Ultrastructural pathology of melanomalytic glaucoma

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SUMMARY A patient presented with a heavily pigmented ciliary body tumour accompanied by diffuse pigmentation of the iris and chamber angle and secondary open angle glaucoma. The case was diagnosed clinically as malignant melanoma and melanomalytic glaucoma, and the eye was enucleated. Routine histopathology, immunohistochemistry, and ultrastructural studies revealed the tumour as being of the spindle cell type with extensive necrosis accompanied by a massive macrophagic response. The iridocorneal angle was heavily infiltrated by large pigmented cells, which were identified as being predominantly melanomacrophages. This is consistent with the few previous descriptions of this condition and confirms the view that the aqueous outflow obstruction is simply a mechanical process. Most of the native trabecular endothelial cells, which had also phagocytosed large quantities of melanin, still maintained their normal relationship to the trabeculae, though there were signs of activation. Immunohistochemistry and electron microscopy revealed the presence of moderate numbers of tumour cells on the iris surface and in the trabecular meshwork distant from the primary tumour site. The importance of these findings to the management of anterior uveal melanomas with secondary open angle glaucoma is discussed.

Secondary open angle glaucoma in cases of malignant melanoma of the anterior uveal tract is due to cellular obstruction of the outflow pathways.¹ Tumour cells which have been shed from the neoplasm may be carried in the aqueous and become trapped within the trabecular meshwork, or the cells may directly proliferate within the chamber angle and meshwork as in ring melanomas. A different pathogenetic mechanism appears to underlie 'melanomalytic glaucoma',² a rare condition in which spontaneous necrosis of an iris or ciliary body melanoma is accompanied by a massive macrophagic response and raised intraocular pressure. Large melanin laden macrophages block the uveal surface and intertrabecular spaces of the outflow system. The term 'melanomalytic glaucoma' was coined by Yanoff and Scheie² because they considered the disease mechanism to be similar to phacolytic and haemolytic glaucoma, in which obstruction of outflow pathways is due to macrophages laden with lens material and haemorrhagic debris respectively.³⁴

In the only previous electron microscopic investigation of the outflow system in a case of melanomalytic glaucoma¹ the majority of the cells causing obstruction were believed to be exogenous macrophages. In addition Van Buskirk and Leure-du Pree¹ observed melanophagocytosis by native trabecular endothelial cells which were also becoming detached from the trabeculae, and they postulated that these cells may have played a role in blocking the aqueous outflow pathways. Tumour cells were not found in the angle or trabecular meshwork in this case.

We here describe a case which was considered on clinical and histopathological grounds to be melanomalytic glaucoma. However, immunohistochemistry and transmission electron microscopy revealed the presence of tumour cells within a predominantly melanomacrophagic infiltration. The aim of the study was to establish morphological criteria for the identification of melanomacrophages, trabecular endothelial cells, and tumour cells within the outflow pathways.

Materials and methods

CLINICAL DETAILS

In June 1982 a 60-year-old Caucasian male presented to his general practitioner with blurred vision in the left eye. He was subsequently seen by an ophthalmologist and was found to have heterochromia iridis, a pigmented mass in the peripheral iris and ciliary body in the superonasal quadrant, and an intraocular
pressure (IOP) of over 50 mmHg. The patient was referred to Glasgow (September 1982), where investigation led to the diagnosis of ciliary body melanoma and melanomalous glaucoma. The tumour encroached locally on to the posterior corneal surface, the iris, and the trabecular meshwork between the 9–11 o'clock positions, but the iris and trabecular meshwork throughout 360° were lined by a carpet-like sheet of pigmented tissue. The lens, vitreous, retina, and choroid appeared normal. The optic disc showed 0-6 cupping. The IOP remained high (>50 mmHg) despite thorough antiglaucoma therapy.

The possibility of conservative management was considered, and a trabeculectomy and iridotomy were performed to reduce the IOP and also to determine the nature of the infiltration of the angle distant from the main tumour mass.

