EXTENDED REPORT

Choroidal dendritic cells require activation to present antigen and resident choroidal macrophages potentiate this response

J V Forrester, L Lumsden, L Duncan, A D Dick

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See end of article for authors' affiliations

Correspondence to: Professor John V Forrester, Department of Ophthalmology, The Medical School, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD, UK; j.forrester@abdn.ac.uk

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Background/aim: The uveal compartment of the eye contains extensive networks of resident macrophages and dendritic cells. These cells are now recognised to have a role in many ocular pathologies. The aim of this study was to isolate, characterise, and compare the function of ciliary body/choroid dendritic cells and macrophages from the normal eye.

Methods: Explants of rat and human ciliary body/choroid were cultured in vitro for various periods of time and cells harvested either from the supernatant fluid or from enzyme digested and washed explants. The cells were then phenotyped by microscopy and flow cytometry, examined by video time lapse photomicroscopy, and analysed functionally in a series of immunoassays.

Results: Two main types of dendritic cell were identified: large veil-like MHC class II^{mid} motile but relatively non-translocatory cells and small MHC class II^{hi} motile and rapidly translocating cells. Tissue macrophages mainly remained associated with the explants in culture but gradually lost their resident tissue marker (ED2) and detached from the explants as clusters of low density, large, CR3 $(ED7)^+$ cells, some of which underwent apoptosis. Video time lapse studies showed dendritic cells constantly interacting with large single cells and cell clusters by traversing the interstices of the cell clusters. In functional studies, freshly isolated dendritic cells were poor presenters of antigen and required activation by short term culture for acquisition of antigen presenting function. In contrast, dendritic cell depleted choroidal cell preparations containing macrophages and other cells failed to present antigen even after short term culture but augmented the antigen presenting function of dendritic cells when tested in co-culture.

Conclusion: At least two types of dendritic cells are present in the normal ciliary body/choroid layer of the eye. It is likely that these cells have different functions based on their motility and potential to migrate to secondary lymphoid tissue either during normal physiological homeostatic processes or during an inflammatory response. The behaviour of resident tissue myeloid cells may decide the outcome of the organism's response to stress, foreign antigen, and ageing processes such as age related macular degeneration.

The immune response to foreign antigens in the eye is less vigorous than in other tissues, a property attributed to ocular immune privilege.[1] The absence of professional antigen presenting cells (APC) from the eye has been proposed as an explanation for this effect but this may only be true in part. Professional APCs are restricted to the perivascular spaces in the retina[2 3] and are infrequent in the central cornea.[4–7] However, there are abundant APCs in the uveal tract forming dense networks of cells in the iris, ciliary body, and choroid.[8–12]

The precise role of uveal tract APCs is unclear. A previous study showed that cells isolated from the choroid could induce a mixed leucocyte response (MLR) in vitro but since the isolated cells contained a mixed population of leucocytes, it was not clear which cells were responsible for this effect.[10] Professional APCs include dendritic cells (DCs), macrophages, and B cells of which DCs are the most potent by 10–100 times.[13–15] Recent work has confirmed that after immunogenic challenge, immature DCs in peripheral tissues and organs transport antigen to lymphoid tissues where they present antigenic peptide on their surface to T cells in the paracortical regions of the lymph node (for review see Itano *et al*[16]). During this process, DCs undergo a phase of ''maturation'' in which they lose their ability to endocytose and process antigen but become much more effective in antigen presentation. There are at least six different types of DC in lymph nodes.[16]

Macrophages are also a heterogeneous group of cells. Blood borne macrophages which form part of an inflammatory exudate are highly phagocytic[17] and can present antigen to sensitised T cells in an ongoing inflammation. In contrast, resident macrophages are poor presenters of antigen and in certain sites may even inhibit DC function under physiological conditions[18–20] thus exerting an immunoregulatory role. Such effects can be abrogated in the presence of granulocyte/macrophage-colony stimulating factor (GM-CSF).[21] Macrophage heterogeneity would therefore appear in some way to be determined by the microenvironment in which these cells find themselves, probably through signals such as cytokines released from parenchymal cells of the tissues.

The present experiments were therefore designed to investigate the antigen presenting function of ciliary body/choroidal DCs (hereafter termed choroidal DC for simplicity) using an organ culture model of in vitro ''migration and maturation.'' The results confirm that freshly isolated choroidal DCs express low levels of co-stimulatory molecules and are poor presenters of antigen. In contrast, these cells migrate rapidly from ciliary body/choroid organ cultures and after 24–48 hours in culture acquire potent antigen

Abbreviations: APC, antigen presenting cells; BSA, bovine serum albumin; DCs, dendritic cells; FBS, fetal bovine serum; mAb, monoclonal antibody; MLR, mixed leucocyte response; NRS, normal rat serum; PBS, phosphate buffered saline

presenting activity for unprimed T cells. Resident choroidal macrophages appear to be less migratory, the majority remaining associated with the organ culture despite some cells rounding up, losing their resident macrophage marker and acquiring some activation markers. Depletion of choroidal cell preparations of dendritic cells removes the antigen presenting function of the macrophage-containing residual cell population. However, this mixed cell population considerably enhances antigen presentation by choroidal and splenic DCs.

MATERIALS AND METHODS

Tissue culture media and reagents

Cells and tissues were cultured in RPMI or GMEM media containing 10% fetal bovine serum (FBS). Cytokines used in this study included recombinant murine GM-CSF, IL4, TNFα, and cKit ligand (Genzyme, Cambridge, MA, USA). Sera were FBS (Gibco, UK) and normal rat serum (NRS).

Retinal antigen was prepared as a hypotonic lysate of bovine retinal tissue as described previously.[22] Retinal S-antigen was prepared as previously described[23] and stored at −20℃ until used.

Animals

For dendritic cell cultures, Lewis rats were used. For MLR and other functional studies (see below) Lewis and DA rats were used. Animal tissues were harvested and prepared according to UK Home Office recommendations.

