

Light-Induced Migration of Retinal Microglia into the Subretinal Space

Tat Fong Ng and J. Wayne Streilein

PURPOSE. To explore the effects of light exposure and deprivation on the distribution and function of microglia in the subretinal space of mice.

METHODS. Using a monoclonal antibody, 5D4, that identifies resting, ramified microglia, the distribution and density of microglia in the retina, and the subretinal space were determined by confocal microscopy and by immunohistochemistry of cryopreserved sections of eyes of albino and pigmented mice exposed to diverse levels of light, ranging from complete darkness to intense brightness. Axotomized retinal ganglion cells were retrograde labeled by fluorescent tracer to determine whether the marker colocalizes to 5D4⁺ cells. Electron microscopy was used to evaluate microglia for evidence of phagocytosis.

RESULTS. 5D4⁺ microglia in pigmented eyes were limited to the inner retinal layers, but in albino eyes 5D4⁺ cells were found in the outer retinal layers and subretinal space as well. The subretinal space of eyes of albino mice raised from birth in complete darkness contained few 5D4⁺ cells, but exposure to light caused the rapid accumulation of 5D4⁺ cells at this site. 5D4⁺ cell density in the subretinal space correlated directly with intensity of ambient light. Retrograde labeling of axotomized ganglion cells resulted in 5D4⁺ cells in the subretinal space that contained the retrograde label. Subretinal microglia contained phagocytized rod outer segment discs. On intense light exposure, 5D4⁺ cells adopted an active morphology, but failed to express class II major histocompatibility complex (MHC) molecules.

CONCLUSIONS. Light exposure induced retinal microglia migration into the subretinal space in albino mice. Subretinal microglia appeared to augment through phagocytosis the capacity of pigment epithelium to take up the photoreceptor debris of light toxicity. The unexpected presence of these cells in the subretinal space raises questions concerning their potential contribution to immune privilege in this space and to the fate of retinal transplants. (*Invest Ophthalmol Vis Sci.* 2001;42:3301-3310)

For many of the blinding diseases of the retina, there are no effective treatments or preventions. In this dire clinical circumstance, retinal transplantation has been advocated as a means of restoring sight. To date, there have been limited reports of successful transplantation of a functioning mammalian retina, in experimental animals or in human beings.¹⁻³ The

obstacles to successful retinal transplantation are obvious and formidable, and they include the potential of foreign retinal grafts to be rejected immunologically. Because the eye is regarded as an immune-privileged site, there is considerable interest in knowing the extent to which immune privilege mitigates the threat of immune rejection of retinal transplants, as it does for corneal transplants. Following a line of investigation initiated more than a decade ago by Luke Qi Jiang et al.,⁴⁻⁶ our laboratory has adopted a systematic approach to understanding the vulnerability of neuronal retinal transplants to immune rejection. One dimension of our approach is to inquire into the existence of immune privilege in the subretinal space—the most likely site in which retinal transplants are to be placed. Another dimension to our approach is to determine the extent to which neuronal retinal tissue functions as an immune-privileged tissue.

In the course of studies into the fate of allogeneic neonatal neuronal retina (NNR) transplants in the eye (anterior chamber, subretinal space), we discovered that microglia indigenous to the graft have important roles to play in dictating graft outcome.⁷ Both syngeneic and allogeneic NNR allografts fulfill portions of their development program when implanted into the anterior chamber and subretinal space of eyes of adult mice. Photoreceptors differentiate, but form rosettes, presumably because there are no retinal pigment epithelial (RPE) cells nearby. As these rosettes form, a significant number of microglia derived from the NNR graft migrate into the rosette's center. At this site, the microglia become activated—that is, they express D-galactose, which binds the lectin derived from *Griffonia simplicifolia*, and they express class II major histocompatibility (MHC) molecules. As proposed originally by Banerjee and Lund,⁸ this activation may coincide with, or even result from, the acquisition of the capacity of these microglia to phagocytize rod outer segments. The symbiosis of activated microglia and photoreceptors in these aberrant rosettes appears to promote the survival of photoreceptor cells in the absence of pigment epithelium and thereby promotes survival of the NNR graft. Thus, graft-derived microglia have a positive effect on graft outcome.

However, activation of microglia in this manner also has a deleterious effect on survival of NNR allografts. Ma and Streilein⁷ have reported that as NNR allografts gradually deteriorate in the anterior chamber, they become infiltrated with recipient-derived CD4⁺ T cells. Simultaneously, donor-derived microglia within the grafts, especially those within rosettes, express extremely high levels of class II MHC molecules. Because microglia are the only cells within the graft that express these molecules, the authors have proposed that effector CD4⁺ T cells that recognize donor antigens on microglia induce rejection, and the graft then deteriorates under the influence of the proinflammatory cytokines that are released locally. These results suggest that not only the ability of neonatal neuronal retinal allografts to sensitize their recipients is due to their content of microglia, but the vulnerability of these grafts to rejection by CD4⁺ T cells is also largely dictated by activated, graft-derived microglia.

To enhance our understanding of the immunobiology of microglia within retinal tissue and in light of their changing

From The Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts.

Supported by National Institutes of Health Grant EY-09595 and the Minda de Gunzburg Research Center for Retinal Transplantation.

Submitted for publication March 29, 2001; revised August 23, 2001; accepted September 5, 2001.

Commercial relationships policy: N.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: J. Wayne Streilein, Schepens Eye Research Institute, 20 Staniford St., Boston, MA 02114.
waynes@vision.eri.harvard.edu

states of activation and expression of cell surface molecules, it is necessary to be able to identify microglia unequivocally, irrespective of their state of activation, and to distinguish them from monocytes and macrophages of peripheral blood origin. It has been reported that resting microglia express the complement receptor CR3 and CD11B and the molecules identified by monoclonal antibody F4/80. In addition microglia, especially when activated, can be stained with GS lectin and anti-MHC class II antibodies.⁹⁻¹² However, none of these reagents permits us to distinguish resting, ramified microglia from blood-derived macrophages. It has recently been reported¹³⁻¹⁵ that a monoclonal antibody, 5D4, that is directed at keratan sulfate proteoglycans (KSPGs), labels a subset of resting, ramified microglia in the central nervous system (CNS) and can distinguish these cells from blood-borne monocytes and macrophages. Not only have we determined that 5D4 can identify resting microglia in the retina, but the availability of this antibody has enabled us to discover that exposure to light has a powerful effect on the distribution and activation of retinal microglia, especially in albino mice. The results of these experiments form the basis of this report.

MATERIALS AND METHODS

Experimental Design

All experimental procedures concerning animals in this study were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All mice were housed and bred in two different rooms. Mice in the light room were maintained in a 12-hour dark-12-hour light cycle (referred to as cyclic light), and the range of exposure per cage was set experimentally between 100 and 500 lux. Mice in the dark room were maintained in complete darkness for the full 24-hour daily cycle. Safe red light illumination was used only during cage changing and experimental examinations.

Mice were killed by an overdose of anesthesia with a mixture of 150 mg/kg ketamine (Ketalar; Parke-Davis, Shawnee, KS) and 6 mg/kg xylazine (Rompun; Haver-Lockhart, Morris Plains, NJ) at the appropriate time points. Whole eye globes were extirpated, and the retina was dissected and fixed as a wholemount in 4% paraformaldehyde in PBS ($n = 5$ at each time point). For selected time points, eyes were cryoprotected in 30% sucrose in PBS and cut into 10- μ m cryosections.

Presence of Microglia in the Subretinal Space

Young adult (postnatal weeks 6 and 7) pigmented (C57BL/6 and DBA/2) and albino (BALB/c, A/J, and C57BL/6J-Tyr^{c2j}) mice were housed in our vivarium under normal light (250 lux outside the cage) and were killed after 1 week. For some groups of C57BL/6 and BALB/c mice, two other light conditions (100 and 500 lux) were also used. In general, the amount of light, in lux units, measured inside the cage ranged between one fifth and nine tenths the amount measured outside the cage. C57BL/6 and BALB/c mice were raised in our animal colony (Schepens Eye Research Institute, Boston, MA), DBA/2 mice were purchased from Taconic Farms (Germantown, NY), and A/J and C57BL/6J-Tyr^{c2j} mice were purchased from Jackson Laboratory (Bar Harbor, ME).