Positive identification of viable tumour cells on a sponge used to collect the aqueous and in the trabecular meshwork led to the decision to enucleate the eye one week later. The globe was fixed in 2% cacodylate buffered glutaraldehyde.

The patient is alive and well at the present time.

PATHOLOGICAL EXAMINATION AND IMMUNOHISTOCHEMISTRY

An oblique pupil-optic nerve block and small segments from the superior and inferior calottes were taken for routine paraffin histopathology (Fig. 1), with conventional staining and bleaching techniques.

The heavy pigmentation of the cells in the anterior segment rendered immunohistochemical studies by the peroxidase-antiperoxidase (PAP) procedure unsatisfactory. Accordingly serial sections were mounted on gelatin and chromalum coated slides and were treated with acidified potassium permanganate (0-25%) and bleached in 2% oxalic acid. The reagents employed were α, antitrypsin (α, AT), α, antichymotrypsin (α, ACT) (Dakopatts, Denmark) and anti-S100 antibody (Dakopatts, Denmark). The antigen-antibody complex was successfully visualised with amino ethyl carbazole, with haematoxylin as a counterstain. DAB, B galactosidase, and 4 chloro-1-naphthol gave unsatisfactory results.

The calottes were cut in meridional slices as shown in Fig. 1.

PREPARATION FOR MICROSCOPY

Representative blocks of limbal tissue were processed in the conventional fashion and embedded in Araldite. Semithin sections (1–2 μm) were stained with toluidine blue. Ultrathin sections (50–90 nm) were stained with uranyl acetate and lead citrate and examined in a Philips 301 transmission electron microscope. The blocks of limbal tissue, cornea, and iris (Fig. 1a) taken for scanning electron microscopy (SEM) were critical point dried, mounted on aluminium stubs, coated with gold, and examined in a JEOL JSM T200.

Results

ROUTINE HISTOPATHOLOGY

The tumour, which measured 6×5×5 mm (Fig. 1b)

Fig. 1 (a) Schematic diagram of the eye to illustrate the position of the tumour in the superonasal quadrant. The cuts made to remove the pupil-optic nerve block for routine histopathology are indicated by the horizontal broken lines. The trabeculectomy site is represented by the broken box. Tissue blocks for SEM were taken from the areas adjacent to the asterisks. The position of the individual meridional slices of limbal tissue taken from the superior and inferior calottes for ultrastructural studies are indicated (A1–5, B1–5, C1–5, D1–5). (b) Macroscopic view of the globe after removal of the superior calotte showing the ciliary body melanoma and adjacent lens opacity, the conjunctival bleb (C) over the site of the trabeculectomy, and fine pigment dusting of the zonular fibres (arrow). N = nasal, T = temporal.
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was a malignant melanoma of spindle cell type. It showed extensive areas of necrosis (Fig. 2) filled with debris and melanomacrophages. Identifiable tumour cells were found only on the posterior corneal surface, in the iris root, and in the pars plana (Fig. 2a). A mixture of macrophages and viable tumour cells (Fig. 2b) extended on to the iris surface and around the chamber angles; on the temporal aspect they had infiltrated the scleral drainage flap, the suprachoroidal space, and conjunctival flap. The trabecular meshwork in paraffin blocks from the superior and inferior calottes contained heavily pigmented cells, which could not be accurately identified even in bleached sections.

The lens showed a secondary cataract (Figs. 1a, 2a), and the retina and optic nerve showed changes typical of secondary glaucoma.

**Immunochemistry**

The reagents (α, AT, α, ACT) for the identification of macrophages showed the profusion of these cells in the chamber angle and within the trabecular meshwork (Fig. 2c). Cells staining positively with anti-S100 antibody could be identified scattered within the inner meshwork and within the infiltrate on the anterior iris surface (Fig. 2d).

