Changes in Murine Hyalocytes Are Valuable Early Indicators of Ocular Disease

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PURPOSE. The distribution, density, and phenotype of hyalocytes or vitreous macrophages in mouse eyes was examined during normal aging and in models of background diabetic retinopathy, retinal vascular proliferation, and exposure to TLR4 and TLR9 ligands.

METHODS. The phenotype and density of hyalocytes were investigated in retinal and ciliary body wholemounts of normal wild-type (WT; C57BL/6) mice at 7, 17, and 120 weeks of age, Ins2Akita mice, transgenic Kimba mice (VEGF-induced retinal neovascularization), and WT mice 24 hours after single intraperitoneal injection with lipopolysaccharide (LPS) or 1 week after three identical doses administered 2 weeks apart. Another group of mice each received a single topical drop of 20 μg CpG-oligodeoxynucleotides (CpG-ODN) to the abraded corneal surface and were euthanized 1 week later.

RESULTS. The data revealed an approximately fivefold increase in the density of preretinal hyalocytes in 120-week-old mice. Some hyalocytes in older eyes contained phagocytosed melanin. Hyalocyte density was doubled in the 3Department of Anatomy and Developmental Biology, School of Biomedical Sciences, Monash University, Wellington Road, Clayton, Victoria 3800, Australia; and the 2Lions Eye Institute, Nedlands, Australia; and the 1Department of Molecular Ophthalmology, Centre for Ophthalmology and Visual Science, University of Western Australia, Crawley, Australia; the 3Department of Anatomy and Developmental Biology, School of Biomedical Sciences, Monash University, Clayton, Australia.

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On the basis of our findings, we hypothesize that hyalocytes may be early indicators of pathogenic processes affecting the retina and vitreous.

Materials and Methods

Animals

We used a range of mice in the present study. These include normal male wild-type (WT) C57BL/6, heterozygous Ins2<sup>2</sup> mice on a C57BL/6 background, 28 age-matched WT littermates, and female transgenic Kimba mice (a model of VEGF-induced retinal neovascularization). 29 We also used transgenic Cx cr<sup>107-797</sup> (heterozygous) knock-in mice (age range, 6–50 weeks) on a C57BL/6 (pigmented) background.

Animals in this study were bred at the Animal Resources Centre (Murdoch, WA, Australia) and maintained on a 12:12-hour light/12-hour dark cycle, with free access to food and water. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by The University of Western Australia and Monash University Animal Ethics Committees.

Ins<sup>2</sup> mutant mice develop type 1 diabetes at 4 weeks of age. Only males carrying a single Ins2<sup>2</sup> allele are established as suitable models of diabetic complications. Our Ins2<sup>2</sup> colony was obtained from Jackson Laboratories (Bar Harbor, ME). Genotyping, blood glucose levels, and HbA<sub>1c</sub> were measured to ensure diabetic control. Ins2<sup>2</sup>-specific primers were forward 5'-CAGACCTGGCACAGGT-3' and reverse 5'-CAGAGGGTATGGCTGGAATGGT-3'. We validated this method against the manufacturer's instructions. The Insulin<sub>2</sub>-specific primers were forward 5'-CAGAGGTAGTGCTACACC-3' and reverse 5'-CAGAGGGTAGCTGGAATGGT-3'.

The Ins2<sup>2</sup> mutation was performed using a thermal cycler (Rotor Gene 3000; Corbett Life Science, Sydney, Australia), in a 10-µL reaction using a DNA extraction kit (TaqlMan Sample-to-SNP; Applied Biosystems, Foster City, CA), according to manufacturer's instructions. The Insulin<sub>2</sub>-specific primers were forward 5'-CACAGCTGGCACTGGAGGT-3' and reverse 5'-CACAGCTGGCACTGGAGGT-3'.

Expression of Ins2<sup>2</sup> was assessed by restriction enzyme digestion protocol with a 100% correspondence between results (restriction enzyme protocol: C57BL/6 Ins2<sup>2</sup> genotype; restriction fragment length polymorphism: C57BL/6 Ins2<sup>2</sup> genotype).