Ciliary body/choroid explant culture

Explants were prepared mostly from rat tissue. A small number of donor human eyes were also used.

Lewis rats were killed by exsanguination and perfusion of the vascular bed with phosphate buffered saline (PBS). Eyes were dissected as described previously.[9] Briefly, the cornea was removed and the iris, lens, and vitreous were removed as one. The retina was floated from the posterior eye cup before cutting its attachment to the optic nerve. The remaining ciliary body/choroid/sclera was dissected into small blocks (1×2 mm), placed in 24 well plates (10–15 blocks per well) and cultured for various periods of time in GMEM with 10% NRS and other cytokines as indicated (see Results).

Donor human eyes were received from the Amsterdam Eye Bank within 24–48 hours of death and dissected as described above. Explants of choroid/sclera 2–4 mm in size were cultured in GMEM with 10% FCS.

Preparation of dendritic cells

Rat and human choroidal dendritic cell preparations were prepared from the non-adherent cell population which had migrated into the supernatant fluid during explant culture (see Results) on metrizamide cushions as described previously.[9] Further purification was performed using magnetic bead separation technology as described by the manufacturers using antibodies against ED2 and MHC class II to coat the cell preparations and sheep ant-rat coated beads (Dynal, UK).

Videomicroscopy time lapse studies

Cell preparations were plated onto glass coverslips in RPMI+10% NRS, mounted in a stainless steel viewing chamber overlaid with a second glass coverslip to a depth of 800 μm, and viewed in an inverted phase contrast microscope (Olympus,UK) fitted with a CCD camera attached to a videorecorder with time lapse facility. Images were captured at a rate of between one and four per minute and re-run at 4× real time speed to give an indication of cell motility.

Immunophenotyping

Immunostaining of tissue sections and cytospin preparations were performed as previously described[9] on eyes from normal Lewis rats after perfusion fixation with cold ethanol. Immunostaining was performed mostly using the ABC immunoperoxidase, immunofluorescence or in some circumstances APAAP staining (as described by Hoey *et al*[24]) and the panel of antibodies shown in table 1.

Flow cytometry

Cell suspensions from explant cultures were also immunophenotyped by flow cytometry, using two colour fluorescence (FACSCalibur,TM, Becton Dickinson). Cells were washed in PBS/1% bovine serum albumin (BSA) and approximately 10^5 cells incubated on ice with combinations of the following mAb (Serotec UK unless otherwise stated) against rat cell surface determinants: OX1 (CD45; common leucocyte antigen), OX6 (rat MHC class II), ED2, ED7, and OX42. In brief, staining was performed sequentially with an initial unconjugated mAb detected after washes in PBS/BSA with sheep-anti-rat $F(Ab)_2$-FITC (Sigma, UK). A second biotinylated or directly conjugated mAb was added in blocking serum (10% normal mouse and 10% normal rat serum) and detected with streptavidin-PE (Caltag, USA). Dendritic cells were isolated according to scatter characteristics and mid-high MHC class II expression. Background levels were set with appropriate isotype mAb control. Analysis was performed on acquisition of 10 000 events using Cellquest software. Simultaneously, cell suspensions were fixed in 4% paraformaldehyde, washed in PBS, and stored in 70% ethanol at −20℃ for future cytospin analysis.

Table 1 Summary of mAb staining of choroid cells

		Fresh cells (digest)		Cultured cells	
Antibody	Stains	Round cells	Dendritic cells	Round cells	Dendritic cells
OX6	MHC II	−	++	−	++
OX17	MHC II	−	++	−	++
OX 62	DC	ND	++*	ND	ND
0X1	CD45	+	+	+	+
OX7	Thy-1	−	+	−	+
ED1	Macs	−	−	−	−
ED2	Res. Macs	++	−	+/−	−
ED7	CR3	+	+	++	++
W3/25	CD4	−	−(+/−)	+	−
OX8	CD8	−	−	−	−
OX42	Microglia	−	+	−	+
R73	TCR	−	−	−	−

*Staining of DC in cultured (undigested) choroidal explants (see fig 1D).

Apoptosis assay

Ethanol fixed cell suspensions from explant cultures were prepared as cytospins and the cells stained using Apotag R plus (Oncor, MD, USA) to demonstrate increased end labelling of DNA as a result of apoptotic fragmentation by tdt catalysed FITC digoxigenin labelling of 3′-OH ends of DNA, and counterstained with propidium iodide (SI).

Mixed lymphocyte responses (MLR)

Low density cells from choroidal explants were tested for antigen presenting capacity in modified syngeneic and allogeneic MLR assays as described previously using choroidal APCs and splenic T cells (>98% pure by magnetic bead isolation). H3-thymidine uptake was counted on a Matrix (Amersham) plate reader in which radioactivity counts are typically one third of counts on a standard liquid scintillation counter.[25]

Cluster formation and polarisation assay

The ability of choroidal dendritic cells to form clusters with T cells was assessed in a simple cluster assay. Briefly, choroidal cells (1×10^4) were incubated with varying concentrations of purified (>98%) T cells from unimmunised animals in 24 well plates and incubated for 24 hours at 37℃. Clusters of lymphocytes were visualised and the cells harvested for immunophenotyping in cytospin preparations.