Temporal Distribution of Subretinal Microglia in Retinas

BALB/c mice ranging in age from day of birth (P0) to 18 months were raised under either complete darkness in a light-tight dark room or conventional light, as provided in our vivarium. Mice were killed at the selected ages, including postnatal days (P)0, P4, P8, P12, P15, and P21 and 6 weeks, 12 months, and 18 months. Wholemount retinas were dissected and examined microscopically after immunostaining. After immunostaining, the number of 5D4⁺ cells was counted in retinal wholemounts. A systematic, randomized sampling method (the frac-

tionator method) with the "forbidden-line" rule¹⁶ was used. The orientation of the retinas was entirely random on the slide. A systematic nonoverlapping series of fields (30-40) was examined across the whole retina at a magnification of $\times 200$. The size of each field was 0.25 mm². The total area sampled from each retina ranged from 7.2 to 9.6 mm² representing approximately 20% to 25% of the area of the retina.

Section of Optic Nerve Followed by Fluorescent Tracer Labeling

Five young adult BALB/c mice (postnatal week 6, raised in the dark room) were anesthetized with intraperitoneally injected anesthesia of mixed ketamine and xylazine (as described earlier). The left optic nerve was surgically exposed within the orbit and transected approximately 1 mm behind the optic disc. Special care was taken when opening the dura mater and when performing the transection of the optic nerve to avoid damaging either the orbital blood vessels or the internal ophthalmic artery. In mouse eyes, this artery lies beneath the optic nerve. This careful surgical procedure insured that that normal retinal circulation was preserved both during and after surgery. A small piece of gelfoam soaked with 5% fluorescent tracer (FluoroGold; Fluorochrome, Denver, CO) was deposited immediately at the transection site. This dye is taken up by severed axons and is quickly transported retrograde into the parent ganglion cell bodies in the retina. It has been demonstrated that approximately 50% of retinal ganglion cells die within 7 days after axotomy¹⁷ and that microglial cells phagocytize membrane particles containing the dye.¹⁸ Two weeks after axotomy, most microglial cells in the ganglion cells layer should be specifically labeled. After axotomy, experimental mice were transferred from an illumination condition of 100 to 500 lux in an effort to induce migration of microglia to the subretinal space. After 1 week of illumination in 500 lux, the animals were killed. Ten-micrometer cryosections were prepared and immunolabeled using 5D4 antibodies.

Immunohistochemistry

The following antibodies, including anti-keratan sulfate proteoglycan (5D4, 1:300; Sekagaku, Tokyo, Japan), macrophage marker (F4/80, 1:100; Caltag, Burlingame, CA), anti-MHC II (I-A^d, 1:100; PharMingen, San Diego, CA), anti-neurofilament M (NF-M, 1:1000; Chemicon, Temecula, CA), anti-inter-retinoid-binding protein (IRBP, 1:100) and anti-zonula-occludens (ZO-1; 1:250; Zymed, San Francisco, CA) were used to label the microglia, retinal ganglion cells, and photoreceptors. Secondary antibodies tagged with Cy-2 (1:100) and Cy-3 (1:1000; Jackson ImmunoResearch, West Grove, PA) directed against the specific primary antibodies were used. Wholemount retinas were first made permeable by incubating in 1% Triton X-100 in PBS for 1 hour and washed three times in PBS. The retinas were then incubated in selective primary antibodies in 0.1% TX-100 overnight and rinsed thoroughly before the application of the secondary antibodies. For double labeling, second primary antibodies were applied after the first secondary antibodies were rinsed off. After the staining, confocal microscopy with a confocal laser scanning microscope (TCS 4D; Leica, Deerfield, IL) was used to examine the double-labeled cells.

Electron Microscopy

Animals were perfused with 0.8% saline and then with half-strength Karnovsky fixative (pH 7.4). Eyes were removed and postfixed in half-strength Karnovsky fixative for 24 hours. The cornea and the lens were removed to prepare a posterior eye cup, which was embedded in Epon-Araldite. Semithin sections (1-2 μ m) were stained with toluidine blue; ultrathin sections (60-90 nm) were stained with uranyl acetate and lead citrate before examination in a transmission electron microscope (EM410; Philips, Eindhoven, the Netherlands).

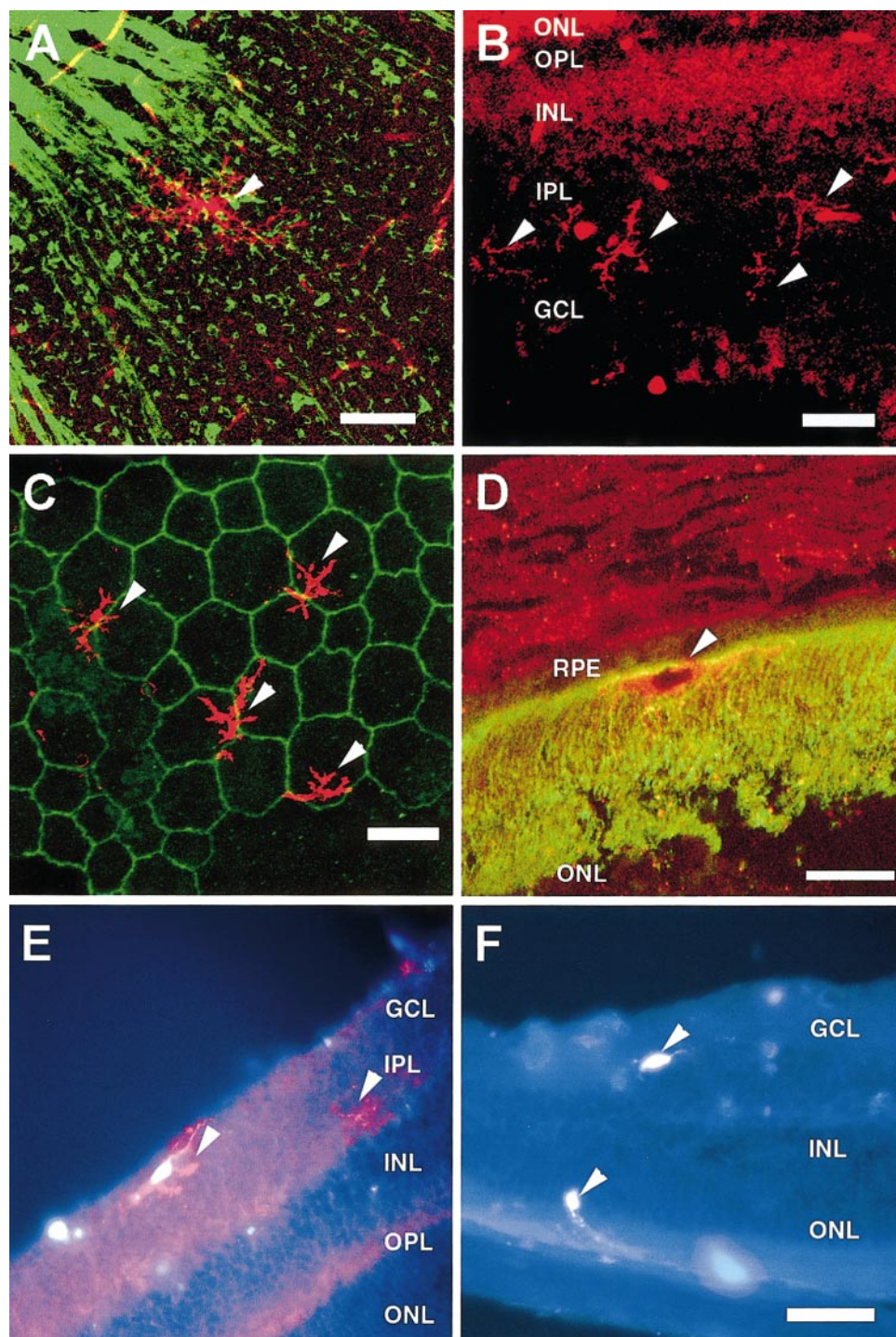


FIGURE 1. Immunolabeling of retinal microglia with 5D4 antibody. *Arrowheads* indicate microglia in all panels. (A) A ramified microglia stained with 5D4 antibody (*red*) in the ganglion cell layer of a whole-mount of retina from adult BALB/c mouse. The section is counterstained with neurofilament antibody (*green*). (B) Several ramified microglia in the retinal ganglion cell layer of a 10- μ m cryosection. (C) Ramified 5D4⁺ microglia resting on the surface of an intact layer of RPE counterstained (*green*) with anti-ZO-1 antibody. (D) A ramified 5D4⁺ cell resting among photoreceptor cells stained with anti-IRBP antibody (*green*). (E) Ramified 5D4⁺ cells double labeled with fluorescent tracer (*yellow*) and 5D4 (*red*) in the ganglion cells layer. (F) A fluorescent tracer (*yellow*)-labeled microglia in the subretinal space. GCL, ganglion cells layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; OPL, outer plexiform layer. Scale Bar, 20 μ m.

