosinophils and polymorphonuclear cells. Eosinophilic amorphous material is sometimes noted here.

Conjectures as to the aetiology of this disease have been numerous. Because of the fever, leucocytosis, enlarged cervical nodes, elevated sedimentation rate and generalized illness of the patient, an infective process has been strongly suggested, but no organism

FIG. 7. At the right side of this micrograph is an apparently mononuclear cell with nucleus and cytoplasm identical with those found in giant cells, and therefore, it is presumed to be a precursor of a giant cell. This cell has many small peripheral cytoplasmic processes which engulf some fragments of extra-cellular fibrillar material (arrow). This is also characteristic of multinucleated giant-cells. A leucocyte is on the left. Lead citrate and uranyl acetate. $\times 7,500$.

FIG. 8. This micrograph demonstrates the ultrastructural details of the extracellular fibrillar material found at the periphery of giant-cells. It is similar to fibrin. It could represent altered internal elastic membrane. Lead citrate, uranyl acetate, and phosphotungstic acid. $\times 53,000$.

have ever been identified with certainty. Certainly no evidence of micro-organisms or virus particles was seen in this ultrastructural study. Because of features in common with polyarteritis nodosa and the association of giant-cell arteritis with polymyalgia rheumatica an allergic or 'autoimmune' aetiology has been postulated. The ultrastructural features of this case are compatible with this mechanism, but do not prove it. The pathognomonic features of multinucleated giant cells, disruption of the internal elastic membrane, and the presence of mucinous or fibrinoid degeneration of the intima near the internal elastica membrane are demonstrated well by electron microscopy.

The intimal proliferative changes found in this patient are identical with those of ordinary atherosclerosis (Geer, McGill, and Strong, 1961; Balis, Haust, and More, 1964; Flora, Pahl, and Nelson, 1967). Although normal internal elastic membrane is seen in this study and no transitional zones of alteration are noted, it is possible that favourable sections of such areas of the membrane were not obtained. The intact membrane comprises only a small portion of the entire wall of the artery, as most of the membrane is absent in the sections examined electron microscopically.

Whether the altered internal elastic membrane stimulated the giant-cell response, or whether the giant cells are responsible for the disruption of the membrane cannot be stated from the present observations, but the latter is unlikely because some instances of disruption of the membrane have been noted by other workers with almost no giant cells present (Harrison, 1948). The fibrillar material surrounding giant cells may represent degenerated internal elastic membrane. It was hoped that phosphotungstic acid staining would delineate elastica clearly, so it could be distinguished from fibrin and other substances. But this stain is not completely specific for elastica. It also stains collagen and probably fibrin to some extent. So it cannot be stated with certainty that all the material surrounding giant cells is indeed altered internal elastic membrane. It probably represents an altered protein which is important in the pathogenesis of giant-cell (temporal) arteritis. Its close resemblance to fibrin (Haust, Wyllie, and More, 1965) could indicate exudation of serum protein and a possible immuno-

logical reaction. Immunofluorescence microscopy and serial ultrastructural observations from the onset of the disease might give valuable information concerning pathogenesis.

SUMMARY

Ultrastructural findings in a case of giant-cell (temporal) arteritis included inflammatory cells within the adventitia, marked intimal proliferation caused by increased elastica and collagen, as well as a large number of myointimal cells. The most distinctive feature was the disruption of the internal elastic membrane with many multinucleated giant cells located in the media and intima adjacent to areas of such disruption. The highly convoluted periphery of giant cells partially engulfed masses of fibrillar material which stained densely with phosphotungstic acid. The nature of this material is uncertain. It may represent altered internal elastic membrane or deposits of fibrin.

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