We validated this method against the conventional PCR and restriction enzyme digestion protec digest with a 100% correspondence between results (restriction enzyme protocol: C57BL/6 Ins2<sup>2</sup> genotype; restriction fragment length polymorphism: C57BL/6 Ins2<sup>2</sup> genotype).

Administration of Systemic TLR4 Ligand, LPS

Ultrapure Escherichia coli lipopolysaccharide (LPS) (strain K12; InvivoGen, San Diego, CA) was diluted to 10 µg/µL in pyrogen-free saline for injection (Pfizer, New York, NY). For the study of short-term effects of acute inflammation, WT C57BL/6 mice were injected intraperitoneally (IP) with LPS (9 µg/g body weight) or pyrogen-free PBS and euthanized 24 hours later. To model recurrent systemic inflammation secondary to bacterial infections, a separate group of C57BL/6 mice received three doses of LPS (9 µg/g body weight) or pyrogen-free controls, at 2-week intervals, starting from 10 weeks of age. A 2-week break was allowed between the final injection and death at 17 to 18 weeks of life. The rationale for 2-week gaps between injections of LPS was to reduce or avoid endotoxin tolerance, a phenomenon characterized by a period of diminished responsiveness to repetitive challenges with bacterial endotoxin. 35

Topical Application of TLR9 Ligand (CpG-ODN)

Mice to be treated with CpG oligodeoxynucleotides (CpG-ODN), a synthetic TLR9 ligand that mimics unmethylated bacterial or viral DNA, were anesthetized by IP injection of ketamine/xylazine, and the epithelium of the central cornea was debrided using a conical rust ring remover with a 0.5-mm burr (Aligerbrush II; Alger Equipment Co., Lago Vista, TX), as previously described. 34 Immediately after epithelial debridement, 20 µg TLR9-specific phosphorothioate CpG 1826 ODN or control ODN (in 1-µL volume) was applied to the corneal surface. Mice were euthanized 1 week later.

Tissue Processing for Immunohistochemistry and Histology

Mice were euthanized by lethal injection of sodium pentobarbitone, and cardic perfusion with 2% paraformaldehyde (PFA) was performed as previously described. 35 Euthanized eyes were postfixed in 2% PFA at 4°C overnight and further stored in phosphate-buffered saline (PBS) at 4°C. Retinal wholemounts, with ciliary body still attached, were prepared as previously documented. 35 Tissue pieces were washed in PBS, incubated in 20 mM EDTA tetrasodium at 37°C for 30 minutes, then blocked for 30 minutes at room temperature (RT) with 3% bovine serum albumin and 0.3% Triton-X solution in PBS. No attempt was made to remove the vitreous membrane. Tissues were treated at 4°C overnight with a single antibody or combinations of antibodies, including monoclonal rat anti-major histocompatibility complex (MHC) class II (M5/114), monoclonal rat anti-F4/80 (Serotec, Raleigh, NC), monoclonal rat anti-CD169 (sialoadhesin; Serotec), rat anti-CD11b (complement receptor 3; BD PharMingen, San Diego, CA), and polyclonal rabbit anti-Iba-1 (ionized calcium binding adapter molecule 1) (Wako, Pure Chemicals Industries, Osaka, Japan). Samples that were dual stained were further incubated in biotin-conjugated anti-rabbit antibody (Vector, Burlingame, CA) for 2 hours at RT followed by Alexa Fluor 594- conjugated anti-rat and streptavidin-conjugated Alexa Fluor 488 (Molecular Probes, Eugene, OR) antibodies for 40 minutes at RT. Primary antibodies were omitted from negative controls, and isotype controls were performed routinely for all antibodies. Multiple PBS washes with agitation were performed between all incubation steps. Retinal and ciliary body wholemounts were placed on slides with the vitreous side up.