RESULTS

Distribution of 5D4-Reactive Microglia in Normal Retina

Eyes of normal, adult BALB/c, A/J, DBA/2, and C57BL/6 mice were enucleated. Wholemounts of retina were prepared, fixed in 4% formaldehyde, and stained with 5D4 antibody. The wholemounts were then observed by confocal microscopy. Cryopreserved sections were also prepared from some eyes to describe the distribution of 5D4⁺ cells according to the various layers of the retina. As displayed in Figures 1A and 1B, extensively ramified microglia with slender cell bodies were de-

tected in the ganglion cell layer of all strains of mice. In eyes of DBA/2 and C57BL/6 mice, this was the primary site of localization, although, very infrequently, 5D4⁺ cells were also present in the inner plexiform layer and inner nuclear layer. It is important to point out that virtually no 5D4⁺ cells were detected in the outer retinal layers or in the subretinal space. By contrast, in the retinas of BALB/c and A/J mice, large numbers of 5D4⁺ cells were found well beyond the ganglion cell, inner plexiform, and inner nuclear layers. Particularly, 5D4⁺ cells were detected within the outer nuclear layer and even among the photoreceptors. In fact, the highest density of 5D4⁺ cells within the retina was found among the rod outer

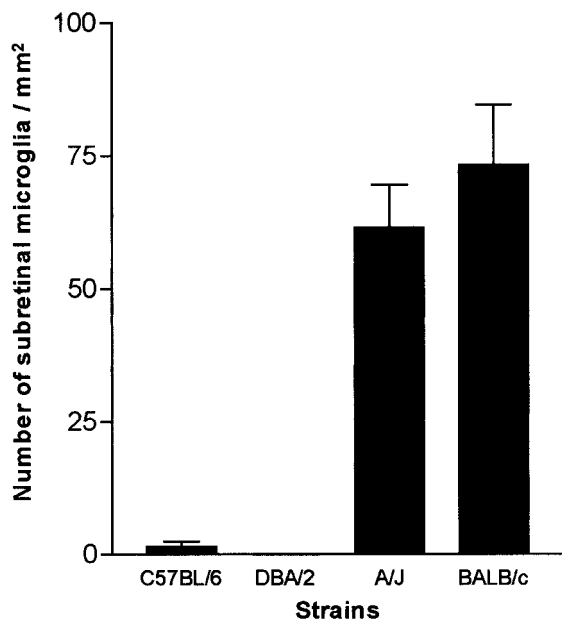


FIGURE 2. Density of 5D4⁺ cells in subretinal space of different strains of adult mice. The density of 5D4⁺ microglia in the subretinal space of retinal wholemounts (five eyes per group) was determined by confocal microscopy for BALB/c and A/J mice (albino) and for DBA/2 and C57BL/6 mice (pigmented). Bars, mean number of cells per square millimeter \pm SEM.

segments and, apparently, within the subretinal space. To further localize these 5D4⁺ cells, wholemounts of BALB/c retinas were counterstained with a monoclonal antibody directed at ZO-1, a component of the tight junctions that unite RPE cells at their apices. As the image displayed in Figure 1C reveals, 5D4⁺ cells were found to be located immediately adjacent to the RPE. To confirm the location of 5D4⁺ cells within the subretinal space, BALB/c retinas were cryopreserved, sectioned, and stained with 5D4 and with an antibody directed at interphotoreceptor retinoid-binding protein, a component of the interphotoreceptor matrix of the subretinal space. 5D4⁺ cells were readily detected within the subretinal space in these sections (Fig. 1D). These results indicate that 5D4 detects resting, ramified retinal microglia, as it does similar microglia in the brain. To show that the 5D4 cells we observed were microglia rather than detached RPE cells, the whole-mount retina was double labeled with antibodies to 5D4 and F4/80. All 5D4-bearing cells were also F4/80⁺ (data not shown). More important, these findings indicate that the distribution of these microglia is not identical in the retinas of all mouse strains. In certain strains, 5D4⁺ microglia are not only present (as expected) in the inner layers of the retina, but also in the outer layers, including the subretinal space.

The density of 5D4⁺ cells within the subretinal space of retina wholemounts was assessed by microscopy in all four strains of mice. The results (Fig. 2) indicate that rare to no 5D4⁺ cells were present within the subretinal space of eyes of C57BL/6 and DBA/2 mice. By contrast, upward of 60 5D4⁺ cells/mm² were detected in the subretinal space of BALB/c and A/J retinas. Because large numbers of 5D4⁺ microglia were found within the subretinal space of eyes of albino mice (BALB/c, A/J) but not within this space in eyes of pigmented mice (C57BL/6, DBA/2), we suspected that distribution of 5D4⁺ microglia in mouse retinas may be influenced by light exposure in relation to intraocular pigment deposition.

Origins of 5D4⁺ Microglia that Accumulate in Subretinal Space on Light Exposure

Because 5D4⁺ microglia are normally present in the inner layers of the retina, it is simplest to speculate that these microglia respond to light exposure and toxicity by migrating into the outer layers and subretinal space. To evaluate the validity of this speculation, we labeled microglia in the inner retinal layers as follows. The optic nerve of BALB/c mice was transected, and a small piece of gelfoam soaked with 5% fluorescent tracer was applied to the transected end. The label was expected to be transported retrograde to ganglion cells of the retina, and when these cells underwent degeneration (as a consequence of the transection), the tracer was released and could be taken up by resident microglia. Therefore, if 5D4⁺ cells containing fluorescent tracer appeared in the subretinal space, this provides prima facie evidence that the cells originated from the inner retinal layers. Adult BALB/c mice, after optic nerve transection and fluorescent tracer labeling, were kept in dim light (100 lux) for 2 weeks and then transferred to bright light (500 lux). After an additional 2 weeks, the manipulated eyes were enucleated, and the distribution of 5D4⁺ and fluorescent tracer⁺ cells in the retina was evaluated. Representative microscopic images are displayed in Figures 1E and 1F. As before, highly ramified 5D4⁺ microglia were present in the inner and outer layers of the retina, and in the subretinal space. Fluorescent tracer⁺ cells were also detected in these locations (Fig. 1F). The double-labeled image showed that some (but not all) of the 5D4⁺ cells in the inner layers of the retina were labeled with fluorescent tracer⁺ (Fig. 1E), indicating that they had phagocytized components of degenerating ganglion cells. More important, some of the 5D4⁺ cells in the subretinal space were also found to contain fluorescent gold tracer. We interpret these results to mean that at least some of the 5D4⁺ cells in the subretinal space of retinas exposed in vivo to bright light arose from the inner layers of the retina and migrated into the outer layers and subretinal space.

Influence of Light Exposure on Microglia Distribution in Eyes of Albino and Pigmented Mice

In our vivarium, average light exposure is approximately 250 lux, and animals are exposed to a 12-hour on-off cycle. Our next experiments were designed to allow us to examine microglia distribution in eyes of mice that were exposed for prolonged intervals to sustained, high-intensity light, and eyes in a different group of mice that were deprived of light exposure for prolonged intervals, including from birth. In the first of these experiments, one group of BALB/c mice, born to mothers that were placed in absolute darkness, were raised in this dark environment until individual groups were killed at 15 days, 3 weeks, and 6 weeks. The density of 5D4⁺ cells in the subretinal space of the enucleated eyes was then assessed as described. Companion BALB/c mice were born to mothers raised under conventional light in our vivarium. Groups of these pups were killed at similar time intervals, and the content of 5D4⁺ cells in the subretinal space determined.

