Senile Macular Degeneration
The Involvement of Giant Cells in Atrophy of the Retinal Pigment Epithelium

Philip L. Penfold, Murray C. Killingsworth, and Shirley H. Sarks

Senile macular degeneration (SMD) is a leading cause of registered blindness in the United States and other Western countries. Loss of central vision develops as a result of atrophy of the retinal pigment epithelium or subretinal neovascularisation. The histopathology of the atrophic form of SMD has not been extensively studied. This paper illustrates at the light and electron microscope level the involvement of giant cells and mononuclear phagocyte series (MPS) cells in the pathology of the atrophic form of SMD. Additional features including pigment clumping and detachment of the retinal pigment epithelium at the advancing edge of the lesion are illustrated. Giant cells and MPS cells are typical features of granulomatous inflammation, and results suggest that they may play a role in the pathogenesis of SMD.


Numerous surveys have shown that senile macular degeneration (SMD) is one of the leading causes of registered blindness in the United States and other Western countries.1 Loss of central vision develops either gradually as a result of atrophy of the retinal pigment epithelium, or rapidly following the complications of subretinal neovascularisation. The majority of cases of SMD are of the atrophic form,1-3 which in the absence of subretinal neovascularisation is considered to be the natural end stage of the disease.4 While in some cases laser photocoagulation provides a means of treating neovascular membranes, no treatment is available for the atrophic form of the disease.

Previous reports have described histiocytic5 and giant cells6'7 in association with breaks in Bruch's membrane and subretinal neovascular membranes. Additionally, we have shown that inflammatory cells including macrophages, fibroblasts, and mast cells play a role in the pathogenesis of the neovascular form of SMD.8'9 Although the ultrastructural features of the normal human eye are well established,10 the atrophic form of SMD has not been extensively examined. Studies based on wax histology1'6 and electron microscopy11 have described some features of the atrophic lesion. Our findings at both the light and electron microscope level confirm the previous results and illustrate additionally the involvement of giant cells and mononuclear phagocyte and series (MPS) cells in the atrophic form of SMD.

Materials and Methods

This study is based on the ultrastructural findings in seven postmortem eyes from six patients demonstrating the atrophic form of SMD. The eyes were taken from male and female patients whose ages at death ranged from 72 to 86 yr. A full ocular examination was performed on all patients including fundus photography and fluorescein angiography. Patients with blood dyscrasia or other forms of ocular disease were excluded. Eyes were enucleated within 6 hr of death by a standard procedure.

For electron microscopy, eyes were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). The eyes were opened in the horizontal plane, and an 8-mm corneal trephine was used to remove the posterior region including the macula and optic nerve. The retina and choroid were dissected away from the sclera, and the trephined disc was then further subdivided to produce blocks suitable for electron microscopy. The blocks were washed in fresh buffer and postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) or 3 hr followed by 2% uranyl acetate staining for 1 hr. Finally the blocks were dehydrated in graded alcohols and acetone and embedded in Spurr's low viscosity resin and cured for 8 hr.
Fig. 1. Figures 1-3 are light micrographs of toluidine blue stained 0.5 μm sections. They were taken at 10-μm intervals at the macula and illustrate the relationship of a giant cell (G) to an area of atrophy of the retinal pigment epithelium. The multinucleate giant cell is closely applied to the inner surface of Bruch’s membrane (B) and is associated with pigment clumps (P); the RPE is entirely atrophied (A) (final magnification ×480).

Initial examination of the material was carried out at the light microscope level using 0.5 μm serial sections stained with toluidene blue. In selected areas ultrathin sections were cut and stained with uranyl acetate and Reynold's lead citrate. Photomicrographs were taken with a Philips 300 Electron Microscope (Philips: Eindhoven, The Netherlands) at an accelerating voltage of 80 kV.

