Localization and Characterization of Major Histocompatibility Complex Class II-Positive Cells in the Posterior Segment of the Eye: Implications for Induction of Autoimmune Uveoretinitis

John V. Forrester,* Paul G. McMenamin,^ Ian Holthouse,† Lynne Lumsden,* and Janet Liversidge*

Purpose. To identify potential antigen-presenting cells in the choroid and retina of the normal rat eye, with a view to proposing a role for such cells in the induction and perpetuation of experimental autoimmune uveoretinitis, a model of human uveocortical inflammation.

Methods. Immunohistochcinical and electron microscopic studies using a panel of monoclonal antibodies were performed on frozen sections of the perfused-fixed normal Lewis rat eye, choroid whole mounts, and cytospin preparations of cells harvested from choroid/ciliary body explant cultures. In addition, time-lapse video recordings of migratory uveal tract cells in culture were taken.

Results. No major histocompatibility complex class II-positive cells were found in the normal Lewis rat retina. However, at least three populations of potential antigen-presenting cells were found in the uveal tissues of the eye: classical dendritic cells expressing high levels of major histocompatibility complex class II antigen; resident dendritiform macrophages, which were negative for major histocompatibility complex class II antigen, but expressed specific macrophage markers (ED2); and blood-borne macrophages (ED1) that had emigrated from the vasculature into the tissue compartment. In addition there were small numbers of cells expressing novel markers such as markers usually found only on macrophage subsets in splenic tissue (ED3) and a recently described marker for veiled dendritic cells (OX62). Dendritic cells and resident dendritiform macrophages closely interacted with each other and with tissue cells, particularly retinal pigment epithelial cells.

Conclusions. The posterior uveal tract is richly populated with classical dendritic cells expressing constitutive high levels of major histocompatibility complex class II antigen. There are also several types of macrophages with the potential to modulate immune responses in the posterior segment. Interactions among these cells and with resident tissue cells such as retinal pigment epithelial cells are probably central to the initiation of (auto)immune responses in the posterior segment of the eye. Invest Ophthalmol Vis Sci. 1994;35:64-77

Experimental autoimmune uveoretinitis (EAU) is regarded as a model for human posterior uveitis. EAU is inducible in a variety of animal models by immunization with purified retinal antigens in complete Freund's adjuvant and is mediated by CD4-positive T lymphocytes. Presentation of processed antigen (peptide) to CD4-positive T cells is performed by antigen-presenting cells (APC) and requires interaction between APC surface major histocompatibility complex (MHC) class II antigen, complexed with peptide, and the T cell receptor on the CD4-positive T cell. Initiation of the immune response in experimental autoimmune uveoretinitis, therefore, requires appropriate APC function; however, the cells that perform this function in the eye have not been identified.

During the last several years, it has been suggested that in various forms of autoimmune disease, aberrant
expression of class II antigens by organ resident non-immune cells may be important in either the induction or the perpetuation of the immune response. In uveitis, class II antigen expression has been observed on retinal pigment epithelial (RPE) cells and retinal endothelial cells both in situ and in vitro, and there has been some evidence that these cells may present antigen in vitro. However, class II expression by nonprofessional APC has not consistently been associated with antigen presentation and indeed on some occasions has been linked with downregulation of the immune response or induction of T cell anergy because of lack of appropriate costimulatory signals. Of the recognized professional APC, dendritic cells are known to be the most potent presenters of antigen by several orders of magnitude and are also the only APC that can present antigen to resting or unstimulated T cells (for review see reference 8). They are therefore regarded as the most likely candidates to initiate an immune response.