The results of these experiments are presented in Figure 3. In mice raised and maintained under conventional light, significant numbers of 5D4⁺ cells were detected in the subretinal space as early as 15 days after birth. The density of these cells continued to increase, reaching more than 70/mm² at 6 weeks. In contrast, fewer than 10 cells/mm² were present in the subretinal space of eyes of BALB/c mice raised and maintained in darkness. Even at 6 weeks of age the density of these cells remained at approximately 10 cells/mm². These results, demonstrating a tight correlation between exposure to light and

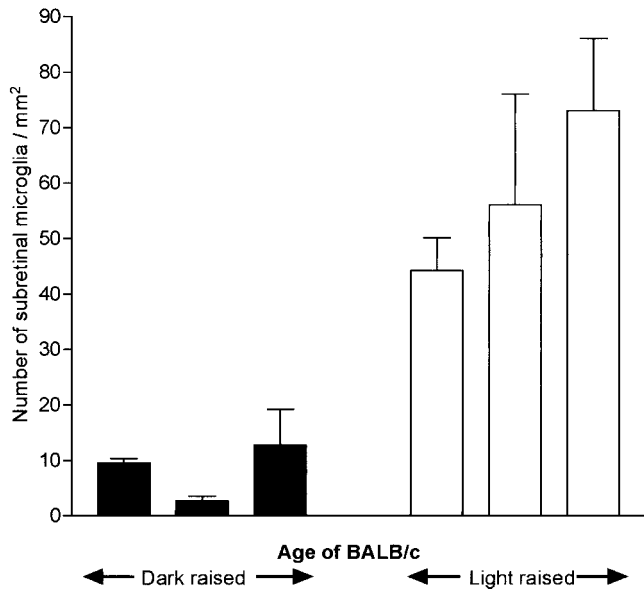


FIGURE 3. Density of 5D4⁺ cells in subretinal space of BALB/c mice raised in total darkness or in conventional light. Pregnant BALB/c females were placed in complete darkness or conventional light (250 lux) in the vivarium. When the pups were born, they were maintained under similar light conditions. Eyes of the pups were enucleated and the density of 5D4⁺ cells in the subretinal space determined at various time points after birth. Bars, mean number of cells per square millimeter ± SEM.

the gradual accumulation of 5D4⁺ cells in the subretinal space of eyes of albino mice, suggest that exposure to light is responsible for the accumulation of microglia within the subretinal space.

If the correlation between exposure to light and 5D4 cell accumulation in the subretinal space is meaningful, it might exist exclusively in albino mice, because pigmented epithelium of the iris, ciliary body, and retina acts to quench nonfocused light exposure, and therefore light toxicity, in the eye. To examine this possibility, BALB/c and C57BL/6 mice that were born to mothers raised under conventional light were maintained under conventional light and killed immediately and at P4, P8, P12, and P15; 3 and 6 weeks; and 12 months. Subretinal 5D4⁺ cell densities were determined as before on retinal wholemounts prepared from the enucleated eyes. As the results presented in Figure 4 reveal, the subretinal space of C57BL/6 mice acquired only rare 5D4⁺ cells throughout development and adult life under ambient light conditions. Only after 12 months did the density of these cells increase to significant levels (approximately 10 cells/mm²). By contrast, the subretinal space of eyes of BALB/c mice began to accumulate 5D4⁺ cells as early as P8 and increased to high levels at day 12, at approximately the time the eyelids were first opening in these pups. The level of 5D4⁺ cells in the subretinal space of adult BALB/c mice was high and remained so for the entire first year of life. The failure of microglia to accumulate in the subretinal space of eyes of pigmented C57BL/6 mice provides further support for the contention that light, perhaps light toxicity, causes the accumulation of microglia in the subretinal space of eyes of albino mice.

In the third experiment in this series, we inquired whether adult mice maintained under different levels of light would display different densities of 5D4⁺ cells in the subretinal space. For these experiments we used BALB/c mice (albino), C57BL/6 mice (pigmented), and C57BL/6J-Tyr^{c2j} mice (albino mutation on a C57BL/6 background). The last strain of mice enabled us to determine whether the important difference between BALB/c

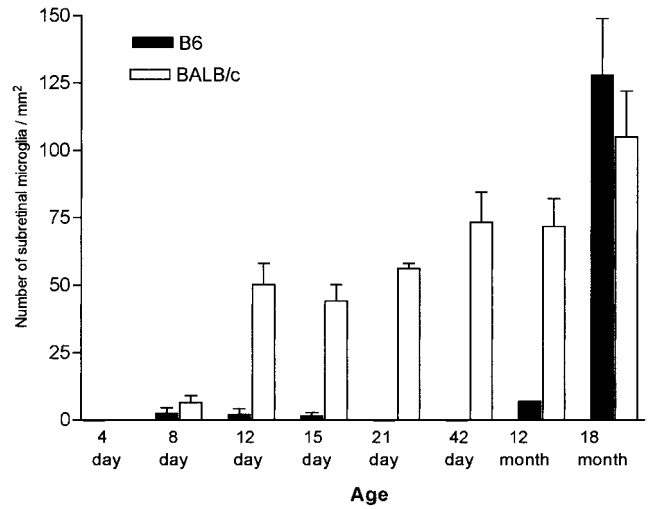


FIGURE 4. Density of 5D4⁺ cells in subretinal space of BALB/c and C57BL/6 mice during postnatal life. Eyes were enucleated in groups (five each) of BALB/c and C57BL/6 mice at various time points after parturition. The mice were housed in conventional light (250 lux) in the vivarium. Bars, mean number of cells per square millimeter ± SEM.

and C57BL/6 mice with regard to density of microglia in the subretinal space was due solely to the presence or absence of melanin pigment. Mice of each strain were born to mothers raised in complete darkness. As adults, raised and maintained in complete darkness, panels of these strains of mice were placed in three different light conditions for 2 weeks: dim (<100 lux), conventional (250 lux), or bright (500 lux). At the end of these intervals, the mice were killed, their eyes enucleated, and the density of 5D4⁺ cells in the subretinal space determined (Fig. 5). Dark-reared mice, both albino and pigmented, placed in dim light acquired very few subretinal microglia after 2 weeks' exposure. Dark-reared BALB/c mice

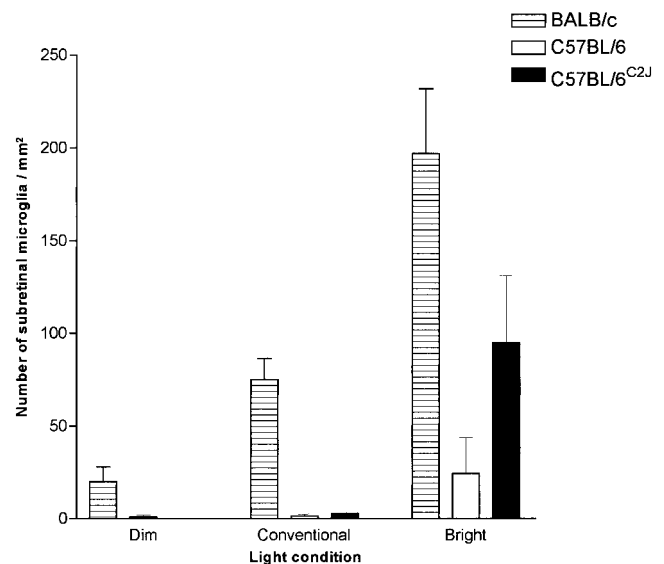


FIGURE 5. Density of 5D4⁺ cells in subretinal space of dark-raised mice after transfer to different levels of light intensity. BALB/c (albino), C57BL/6 (pigmented), and C57BL/6-Tyr^{c2j} (albino) mice were born to dark-conditioned mothers and maintained in the absence of light until adulthood (8 weeks). The mice were then transferred into light environments of different intensity (dim: 100 lux, 12-hour on-off cycle; conventional: 250 lux, 12 hour on-off cycle; bright: 500 lux, continuous light). Bars, mean number of cells per square millimeter ± SEM.

placed in conventional light for 2 weeks acquired significantly higher levels (>50 cells/mm², *t*-test, $P = 0.004$) of 5D4⁺ cells in the subretinal space, compared with dark-reared C57BL/6 and C57BL/6J-Tyr^{c2J} mice that displayed very few 5D4⁺ cells in the subretinal space after 2 weeks' exposure to conventional light. Under bright light for 2 weeks, the density of 5D4⁺ cells rose greatly in BALB/c and C57BL/6J-Tyr^{c2J} subretinal spaces (>180 and >90 cells/mm², respectively; *t*-test, $P = 0.06$), compared with the density of these cells in the subretinal space, which remained low in C57BL/6 mouse retinas (between 10 and 20 cells/mm²; *t*-test, $P = 0.002$). On the one hand, these results further support the hypothesis that light exposure (perhaps causing toxicity) induces 5D4⁺ microglia to appear in the subretinal space of retinas of albino, but not pigmented, mice. On the other hand, the accumulation of 5D4⁺ cells was more impressive in the subretinal space of BALB/c than in C57BL/6J-Tyr^{c2J} retinas (both strains are albinos). This suggests that genetic factors beyond melanin pigmentation may mean that factors in addition to light influence the magnitude of this light-induced effect.