Presentation of antigen by dendritic cells to naive T cells was assayed in a polarisation assay.[26 27] This assay detects T

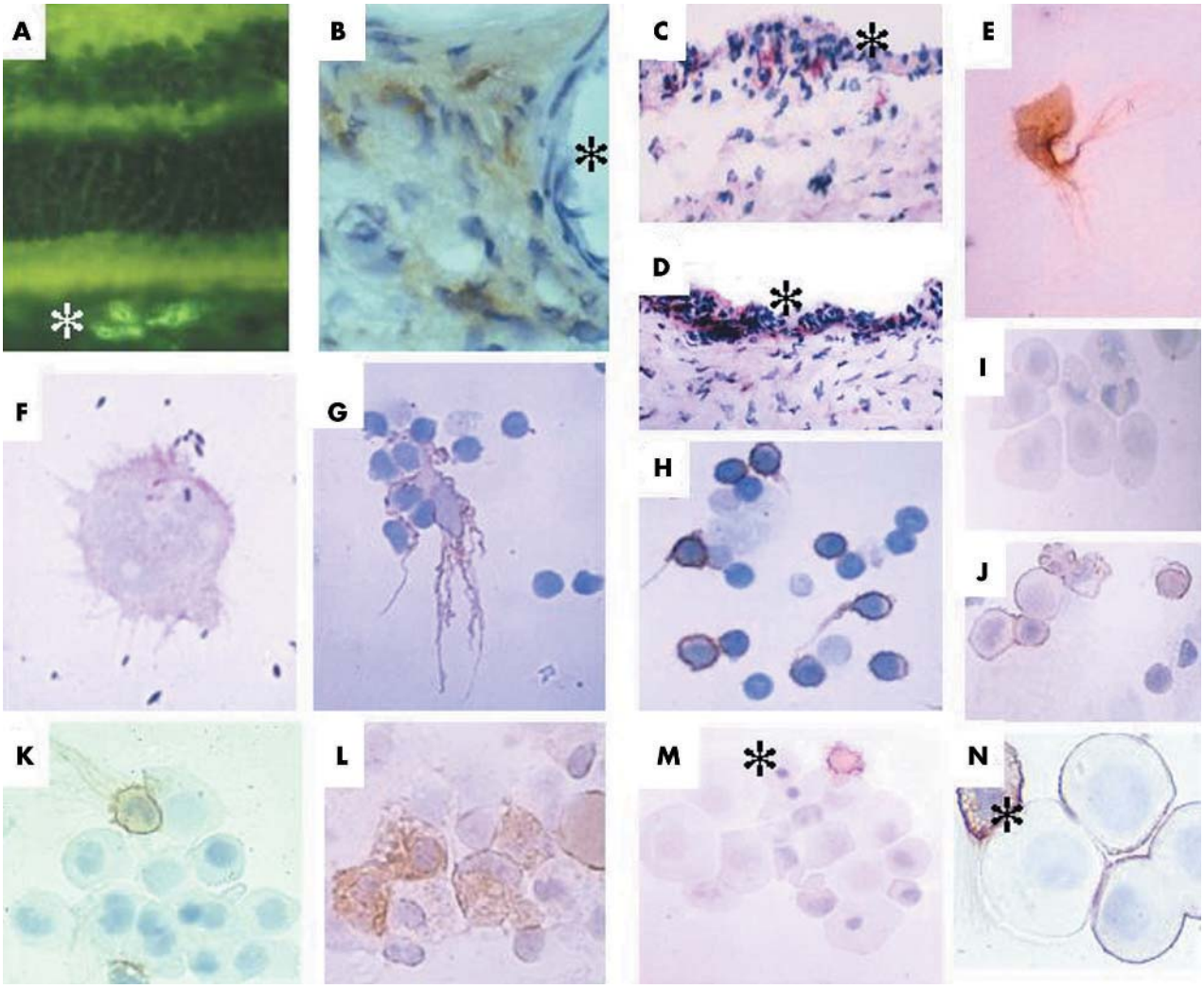

Figure 1 Immunohistochemistry of choroidal dendritic cells in sections from normal rat eyes and in cytospin preparations after culture in vitro (see Methods). (A) Immunofluorescence of section of rat eye stained for MHC class II (OX6); note MHC class II positive cell in the choroid (*). Photoreceptors and bipolar cells also show autofluorescence caused by retinal chromophores (see Brissette-Storkus *et al*[9]). (B) Immunoperoxidase stained section of choroid showing brown stained MHC class II (OX6) positive dendritic cells surrounding a vein (*marks the lumen). (C) APAAP stain of choroid-retinal pigment epithelium (RPE) explant with ED2$^+$ (red) cells present in the RPE layer (*). (D) APAAP stain of choroid-RPE explant showing OX62$^+$ (red) cells present in the RPE layer (*) and in the choroidal stroma. (E) Cytospin preparation of immunoperoxidase-stained culture of rat MHC class II$^+$ dendritic cell showing extensive veiled phenotype. (F) Cytospin APAAP preparation of cultured human dendritic cell showing moderate positivity for B7 antigen; the rod-like structures also present in the figure are photoreceptor fragments. (G) Cytospin preparation of large veiled MHC class II positive (immunoperoxidase) rat dendritic cell clustered with T cells in co-culture (see Methods). (H) Cytospin preparation of small intensely MHC class II positive dendritic cells with long tails forming small T cell clusters after co-culture. (I) Cytospin preparation of large round low densitiy MHC class II negative choroidal cells forming clusters in culture. (J) similar preparation to (I) stained for ED7 antigen (immunoperoxidase). (K) Cytospin preparation of large cell cluster harvested from choroid-RPE explant by washing, and showing two MHC class II positive dendritic cells in contact with the MHC class II negative large cells. (L) Cytospin preparation of a cluster of large cells freshly harvested from collagenase digested choroidal-RPE explant and showing strong ED2 positivity in a proportion of the cells. (M) Cytospin preparation of a cluster of large cells some of which contain pyknotic nuclei (*), in contact with a single MHC class II positive dendritic cell. (N) Similar preparation to (M) but at higher magnification showing a single ED7 positive dendritic cell (*) in contact with four large cells, three of which are also ED7 positive.