Dendritic cells or their equivalent have been identified in several ocular tissues such as the conjunctiva, cornea, and anterior chamber angle as well as the anterior uveal tract. Most of these tissues, however, are linked either directly or indirectly (via aqueous veins) to a lymphatic system and are thus likely to be involved in lymphocyte trafficking to and from regional lymph nodes. Dendritic cells are normally absent from the brain, and it is likely that they are also lacking from the retina. The question, therefore, of how retinal antigenic peptides are presented to circulating autoreactive lymphocytes in experimental autoimmune uveoretinitis remains unclear. Recent studies have suggested that microglial cells, considered the resident macrophages in the central nervous system, may be important in antigen presentation in neural tissue but direct evidence of their role is awaited. MHC class II-positive cells have been reported in the choroid in inflammatory disease but because of difficulties with morphologic preservation of frozen choroidalretinal tissue, it is not clear from these reports whether the identified class II-positive cells were in the choroid or at the choroidretinal interface, and thus represented class II-positive RPE cells. Experience in other tissues has shown that the dendritiform shape and networklike arrangement are not revealed unless plan views of the tissue are taken. In addition, because the choroid tissue in these studies contained a predominance of blood borne cells, it remains possible that the class II-positive cells represented circulating monocytes or T/B cells.

In this study we circumvented several of these problems in an attempt to localize and characterize the class II-positive cells in the uveal tract and retina. We present evidence that these cells are true dendritic cells and resemble the interdigitating dendritic cells of splenic tissue. In addition we characterized several other "immune" cells in the choroid, including the non-class II-positive dendritiform macrophage. We suggest that these cells are important in the regulation of (auto)immune uveoretinal inflammatory disease.

**MATERIALS AND METHODS**

**Animals**

Adult Lewis rats (200 to 250 g) of both sexes were obtained from Olac Ltd. (Slough, UK), housed and fed and experiments were conducted according to Home Office (UK) regulations and to the guidelines described in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Each experimental section detailed here was conducted on several (at least six) occasions with four to eight rats used each time apart from the electron microscopy when three animals were used.

**Preparation of Tissues for Immunohistochemistry and Immunofluorescence**

To remove circulating class II-positive cells from the tissues, animals were perfused through the left ventricle by intracardiac catheterization with 200 to 300 ml of cold phosphate-buffered saline containing calcium and magnesium (PBS+, pH 7.4) followed by perfusion fixation with 100 ml cold ethanol. Samples of spleen, liver, lymph node, trabecia, and both eyes were then removed and immersed in cold ethanol for 30 minutes. The tissues were rehydrated in three changes of cold PBS+ (1 hour each) and then left overnight in PBS+/OCT at 4°C. Before rehydration the eyes were incised at the limbus to permit adequate rehydration without distortion of the normal ocular architecture. The tissues were then transferred to foil cups containing OCT and snap frozen in liquid N2-cooled isopentane. They were then stored at —80°C until used.

Whole-mount preparations of iris, ciliary body, and choroid were also prepared for immunocytochemistry and immunofluorescence. The tissues of anesthetized animals were perfused with 200 to 300 ml of PBS+ to remove circulating cells and the eyes were dissected by removal of the cornea, lens, and retina. The iris was removed with fine scissors and the choroid was dissected from the sclera as a single sheet using a flat, rounded blade. Both tissues were then floated onto small pieces of Millipore (Watford, UK) filter for immunocytochemistry or onto 200 mesh electron microscopy grids for immunofluorescence. The tissues were fixed for 20 minutes in cold ethanol and rehydrated in PBS+ before immunostaining.

**Tissue/Cell Culture**

Eyes were removed under sterile conditions from animals that had been perfused with 300 ml of warm
beled streptavidin (1:100 in PBS+ in bovine serum albumin). After washing with PBS+, the slides were incubated in biotinylated peroxidase incubation as for light microscopy. The tissues were then fixed in 2.5% buffered glutaraldehyde, and postfixed in 1% osmium tetroxide and embedded in araldite. No counterstain was used. Sections were cut, viewed in a Phillips electron microscope (Phillips Analytical, Cambridge, UK), and intentionally underexposed to highlight the immunostaining.

**Light Microscopy**

Tissue sections and whole-mount preparations were examined by light microscopy and immunofluorescence microscopy using a Leitz Orthoplan direct microscope. Samples stained for fluorescence were also examined in a BioRad confocal microscope (BioRad, Henel Hempstead, UK). This had the advantage that thick sections of tissue (> 20μm) and whole-mount preparations could be viewed without interference from out-of-focus fluorescent images. Direct measurements of areas of tissues could be made using the image analysis software. This permitted a rapid and accurate assessment of the number of cells per unit area expressing surface markers for the antigens studied. In addition, a concept of the shape of individual cells in three dimensions could be obtained by preparing a "Z series" of images of the cell, that is, a series of optical sections at fixed distances through the cell, usually at 2 to 4 μm. This also allowed an approximate estimate of cell size.