Influence of Change in Light Intensity on Maintenance of 5D4⁺ Cell Density in the Subretinal Space

Microglia are mobile cells, and this feature caused us to wonder whether a change in light exposure would cause a commensurate change in 5D4⁺ cell density in the subretinal space. To examine this matter, BALB/c mice that had been raised in complete darkness were placed, as adults, in bright light (500 lux, continuous exposure). Groups of animals were killed after 1, 2, 3, 7, and 14 days' exposure to this level of light, and a density of 5D4⁺ cells in the subretinal space was determined. In companion experiments, adult BALB/c mice that were raised and maintained under conventional light (250 lux, 12-hour light-dark cycle) were placed in complete darkness. Groups of these mice were killed after 1, 2, and 3 weeks of complete darkness, and the density of subretinal 5D4⁺ cells determined. The results of these experiments are presented in Figure 6. Dark-raised mice suddenly exposed to bright light (Fig. 6A) rapidly acquired 5D4⁺ microglia in the subretinal space, reaching levels in excess of 50 cells/mm² at 1 week, and greater than 200 cells/mm² at 2 weeks. Mice raised in conventional light and suddenly placed in complete darkness (Fig. 6B) displayed little if any change in the density of microglia in the subretinal space. In the third experiment of this series, adult BALB/c mice maintained for 2 weeks under bright light (continuous 500 lux) were placed in complete darkness and their retinas examined for 5D4⁺ cells in the subretinal space after 2 weeks. Whereas the density of 5D4⁺ cells in the subretinal space after 2 weeks of bright light was almost 200 cells/mm², the density of these cells in the subretinal space after 2 weeks of darkness decreased to approximately 100 cells/mm² (Fig. 6B). Together, these results support the view that the density of microglia in the subretinal space of eyes of albino mice is directly related to the intensity of ambient light exposure. At the extremes—animals maintained in complete darkness versus those maintained in bright light—changes in ambient light were reflected in significant changes in microglia density in the subretinal space. By contrast, for animals maintained in conventional light, exposure to complete darkness had very little immediate effect (within 3 weeks) on the density of 5D4⁺ cells in the subretinal space.

Evidence for Phagocytosis by Microglia in the Subretinal Space

The presence of cells with the potential for phagocytosis in a site in which phagocytosis by RPE (of effete rod outer seg-

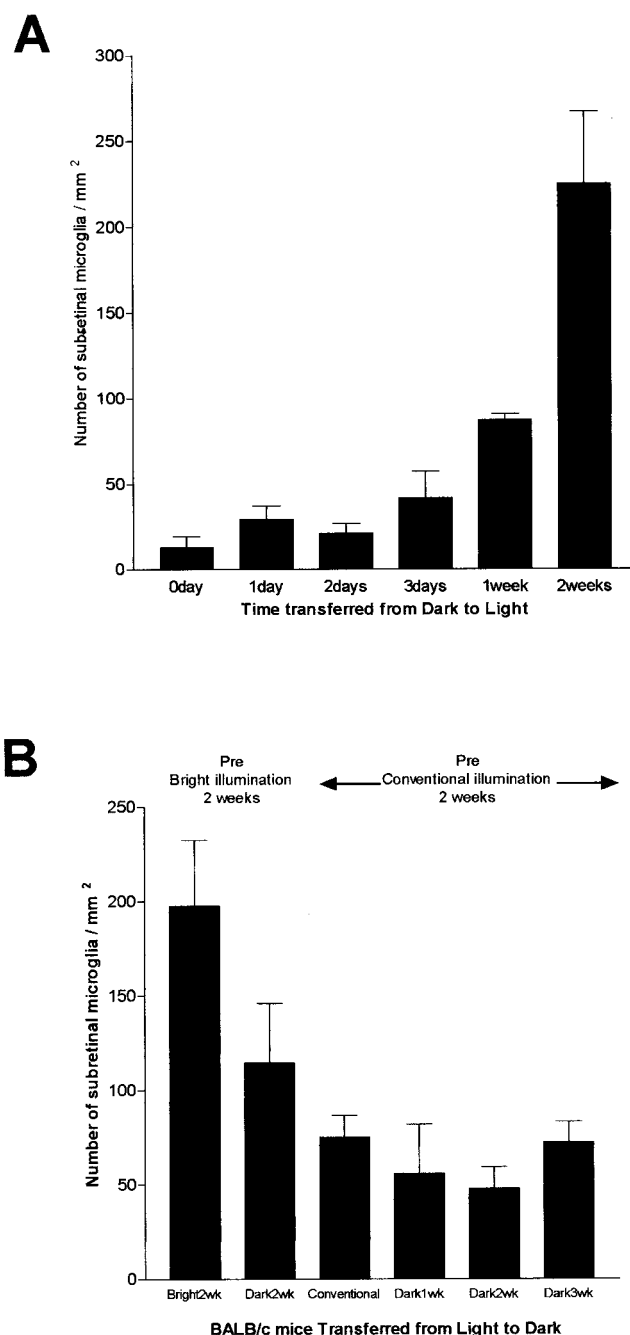


FIGURE 6. Change in 5D4⁺ cell density in subretinal space after shifting mice from dark to light conditions and vice versa. (A) BALB/c mice were raised in complete darkness. As adults, the mice were transferred into bright light (500 lux, continuous). (B) Adult BALB/c mice raised in conventional light were exposed for 2 weeks to bright light (500 lux, continuous). The mice were then transferred into an environment of complete darkness. Eyes were enucleated from both sets of mice at periodic intervals, and the density of 5D4⁺ cells in the subretinal space was determined. Bars, mean number of cells per square millimeter \pm SEM.

ments) occurs constitutively led us to inquire whether microglia in the subretinal space of albino eyes are capable of phagocytosing rod outer segments after light exposure. Retinas from eyes removed from BALB/c mice exposed to conventional and bright light were analyzed by electron microscopy. As revealed in Figure 7, microglia adjacent to rod outer segments

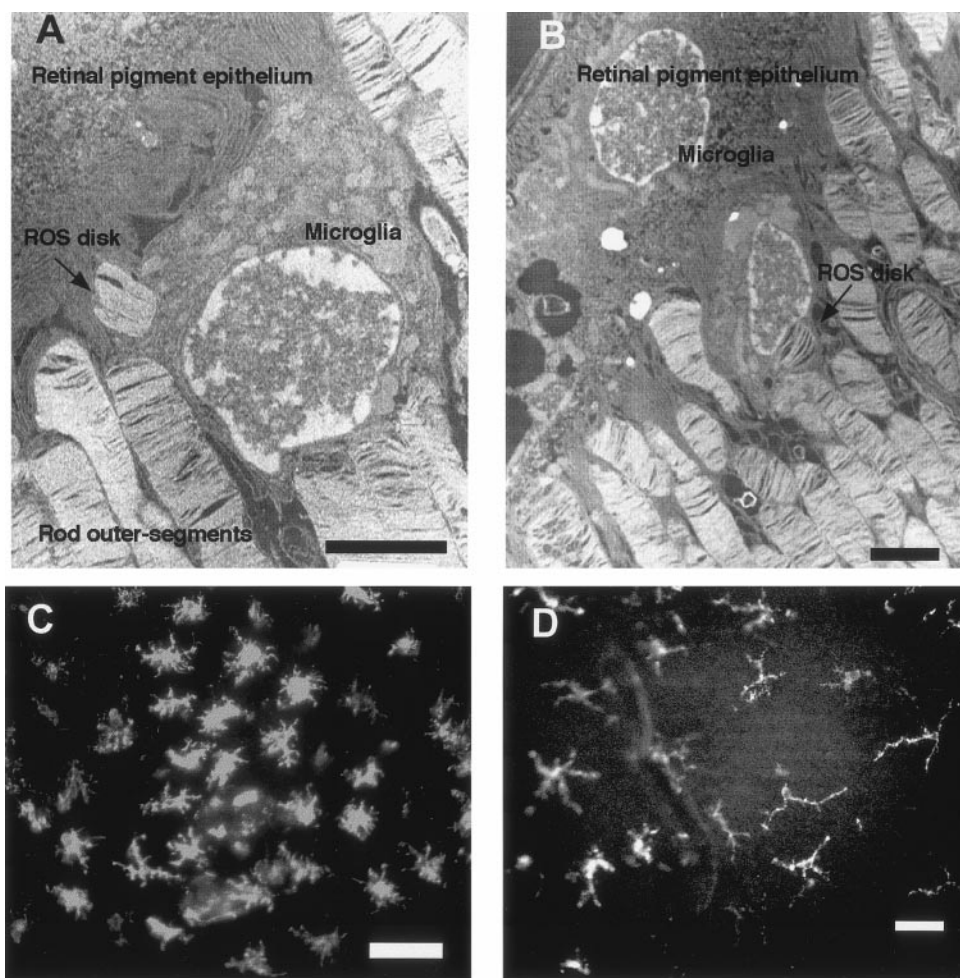


FIGURE 7. Evidence that subretinal $5D4^+$ cells are phagocytic. Eyes were enucleated from adult BALB/c mice that had been exposed to bright light (500 lux, continuous) for 2 weeks. (A) Electron micrographic image of layers of retina and RPE. A microglia is present between photoreceptor cells, and its cytoplasm contains phagocytized rod outer segment (ROS) discs. (B) Electron micrographic image of microglia with extensive profile of phagocytized ROS discs. The cell is adjacent to an RPE cell. (C) Light microscopic image of $5D4^+$ cells in the subretinal space. The cells have large, round cell bodies and short, stubby dendrites. (D) Light microscopic image of $5D4^+$ cells in the subretinal space of a BALB/c mouse that was placed in complete darkness for 2 weeks after a 2-week exposure to bright light. $5D4^+$ cells have slender cell bodies and extensively ramified dendrites. Scale bar, (A, B) $3 \mu\text{m}$; (C, D) $20 \mu\text{m}$.

