Retinal Microglia and Uveal Tract Dendritic Cells and Macrophages Are Not CX3CR1 Dependent in Their Recruitment and Distribution in the Young Mouse Eye

Jelena Kezic,1 Heping Xu,2 Holly R. Chinnery,1 Connor C. Murphy,3 and Paul G. McMenamin1

PURPOSE. The chemokine receptor CX3CR1 is expressed by monocyte-derived dendritic cells (DCs) and macrophages. CX3CR1 mediates leukocyte migration and adhesion in homeostatic and inflammatory conditions. Mice lacking Cx3cr1 have altered distribution and function of DC subpopulations in some tissue microenvironments. The present study compares the distribution of monocyte-derived cells in the normal retina and uveal tract as a prelude to the investigation of the role of CX3CR1 in murine models of ocular disease.

METHODS. Transgenic mice in which either one (Cx3cr1+/-, heterozygous) or both (Cx3cr1+/+, homozygous) copies of the Cx3cr1 gene have been replaced by the enhanced green fluorescent protein (eGFP) reporter gene were used to investigate the role of Cx3cr1 expression on macrophages and DCs in the normal uveal tract and retina. Chimeric mice were used to investigate turnover of these cells in the normal, uninflamed eye.

RESULTS. Confocal analysis found no significant differences in the density, phenotype or morphology of eGFP+ cells between Cx3cr1-/- and Cx3cr1+/+ mice in immunostained iris, ciliary body, or choroidal and retinal wholemounts. Flow cytometry also failed to detect any difference in the density or cell shape of eGFP+ cells between Cx3cr1+/- and Cx3cr1+/+ mice. Chimeras revealed 73% turnover of monocyte-derived cells in the iris and 63% in the choroid by 6 weeks after transplantation.

CONCLUSIONS. These data illustrate that homing or migration of DCs and macrophages to the uveal tract and retina in normal young mice is not Cx3cr1 dependent and provide a solid foundation for future studies of monocyte-derived cells and the role of Cx3cr1 in models of ocular disease. (Invest Ophthalmol Vis Sci. 2008;49:1599–1608) DOI:10.1167/iovs.07-0953

From the 1School of Anatomy and Human Biology, The University of Western Australia, Crawley, Western Australia; the 2Department of Ophthalmology, University of Aberdeen, Aberdeen, Scotland, United Kingdom; and the 3Department of Ophthalmology, Royal Perth Hospital, Perth, Western Australia.

Supported by a Small Grant from the University of Western Australia. All confocal microscopy was performed using facilities at the Centre for Microscopy, Characterization and Analysis, The University of Western Australia, which are supported by University, State, and Federal Government funding.

Submitted for publication July 27, 2007; revised November 2, 2007; accepted February 15, 2008.

Disclosure: J. Kezic, None; H. Xu, None; H.R. Chinnery, None; C.C. Murphy, None; P.G. McMenamin, None

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Corresponding author: Paul G. McMenamin, School of Anatomy and Human Biology, The University of Western Australia, Crawley (Perth), 6009, Western Australia; mcmenamin@anhb.uwa.edu.au.

Regulatory processes aimed at reducing potentially harmful bystander effects of immune-mediated responses appear to be more developed in delicate tissues such as the eye and brain.1,2 Since the original descriptions and characterization of macrophages and dendritic cells (DCs) in the uveal tract of the eye,3–5 and microglia in the retina,6,7 it has become clear that these cells are critical in regulating the afferent and efferent arms of cell-mediated ocular immune responses, including uveoretinitis.8–10 More recently, ocular macrophages and DCs are being recognized for their role as effectors and regulators of innate immune responses.10

After recent evidence that the pathogenesis of age-related macular degeneration (AMD) may have an inflammatory component,16–20 attention has been focused on the role of resident or recruited choroidal macrophages at the choroidal-retinal interface.21,22 This focus mirrors similar renewed interest in the role of macrophages in other nonocular diseases, including atherosclerosis23 and obesity.24