Results

Giant cells were found in 4 of the 7 atrophic eyes examined histologically. Figures 1–3 are light micrographs taken at 10-μm intervals from a single specimen at the macula. They demonstrate the relationship of a giant cell to the edge of the atrophic area (see figure legends). The same giant cell, at different stages of sectioning, is shown in each case. The figures illustrate the changing form of the multi-nuclear structures within the giant cell. Giant cells appear to form at the edge of the area of atrophy, usually associated with clumps of pigment. The photoreceptors showed progressive degeneration related to their proximity to the atrophic area. At the edge of the atrophic area, the external limiting membrane and photoreceptors disappeared entirely, together with the outer nuclear layer and retinal pigment epithelium (RPE). Within the lesion the outer plexiform layer was in direct contact with residual pigmented material and macrophage series cells lying on Bruch’s membrane. In none of the specimens examined were breaks in Bruch’s membrane discovered.

The ultrastructural features of the giant cell shown in Figures 1–3 are illustrated in Figures 4–8. A second example of a multinucleate giant cell is shown in Figures 9–10, taken from a second specimen in which the atrophic lesion was at a more advanced stage. In all cases the giant cells were in close contact with Bruch’s membrane, although there was no evidence to suggest that Bruch’s membrane was being degraded by them. The giant cells contained internalised pigment associated with secondary lysosomes (Figs. 3, 8, 10). The pigment appeared to be melanin-derived from degenerate retinal pigment epithelium. Figure 4 is a low magnification electron micrograph of the giant cell shown in Figure 2 and confirms that the giant cell is a single cell. Figures 5 and 6 illustrate the granular nature of the cytoplasm and the fimbriate nature of the plasma membrane. The close association between the giant
The relationship of the same giant cell as in Figure 1 (G) to the edge of the area of atrophy is illustrated in this figure. Within the area of atrophy (A) the photoreceptors are completely degenerate. At the edge of the area of atrophy (E) degenerate photoreceptors (R) are apparent. The giant cell is closely associated with macrophage series cells (M). Figures 4, 5, 6, 7, and 11 present an ultrastructural view of the giant cell and macrophage series cells taken immediately after the 0.5-μm section shown in this figure (final magnification ×480).

The edge of the area of atrophy (E) is still apparent (final magnification ×480).
Fig. 4. A low power electron micrograph of the giant cell (G) corresponding to the light micrograph shown in Figure 2. At this level the plasma membrane appears fimbriated (F). Only one nuclear lobe (N) is apparent, although the cell was multinucleate in earlier sections (Fig. 1). A cell process and nucleus from a macrophage (M) are partially enclosed in the cytoplasm of the giant cell (see Fig. 6). The cells are closely applied to the retinal surface of Bruch's membrane (B). There is no apparent thinning of Bruch's membrane associated with the giant cell (final magnification ×2856).

cell and the macrophage series cell shown in Figure 6 and the pigment content of the giant cell shown in Figure 8 are discussed below in the context of their possible role in giant cell formation.

Mononuclear phagocyte series (MPS) cells were found in all of the seven specimens examined at the light and electron microscope level. The MPS cells were

Fig. 5. A higher magnification view of the tip of the giant cell relating to the outer edge of the area of atrophy. The fimbriate plasma membrane (F) is associated with bundles of microfilaments (MF) seen in cross section (final magnification ×6000).

Fig. 6. A higher magnification view of the tip of the giant cell relating to the inner edge of the area of atrophy. Numerous lysosomal granules (L) are discernible within the giant cell cytoplasm. The plasma membrane of the macrophage (M) and the giant cell are clearly distinct at this level of resolution (final magnification ×6150).
almost always situated between the basement membrane of the RPE and the inner collagenous layer of Bruch's membrane. Individual MPS cells were occasionally found similarly situated outside the area of atrophy. Within the atrophic lesion they were usually present in the form of a closely related monolayer of cells. In most cases MPS cells were apparent in the first
sections taken from each specimen. Subsequent serial sectioning revealed that these cell types occurred throughout the area of atrophy. While some cells in this area were clearly MPS cells (Fig. 11), other cell types were of uncertain lineage (Fig. 12) and were referred to as melanophages.

Low numbers of fibroblasts associated with areas of collagen deposition were apparent in some specimens (Fig. 13). These cells tended to appear individually; there was no evidence of large multilayered arrangements of fibroblasts and collagen usually associated with disciform lesions. Packages of non-nucleated pigmented material were frequently observed at the edge of the atrophic lesion (Figs. 12, 14). Individual cells, at least some of which were detached RPE cells, were observed in the subretinal space (Fig. 14). The potential relationship of these observations to the pathogenesis of SMD is considered in the discussion.