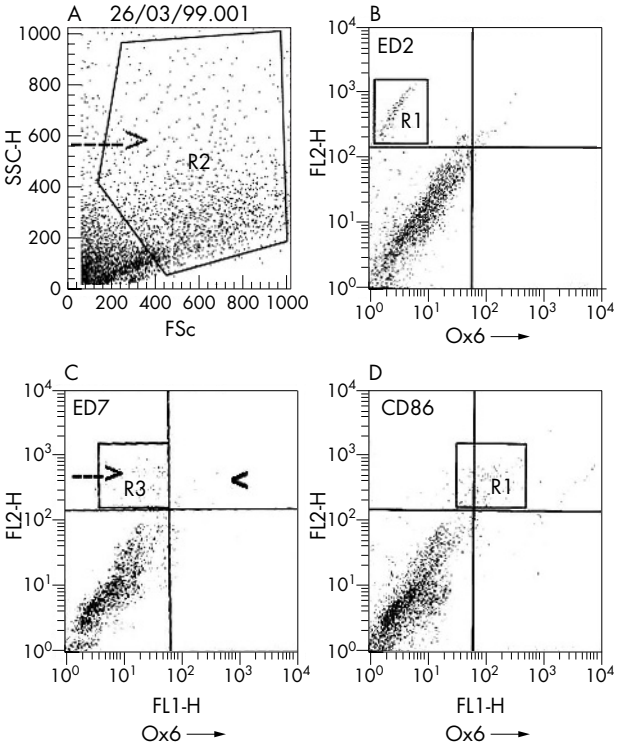

Figure 2 Flow cytometry of choroidal cells obtained by harvesting the supernantant from choroidal-RPE explant cultures. (A) Scatter plot demonstrating two populations of cells, one of small cells (outside gate), and the second of larger cells represented by arrow and liberal gate applied around cells with large forward scatter (FSc). Approximately 20% of the large cells were MHC class II positive (OX6). (B) Dot-plot of FL-1-ED2 *v* FL2-OX6 (MHC class II) indicating that ED2 positive cells were almost exclusively OX6 negative. (C) Dot plot of FL1-ED7 *v* FL2-OX6 (MHC class II) demonstrating two population of $ED7^+$ MHC class II^+ (arrowhead) and $ED7^+$ MHC class II^- cells (arrow). Backgating region (R1) throughout confirmed the cells lay in R2 region of scatter plot, representing large low density cell population. (D) Dot plot of FL1-CD86 *v* FL2-OX6 (MHC class II) demonstrating dual expression of large low density cell population.

cell activation and cluster formation by induction of round to polarised shape change in lymphocytes.[28] Briefly, choroidal DCs (1×10^4) were incubated with purified (>98%) T cells (1×10^6) in the presence of different concentrations of retinal S antigen for 30 minutes at 37℃ in a gently rotating test tube after which an equal volume of 1.0% glutaraldehyde in PBS was added to fix the cells while in suspension. Controls included cells without antigen, cells without DCs, and cells incubated at 0℃. The cells were plated on slides and the percentage of polarised cells in the population was estimated visually by counting.

RESULTS

Characterisation of choroidal dendritic cells and macrophages

The rat choroid contains interdigitating networks of MHC class II^+, $ED2^-$ dendritic cells and MHC class II^-, $ED2^+$ resident tissue macrophages.[9] Both cell types possess extensive elongated processes in situ (fig 1A) and are located predominantly around the vessels (fig 1B). Dendritic cells appear to be variable in size while the tissue macrophages appear to be uniformly very large cells. Double staining studies have, however, shown that these two populations appear to be quite distinctive with <1% of the cells expressing both MHC class II and ED2 markers.[9] Occasional dendritic cells in tissue sections were weakly positive for CD4 (W3/25) but in general they were strongly CD1 positive (data not shown). No $CD8^+$ cells were detected. A small proportion of macrophages also showed positive staining for ED1 and ED3 (sialoadhesin) in the normal choroid.[9] However, staining for CR3 (OX42, ED7/8) was generally absent from normal choroidal sections. No positive staining for the TCR antibody, or B cells was found (data not shown, see Forrester *et al*[9]).

Histological examination of explants of choroid-RPE cultured for 24–48 hours showed some swelling of choroidal tissue with prominent staining and rounding up of $ED2^+$ macrophages (fig 1C). $CD1^+$, $OX62^+$ DCs appeared to be fewer in number than in the intact tissue but had not changed in size (fig 1D). Many non-APC type cells such as endothelium and RPE cells expressed MHC class II as has been shown previously for cultured cells of this type[29 30] (data not shown).

Cytospin preparations of the population of low density cells from the explant culture supernatant showed that a significant proportion (20%) had the appearance of large veiled moderately MHC class II^{mid} dendritic cells (fig 1E) which were also weakly B7 positive (fig 1F). These cells formed clusters when incubated with T cells (fig 1G). However, there was also a second population of smaller, strongly MHC class II^{hi} dendritic cells which were more frequently observed when the choroidal cells were co-cultured with T cells (see below) (fig 1H). These cells also strongly expressed the CR3 antigen (see below). Other cells included in the low density cell fraction from the explant supernatant were a population of very large round cells (30–40%) with vacuolated cytoplasm, which were MHC class II−, ED2+/− (fig 1I). Some of these cells had an epithelial morphology and were probably detached clusters of ciliary body epithelial cells (fig 1I). However, these large cell clusters also contained cells which strongly expressed the CR3 antigen (ED7) characteristic of macrophages (fig 1J). Examination of the high density cell fraction (pellet) from the metrizamide separation indicated that RPE cells present in the harvested supernatant generally separated into this fraction (data not shown).

Flow cytometry of the cells from the cultured explant supernatant displayed two populations of cells; one was a population of large MHC class II^+ cells accounting for around 20–30% of the total population of low density cells (fig 2A). Cell surface phenotype of gated large low density cells confirmed that MHC class II^+ cells were ED2 negative (fig 2B) and after culture a proportion of cells expressed low/moderate levels of CD86 (fig 2D). A small subpopulation were MHC class II^+ $ED7^+$ but most $ED7^+$ cells were MHC class II^- (fig 2C). There were no T cells (R73) or B cells (OX33) detected (data not shown).