Cultured cells from choroid/ciliary body explants were also examined in small petri dishes by time-lapse video microscopy on the warm stage (37°) of an Olympus microscope which was fitted with a Sony charge-coupled device television camera (Sony UK Ltd., Staines, UK).

**Electron Microscopy**

Tissue blocks and whole-mount preparations from Lewis rats were incubated with OX6 monoclonal antibody diluted 1:50 in PBS+ for 1 hour, fixed in periodate-lysine-paraformaldehyde for 30 minutes, washed and processed through second antibody, sheep anti-mouse monoclonal antibody, and peroxidase incubation as for light microscopy. The tissues were then fixed in 2.5% buffered glutaraldehyde, and postfixed in 1% osmium tetroxide and embedded in araldite, No counterstain was used. Sections were cut, viewed in a Phillips electron microscope (Phillips Analytical, Cambridge, UK), and intentionally underexposed to highlight the immunostaining.

**RESULTS**

**Immunostaining of Tissue Sections**

Sections of normal iris, ciliary body, and choroid contained MHC class II-positive cells in the stroma of each tissue (Fig. 1, B to G). Most of the cells had either

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<td>OX62</td>
<td>Veiled dendritic cells</td>
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Table 1. Monoclonal Antibodies Used for Immunostaining of Ocular Cells
FIGURE 1. MHC class II–positive cells in frozen sections of iris, ciliary body, and retina/choroid after perfusion with phosphate-buffered saline–alcohol (see Methods). (A) Control tissue, stained with irrelevant monoclonal antibody, to identify tissue layers. GCL = ganglion cell layer; INL = inner nuclear layer; ONL = outer nuclear layer; ROS = photoreceptor rod outer segments; RPE = retinal pigment epithelium; Ch = choroid; Sc = sclera. (B) Iris tissue containing class II–positive cells (arrows). LC = lens capsule (C) class II–positive cells in the choroid (arrows) (D) High power view of class II–positive cells in the choroid (arrows). Note fine cytoplasmic processes and nonstaining RPE layer (curved arrow) (E) Extended ciliary body processes showing numerous intraepithelial (arrows) and stromal (arrowheads) class II–positive cells. (F) Oblique section of outer retina/choroid showing several dendritic cells in choroidal tissue. Note close interaction of dendritic cell processes (long arrow) with RPE cells (short arrows). (G) Section of peripheral retina/pars plana of the ciliary body showing an elongated, class II dendritic cell with fine extended processes (arrow).
a dendritic appearance with short, thick processes or had very long, veil-like processes that gave the cell somewhat indeterminate borders (Fig. 1D). Other cells were round with dense immunostaining. Cells in the ciliary body were distributed in the epithelium with fewer cells in the stroma (Fig. 1E). Oblique sections through the choroid indicated that class II-positive staining cells were numerous and occurred at all levels within the tissue from the suprachoroidal space to the immediate subpigment epithelial region (Fig. 1F).

Dendrites appeared to interject with the lower surface of the RPE but did not penetrate into the subretinal space. The density of class II-positive cells appeared to be slightly greater in the peripheral than in the posterior choroid with a preponderance of cells associated with major vessels. In addition, some very elongated cells with fine processes were observed in the peripheral choroid (Fig. 1G). No staining was observed on resident ocular cells such as retinal pigmented or ciliary pigmented and nonpigmented epithelial cells (Fig. 1, C and D). Controls using no primary antibody or an irrelevant antibody of similar isotype showed no background staining (Fig. 1A). At this stage, cells that expressed such high levels of class II and had a dendritiform structure were considered to probably represent true dendritic cells.