Transgenic mice in which either one (Cx3cr1+/-, heterozygous) or both (Cx3cr1+/+, homozygous) copies of the Cx3cr1 gene have been replaced by the enhanced green fluorescent protein (eGFP) reporter gene enable the visualization of Cx3cr1-bearing monocyte-derived DCs and macrophages in a range of tissues both ex vivo and in vivo.26–28 In addition, this transgenic mouse model has allowed examination of the specific role of CX3CR1 in the normal homing, recruitment, and subsequent differentiation of monocyte-derived DCs and macrophages in various normal and diseased tissues.23,26,29 There is emerging evidence that CX3CR1high LFA-1high Ly6c+ (GR1+), and CCR2+ macrophage-derived cells constitute a subpopulation of resident macrophages in resting and inflamed tissues.29 So-called inflammatory monocytes that express the cell surface protein Ly6c+ (GR1+), the chemokine receptor CCR2, and the adhesion molecule l-selectin are selectively recruited to inflammatory sites and lymph nodes and can replenish resident monocyte-derived populations of macrophages and DCs in the skin, digestive system, and lung.29

The chemokine CX3CL1 can occur in both a membrane-bound form, which sits atop a mucinlike stalk, as well as a soluble form after proteolytic cleavage.30 CX3CR1 is the sole receptor for this chemokine and is expressed by all monocyte-derived cells—namely, DCs, natural killer cells, and macrophages.31,32 Membrane bound CX3CL1 mediates adhesion of CX3CR1-bearing cells while soluble CX3CL1 acts as a chemoattractant directing the migration of CX3CR1+ NK cells, monocytes, DCs, macrophages and subpopulations of T cells in both homeostatic and inflammatory conditions.26,31,35,34

Previous studies have shown that in mice lacking CX3CR1, the capacity of subpopulations of monocyte-derived DCs to home to epithelial surfaces of the small intestine26 and cornea35 is markedly impaired. As part of a series of experiments investigating the role of CX3CR1 in murine models of ocular disease, we wanted to determine whether CX3CR1 deficiency influences the ability of DCs and macrophages to populate the...
internal compartments of the normal eye, including the uveal tract and retina.

Analysis of the distribution of eGFP+ cells in Cx3cr1Δ/Δ and Cx3cr1-/- mice in the present study showed no significant differences in cellular morphology or number of macrophages and DCs between young homozygous and heterozygous mice, demonstrating that CX3CR1 does not play a role in the homeostatic recruitment of DCs and macrophages to normal young murine uveal tissues or the retina. Turnover studies also revealed different rates of replenishment of tissue-resident macrophages and DCs in the retina and uvea in homeostatic conditions. The present study provides essential baseline data for future investigations of the role of CX3CR1 in models of uveitis and retinal degeneration.

METHODS

Animals

Female transgenic Cx3cr1Δ/Δ mice aged between 6 and 12 weeks, in which either one (Cx3cr1Δ/+) or both (Cx3cr1Δ/Δ) copies of the Cx3cr1 gene were replaced by enhanced green fluorescent protein (eGFP) were used in the present study. The transgenic mice which were on either a BALB/c or C57Bl/6 background were established from a primary colony provided by Steffen Jung (Weizmann Institute of Science, Rehovot, Israel). Wild-type (WT) mice of either strain acted as control subjects. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Creation of Chimeras for Turnover Studies

Recipient BALB/c WT mice were irradiated with two doses of 600 Gy 14 hours apart. Donor Cx3cr1Δ/Δ mice were euthanized and femurs and tibia harvested. After removal of the proximal and distal ends of the bone, the shafts were centrifuged at 10,000 rpm for 30 seconds at 4°C. The pellet was resuspended in RPMI media (N6396; Sigma), and the cells were resuspended again, and the live cells were counted by trypan blue exclusion. The cells were resuspended and diluted as appropriate. Recipient mice received an injection of 3 to 5 × 10⁶ bone marrow cells (in 150 μl) into the lateral tail vein (~2–3 hours after a second dose of irradiation). Antibiotics were given to recipient mice (neomycin trisulfate salt hydrate; Sigma-Aldrich, St. Louis, MO) for 7 days before and 2 weeks after irradiation. Chimeric animals were killed at 2, 4, and 6 weeks after transplantation (n = 6 per time point) for collection of ocular tissues to examine the recruitment of donor Cx3cr1Δ/Δ cells into WT host ocular tissue. Turnover of eGFP+ cells in the iris and choroid was calculated by counting eGFP+ cells at each time point and estimating this as a percentage of the density of eGFP+ cells in the normal Cx3cr1Δ/Δ iris and choroid.