Cytospin preparations of the low density population of cells obtained from washes of the explant indicated that a much larger proportion (>80%) of large MHC class II^-, round cells remained associated with the explants and had not migrated into the culture supernatant. Some of these cells were weakly $ED2^+$, and expressed ED7, but many cells in clusters did not stain with any antigen. Some had an epithelial morphology while others may have been residual choroidal cells such as melanocytes.

Many of the clusters contained one or two dendritic cells (fig 1K). Collagenase digestion (1–2 hours at 37℃) of choroidal explants allowed the harvesting of fresh, non-cultured large round cells from washes of the explants. On cytospin preparations, some cells within the clusters were strongly ED2 positive (fig 1L). Prolonged culture of explants (up to 9 days) showed that the large round cells mostly remained in clusters as low density, non-adherent cells, containing many cytoplasmic granules and a large nucleus. In this condition, $ED2^+$ cells persisted. However, with time an increasingly large number of these cells appeared to lose their

cytoplasmic granules, become less dense, lose their marker for ED2, and detach into the culture supernatant. Some cells showed signs of apoptosis with pyknotic nuclei (fig 1M) but the majority remained viable and strongly expressed ED7 suggesting activation (fig 1N, fig 2D). Several other markers were examined in freshly isolated *vs* cultured low density cells the results of which are shown in table 1.

Video time lapse studies of choroidal DC and macrophage motility

The low density cells harvested from choroidal explant culture supernatant were plated onto coverslips in small flat chambers and examined by inverted phase contrast microscopy using video time lapse equipment (see Methods).

Dendritic cells were identified by their typical undulating motile behaviour without making significant translocations. Such cells appeared to contact the substrate by one or two fine dendritic processes, which interchanged at regular intervals with typical filopodial protrusions from the cell surface (fig 3 and video clips 1 and 2 (on *BJO* website, www.bjophthalmol.com/supplemental)). Dendritic cells sometimes occurred in small groups of two or three cells adopting highly interactive behaviour within the group. In addition, several large round cells were observed which appeared relatively non-motile (fig 3 and video clips). Some of these cells extended broad based pseudopodia, appeared active and translocated slowly on the tissue culture surface. Dendritic cells appeared to extend filopodia to contact the large round cells and induce behavioural changes and even appear to ''transport'' the large cells for several micrometres across the culture dish (see video clips).

Highly motile translocating cells were also observed, some of which were quite small (<10 μm in diameter see video clip 2) while others were larger than dendritic cells. Within some cell clusters, one or two motile cells could be observed which appeared to migrate in and out of the interstices of the clusters (see video clip 1). Cytospin analysis indicated that such clusters were composed of presumed non-staining ciliary body epithelial cell aggregates, containing ''activated'' $ED7^+$ macrophages (see figs 1N and 2C). Some cells showed signs of apoptosis (fig 4) as shown by their pyknotic nuclei, lack of cytoplasmic granules, and staining for annexin V. These clusters usually contained one or more MHC class II^+ dendritic cells whose dendrites appeared to be closely wrapped round their cell bodies (see figs 1K, N and 4).

Functional studies

Lewis rat ($RT1^l$) dendritic cells were harvested from choroidal explant cultures (48 hours in GMEM plus 10% NRS) and showed strong MLRs when tested against purified spleen T cells from DA rats ($RT1^h$) (see Methods). The response was considerably stronger than that seen with splenic DC from Lewis rats (fig 5A). In contrast with cells obtained from cultured explants, dendritic cells ''freshly'' obtained by collagenase digestion of choroidal explants failed to induce an allogeneic MLR response (fig 5B). A strong syngeneic MLR was also observed with choroidal DCs from cultured explants (see below). Culturing explants in murine rGM-CSF or rIL-4 did not increase the antigen presenting capacity of the choroidal dendritic cells in the MLR assay (fig 5C). Similarly, culturing the explants in TNFα or stem cell factor (cKit) had no effect on the MLR (data not shown).

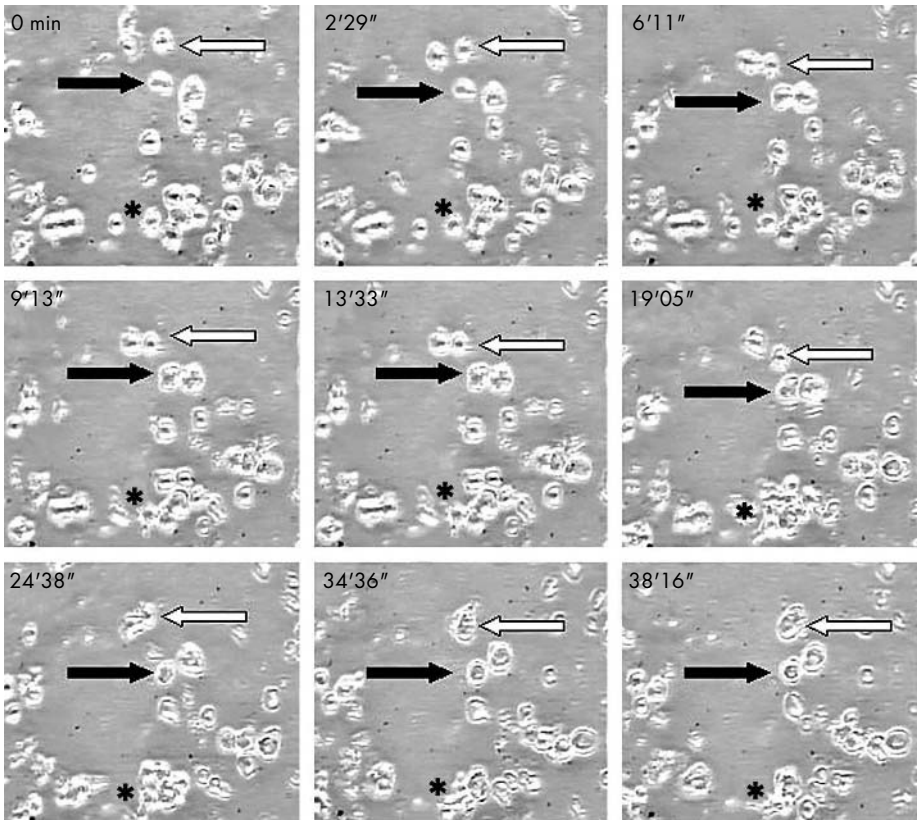

Figure 3 Time lapse video microscopy. Single shots from a video time lapse photographic sequence of choroidal dendritic cells in culture. Black arrow indicates a motile but non-translocating cell while white arrow identifies two translocating cells which contact and separate from the non-translocating cell. (*) identifies a large motile cluster of cells.