To confirm the phenotype of the class II–positive cells in uveal tissue, sections were stained with antibodies to other cells with the ability to express class II antigens namely macrophage-lineage cells (the ED series of antibodies), pan T cell (OX19/OX52), activated T helper cells and macrophage subset (W3/25) and B cells (MARD antibody; Table 1). Currently there is no antigens namely macrophage-lineage cells (the ED series of antibodies) to probably represent true dendritic cells.

Two antibodies are currently under study as dendritic cell markers, ie, OX62 and ED17, and these were included in this study. Sections of choroidal tissue that contained cells staining positively for class II dendritiform cells, also contained cells positive for ED1, although much more weakly, and for ED2. The distribution of the cells was different for the class II–positive cells (Fig. 2A), compared to the ED1- and ED2-positive cells. In addition, ED1-positive cells did not conform to a typical dendritic pattern being generally rounder (Fig. 2B), although ED2-positive cells did exhibit extensive but rather poorly defined processes (Fig. 2, C and D). ED-positive cells were usually less numerous in tissue sections, and were distributed around blood vessels or were located near the retinal periphery/pars plana region of the eye (Fig. 2, C and D). ED2-positive cells were often very elongated with veil-like processes and were sometimes difficult to distinguish morphologically from MHC class II (OX6-positive) cells. ED2-positive cells were also present in the ciliary body and iris (Fig. 2E). Small numbers of ED3-positive cells were also observed at the pars plana and in the posterior choroid (Fig. 2F). Double antibody experiments showed that the ED-positive staining cells formed separate populations from cells staining with class II antibody (Fig. 2H) and only very rarely were cells stained with more than one antibody.

Staining for B cells, CD4-positive and CD8-positive T cells, and pan T cells was uniformly negative. Although no W3/25 positive T cells were observed, weak staining of dendritic-shaped cells was observed in serial sections of tissues stained positively for class II cells (Fig. 2G). This is consistent with the known weak staining of dendritic cells for W3/25. Occasionally cells staining positively for CD11/CD18 adhesion integrins (ED7,8) were observed as were a few cells staining for OX62. No immunostaining was observed in retinal tissue using any of these antibodies.

Conventional and confocal fluorescent microscopy of tissue sections confirmed our findings. In addition, the extent of the dendritic projections on the class II–positive cells was made clear (Fig. 2I). Some cells spanned the thickness of the choroid but most were located around blood vessels. An intimate relationship of some dendritic cells was observed with the RPE, which remained uniformly nonfluorescent (see Fig. 2I).

Confocal scanning laser microscopy of ciliary body and choroidal sections revealed the extensive nature of the cytoplasmic projections on class II–positive cells (Fig. 3A) and ED2-positive cells (Fig. 3B). In general, the cellular processes appeared significantly greater on class II–positive cells than on ED2-positive cells using this technique (see also Fig. 3, C and D).