(Fig. 7A), and within the interphotoreceptor matrix near pigment epithelial cells (Fig. 7B) contained numerous profiles of phagocytized rod outer segments. Moreover, the configuration of microglia in the subretinal space was different from that of cells in the inner retina. In retinas removed from eyes of mice exposed to bright light, $5D4^+$ cells in the subretinal space displayed large, round cell bodies and short, stubby dendrites (Fig. 7C). However, when similar mice were suddenly exposed to darkness for 2 weeks, the $5D4^+$ cells in the subretinal space displayed small, slender cell bodies, and longer, more ramified dendrites. These results indicate that, especially in bright light, microglia in the subretinal space are capable of phagocytizing rod outer segments, and their morphologic configuration suggests that the cells have been activated.

The activation implied by the morphology of subretinal microglia in retinas exposed *in vivo* to bright light caused us to determine whether class II MHC molecule expression had been induced on these cells. Class II MHC molecules play a central role in presentation of antigens to T lymphocytes and therefore are involved in transplantation immunology and rejection. Retinal wholemounts obtained from eyes of adult BALB/c mice exposed for 2 weeks to bright light were examined immunohistochemically with confocal microscopy, using antibodies directed at I-A^d and $5D4$ for counterstaining. Despite their morphologic appearance, activated $5D4^+$ cells displayed no evidence of I-A^d expression. Thus, this form of microglia activation was not associated with upregulation of MHC class II molecules.

DISCUSSION

Microglia within the CNS and the retina can adopt a diverse range of functional properties. At present, there is no single reagent that can reliably distinguish all forms of microglia from macrophages. Using immunohistochemical methods, identification of resting microglia with extensively ramified dendrites has been particularly difficult. Consequently, the monoclonal antibody $5D4$ is an important addition to the repertoire of microglia-identifying reagents. This antibody readily detects resting, ramified microglia.¹³⁻¹⁵ Use of $5D4$ in our studies of the murine retina has revealed dimensions to the biology of microglia that we had not anticipated. On the one hand, $5D4$ proved fully capable of marking ramified microglia in the inner layers of the adult murine retina, where we expected to find them. On the other hand, $5D4$ identified additional microglia that were present in the outer layers of the adult retina, between photoreceptors, and within the subretinal space adjacent to RPE. This unexpected distribution of $5D4^+$ cells was observed in adult retinas of albino, but not pigmented, mice obtained from our vivarium.

The data presented lead us to suggest that ambient light exposure can be a factor in determining the differential distribution of microglia in the retina of different strains of pigmented and albino mice. In the retinas of albino mice that were born, raised, and maintained as adults in complete darkness, very few microglia were detected beyond the inner layers of the retina. However, simply placing these mice in an environment with cyclic bright light (500 lux) led within 1 week to the

appearance of large numbers of 5D4⁺ cells in the subretinal space. At the end of 2 weeks of bright light exposure, an even higher density of 5D4⁺ cells was found in the subretinal space. Morphologic examination of these cells revealed that they were activated and that they contained profiles of rod outer segments in phagolysosomes. Together, these results suggest that light toxicity promotes the appearance of 5D4⁺ cells in the subretinal space of albino eyes, and in this site the cells participate, along with pigment epithelial cells, in phagocytosis of shed rod outer segments—a consequence of acute light toxicity.

Determining the histogenic origin of microglia and macrophages in the brain and retina is a continuing challenge. A large body of literature^{9–12} indicates that microglia in the CNS share some common markers with monocytes, and most investigators favor the view that microglia share a common bone marrow (hematopoietic) origin with blood-borne monocytes, rather than the former originating from neuroectodermal origin, as do all other glia. Ling and Wong¹⁰ reported that the drastic increase in ramified microglia in the rodent CNS during the first postnatal week coincides with a sharp decrease in bone marrow-derived amoeboid cells. This correlation suggests that the amoeboid cells transform to become ramified microglia. Eglitis and Mezey¹⁹ demonstrated that hematopoietic cells can differentiate into microglia in the brains by transplanting genetically tagged bone marrow cells to adult female mice. However, a recent experiment by Kurz and Christ²⁰ demonstrated that avian CNS microglia precursors do not penetrate through the wall of embryonic CNS vessels, but arise from migratory macrophages that come from the pial surface and proliferate inside the CNS to generate all microglia found in avian embryos. These findings are further supported by the studies of Alliot et al.²¹ who demonstrated that microglia progenitors originate from the yolk sac and proliferate in the brain.

In 1984, Sanyal et al.²² demonstrated that there was an accumulation of β -glucosaminidase⁺ cells in the subretinal space of albino mice, but not in pigmented mice. They suggested that the β -glucosaminidase⁺ cells are macrophages derived from the blood and that the cells function to supplement the phagocytic activity of RPE. In other reports, wandering phagocytes have been found at the interface of photoreceptors and RPE, suggesting that the phagocytes may be responsible for removing shed photoreceptor outer segments.^{23–25} It has also been demonstrated that macrophages can be found in the subretinal space of eyes of developing eutherian mammals and marsupials²⁶ and fetal humans,²⁷ but only occasionally in eyes of the adult opossum.^{26,28} These investigators suggest that fetal subretinal macrophages migrate from the ciliary body, invading the neuronal retina and becoming resident microglia.

Our results suggest a different scenario to account for the microglia we found in the subretinal space of adult mouse eyes. We adopted a strategy similar to that of Thanos¹⁸ and Thanos et al.²⁹ in which transection of the optic nerve and retrograde labeling of ganglion cells with fluorescent tracer allowed us to demonstrate that selectively labeled retinal microglia from the ganglion cell layer migrated to the outer nuclear layer. Thus, we found that at least a portion of the 5D4⁺ cells in the subretinal space of eyes of light-exposed albino mice originated as microglia from the inner layers of the retina. Because not all microglia in the inner retinal layers nor all subretinal 5D4⁺ cells in this experiment contained the fluorescent tracer label, we cannot be certain that all subretinal 5D4⁺ cells represent microglia that have migrated from the inner retinal layers. However, Wilms et al.³⁰ have reported that the 5D4 antibody does not stain blood-derived macrophages directly nor does the antibody stain these cells when they

acquire a dendritic morphology after coculture with astrocytes. Therefore, we suspect that the retina itself (the inner layers) is the predominant, if not the only, source of the 5D4⁺ cells we observed in the subretinal space of light-exposed albino mice and that the majority of these cells are microglia.

Other possible sources of these cells to consider are perivascular microglia^{31,32} and macrophages from the choroid.²³ However, neither of these cell types would be labeled by the fluorescent tracer in this study. Of note, it has been reported that perivascular microglia are replaced regularly from the bone marrow and that they constitutively express CD4 and MHC II molecules.^{32,33} Taking our results together with the finding of McMenamin³⁴ that murine choroidal macrophages constitutively express MHC II molecules, the likelihood is small that the subretinal microglia we detected were either perivascular microglia or choroidal macrophages. In our study, subretinal microglia did not express MHC II molecules. However, we are aware of the possibility that putative immunosuppressive factors in the subretinal space could downregulate MHC II expression on macrophages that may migrate into this immune-privileged site. It is relevant that the turnover rate of microglia has been reported to be very slow,³⁵ an observation that further indicates that the microglia we observed in the subretinal space arrive through migration, rather than from *in situ* proliferation of precursor cells.