**Ultrastructural Pathology**

**Tumour morphology**

In both blocks taken from the edge of the tumour (A1 and A2 in Fig. 1) islands of viable spindle B melanoma cells were found along the posterior corneal surface (Fig. 3a). The tumour cells had a low melanin content, which contrasted with the heavily pigmented macrophages, and showed the characteristic features of spindle melanoma cells, namely a large ovoid nucleus, which was sometimes indented, with a variable chromatin pattern and coarse nucleolomema, a large nuclear-cytoplasmic ratio, premelanosomes of types I–III, lipid inclusions, and

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**Fig. 2** (a) Light micrograph of routine histological preparation (H and E) showing the heavily pigmented tumour in the ciliary body and iris. Islands of identifiable tumour cells were noted at the positions indicated (arrows). (b) Bleached preparation of the tumour showing spindle melanoma cells among larger rounded cells. (c) Immunohistochemical demonstrations of large numbers of α, antichymotrypsin-positive cells (macrophages), and (d) S100 positive cells (tumour cells) in bleached paraffin sections of the chamber angle from the inferior calotte. Note the occasional S100 positive cell in the uveal meshwork (straight arrow) and on the iris (curved arrow) beneath the rounded macrophages. T = trabecular meshwork, cb = ciliary body, ut = uveal trabeculae. Magnifications: (a) ×12.5, (b) ×260, (c) ×63, (d) ×260.
abundant free ribosomes (Fig. 3a). Within the necrotic areas of the tumour there were large quantities of free melanin granules, lipid-like inclusions, lysed cell organelles, and membranes (Fig. 3b). Large rounded melanomacrophages were identified within but more especially around the necrotic zones (Fig. 3b), and there was evidence of death of macrophages in situ. This was manifest as rupture of plasma membranes and nuclear fragmentation, but in addition large compound melanophagolysosomes identical to those found within the cytoplasm of macrophages were distributed in the extracellular space among the rest of the cell debris (Fig. 3b).

In other areas of the tumour viable melanoma cells were interspersed among large numbers of giant melanomacrophages (Fig. 4a). There was a wide variation in the morphology of the melanoma cells. The melanin granules occurred either as oval individual premelanosomes in various stages of maturity, or as uniformly electron-dense, round, mature melanin granules (Figs. 4b-d). Even in those cells containing mature melanosomes there was a great deal of variation in their density within the cell cytoplasm (Figs. 4a, d). The melanin within the macrophages was characteristically within large (0.3–7.0 μm) membrane bound lysosomal complexes (Fig. 4a). These melanophagolysosomes contained numerous melanosomes in various stages of melanogenesis, but occasionally there were also individual melanosomes within some macrophages.

The anterior surface of the iris in all the specimens studied was carpeted by large, globular, pigment laden macrophages several layers thick resting on a layer of tumour cells (Fig. 5).

Chamber angle and aqueous outflow pathways
The iridocorneal angle recess in all the blocks investigated by light microscopy and scanning and transmission electron microscopy contained large numbers of giant globular melanomacrophages. This was particularly evident in the blocks from the inferior angle (Figs. 6, 7). In the majority of the blocks studied these cells obstructed the intertrabecular spaces on the inner surface of the uveal meshwork (Figs. 6, 7a). The anterior ciliary muscle face and iris root as well as all the available inter- and intratrabecular spaces in the trabecular meshwork were also penetrated by heavily pigmented macrophages (Figs. 6a, b), which often assumed very large proportions (up to 50 μm diameter). Complex microvillous processes and ruffles were numerous on the surface of these cells and were observed surrounding extracellular melanosomes (Figs. 7b, c). Their cytoplasm contained large quantities of phagocytosed melanin within compound membrane-bound phagolysosomes (Figs. 7a, b, c). The large pale nucleus of the macrophage was eccentrically located and occasionally contained two nucleoli. Frequently the only identifiable cytoplasmic organelles apart from melanin were the Golgi apparatus (Fig. 7b) and some small swollen mitochondria; binucleate macrophages were occasionally observed (Fig. 7a). Lysis of
macrophages was observed as rupture of the cell membrane, which was lined internally by melanosomes. Melanin granules were extruded through the disrupted membrane (Fig. 8) and released into the anterior chamber and outflow system.