Examination of Wholemount Tissue and Quantitative Analysis of Iba<sup>+</sup> Cells in Retinal Tissue Mounts

Stained specimens were examined by conventional epifluorescence microscopy (BX60; Olympus, Tokyo, Japan) or confocal microscopy (TCS SP2; Leica, Wetzlar, Germany). For qualitative and quantitative analysis, the focal plane on epifluorescent microscopy was maintained at the level of the retinal inner limiting membrane. When examining ciliary processes, confocal imaging was limited to the vitread surface of the whole-mounted specimen to reduce the possibility of imaging intraepithelial and stromal macrophages in the ciliary body or the retinal microglia. Z-profiles (2-µm step size) of the entire retinal thickness were generated to further define the location of immunofluorescent cells. Scientific image processing and analysis software (Imaris; Bitplane, Zurich, Switzerland) was used to create 3D visualizations and z-profiles. For counts of Iba<sup>+</sup> hyalocytes, three random images were taken from each retina at 20× magnification. Final compilation of z-profile images was performed using microscopy image analysis software (Imaris 7.1.1; Bitplane, Zurich, Switzerland). Mean cell density per square millimeter (±SEM) was calculated and compared between groups of interest using unpaired Student's t-test (Prism; GraphPad, San Diego, CA). P < 0.05 was considered statistically significant.

Results

Characterization and Quantitation of Hyalocytes in the Normal and Aging Mouse Eye

Examination of immunostained retinal tissue wholemounts from young (7-week-old) WT C57BL/6 mice, in which no effort...
phenotype (F4/80\(^+\) Iba-1\(^+\) CD169\(^+\) CD11b\(^-\)) similar to that of young eyes but were frequently observed to contain variable amounts of phagocytosed melanin granules (Fig. 1F). Quantitative analysis revealed no statistically significant difference in the density of Iba-1\(^+\) preretinal hyalocytes between 7-week-old WT and 17-week-old WT mice, but there was a significant (P < 0.001) increase by 120 weeks of age (48.1 ± 5.6 cells/mm\(^2\); Fig. 1G).

**Effects of Early Diabetes on the Phenotype and Density of Preretinal Hyalocytes**

In light of the pathologic processes that manifest in the vitreous during diabetic retinopathy, we chose to investigate hyalocytes in the Ins2\(^{Akita}\) mouse model of background proliferative retinopathy. In 7-week-old Ins2\(^{Akita}\) mice after 3 to 4 weeks of uncontrolled hyperglycemia, there was a significant increase in the density of Iba-1\(^+\) preretinal hyalocytes (24.8 ± 1.9 cells/mm\(^2\)) (Fig. 2). The morphology, distribution, and immunophenotype of the cells were similar to those of age-matched WT mice.

**VEGF-Driven Proliferative Retinopathy in Mice Causes a Marked Increase in Preretinal Hyalocytes**

We wanted to establish whether hyalocytes are responsive to vascular proliferative disease. To this end, we chose the Kimba mouse model of VEGF-driven retinal degeneration.\(^{31,32}\) Retinas from 7-week-old Kimba mice revealed abnormally large numbers of small, round, or pleomorphic Iba-1\(^+\) F4/80\(^-\) myeloid cells resembling hyalocytes distributed relatively uniformly in the preretinal compartment. A significant subpopulation of these cells were MHC II\(^+\) (Fig. 3A), which is not a characteristic of hyalocytes in healthy mice. Quantitative analysis revealed a statistically significant increase in the density of preretinal Iba-1\(^+\) hyalocytes or myeloid cells in Kimba mice (84.1 ± 17.9 cells/mm\(^2\)) (Fig. 3B).

**Effects of Single and Repeated Episodes of Endotoxemia on Hyalocytes in the Anterior and Posterior Vitreous**

Although it is well accepted that anterior uveitis can accompany systemic exposure to LPS,\(^{36}\) there is little to suggest there may be retinal or vitreal changes in this model. Thus we chose