**Immunostaining of Whole-Mount Preparations**

An estimate of the distribution and density of class II–positive cells was obtained from the whole mount preparations of iris, ciliary body, and choroid (Fig 4). In general, the findings from the tissue sections were confirmed in the whole mounts. An extensive network of dendritiform cells was observed with the antibody to MHC class II (Fig. 4, A and B) whereas cells stained with the ED1 antibody were rounded with clear staining of the cell membrane and intracellular granules (Fig. 4C). In contrast, many of the cells staining positively with ED2 antibody also had a dendritiform structure although as with the tissue sections, their veil-like processes were less well defined than the processes on cells that stained with class II antibody (Fig. 4, D and E). A population of round cells was also observed in the whole mounts of choroid that stained positively although weakly with the ED3 antibody (Fig. 4F), while a sparse population of cells stained positively with OX62 antibody (Fig. 4G). No cells staining with ED17 antibody were observed in the choroid but
FIGURE 2. Identification of different cell types using a range of monoclonal markers. (A) Dendritic MHC class II-positive cells in the choroid (arrows). (B) Weakly staining, round-shaped ED1-positive cells in the choroid (arrows). (C) Dendritic-shaped ED2-positive cells in choroid (arrows). (D) ED2-positive dendritiform cells at the peripheral retina/pars plana region (arrows). (E) ED2-positive cells dendritiform cells in the iris stroma. (F) Weakly staining ED8-positive cells at the peripheral retina/pars plana region (arrow) Ret = retina; Ch = choroid. (G) W3/25 weakly staining cells in the choroid (arrows). (H) Double-stained section of retina/choroid/sclera for ED2-positive (red, arrow) and MHC class II (blue, arrowhead) cells. Two discrete populations of cells are observed in the choroid; no double stained cells were noted. Note large numbers of ED2-positive macrophages in the extraocular muscle (M). (I) Fluorescent stain for antibody to class II showing extent of cytoplasmic processes on dendritic cells in the choroid. RPE = retinal pigment epithelium. Note endogenous fluorescence in the rod outer segments (ROS). Scl = sclera.
FIGURE 3. Confocal scanning laser microscopy of dendritiform cells in ciliary body/choroid tissue. (A) Projected image of class II-positive, intraepithelial ciliary body dendritic cell (arrow). (B) Optical sections (1 to 9) taken at 2 μm intervals through an ED2-positive cell in a section from the pars plana region of normal rat eye. 1, (top) and 9, (bottom) of the cell. Note generally rounded cell body with cellular projections in two main directions (arrows). (C) Optical sections (1 to 9) at 4 μm intervals of a class II-positive dendritic cell in a choroidal whole mount. Note the extensively convoluted and veil-like cellular architecture with cellular projections in several directions. 1, (top) and 9, (bottom) of the cell. (D) Single optical section (2 μm) of cultured class II-positive dendritic cell, prepared as a cytospin specimen (see text for details). Note extensive trailing processes and cupped leading edge of cell, which embraces an unstained small mononuclear cell (arrow).

glial cells in the retina stained strongly positive (data not shown). Because the relative proportions of the different types of dendritic cells in the choroid was of interest, the density distribution of the positively staining cells for OX6 and ED2 was determined by analysis of the confocal image using a software program provided by the manufacturers of the confocal microscope (BioRad). The results are shown in Table 2. In general, dendritic class II-positive, ED2-negative cells were about twice as frequent as "dendritic-like" class II-negative, ED2-positive macrophages.

Confocal microscopy of the whole mounts also permitted a pseudo-three-dimensional conceptualization of individual dendritic cells to be made using the Z series program of the scanning laser microscope system (Fig. 3, B and C). Optical sections taken at 2 and 4 mm intervals through the cells revealed the highly convoluted surface of the cells within the tissue and showed the number and length of the projections. MHC class II-positive dendritic cells generally extended from tip to tip for more than 50 μm, whereas ED2 staining cells had fewer projections, and were normally about 30 μm long.

**Tissue/Cell Culture Studies**

Explants of choroid and ciliary body were cultured for 2 to 5 days in Glasgow's modification of Eagle's Minimum Essential Medium with 10% fetal calf serum as described in Materials and Methods. The cells were then harvested and cytospin preparations were stained
Dendritic Cells and Macrophages in the Uveal Tract

FIGURE 4. Immunostaining of choroidal whole-mount preparations. (A) Network of class II-positive (OX6) cells showing typical dendritic processes. V = blood vessel. (B) High-power view of class II-positive cells with dendriform shape (arrow). (C) ED1-positive cells. Most cells are rounded with occasional processes (arrow). (D) ED2-positive cells showing densely staining rounded cell body, and shorter dendritic processes (arrows) than class II-positive cells. (E) High-power view of ED2-positive cells showing characteristic round cell body and trailing processes (arrow). (F) ED3-positive cells in choroidal whole mount showing moderate to weak staining, and generally rounded structure (arrows). (G) OX62-positive cells in choroidal whole mount showing infrequent distribution and rounded structure (arrows).

with the panel of antibodies. Within 24 hours of culture, cells had migrated from the edge of the explant into the tissue culture medium. Several cell types were observed: large phase-lucent round cells, small round cells, some very small migratory cells that continuously extended dendriform protrusions as seen by time-lapse video photomicroscopy, and occasional single or groups of flat hexagonal cells that were assumed to be ciliary body epithelial cells. Some larger cells displayed classical dendritic "undulating" movements of their pseudopodial extensions in time-lapse studies from their earliest migration from the explant; in contrast,
TABLE 2. Density of Cells in Whole Mounts of Choroid