Those giant melanomacrophages which had penetrated into the trabecular meshwork had a more complex configuration, as they conformed to the intertrabecular spaces in the uveal, corneoscleral, and cribriform layers (Figs. 6a, 7a, 9a). In all the blocks the meshwork also contained melanoma cells and endogenous pigmented trabecular endothelial cells (TECs) (Fig. 9b). Melanoma cells were found either singly or in small aggregates and were present in reasonable numbers within the outflow tissues both close to and distant from the main tumour mass (Fig. 9b).

A large proportion of the melanin seen in the trabecular meshwork by light microscopy was found to be present within the TECs. The melanin granules
Fig. 5 (a) Scanning electron micrograph of the anterior iris surface, which is carpeted by large numbers of rounded melanomacrophages. (b) higher magnification shows that some cells have the characteristic 'ruffled' plasma membrane of macrophages (straight arrow). Other cells have rounded granules, probably melanin, both on their cell surface (curved arrow) and directly beneath the plasma membrane, giving the cell a nodular appearance. Magnifications (a) × 130, (b) × 2500.

Fig. 6 (a) Light micrograph (block D1) and (b) SEM of the iridocorneal angle in the inferotemporal quadrant. Large numbers of giant melanomacrophages (MP) fill iridocorneal angle and obstruct the openings in the uveal meshwork as well as most of the intertrabecular spaces in the corneoscleral meshwork and cribriform layers. Note the globular appearance of these cells while still in the anterior chamber; however, they assume more complex shapes as they pass between the trabeculae (arrowheads). A binucleate macrophage is indicated (arrow). SC=Sclermm's canal, ut=uveal trabeculae. Magnifications: (a) ×300, (b) ×1000.
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Fig. 7 Electron micrographs of the giant melanomacrophages in the iridocorneal angle and uveal meshwork (block D). (a) Clearly illustrates the extent to which the openings in the uveal meshwork are obstructed. Some uveal trabeculae are denuded of TEC cover (small arrows). In some cases they are surrounded by macrophages (MP) or tumour cells (large arrow). Some TECs have prominent nucleoli. (b) and (c) Details of the melanin laden macrophages. Microvillous projections of the macrophages are clearly visible and at higher magnification appeared to be in the process of phagocytosing a free melanosome. An area of membrane specialisation whose function is unknown was associated with this area of the cell. Intracytoplasmic melanin is mostly within large complex membrane bound phagolysosomes (arrow) which contained stage III and mature melanosomes. Magnifications: (a) ×900, (b) ×2800, (c) ×15,000.

In regions close to the tumour and in the inferior quadrants, where the obstruction by macrophages and tumour cells was most marked, there were denuded and fused trabeculae (Fig. 9a). The connective tissue components of the trabeculae showed the normal morphological appearance of an eye in the sixth decade of life.

The TECs of the cribriform layer and to a less extent the lining endothelium of Schlemm's canal had also been active in phagocytosis of melanosomes (Fig. 10b). There was a paucity of giant vacuoles in the lining endothelium of Schlemm's canal, which is hardly surprising in view of the previous drainage procedure. The presence of red blood cells and plasma (Figs. 6a, 10b) in the lumen of the canal suggested that there had been a hypotensive situation in the anterior chamber at the time of fixation, causing a backflow of blood into Schlemm's canal.

There were some regions of Schlemm's canal especially close to the tumour which were closed by apposition of the inner and outer walls.
degree of tumour spread in such an eye might be aided by fine needle aspiration cytology or by biopsy, as in the present case. In either instance the identification of the various cell types may require routine paraffin histology to be supplemented by immunohistochemistry and electron microscopy. The present case provided an opportunity to document the morphological characteristics of the cellular infiltrate of the chamber angle in a case of melano-mytic glaucoma.