Tissue Collection and Processing for Immunohistochemistry

After enucleation, the eyes were fixed in either 4% (uveal tissue studies) or 2% (retinal studies) paraformaldehyde and stored at 4°C until further processing. From each eye, the iris, ciliary body, choroid, and retina were dissected and cut into quadrants as previously documented. Tissue pieces were washed in PBS, incubated in 20 mM EDTA tetrasodium at 37°C for 30 minutes, then incubated with a 0.2% solution of Triton-X in PBS with 2% bovine serum albumin at RT for 10 minutes to assist in antibody penetration. Tissues were treated at 4°C overnight with rabbit anti-eGFP (1:200; Chemicon, Temecula, CA) and with PBS before incubation at RT for 60 minutes with Alexa Fluor 488 conjugated goat anti-rabbit (1:100; Invitrogen-Molecular Probes, Eugene, OR). Further washes with PBS were followed by overnight incubation (4°C) with a range of monoclonal antibodies (mAbs) including anti-MHC class II (M5/114; 1/200; BD Pharmingen, San Diego, CA), anti-CD169 (Sera; 1/100; AbD Serotec, Kidlington, UK), anti-CD68 (1/100; Serotec), anti-CD45 (1/100; Serotec), anti-F4/80 (1/50; CL-A3-1, Serotec), anti-CD11b (1/100; BD Pharmingen), vascular endothelial cell marker PECAM-1 (CD31; BD Pharmingen), and anti-CD11c (1/100, HL3 clone; BD Pharmingen), as well as isotype controls (IgG2a and IgG2b, 1/100; BD Pharmingen). Tissues were then treated with biotinylated goat anti-rat IgG (1/100; GE Healthcare, Piscataway, NJ) at RT for 60 minutes, washed with PBS and incubated with streptavidin-Cy3 (1/100; Jackson ImmunoResearch Laboratories, West Grove, PA) at RT for 60 minutes. DAPI (4′,6-diamidino-2′-phenylindole dihydrochloride; Roche Diagnostics, Mannheim, Germany) was added at RT for 7 minutes as a nuclear stain. Stained wholemount tissues were mounted onto slides (retinas were mounted with the vitreous side face up) using aqueous mounting medium (Immumount; Thermo Shandon, Runcorn, UK) and coverslipped.

Flow Cytometry

Single-cell suspensions from choroidal and retinal tissues were prepared for flow cytometric analysis. Choroid and retina from three mice were dissected and pooled. Samples were then incubated with 0.2% (wt/vol) collagenase A-EDTA in DMEM culture medium containing 5% fetal calf serum (FCS) for 2 hours with rotation (70 rpm) at 37°C. The solution was filtered through a 70-μm cell strainer, and the filtrate was collected. After they were washed, the single cells were resuspended in fluorescence-activated cell sorter (FACS) buffer (1%BSA/PBS/10 mM NaN3) and aliquots were prepared for further staining. Aliquots of choroidal and retinal single-cell suspensions were first blocked with 5% normal rat serum for 15 minutes and then stained with directly conjugated monoclonal antibodies for mouse CD45 (1/100; ICA, Ly-5, BD Bioscience), CD11b, CD11c, I-A/E (1/100; M5114; BD Bioscience), and F4/80. Biotin-labeled antibodies were detected by addition of SA-APC or SA-PE (1:100; BD Biosciences). Samples were kept on ice throughout the experiment. All antibodies were diluted in PBS containing 1% BSA. Negative controls and single fluorochrome controls were performed to allow accurate compensation. Monochrome-isotype control antibodies were used to ensure the specific staining of each antibody. All samples were analyzed by flow cytometry (CELLQuestPro software; BD Biosciences). eGFP+ cells were gated and further analyzed for other surface antigen expression.