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<td>ED2</td>
<td>Resident macrophage</td>
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<tr>
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<td>Dendritic cells</td>
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other cells appeared initially rounded when they emerged from the explant and only developed the characteristic cell motility pattern after several hours of culture. None of these cells were initially adherent to the plastic tissue culture dish and after several days of culture only the occasional “epithelial” cells had adhered to the dish in a typical epithelial colony. The nonadherent cells, which were in the large majority, were harvested from the dishes for cytospin preparations.

Cells harvested directly from the explant cultures (24 to 48 hours) included all the cell types described earlier. Cytospins immunostained for class II antigen revealed positive staining on three types of cells: large or very large cells with extensive veil-like processes, smaller more compact cells also with extensive but shorter processes, and round cells with indistinct processes. The large veiled cells were extremely varied in their shape and their processes were frequently in contact with and even enveloping smaller round shaped non-class II–positive cells and occasionally also with nonstaining epithelial cells (Fig. 5, A to C). Clusters of cells were often observed with one or two class II–positive cells in contact with several nonstaining cells (Fig. 5B). The cell processes were often several times the length of the cell body and extremely attenuated as seen both by phase contrast microscopy of peroxidase-stained cells (Fig. 5C) and by confocal fluorescent scanning laser microscopy (Fig. 3D). Other cells were smaller and had shorter, thicker processes that conformed more to the classic descriptions of dendritic cells (Fig. 5A) and were also distinguished from small cells with fine processes.

Staining of cytospins with ED1 monoclonal antibody showed moderate intracytoplasmic positivity of round, usually small, MHC class II–negative cells (Fig. 5D,E). These cells frequently were in contact with larger MHC class II–positive cells (Fig. 5B). In contrast, ED2 staining was observed on cells with extensive processes although these cells were consistently smaller than the large veil cells, which stained positively for class II (Fig. 5, F and G). In addition, cells with the classic morphology of dendritic cells (shorter, thicker processes; see Fig. 5A) were negative for ED2. A significant proportion of cells in these preparations showed no staining for any of these three markers. Staining for other markers in the above panel of monoclonal antibodies was negative.

The results of the immunostaining experiments are summarized in Table 3. Throughout the experiments, there was minimal variation in the intensity of staining as arbitrarily assessed in the table. Consistency of staining was attributed to the care taken in the perfusion fixation technique (see earlier).

Electron Microscopy

Electron microscopy of sections of choroid preincubated with OX6 antibody revealed many positively stained cells throughout the tissue. Immunostaining was particularly evident along the extensive dendritic cell processes (Fig. 6A). Positively stained cells were observed in close association with other mononuclear cells, with vessels and with the basement membrane of the RPE with which they interconnected via fine cell processes (Fig. 6B).

DISCUSSION

This study addressed the question of how antigens and particularly retinal autoantigens can be presented within the posterior segment of the eye. Several previous studies have been directed toward identifying potential antigen-presenting cells in the intraocular compartments, particularly the uvea and retina. During inflammation, MHC class II–positive cells have been observed in these tissues, but most of these cells are components of the inflammatory cell exudate. Studies of the normal eye have suggested that the iris and ciliary body contained no resident antigen-presenting cells and this was proposed as an explanation for the immunologically privileged status of the anterior chamber of the eye, demonstrated most clearly by the phenomenon of anterior chamber-associated immune deviation.16