By conventional light microscopy the case bears many similarities to that described by Yanoff and Scheie except for the fact that serial sections revealed small foci of surviving tumour cells. While a specific antimelanoma antibody for immunohistochemical studies is not yet available, the use of anti-S100 antibody revealed a positive reaction in some cells distant from the primary tumour site. This confirms the results obtained by electron microscopy and it supports the evidence of a previous immunohistochemical study. The massive predominance of melanomacrophages, identified by conventional macrophage markers and electron microscopy, in the chamber angle and the trabecular meshwork, however, is consistent with the concept that aqueous outflow obstruction in this disease is simply a mechanical process.

The giant melanomacrophages in the present case shared many of the characteristics of the type I clump cells (of Koganei), namely cytoplasmic pseudopodia (or ruffles) around their circumference, lack of a basement membrane and cellular attachments, an eccentrically located nucleus, and an electron dense cytoplasm containing large complex melano-phagolysosomes and lipid inclusions. These are not features of neuroepithelial cells, which were once thought to be the precursors of all clump cells. Greater than normal numbers of clump cells have been observed in pigmented glaucoma, though these authors considered some of them to be pigment epithelial in origin owing to the larger size of the melanosomes. However, it is possible that melanosomes, released from degenerating iris pigment epithelium, could have been phagocytosed by macrophages, which may as a consequence appear similar to activated pigment epithelial cells. An increase in clump cells was also observed in an experimental animal model of debris clearance from the iris and iridocorneal angle. It seems therefore that macrophages are important to the clearance of released pigment in the anterior chamber, whether it originates from atrophy of the pigment epithelium or from a necrotic uveal melanoma. What is unclear is whether all these cells are derived from the resident population of histiocytic macrophages within the iris (clump cells) and trabecular meshwork or whether

Discussion

In general a raised intraocular pressure in a patient with an iris or ciliary body melanoma is a contra-indication to conservative management, though many separate mechanisms may be responsible for the raised intraocular pressure in these cases. If melano-mytic glaucoma is due only to blockage of trabecular tissue by macrophages engorged with melanin and not by tumour cells, treatment of the case by local surgical resection of the tumour may be justified. In the case reported here diagnostic trabeculectomy demonstrated the presence of viable tumour cells in the aqueous and in the angle remote from the tumour, indicating enucleation as the appropriate therapy. When a patient with a heavily pigmented ciliary body tumour presents with diffuse pigmentation of the iris and chamber angle, the following diagnoses should be considered—melanocyto ma, diffuse spread of malignant melanoma cells, or melano-mytic glaucoma. Establishment of the diagnosis and assessment of the
they are blood borne. In the present case it would seem from the numbers of macrophages involved in the response that they are most probably blood borne.

It is generally accepted that obstruction of the outflow pathways by macrophages is an important aetiological factor in many forms of secondary open angle glaucoma, including haemolytic, phacolytic,

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**Fig. 9** Electron micrographs of the corneoscleral meshwork at a site close to (a) block A, and remote from the main tumour mass (b) block D. In (a) note the variable morphological appearance of the melanoma cells (M), the fusion of adjacent denuded trabeculae, and the lack of intertrabecular spaces. Accumulations of long spacing collagen in the cortical zone of the trabeculae are evident (arrows). The TECs show some signs of activation, particularly increased quantities of rough endoplasmic reticulum (ER). In (b) note the presence of several melanoma cells (M) in the intertrabecular space. A mononuclear cell (H), probably histiocytic in nature, can be seen between the basement membrane of a TEC and the cortical zone of the trabeculae. Magnifications: (a) ×4000, (b) ×9000.
and ghost cell glaucoma.\textsuperscript{4} It seems likely from the absence of large quantities of free melanin in the tissues in the present case that outflow obstruction was due to cellular infiltration rather than an accumulation of particulate material.

The finding in the present and previous studies\textsuperscript{3} of melanin phagocytosis by TECs clearly confirms the accepted view that these cells have a large phagocytic potential.