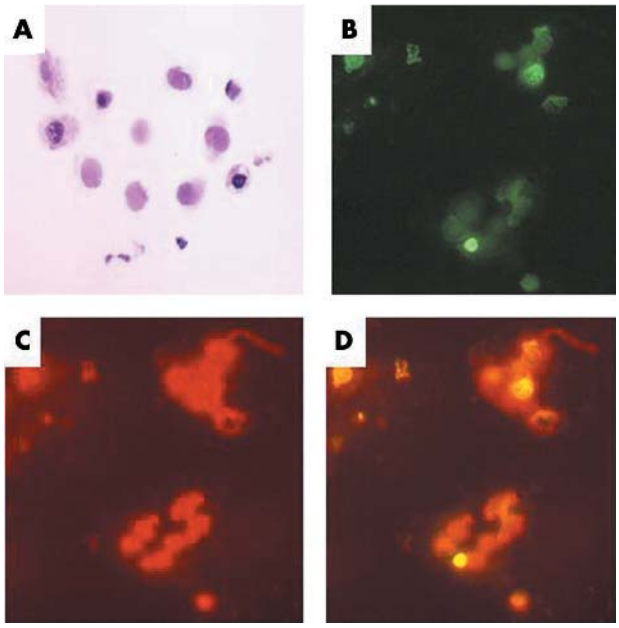

Figure 4 Fixed cytospin preparations of cluster of large round cells from low density fraction of cells harvested from cultured choroidal explants. Some cells in the cluster demonstrate evidence of apoptotic cell death. (A) Giemsa stain: note pyknotic (*) and fragmented (**) nuclei (B) Apotag R stain (FITC, green label: see Methods). (C) propidium iodide (red) stain of same section as (B). (D) Dual exposure of images in (B) and (C).

Choroidal dendritic cells from cultured explants were also effective in presenting retinal antigen to T cells from draining lymph nodes of retinal antigen immunised rats in a dose dependent fashion (fig 6). In addition, in a polarisation assay, which is a measure of T cell priming (see Methods) choroidal dendritic cells were effective in presenting bovine retinal S-antigen in a dose dependent manner to naive, unstimulated T cells (fig 7).

Comparison of choroidal macrophages *v* dendritic cells as antigen presenting cells

The functional studies reported above were performed with cells which had migrated from the explants to form a non-adherent, low density cell population after 24–48 hours of culture. These cells comprised non-staining large round cells (presumed epithelial cells), $ED2^{+}ED7^{+}$ macrophages and typical MHC class II^{+} dendritic cells (see above). Since it was possible that both the macrophage and the dendritic cell populations were responsible for the MLR, further purification of the cell populations was undertaken using negative and positive selection with antibody coated magnetic beads (see Methods).

Cells recovered by positive selection using anti-MHC class II antibody coated beads were >80% MHC class II positive, had the typical appearance of dendritic cells in cytospin preparations, and induced strong MLRs (fig 8). In contrast, $ED2^{+}$ cells and ED2-MHC class II cells (both prepared by negative separation) failed to induce a significant MLR.

Resident tissue macrophages enhance antigen presentation by spleen dendritic cells and promote a strong syngeneic MLR

Unlike Langerhans cells[31] and marrow derived dendritic cells[14] choroidal dendritic cells did not require cytokines such as GM-CSF, IL-4, or cKit to become efficient APCs during their emigration from 48 hours' culture of choroidal explants (fig 7). This suggests that cells within the explant were available to act as sources of these dendritic cell survival