High light intensity has been demonstrated to cause irreversible damage to retinas of albino rodents.^{36–38} Constant light of high intensity can cause most of the photoreceptors to disappear within 1 week in these rodents.³⁹ Similarly, cyclic light as well as 270 lux has been shown to cause a significant decrease in the thickness of the outer nuclear layer of albino rats.⁴⁰ However, contrasting results were observed by Penn et al.⁴¹ who reported that cyclic light as high as 800 causes the outer nuclear layer to decrease at a rate of only 0.009 mm²/week in albino rats. By contrast, Danciger et al.⁴² found no significant reduction in the outer nuclear layer in BALB/c mice kept in cyclic light until 4.5 months of age. One reason for these contrasting results may relate to genetically determined strain differences among albino mice, only some of which have limited degeneration while aging in normal cyclic light.^{43,44}

Intense light may not be the only factor implicated in migration of microglia within the retina; aging has also been considered. The thickness of the outer nuclear layer is reduced in aged rodents,^{45,46} and it is possible that aging photoreceptors place a greater burden on the RPE, a burden that directly or indirectly leads to chemoattraction of microglia from the inner nuclear layer to the subretinal space.¹⁸ Perhaps this explains why we found many microglia in the subretinal space of pigmented, aged C57BL/6 mice (18 months old).

The unexpected discovery of large numbers of microglia in the subretinal space of eyes of albino mice adds a new variable to the concept of immune privilege in this space. Several reports have documented that the subretinal space is an immune-privileged site, accommodating prolonged acceptance of allogeneic grafts of tumor cells and of neonatal neuronal retina.^{4,47} Moreover, injection of antigens into the subretinal space readily induces a systemic form of immune deviation that resembles anterior chamber-associated immune deviation (ACAID).⁴⁸ The studies on which these observations rest were all conducted in albino recipient animals. Because these animals were housed under conventional light in the vivarium, the subretinal space undoubtedly contained 5D4⁺ microglia. Thus, the immune privilege and immune deviation that have been described in the subretinal space occur when the space is already contaminated by microglia. Because these 5D4⁺ microglia are class II negative, they may not function *in situ* as antigen-presenting cells, until or unless trauma to, or inflammation in, the subretinal space alters them locally.^{15,49} It is also

possible that manipulation of the subretinal space induces these cells to migrate to distant sites (secondary lymphoid organs) where their expression of class II molecules may be upregulated by factors in the local microenvironment, thereby conferring on them the capacity for antigen presentation.

Our present experiments give little insight into the immunologic meaning of the presence of activated microglia in the subretinal space. We found that class II MHC molecules were not expressed on these cells, even when the retinas were harvested from albino mice exposed for 2 weeks to continuous bright light. When antigens injected into the anterior chamber of the eye induce ACAID, indigenous bone marrow-derived cells within the stromae of the iris and ciliary body are believed to function as the relevant antigen-presenting cells.⁵⁰⁻⁵² Because immune deviation also occurs after injection of antigen into the subretinal space, it is possible (at least in albino mice) that 5D4⁺ microglia in the subretinal space function as the relevant antigen-presenting cells. If true, it could be predicted that antigens injected into the subretinal space of eyes of pigmented mice may fail to induce immune deviation. Alternatively, it has been reported that the immune privilege extended in the subretinal space to allogeneic tumors is not permanent,⁴⁷ whereas allogeneic tumor cells injected into the anterior chamber grow progressively and are never rejected.⁵³ Is the reason for the truncated privilege described in the subretinal space that 5D4⁺ microglia are present and act to thwart allograft acceptance? If true, it could be predicted that allogeneic tumor cells injected into the subretinal space of pigmented mice would experience indefinite immune privilege. These predictions hint at the range of immunologic experiments that must follow the discovery of microglia in the subretinal space.

By examining for the presence of microglia in the subretinal space of eyes of albino and pigmented mice, we not only learned that light exposure promotes the migration of microglia into the outer retinal layers, but that melanin pigment within the uveal tract is a powerful inhibitor of this process. We presume that pigment both reduces the amount of light that enters the eye and absorbs scattered light within the globe, thereby shielding the photoreceptors from the deleterious effects of excess photic energy. C57BL/6 mice with a genetic mutation that prevents melanin synthesis are albinos. We found that light exposure promotes the penetration of 5D4⁺ microglia into the subretinal space of the eyes of these mice. The finding that light-induced migration of 5D4⁺ cells into the subretinal space is less robust in C57BL/6J-Tyr^{c2J} mice than in BALB/c mice indicates that light alone may not be the only variable influencing this process.

Our results indicate that, during ontogeny, microglia were not present in the subretinal space of murine eyes, a finding that differs from that reported in humans. After exposure to ambient light (and before the eyelids open at approximately 12-14 days), microglia began to accumulate in the subretinal space of eyes of albino pups. A steady state of 5D4⁺ cell density was reached when the mice were young adults, and this density was maintained for the entire first year of life, if ambient light levels were maintained at a relatively constant level. It has been hypothesized that the constant renewal process among photoreceptor rod outer segments is designed to provide a plasticity that is needed to accommodate to shifting light levels in the environment.^{41,54,55} Animals adjust the light absorption capabilities of their retinas by changing the length of rod outer segments so that length is inversely proportional to light intensity.⁵⁶ Irrespective of the light-intensity level, through time the retina adjusts. Thus, there is no difference in the rate of degradation of outer segment discs in phagosomes of RPE cells between albino and pigmented mice. This point is supported by our observations that 5D4⁺ micro-

glia in the subretinal space of mice acutely exposed to cyclic bright light displayed an activated morphology, but that after several weeks of continuous exposure, the subretinal microglia readopted a ramified appearance with slender cell bodies. Indirectly, this observation also supports the contention that microglia in the subretinal space are functioning as scavengers for the products of light toxicity, in a sense supplementing the functions already displayed by RPE. It may well be that the RPE in albino mice is especially vulnerable to light damage, and when the phagocytic capacity is disturbed by the toxic effects of light, phagocytic microglia come to their aid.

Aside from their apparent role in phagocytosis of shed rod outer segments, the functional properties of microglia in the subretinal space are largely unknown. As mentioned, we were interested in determining the extent to which subretinally disposed microglia participate in immune privilege and allograft rejection. The possibilities are not limited to immune functions, however. For example, we wonder whether activated microglia in the subretinal space may be deleterious to photoreceptor cells or to RPE. We do not know whether light damage in pigmented human eyes leads to microglia accumulation in the subretinal space. It is not inconceivable that the ease with which microglia can be induced to migrate into the subretinal space is inversely proportional to the degree of melanin pigmentation of the uveal tract. Perhaps intense light exposure in individuals with lightly pigmented eyes promotes migration of microglia into the subretinal space where further activation of these cells may have a decidedly negative effect on retinal function.

Acknowledgments

The authors thank Alice Adler for her generous gift of antibodies against IRBP, Pat Pearson for lending her expertise in electron microscopy, Marie Ortega for superb support with animal husbandry and facilitation of light-dark housing conditions, Jacqueline Doherty for expert managerial assistance, and Michael Young for many helpful discussions.

References

1. Young MJ, Lund RD. Parameters of transplant-mediated pupilloconstriction in rats with unilateral olivary pretectal lesions. *J Comp Neurol.* 1997;388:327-335.
2. Radner W, Sadda SR, Humayun MS, et al. Light-driven retinal ganglion cell responses in blind rd mice after neural retinal transplantation. *Invest Ophthalmol Vis Sci.* 2001;42:1057-1065.
3. Woch G, Aramant RB, Seiler MJ, et al. Retinal transplants restore visually evoked responses in rats with photoreceptor degeneration. *Invest Ophthalmol Vis Sci.* 2001;42:1669-1676.
4. Jiang LQ, Jorquera M, Streilein JW. Subretinal space and vitreous cavity as immunologically privileged sites for retinal allografts. *Invest Ophthalmol Vis Sci.* 1993;34:3347-3354.
5. Jiang LQ, Jorquera M, Streilein JW, et al. Unconventional rejection of neural retinal allografts implanted into the immunologically privileged site of the eye. *Transplantation.* 1995;59:1201-1207.
6. Jiang LQ, Streilein JW. Immunity and immune privilege elicited by autoantigens expressed on syngeneic neonatal neural retina grafts. *Curr Eye Res.* 1992;11:697-709.
7. Ma N, Streilein JW. Contribution of microglia as passenger leukocytes to the fate of intraocular neuronal retinal grafts. *Invest Ophthalmol Vis Sci.* 1998;39:2384-2393.
8. Banerjee R, Lund RD. A role for microglia in the maintenance of photoreceptors in retinal transplants lacking pigment epithelium. *J Neurocytol.* 1992;21:235-243.
9. Banati RB, Graeber MB. Surveillance, intervention and cytotoxicity: is there a protective role of microglia? *Dev Neurosci.* 1994; 16:114-127.
10. Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. *Glia.* 1993;7:9-18.