Fig. 10  Electron micrographs of the trabecular meshwork in melanosomatic glaucoma. In (a) block A\textsubscript{3}, the TECs of the corneoscleral meshwork have phagocytosed large quantities of melanin granules, which are either in small secondary lysosomes or individually within the cytoplasm. Contact between adjacent TECs and with the connective tissue cores of the trabeculae (T) are maintained. Note the large nucleolus of the TEC. Part of a large necrotic macrophage (MP) within an intertrabecular space is also visible. (b) Block D\textsubscript{3} illustrates the phagocytic capacity of TECs in the cribriform layer and the lining endothelium of Schlemm's canal (arrow). Again note the large nucleolus in the TEC. Magnifications: (a) $\times$10 288, (b) $\times$5621.
capability which is essential to the function of the primate trabecular meshwork as a self-cleansing filter. Melanin phagocytosis by TECs has also been observed in pigmented glaucoma, a condition characterised by depigmentation of the iris stroma and epithelium associated with pigment dispersion in the anterior chamber. Evidence of TEC activation has been reported following traumatic hyphaema, and it would seem likely that this would also occur in haemolytic and phacolytic glaucoma, though further ultrastructural studies of these conditions are required to confirm this suggestion. In the present case of melanomalytic glaucoma the TECs showed slight evidence of increased metabolic activity and protein synthesis, namely prominent nucleoli and Golgi apparatus and increased amounts of rough endoplasmic reticulum. It did not appear from the morphological evidence that many of the TECs had become totally detached from their trabeculae and neighbouring cells and assumed the role of mobile 'endothelial macrophages' which has been observed following exposure of the outflow tissues to haemorrhagic debris. This may be due to differences in the character of the phagocytosed debris or the manner and length of exposure of the material to the cells of the trabecular meshwork. For example, in the present case the TECs may have only recently come into direct contact with free melanosomes as a result of macrophage lysis in situ. Van Buskirk and Leure-du Pree did not observe such cell lysis in their investigation of melanomalytic glaucoma. The case studied by these authors differed from the present one in that the patient was only known to have had secondary open angle glaucoma for two weeks prior to enucleation, whereas in our case four months had elapsed since initial diagnosis of raised IOP. It seems possible, therefore, that macrophage lysis in situ may be a feature of the later stages of the disease. Although Van Buskirk and Leure-du Pree observed 'endothelial macrophages,' they did state that there was difficulty in differentiating these from histiocytic macrophages.

The ultrastructure of TECs after activation approximates to those of tumour cells of low grade malignancy. The positive identification of melanoma cells within the trabecular meshwork at sites in the circumference of the chamber angle distant from the tumour mass is an important finding of the present study which has not been reported in previous studies. In the present case it seems unlikely that these cells had a role in obstruction of the aqueous pathways. However, melanoma cells in the outflow system may increase the risk of metastatic spread. Shields and Klintworth, in a histological study of 11 cases of anterior uveal melanomas, found some evidence of tumour cell infiltration of the iridocorneal angle in six, of which four also had raised IOPs. The mechanism of the glaucoma in their cases was secondary to direct tumour invasion of the outflow system and did not appear to be melanomalytic, though they positively identified melanin laden macrophages in the angle of three cases.

It is obvious that the outflow pathways present a possible route for tumour cells to enter the general circulation, and Shields and Klintworth have emphasised that indeed increased IOP may accelerate the extracellular spread of cells. They observed that three out of the four fatalities had glaucoma associated with their ciliary body melanomas. It is clear from our findings that tumour cells may be present in the angle distant from the tumour in melanomalytic glaucoma and may therefore be an important factor in the management of such cases.

We thank Professor W S Foulds for his kind co-operation with the study of this case. We are grateful to Mrs C Morris for artistic help and to Miss B Robinson and Mrs L Peedle for secretarial assistance.

References


Accepted for publication 21 February 1986.