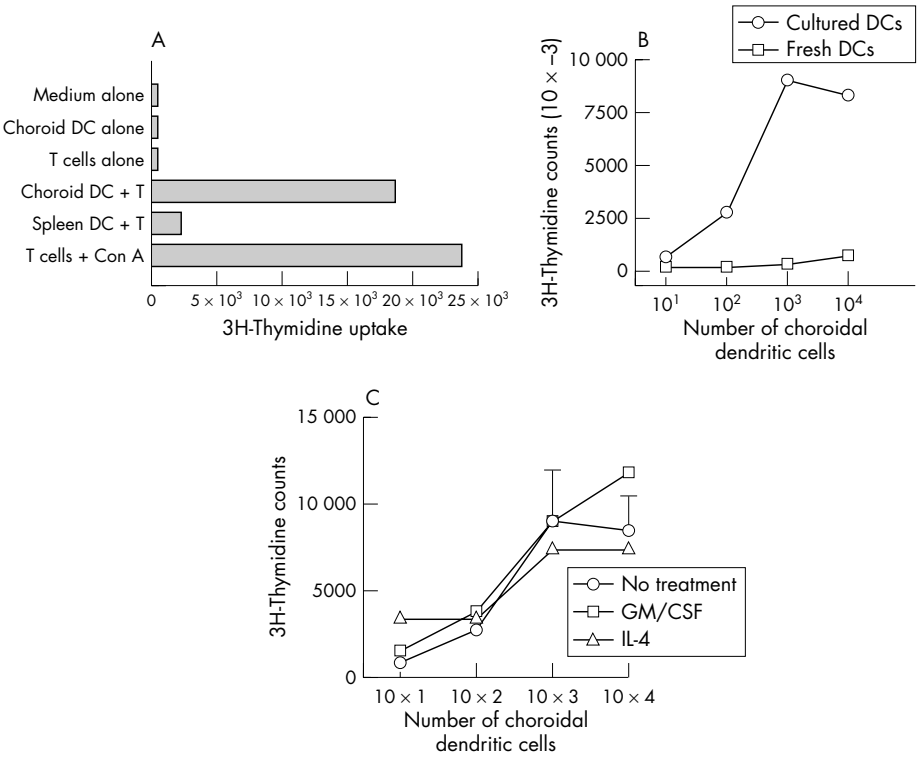

Figure 5 Functional studies—mixed leucocyte response (MLR). (A) Modified allogeneic mixed leucocyte response (MLR) by DA rat splenocytes in response to co-culture with rat Lewis choroidal dendritic cells. (B) The effect was dose dependent and was not inducible by freshly harvested cells, only by dendritic cells after 12–24 hours of culture. (C) Allogeneic MLR response induced in DBA rats by Lewis choroidal dendritic cells harvested from explants which were cultured in the absence of additional cytokine or in the presence of GM-CSF alone or GM-CSF plus IL-4.

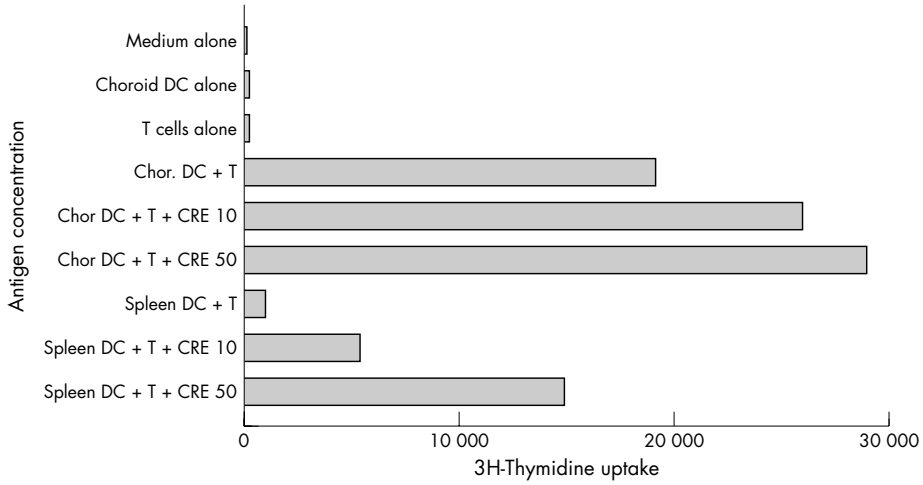

Figure 6 Functional studies—antigen presentation (H3-thymidine uptake assay). Presentation of retinal antigens to draining lymph node cells from Lewis rats sensitised to retinal antigen by immunisation with S-antigen rich crude retinal extract (CRE) in complete Freund's adjuvant 9 days before assay. Note strong syngeneic response induced by choroidal DCs in the absence of antigen.

factors, especially GM-CSF. RPE cells have been shown to act as sources of GM-CSF in vitro[32] but it is also possible that resident tissue macrophages provide this function. Accordingly, the effect of choroidal resident tissue macrophages were tested for their ability to modulate the MLR induced by spleen DCs. Addition of MHC class II depleted, $ED2^+$ cell enriched choroidal explant cells, which were ineffective in the MLR assay alone, considerably enhanced the MLR induced by spleen DCs (fig 9). In addition, they promoted strong syngeneic MLRs by choroidal DCs in contrast with the low syngeneic responses seen with spleen DCs alone (data not shown).

DISCUSSION

The results of this study confirm the presence of MHC class II+, OX62+ motile dendritic cells (DCs) in the rat ciliary body/choroid and similar cells in the human eye. In situ, and when freshly harvested, choroidal DCs appear immature, showing low levels of co-stimulatory molecules (B7) and activation markers (CR3). Choroidal DCs migrate readily from cultured explants of choroidal tissue and can be harvested as non-adherent cells from the culture supernatant. Highly purified populations of choroidal dendritic cells (>80% pure) can then be prepared using magnetic bead isolation techniques.

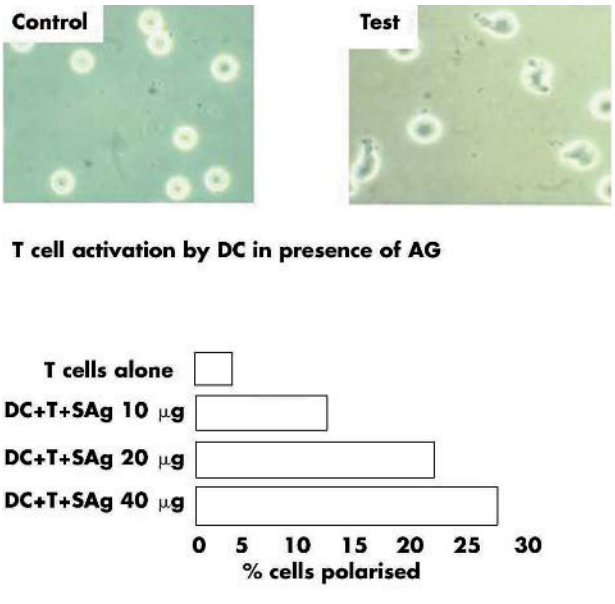

Figure 7 Functional studies—antigen presentation (polarisation assay). Priming response of naive T cells in the presence of choroidal dendritic cells (DCs) (see Methods). Control lymphocytes remain round while test lymphocytes cultured with DC in presence of antigen (AG) undergo a polarised shape change. Note dose dependent nature of the response to purified retinal S-antigen antigen.