11. Perry VH. A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. *J Neuroimmunol.* 1998;90:113-121.
12. Thanos S, Moore S, Hong Y. Retinal microglia. *Prog Retinal Eye Res.* 1996;15:331-361.
13. Bertolotto A, Catterson B, Canavese G, et al. Monoclonal antibodies to keratan sulfate immunolocalize ramified microglia in paraffin and cryostat sections of rat brain. *J Histochem Cytochem.* 1993;41:481-487.
14. Bertolotto A, Manzardo E, Iudicello M, et al. Keratan sulphate is a marker of differentiation of ramified microglia. *Brain Res Dev Brain Res.* 1995;86:233-241.
15. Jander S, Stoll G. Strain-specific expression of microglial keratan sulfate proteoglycans in the normal rat central nervous system: inverse correlation with constitutive expression of major histocompatibility complex class II antigens. *Glia.* 1996;18:255-260.
16. Mayhew TM. A review of recent advances in stereology for quantifying neural structure. *J Neurocytol.* 1992;21:313-328.
17. Mansour-Robaey S, Clarke DB, Wang YC, et al. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. *Proc Natl Acad Sci USA.* 1994;91:1632-1636.
18. Thanos S. Sick photoreceptors attract activated microglia from the ganglion cell layer: a model to study the inflammatory cascades in rats with inherited retinal dystrophy. *Brain Res.* 1992;588:21-28.
19. Eglitis MA, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci USA.* 1997;94:4080-4085.
20. Kurz H, Christ B. Embryonic CNS macrophages and microglia do not stem from circulating, but from extravascular precursors. *Glia.* 1998;22:98-102.
21. Alliot F, Godin I, Pessac B. Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res Dev Brain Res.* 1999;117:145-152.
22. Sanyal S, De Ruiter A, Dees C. Light dependent accumulation of macrophages at the photoreceptor-pigment epithelial interface in the retina of albino mice. *Experientia.* 1984;40:852-854.
23. O'Day WT, Young RW. Rhythmic daily shedding of outer-segment membranes by visual cells in the goldfish. *J Cell Biol.* 1978;76:593-604.
24. Braekvelt CR. Wandering phagocytes at the retinal epithelium-photoreceptor interface in the teleost retina. *Vision Res.* 1980;20:495-499.
25. Braekvelt CR. Further observations on the presence of wandering phagocytes within the teleost retina. *Anat Anz.* 1985;160:45-54.
26. McMenamin PG. Subretinal macrophages in the developing eye of eutherian mammals and marsupials. *Anat Embryol (Berl).* 1999;200:551-558.
27. McMenamin PG, Loeffler KU. Cells resembling intraventricular macrophages are present in the subretinal space of human foetal eyes. *Anat Rec.* 1990;227:245-253.
28. Herman KG, Steinberg RH. Phagosome degradation in the tapetal retinal pigment epithelium of the opossum. *Invest Ophthalmol Vis Sci.* 1982;23:291-304.
29. Thanos S, Pavlidis C, Mey J, et al. Specific transcellular staining of microglia in the adult rat after traumatic degeneration of carboxyanine-filled retinal ganglion cells. *Exp Eye Res.* 1992;55:101-117.
30. Wilms H, Wollmer MA, Sievers J. In vitro-staining specificity of the antibody 5-D-4 for microglia but not for monocytes and macrophages indicates that microglia are a unique subgroup of the myelomonocytic lineage. *J Neuroimmunol.* 1999;98:89-95.
31. Hume DA, Perry VH, Gordon S. Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. *J Cell Biol.* 1983;97:253-257.
32. Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science.* 1988;239:290-292.
33. Hickey WF, Vass K, Lassmann H. Bone marrow-derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. *J Neuropathol Exp Neurol.* 1992;51:246-256.
34. McMenamin PG. Optimal methods for preparation and immunostaining of iris, ciliary body, and choroidal wholemounts. *Invest Ophthalmol Vis Sci.* 2000;41:3043-3048.
35. Lawson LJ, Perry VH, Gordon S. Turnover of resident microglia in the normal adult mouse brain. *Neuroscience.* 1992;48:405-415.
36. Noell WK, Walker VS, Kang BS, et al. Retinal damage by light in rats. *Invest Ophthalmol.* 1966;5:450-473.
37. Gorn RA, Kuwabara T. Retinal damage by visible light: a physiologic study. *Arch Ophthalmol.* 1967;77:115-118.
38. Kuwabara T, Gorn RA. Retinal damage by visible light: an electron microscopic study. *Arch Ophthalmol.* 1968;79:69-78.
39. LaVail MM, Gorrin GM, Repaci MA, et al. Genetic regulation of light damage to photoreceptors. *Invest Ophthalmol Vis Sci.* 1987;28:1043-1048.
40. Semple-Rowland SL, Dawson WW. Cyclic light intensity threshold for retinal damage in albino rats raised under 6 lx. *Exp Eye Res.* 1987;44:643-661.
41. Penn JS, Tolman BL, Thum LA, et al. Effect of light history on the rat retina: timecourse of morphological adaptation and readaptation. *Neurochem Res.* 1992;17:91-99.
42. Danciger M, Matthes MT, Yasamura D, et al. A QTL on distal chromosome 3 that influences the severity of light-induced damage to mouse photoreceptors. *Mamm Genome.* 2000;11:422-427.
43. Sanyal S, Hawkins RK. Development and degeneration of retina in rds mutant mice: effects of light on the rate of degeneration in albino and pigmented homozygous and heterozygous mutant and normal mice. *Vision Res.* 1986;26:1177-1185.
44. Saari JC, Nawrot M, Kennedy BN, et al. Visual cycle impairment in cellular retinaldehyde binding protein (CRALBP) knockout mice results in delayed dark adaptation. *Neuron.* 2001;29:739-748.
45. Weisse I, Stotzer H, Seitz R. Age- and light-dependent changes in the rat eye. *Virchows Arch A Pathol Anat Histol.* 1974;362:145-156.
46. Lai YL, Jacoby RO, Jonas AM. Age-related and light-associated retinal changes in Fischer rats. *Invest Ophthalmol Vis Sci.* 1978;17:634-638.
47. Wenkel H, Chen PW, Ksander BR, et al. Immune privilege is extended, then withdrawn, from allogeneic tumor cell grafts placed in the subretinal space. *Invest Ophthalmol Vis Sci.* 1999;40:3202-3208.
48. Wenkel H, Streilein JW. Analysis of immune deviation elicited by antigens injected into the subretinal space. *Invest Ophthalmol Vis Sci.* 1998;39:1823-1834.
49. Jander S, Stoll G. Downregulation of microglial keratan sulfate proteoglycans coincident with lymphomonocytic infiltration of the rat central nervous system. *Am J Pathol.* 1996;148:71-78.
50. Wilbanks GA, Mammolenti M, Streilein JW. Studies on the induction of anterior chamber-associated immune deviation (ACAID). II: eye-derived cells participate in generating blood-borne signals that induce ACAID. *J Immunol.* 1991;146:3018-3024.
51. Wilbanks GA, Streilein JW. Studies on the induction of anterior chamber-associated immune deviation (ACAID). I: evidence that an antigen-specific, ACAID-inducing, cell-associated signal exists in the peripheral blood. *J Immunol.* 1991;146:2610-2617.
52. Wilbanks GA, Streilein JW. Macrophages capable of inducing anterior chamber associated immune deviation demonstrate spleen-seeking migratory properties. *Reg Immunol.* 1992;4:130-137.
53. Niederkorn J, Streilein JW, Shaddock JA. Deviant immune responses to allogeneic tumors injected intracamerally and subcutaneously in mice. *Invest Ophthalmol Vis Sci.* 1981;20:355-363.
54. Penn JS, Williams TP. Photostasis: regulation of daily photon-catch by rat retinas in response to various cyclic illuminances. *Exp Eye Res.* 1986;43:915-928.
55. Schremser JL, Williams TP. Photoreceptor plasticity in the albino rat retina following unilateral optic nerve section. *Exp Eye Res.* 1992;55:393-399.
56. Schremser JL, Williams TP. Rod outer segment (ROS) renewal as a mechanism for adaptation to a new intensity environment. I: rhodopsin levels and ROS length. *Exp Eye Res.* 1995;61:17-23.