FIGURE 1. The total number of eGFP+ cells (per square millimeter) were quantified in the iris (A) and choroid (B) of heterozygous and homozygous mice and compared by using Student’s t-test. No significant differences were noted.
Examination of Wholemount Tissue

Stained specimens were examined by both conventional epifluorescence microscopy (Olympus, Tokyo, Japan; DMRBE, Leica Microsystems, North Ryde, NSW, Australia) and confocal microscopy (TCS SP2; Leica). Confocal microscopy was used to characterize eGFP/CX3CR1$^{+}$ cells or immunopositive cells in the iris/ciliary body, choroid, and retina. Images of the entire tissue wholemount were produced by performing Z-stacks of the tissue from the internal to external aspect at increments ranging from 0.4 to 0.8 μm. Z-stacks of choroidal wholemounts did not include the full thickness of retinal pigment epithelium, because of the presence of background autofluorescence. Image-analysis software (Photoshop, ver. 7.0; Adobe Systems, San Diego, CA) was used to perform final image processing.

Quantitative Analysis of eGFP$^{+}$ Cells in the Uveal Tract of Heterozygous and Homozygous Mice

The number of eGFP$^{+}$ cells in the iris and choroid of both heterozygous ($n = 6$) and homozygous ($n = 6$) mice was quantified by two masked observers using Z-stacks acquired from confocal microscopy. Cells were counted in a $375 \times 375$-μm frame in a minimum of six randomly selected areas of each tissue. The mean cell density (per square millimeter) was calculated (Image ProPlus; ver. 5.1) and compared in heterozygous and homozygous mice using Student’s $t$-test (Prism; GraphPad Software, San Diego, CA). $P < 0.05$ was considered to be statistically significant.

RESULTS

Influence of the Absence of CX3CR1 on the Ability of DCs and Macrophages to Populate the Normal Murine Uveal Tract

The total density of eGFP$^{+}$ cells in the iris (Fig. 1A) and choroid (Fig. 1B) did not differ between heterozygous and homozygous Cx3cr1$^{gfp}$ mice. Flow cytometry of choroidal preparations concurred with these observations (Cx3cr1$^{+/+}$, 15.57% eGFP$^{+}$ cells; Cx3cr1$^{+/gfp}$, 16.67% eGFP$^{+}$ cells). In light of the demonstration in studies conducted in our laboratory$^{35}$ that some corneal epithelial DCs in Cx3cr1$^{gfp/gfp}$ lack cellular extensions, we compared the cell shape of eGFP$^{+}$ cells in heterozygous and homozygous mice. Thus, eGFP$^{+}$ cells in the iris were classified and quantified as either pleomorphic (round-irregular) or dendriform (possessing one, two, or more large thin...
branched dendritic processes). Most of the cells in both heterozygous and homozygous mice were of dendriform morphology (Cx3cr1<sup>gfp</sup>/<sup>+/−</sup>, 67%; Cx3cr1<sup>gfp</sup>gfp, 70%) and no differences were observed between the groups in either pleomorphic or dendriform cells (data not shown).

**Phenotypic Characterization of eGFP<sup>+</sup> Cells in the Iris and Ciliary Body**

The expression of eGFP by all monocyte-derived cells in the normal ocular tissues of Cx3cr1<sup>gfp</sup> mice afforded us a unique opportunity to confirm previous investigations of macrophages and DCs in the mouse uveal tract. In iris wholemounts of homozygous and heterozygous mice, a regular network of evenly spaced eGFP<sup>+</sup> cells displaying mixed morphologic characteristics was present throughout the stroma (Fig. 2A). In addition, a novel population of cells not previously appreciated, was clearly demonstrable on the posterior iris surface (Figs. 2B, 2C). This newly discovered population of eGFP<sup>+</sup> cells were CD169<sup>+</sup>, MHC Class II<sup>+</sup>, CD45<sup>+</sup>, and CD11b<sup>+</sup> (data not shown). Isotype controls (IgG2a and IgG2b) were negative (Fig. 2D). A large proportion of eGFP<sup>+</sup> cells in the iris coexpressed MHC Class II (68%, Fig. 2E), with dendriform cells tending to display stronger expression. Staining with CD11c did not produce consistent immunostaining results in any tissue. The majority of eGFP<sup>+</sup> cells coexpressed the macrophage marker CD169 (Figs. 2F, 2G). All eGFP<sup>+</sup> cells coexpressed CD68 (Fig. 2H, 2I), CD11b (Fig. 2J, 2K), and CD45 (Fig. 2L). In light of the recent discovery of Cx3cr1<sup>+</sup> cells patrolling the lumen of blood vessels in the intestine and skin, we performed staining with the vascular endothelial cell marker PECAM-1 to determine whether any of the eGFP<sup>+</sup> cells in our tissues were intravascular. The analysis demonstrated that while many eGFP<sup>+</sup> cells were closely related to vessels, they all appeared to have a perivascular distribution with no cells observed within the lumen of the iris vessels (Fig. 2M).