Recently, however, class II–positive cells have been detected in the normal nonperfused mouse iris and ciliary body17,10 and it has been suggested that these cells were in some way responsible for anterior chamber-associated immune deviation. Williamson et al18 noted that most of the class II–positive cells were rounded and lacking in processes with dendritiform shapes being observed only occasionally. They concluded from their observations that most of the cells were bone marrow derived macrophages although no morphologic evidence was provided. In contrast, Knisely et al13 used improved fixation techniques to show that there was indeed a large network of class II–positive dendritic-shaped cells in the iris akin to the Langerhans cell population in the skin, but that very few in any of these cells stained positively for the Mac-1 macrophage marker. More recently, McMenamin et al16 showed that there were two separate populations...
FIGURE 5. Immunostaining of cytospin preparations of cells that migrated from cultured chorioidal choroid explants. (A) Class II-positive "typical" dendritic cells. Note small nonstaining mononuclear cells (arrows). (B) "Cluster" of cells containing a large class II-positive dendritic cell closely interdigitated with a group of nonstaining round cells. From other preparations the granular round cells (arrows). (C) Very large class II-positive veil cell with extensive cytoplasmic processes, the limits of which are demarcated by the arrows. (D) Two small, round ED1-positive cells (arrows). (E) Fluorescent stain of two ED1-positive cells to show brightly fluorescent granules in the cytoplasm. The nonfluorescent area in the round (arrowed) cell is occupied by the typical horseshoe shaped nucleus (B). (F) ED2-positive cells with trailing processes (arrow). (G) Phase contrast view of a group of ED2-positive cells, revealing the extent of the cytoplasmic processes.
of cells positive for macrophage and MHC class II markers respectively. To date there are no similar data relating to potential APC in the choroid or retina.

In this study we examined perfused normal rat eyes by several methods to determine the nature and distribution of class II-positive cells in the uveal tract with particular reference to the choroid. In this way we removed any class II-positive cells that may have been present in the circulation and have focused only on those cells present in the tissue compartment. We observed at least three populations of cells: large dendritic MHC class II-positive, ED2-negative cells that behave in culture like classic dendritic antigen-presenting cells with processes sometimes of enormous length; a mixed population of large- and medium-sized MHC class II negative, ED2-positive dendritic cells that possess many extremely fine but shorter processes; and a group of MHC class II-negative, ED1-positive, round cells. In addition, a population of weakly staining ED3-positive, small, round cells was observed, particularly in choroidal whole mounts. Also, a less dense network of OX62 positive cells was observed that did not appear to correspond in distribution to the other dendritic cell populations. Recently, OX62 antibody has been shown to bind to a subset of veiled dendritic cells in cell populations from the afferent lymph and to a further subset of γδ T cells in the epidermis and the lamina propria of the gut.20

In this study, pan T cell markers were negative, which excludes the latter type of cell. It therefore appears that veiled dendritic cells represent a subpopulation of dendritic cells in the choroid. It is possible that these cells are important in trafficking from the choroid to central lymphoid tissue as they are in the skin but further studies are necessary to resolve this issue.

ED17 staining was negative in the choroid, but interestingly, stained a subset of retinal glial cells. This antigen has not yet been fully characterized but preliminary evidence suggests that it is a marker for a 55 kD molecule on macrophage cell membranes (C. Dijkstra, personal communication). Its role in the retina is not yet known.

Double staining procedures for class II/ED1 and class II/ED2 cells indicated that each group of cells represented a discrete population and only rarely were double-positive-stained cells observed. Class II-positive dendritic cells appeared to be more numerous than ED2-positive dendritic cells. Occasionally, small, densely staining class II-positive dendritic cells were observed in the cytopsins and these appeared in culture to be very actively motile cells; their counterparts in the tissue sections, however, were difficult to identify although it is likely that they represent another type of dendritic cell.

The antigen recognized by ED1 antibody occurs on most cells of the monocyte-macrophage lineage whereas ED2 recognizes resident tissue macrophages that in vitro have developed adherence to the matrix.20 It is probable that the ED2-positive cells present in the choroid represent a population of long-lived resident choroidal macrophages involved in immune recognition. Morphologically, choroidal ED2-positive cells have certain similarities to veiled dendritic cells, the migratory phenotype of dendritic cells that occurs in the afferent lymph during passage from peripheral sites to the lymph node as indicated earlier.8 Veiled dendritic cells, however, are not normally ED2-positive and it is likely that the choroidal counterpart of the trafficking veiled cell is the OX62-positive dendritic cell (see earlier).

The function of the different cell populations in the choroid and indeed the uveal tract generally, is

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<td>OX62</td>
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Staining is arbitrarily graded on a scale of 0 to 3 (- to ++++) based on the number of cells and the intensity of staining.