**Discussion**

Multinucleate giant cells are thought to arise as result of macrophage fusion. Their function is uncertain at the present time, although their formation appears to be related to the presence of a poorly degradable irritant or stimulus. They probably form as a consequence of attempted simultaneous phagocytosis, during which two or more macrophages attempt to ingest the same material. Giant cells are a common feature of granulomatous inflammation. The results of this study il-
Fig. 14. Unattached melanin-containing cells (MC) were often observed in the subretinal space associated with areas of atrophy. Also illustrated here are the remains of the photoreceptors (R), an intact layer of retinal pigment epithelial cells (RPE), Bruch's membrane (B), and the choriocapillaris (CC) (final magnification X1572).

Illustrate many of the processes typical of giant cell formation. All specimens of the atrophic form of SMD examined in this study contained MPS cells, situated upon the inner collagenous layer of Bruch's membrane. They were usually arranged in a continuous line, which often terminated with a giant cell within the atrophic lesion. The giant cells contained areas of phagosome formation surrounded by interdigitating cell processes. It is tempting to speculate as a result of these observations that the MPS cells and subsequently giant cells formed in response to an inflammatory stimulus associated with Bruch's membrane. Strong candidates for the provision of such an inflammatory stimulus would be the basal laminar deposit (BLD) defined by Sarks, undegraded pigment derived from the RPE and/or a constituent of Bruch's membrane itself.

The giant cells described in this report appear to be derived from MPS cells rather than cells of other lineages, for example RPE cells. This proposal is supported by a number of observations. Giant cells of known MPS cell origin have distinctive ultrastructural features. In addition to being multinucleate, they typically have a fimbriate plasma membrane, prominent bundles of microfilaments, and numerous lysosomal bodies and membrane bound vesicles. These features have been identified in Figures 1, 5, and 6. Mechanisms whereby macrophages fuse to form giant cells have been widely studied. The cell types involved in the process of fusion are indicative of the origin of the giant cell. The cells implicated in the formation of the giant cell, shown in Figures 1–6, 11, have many of the features of macrophages. The cell described in Figure 11 has no melanin granules, numerous mitochondria, and extensive amounts of rough endoplasmic reticulum; the nucleus has peripheral and central condensations of heterochromatin, the lysosomal granules are small, occasionally dumbbell-shaped, and resemble lysosomal granules found in the giant cell (Figure 6). We believe that these cells are distinct from RPE cells in that they do not contain melanin, contain a distinctive form of lysosomal granule and a different distribution of heterochromatin and subcellular organelles. We intend to apply macrophage specific antisera and immunohistochemical techniques to define further the cell types involved.

A prominent histological feature of geographic atrophy, also visible ophthalmoscopically, is the accumulation of pigment bordering the lesion. A number of factors may contribute to the appearance of these formations. Layering and detachment of the RPE was most pronounced in this region. Pigment containing MPS cells and giant cells were frequently observed related to the outer edge of the lesion. Accumulations of pigment and associated debris may adversely affect photoreceptor survival and thus accelerate the rate of spread of the degenerative process. These findings may be related to clinical observations suggesting that geographic atrophy often develops in areas of coarse pigmented mottling.

SMD occurs as a spectrum of histopathologic forms. At one end of the spectrum is the disciform lesion in which the most dominant features are subretinal neovascularisation, breakdown of Bruch's membrane, and fibrous tissue formation. At the other extreme is the atrophic lesion described here. Intermediary forms also occur in which both neovascularisation and atrophic degeneration are present simultaneously. In most cases, however, the disciform response is clearly distinguishable from the atrophic lesion by the absence of fibrovascular tissue formation. We have shown elsewhere that giant cells and MPS cells are involved in the pathogenesis of the disciform lesion. The precise role of these cell types in the pathogenesis of the atrophic form of SMD remains to be elucidated. However, their close association with the boundary of the area of atrophy suggests that they may actively influence the rate of development of the atrophic lesion.

Key words: macular degeneration, geographic atrophy, giant cell, macrophage, ultrastructure
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References