Confocal analysis of ciliary body wholemounts in both heterozygous and homozygous mice revealed a dense network of dendriform and pleomorphic eGFP<sup>+</sup> cells (Fig. 3A). Isotype controls (IgG2a and IgG2b) were negative (Fig. 3B). A major proportion of eGFP<sup>+</sup> cells were MHC Class II<sup>+</sup> (Fig. 3C). This positivity was particularly evident in the population of highly dendriform intraepithelial cells situated within the ciliary processes (Fig. 3D). Confirmation of this location was made possible by reference to nuclear staining (DAPI; data not shown). Whereas most eGFP<sup>+</sup> cells in the ciliary body stroma coexpressed CD169, the highly dendriform intraepithelial cells...
were CD169\textsuperscript{lo} or CD169\textsuperscript{−} (Figs. 3E, 3F). eGFP\textsuperscript{+} cells in the ciliary body stroma were CD68\textsuperscript{lo} (Figs. 3G, 3H) in comparison to the level of expression observed in the iris population. All eGFP\textsuperscript{+} cells were CD11b\textsuperscript{+} (Figs. 3I, 3J) and CD45\textsuperscript{+} (Figs. 3K, 3L).

**Phenotypic Characterization of eGFP\textsuperscript{+} Cells in the Choroid of Cx3cr1\textsuperscript{gfp} Mice**

Large numbers of pleomorphic and dendriform cells were observed in the choroid of both heterozygous and homozygous Cx3cr1\textsuperscript{gfp} mice (Fig. 4A). The elongated and pleomorphic cells displayed a more perivascular orientation than was evident in the iris (Fig. 4A). Isotype controls were negative (Fig. 4B). The majority of eGFP\textsuperscript{+} cells were MHC Class II\textsuperscript{+} (90%; Fig. 4C), with high expression on the fine cellular processes of the more dendriform cells (Fig. 4D). All eGFP\textsuperscript{+} cells were CD169\textsuperscript{+} (Figs. 4E, 4F), CD68\textsuperscript{+} (Figs. 4G, 4H), CD11b\textsuperscript{lo} (Figs. 4I, 4J), and CD45\textsuperscript{+} (Figs. 4K, 4L).

**Retinal Microglia in Cx3cr1\textsuperscript{gfp} Mice**

Microglial cell populations in heterozygous (Fig. 5A) and homozygous (Fig. 5B) mice were compared in retinal whole-mount preparations by generating Z-series which included all retinal layers. No differences were observed in the overall distribution or topography of eGFP\textsuperscript{+} microglia in these normal young mice.

Microglia reside as well recognized populations in the nerve fiber/ganglion cell layer (GCL), inner plexiform layer (IPL), and outer plexiform layer (OPL) of the retina.\textsuperscript{8,39,40} Therefore, in the present study, images were collected at each of the aforementioned retinal layers to compare morphology and distribution of microglial subpopulations in heterozygous and homozygous mice. Microglia in the GCL (Figs. 5C, 5D) possessed fusiform cell bodies and two to four elongated, ramified processes. No morphologic differences between microglial populations in this layer was noted between heterozygous (Fig. 5C) and homozygous (Fig. 5D) mice. At the level of the IPL (Fig. 5E, 5F), dense networks of microglia possessed rounded cell bodies and displayed more highly ramified morphology, with no differences observed between heterozygous (Fig. 5E) and homozygous (Fig. 5F) mice. Microglia in the OPL were similar to those in the IPL in morphology with slightly lower density. There were no differences between heterozygous and homozygous mice (data not shown).