* Explant cells are cells harvested from cultures of choroidal explants (see Methods) and prepared as cytopsins.
Dendritic Cells and Macrophages in the Uveal Tract

FIGURE 6. Electron micrographs of choroidal dendritic cells immunostained with OX6 monoclonal antibody against MHC class II antigen. (A) Note the densely staining cell processes that span considerable distances within the tissue. (B) Processes from an elongated dendritic cell approach to within contact distance of the basement membrane of the retinal pigment epithelium (arrow). Bar = 10 μm.

unknown. It is likely that the typical class II dendritic cells are short-lived bone marrow-derived cells that will have a sentinel role in antigen capture and sampling. Both these cells and the longer-lived ED2-positive macrophages may have a major role in regulating/suppressing autoimmune responses to the many potentially immunogenic autoantigens present in the outer retina.21 Several of these antigens are “processed” in the RPE cell during the normal mechanism of rod outer segment renewal.22 It is therefore possible that autoantigenic peptides may be exocytosed from RPE cells and be taken up by dendritic cells during a period of intimate contact between the dendritic cell and the RPE cell on its basal aspect (Fig. 1). Presentation of antigenic peptide to potentially autoreactive T cells would result in autoimmune responses unless there were regulatory mechanisms in place, and it is possible that resident macrophages mediate a suppressive effect on dendritic cell-inducible antigen presentation as occurs in other tissue such as the lung.23,24 Similarly, class II-positive dendritic cells and ED2-positive macrophages in the iris and ciliary body may have a related function in anterior chamber-associated immune deviation. What cell subserves this function is, however, not clear. Studies examining the functional activity of these cells are few. However, in the study by Williamson et al,18 suppressive function was attributed to the Mac-1 positive, bone marrow-derived macrophages.

It is also possible that macrophages (or macrophage subsets) have a separate role in assisting dendritic cells to perform their antigen presentation function. Dendritic cells are recognized to be poorly phagocytic and generally require a close interaction with macrophages for full processing of antigen, particularly insoluble antigen.8 The role of the ED-positive cells may therefore be to cooperate with the dendritic cells in this process. Therefore, although there is no evidence as yet, resident ED2-positive cells might have a negative or suppressive role in choroidal dendritic cell antigen presentation (ie, they might have a role in maintaining homeostasis), whereas the more short-lived ED1 positive cells might cooperate with dendritic cells to promote antigen presentation.

The function of the ED3-positive cells in the choroid is least evident. These cells are not found in the peripheral tissues and usually occur in lymphoid organs such as the spleen. Their role in the spleen is unknown but they may have a suppressive function because they interact preferentially with B cells.25 In culture, the frequency of ED3 expression increases, indicating that it is a relatively labile membrane protein.26 ED3 expression is inducible by T lymphocyte products and by dexamethasone, which also supports a suppressive function for these cells.27

Finally, in the inflammatory situation it is likely that these relationships are altered. Tissue destruction and autoantigen release from the damaged retina, as occurs in experimental autoimmune uveoretinitis, is likely to lead to a large increase in the level of antigen...
processing and presentation both by resident choroidal macrophages and dendritic cells, and by increased numbers of typical bone marrow–derived dendritic cells emigrating from the circulation. Under these circumstances the normal “suppressive” mechanisms associated with immune privilege will be overridden. Because all the components for induction of an immune response are present in the uveal tract, there is little need to invoke mechanisms of antigen presentation by resident nonimmune ocular cells such as RPE cells and ciliary body epithelium although there is little doubt that these and other cells such as Müller cells, can modulate the response in vitro and may play a coregulatory role in vivo.

In conclusion, we have shown that the choroid contains a rich network of recognized antigen-presenting cells including dendritic cells, which have the ability to present antigen to naive T cells as well as macrophages of various types. The interrelationships among these cells is probably central to the induction of (auto)immune responses within the eye and alternative mechanisms involving ocular tissue cells are probably of less importance.

**Key Words**

uveal, dendritic cells, macrophages, antigen presentation

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