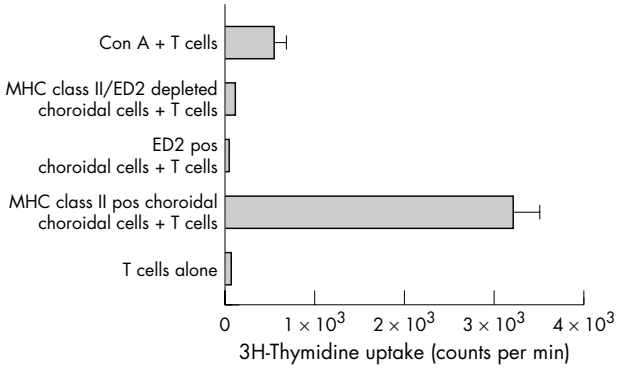

Figure 8 Functional studies—mixed leucocyte response (MLR). Purified MHC class II positive cells (magnetic bead separation see Methods) induced a strong MLR but purified ED2 positive cells failed to induce a significant MLR.

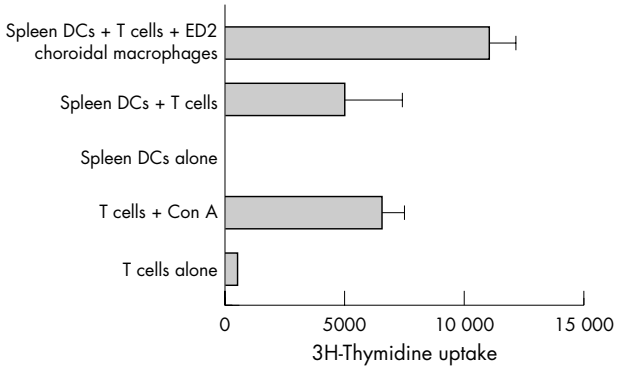

Figure 9 Functional studies—mixed leucocyte response (MLR). Augmentation of splenic DC induced MLR response by $ED2^+$ enriched, MHC, class II depleted choroidal cells.

Choroidal DCs are of two types: large veiled, MHC class II^{mid} DCs (fig 1E) and small, MHC class II^{hi} cells (fig 1G). The latter cell type was more frequently observed when the choroidal cells were co-cultured with T cells with which they readily formed clusters (fig 1G) and is probably a manifestation of their maturation. In contrast, the large veiled cells could frequently be harvested as single cells (fig 1E). Heterogeneity in dendritic cells populations is well recognised. DCs in tissues are derived from at least two types of bone marrow derived precursor cell: (a) the $CD34^+$ haematopoietic precursor, and (b) the $CD14^+$ monocyte. Both these cell types appear to occur in the choroid of the eye, the large veil cell being the equivalent of the dermal dendritic cell while the smaller, MHC class II^{hi} cell resembles the $CD14^+$ blood monocyte derived DC.

The current study confirms and extend a previous study of choroidal dendritic cells[10] showing that, in the resting state, choroidal DCs are poor presenters of antigen, but can become potent APCs if activated[10]—for example, after culture in vitro. For the first time, we also demonstrate using the polarisation assay that choroidal DCs, after activation by culture in vitro, can prime naive T cells. In contrast, choroidal macrophages are poor presenters of antigen but can augment the antigen presenting function of DCs (fig 9). Thus, choroidal macrophages resemble their counterparts in the iris, unlike lung macrophages, which appear to have a suppressive effect on the immune response.[33]

What is the role of choroidal DCs in the resting state? Recent studies have suggested that iris antigen presenting cells do not migrate readily to the draining lymph node.[34 35] However, indirect studies suggest that antigen is carried to the draining lymph node via the lymphatics[36] and there is evidence that under physiological conditions the role of tissue traversing dendritic cells may be to maintain homeostasis and prevent T cell activation. The half life of iris dendritic cells is only a few days indicating considerable cell trafficking.[12]

A tolerising role for ''sentinel'' tissue dendritic cells has been shown at other sites[37] and may be under the control of cytokines such as monocyte chemotactic protein-1 (MCP-1).[38] Interestingly MCP-1 is produced constitutively by RPE cells and is upregulated in response to inflammatory stimuli, perhaps as a homeostatic regulatory response.[39] Furthermore, mice in which the gene for MCP-1 gene has been deleted, develop a retinal degeneration with considerable similarity to age related macular degeneration (AMD).[40] It is possible that AMD occurs by default as a result of dysregulation of choroidal dendritic cell homeostatic function.[41] Thus, in the presence of pro-inflammatory signals, such as GM-CSF, IL-1, or TNFα and the absence of immunomodulatory cytokines such as MCP-1, choroidal DCs may lose their tolerogenic function and become potent APCs as demonstrated here.

Choroidal macrophages may have similar dual roles to dendritic cells as agents of both pro-inflammatory or anti-inflammatory responses. Under physiological conditions they probably sustain a housekeeping role, removing dead and dying cells in the normal process of cell turnover, and they may even downregulate the immune response.[18–20] Thus, in this study they appeared in close association with presumed epithelial cell clusters some of which appeared to be undergoing apoptosis. However, when activated, they secrete pro-inflammatory cytokines and, while still performing poorly as APCs, they synergistically promote antigen presentation by dendritic cells (fig 9).

Interest in the function of choroidal dendritic cells and macrophages has increased because of their possible role in several disorders of the posterior segment of the eye including ocular inflammatory diseases, retinal degenerations, and particularly AMD. The present study shows that it is possible to prepare enriched populations of these cells from the eye. This should permit study of their function in diseases such as AMD.

The choroidal videos can be viewed on the *BJO* website (www.bjophthalmol.com/supplemental).

Authors' affiliations
J V Forrester, L Lumsden, L Duncan, Department of Ophthalmology, University of Aberdeen, UK
A D Dick, Division of Ophthalmology, University of Bristol, UK

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