Flow cytometric analysis concurred with the retinal whole-mount studies and revealed no significant differences in the

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**Figure 4.** Confocal analysis of eGFP\textsuperscript{+} cells in choroidal wholemounts of naïve heterozygous and homozygous mice. Pleomorphic eGFP\textsuperscript{+} cells were largely perivascular in their orientation (A). Isotype controls were negative but displayed mild autofluorescence of the retinal pigment epithelium (B). eGFP\textsuperscript{+} cells were variable in their expression of MHC Class II (C, D). All eGFP\textsuperscript{+} cells were CD169\textsuperscript{+} (E, F), CD68\textsuperscript{−} (G, H), CD11b\textsuperscript{lo} (I, J), and CD45\textsuperscript{+} (K, L).
that had received bone marrow from CεGFP expression of surface antigens, by using various mAbs. Most heterozygous and homozygous mice were further analyzed for both the vitreal aspect and in the deeper layers of the retina.

The turnover of eGFP cells in homozygous and heterozygous mice in the uveal tract and retina, indicating a lack of dependency of Cx3cr1 on the homing of uveal tract macrophages and retinal microglia in normal young mice. This contrasts with the recently demonstrated Cx3cr1-dependent homing of DCs to the corneal15 and intestinal epithelium.26 Although various chemokines such as MIP-1α (macrophage inflammatory protein-1α), MIP-1β, MCP-1 (monocyte chemoattractant protein-1), RANTES (regulated on activation normal T-cell expressed and secreted), and the chemokine receptor CCR5 have been implicated in directing the migration of monocyte-derived cells in various ocular inflammatory conditions and disease states,35-37 less is known about which chemokines or chemokine receptors play a role in the homing of these cells to ocular tissues in the steady state. It is likely that chemokines such as MCP-1 (Ccl-2) play a more direct role in the homing of macrophages to the uveal tract and retinal tissues, since mice deficient in Ccl-2 or its receptor (Ccr-2) acquire defects in macrophage recruitment to various tissues,46 including the retina.59

The present study, while confirming previous investigations of the distribution and phenotype of monocyte-derived cells in the murine iris, ciliary body, choroid, and retina also served to delineate a previously unrecognized population of macrophages on the posterior surface of the mouse iris. These cells were immunophenotypically identical with iris stromal macrophages, but differed slightly in their morphologic appearance and closely resembled hyalocytes, the “specialist” macrophages of the vitreous.50-52 Part of the motivation for a thorough examination of monocyte-derived macrophages in the normal choroid and retina using the present model was due to reawakening interest in the role of these cells as potential contributors to the pathogenesis of age-related macular degeneration (AMD).21-22 AMD is characterized pathologically by the accumulation of basal laminar deposits internal to the RPE basement membrane and membranous debris that accumulates to form basal linear deposit and soft drusen, both of which are external to the RPE basement membrane. Previous reports have suggested that increased choroidal macrophages are associated with the pres-
ence of basal laminar deposits and membranous debris. However, the influence of the progressive accumulation of these deposits on the number and activation states of both recruited and resident choroidal macrophages at each stage of degeneration is poorly understood. Immunoelectron microscopic studies of the rat choroid have revealed that macrophages and MHC class II+ putative DCs are located directly beneath Bruch’s membrane and in the intercapillary piliars, however, their function and turnover rate and the factors mediating their migration, retention, and possible egress in this tissue microenvironment are only now coming under scrutiny. A recent elegant study of knockout mice lacking the monocyte chemotactic protein-1 (MCP-1 or Ccl-2) and its cognate receptor Ccr-2 showed the development of sentinel clinical and morphologic features of wet (i.e., accompanied by neovascular changes) and dry AMD with increasing age. It has been postulated that a defect in the housekeeping role of choroidal macrophages in degrading deposits of complement...
and IgG is present in ageing Ccr-2 or Ccl-2 knockout mice, further fueling speculation that drusen deposits at the choroidal-retinal interface in human AMD are the result of defective clearance or scavenging by resident macrophages or even DCs.18,21,22

In the present study, flow cytometric analysis performed on eGFP+ cells from retinal cell preparations revealed a phenotype (F4/80+, CD45−, MHC Class II+, and CD11c−) consistent with previous studies of retinal microglia40,42,55 and also other investigations that have used the Cx3cr1fl/fl model in CNS tissues.27,44 In light of interest in the role of Cx3cr1 in the homing of monocyte-derived cells and neural repair and the potential role of Cx3cr1 in AMD,56 we sought to investigate whether there was any difference between retinal microglia in heterozygous and homozygous mice. Both immunohistochemical and flow cytometric analysis revealed no significant differences in the distribution and density of resident populations of microglia in the retina of normal young mice. This result demonstrates that Cx3cr1−/− macrocyte precursors of microglia are not hampered in their homing to this neural environment in homeostatic conditions. Confirmation that continual renewal or turnover of microglia, albeit at a slower rate than in the uveal tract, is occurring was provided by our novel chimera model.57 In our study, Cx3cr1fl/fl cells may have been recruited earlier in BALB/c mice due to the possible strain-related differences in the integrity of the blood-retinal barrier (BRB).58 Although no differences in microglia populations were noted in normal young Cx3cr1fl/fl mice during this study, we observed an accumulation of photoreceptor-laden microglia in the subretinal space in older animals. Since submission of this manuscript, a very detailed analysis of this phenomenon has been published that suggests a possible dysfunction in the retinas of aged transgenic albino mice that lack Cx3cr1.56

The chemokine receptor CX3CR1 has recently been shown to be critically involved in the normal homostatic recruitment of monocyte-derived cells in a variety of tissues, and CX3CL1-mediated leukocyte chemotaxis and adhesion have been implicated as a key player in a wide range of inflammatory conditions such as atopic dermatitis, collagen-induced arthritis, and autoimmune myositis.59–61 In addition, since the presence of constitutive CX3CL1 has now been confirmed in several different tissues including that of the CNS62,63 and human ocular tissues such as the iris and choroid,64 it seems reasonable to speculate that the CX3CL1/CX3CR1 dyad may be involved in regulating both the development and progression of various CNS and ocular inflammatory disorders. Indeed, recent data suggest Cx3cr1 may have a role in regulating NK cell migration into the CNS,65 whereas the expression of both CX3CL1 and its receptor has been recently demonstrated in experimental autoimmune anterior uveitis66 and an association has been reported between CX3CR1 polymorphisms and the increased risk of AMD.67,68 and retinal vasculitis.69 The most recent data indicate that homozygosity in the M280 allele of CX3CR1 was consistently more frequent in patients with AMD, suggest-

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<tr>
<th>Tissue</th>
<th>eGFP+ Cell Density in Cx3cr1fl/fl Mice (A)</th>
<th>eGFP+ Cell Density in 6-Week Chimera (B)</th>
<th>% Turnover at 6 Weeks (B/A × 100)</th>
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<tbody>
<tr>
<td>Iris</td>
<td>651.53 ± 39.24</td>
<td>474.75 ± 18.68</td>
<td>73</td>
</tr>
<tr>
<td>Choroid</td>
<td>524.4 ± 28.82</td>
<td>328.55 ± 14.22</td>
<td>63</td>
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Counts (per square millimeter) were calculated as a percentage by dividing the density of donor Cx3cr1fl/fl+ cells by the normal density of eGFP+ cells in Cx3cr1fl/fl+ mice.
ing that impaired migration and accumulation of microglia in the subretinal space may be a primary occurrence in AMD and not a secondary phenomenon as previously believed. The Cx3cr1<sup><i>−/−</i></sup> mice offer exciting possibilities as a tool to investigate the role of CX3CR1 and various monocyte-derived cells in models of ocular diseases such as EAU and AMD.

**Acknowledgments**

The authors thank Wally Langdon (Faculty of Medicine, Dentistry and Health Sciences, University of Western Australia (UWA)) for assistance and advice in setting up bone marrow chimeras; Rajin Nathan and Karen Waldock for performing all mouse irradiations in the Department of Radiation Oncology (QEI Medical Centre, UWA); and Valentina Voigt (Lions Eye Institute) for providing technical assistance with tail vein injections for creation of bone marrow chimeras.

**References**


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