**Figure 1.** Hyalocytes in the normal retina. (A-D) The superficial retina of young WT C57BL/6 mice is visualized by confocal microscopy of immunostained wholemounts. Note the pleomorphic F4/80\(^+\) (A), Iba-1\(^+\) (B), and CD169\(^+\) (D) hyalocytes randomly distributed on the retinal surface and how they differ in morphology and immunophenotype (F4/80\(^-\) Iba-1\(^-\)) (C, arrow) from the ramified microglia (Iba-1\(^+\) F4/80\(^-\)) in the underlying retina. A z-profile from an Iba-1 (red) stained retinal wholemount of a Cx3cr1\(^{gfp/\text{H11001}}\) mouse (E), as visualized with scientific image processing and analysis software, illustrates the distribution of hyalocytes on the vitread (vit) aspect relative to the microglia in the plexiform layers (IPL, inner plexiform layer; OPL, outer plexiform layer) (Supplementary Video S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8601/-/DCSupplemental, for layer by layer visualization). Hyalocytes are more reactive with the Iba-1 because of their superficial location. Hyalocytes of increasing age accumulate intracellular pigment granules (F) and increase in density (G), 7 weeks, n = 14; 17 weeks, n = 8; 120 weeks, n = 4. Error bars represent SEM. ***P < 0.001.

had been made to remove the vitreous body, revealed a population of F4/80\(^+\) (Fig. 1A), Iba-1\(^+\) (Figs. 1B, 1C), CD169\(^+\) (Fig. 1D), CD11b\(^-\) hyalocytes or vitreous macrophages. These cells, characterized by a few stout processes or broad filopodia and blebs, were distributed randomly across the inner retinal surface at a density of 11.2 ± 1.1 (SEM) cells/mm\(^2\). The location of these preretinal hyalocytes did not overly relate to the retinal vessel topography or the underlying Iba-1\(^+\) microglia from the ganglion cell layer or the inner plexiform layer (Figs. 1A, 1B), and their superficial locations relative to the well-recognized layers of microglia in the inner plexiform and outer plexiform layers were verified by z-profile analysis (Fig. 1E; Supplementary Video S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8601/-/DCSupplemental).

In light of the interest in the role of myeloid cells in a number of age-related disorders in the eye, including AMD, and the search for useful mouse models of this disease (see Ref. 24 for review) we also examined hyalocytes in WT C57BL/6 mice of 17 and 120 weeks of age. In aged mice, hyalocytes had a

**Figure 2.** Quantitative analysis of Iba-1\(^+\) hyalocyte density in the retinas of 7-week-old WT C57BL/6 mice compared with Ins2\(^{Akita}\) (AK) mice. n = 7 mice/group. Error bars represent SEM. ***P < 0.001.
to investigate the effects of either single or multiple IP injections of TLR4-specific LPS on hyalocyte phenotype and density in the mouse eye. In addition to being located in the vitreous throughout the extent of the retinal surface, hyalocytes are distributed in the anterior portion of the vitreous adjacent to the ciliary body. Previous studies have characterized the extensive network of resident tissue macrophages and DCs within the stroma and epithelial layers of the ciliary body in mouse, rat, and human eyes. However, in these species, there are also anterior hyalocytes, a subpopulation of vitreal macrophages, localized on the inner surface of the ciliary body. The present study, qualitative confocal analysis of ciliary body tissue wholemounts from young WT and Cx3cr1gfp/− mice revealed an abundant population of F4/80+ CD169+Iba1+ cells on the vitreal aspect of the ciliary body adjacent to the anterior vitreous membrane. This distribution and cell morphology corresponded closely to the anterior hyalocytes described by previous authors, which were in continuity posteriorly with identical cells on the vitreal aspect of the retina (periretinal hyalocytes). Some of the anterior hyalocytes in normal eyes coexpressed MHC II (Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11- 8601/-/DCSupplemental).

Twenty-four hours after systemic LPS exposure, irregular accumulations of F4/80+Iba1+ cells were evident at the posterior margins of the ciliary processes extending over the adjacent inner retinal surface (Fig. 4B). There appeared to be fewer F4/80+ cells in the clefts and apices of the ciliary processes than seen in normal or control eyes (PBS IP injection) (Fig. 4A). After multiple systemic LPS injections, there was a much greater abundance of MHC class II+ cells on the apical aspect of the ciliary processes than in normal eyes, and these cells appeared to spill over onto the peripheral inner retinal surface (Fig. 4C). Some of these MHC class II+Iba1+ hyalocytes were larger than normal and were characterized by complex lamellipodia and a veiled appearance (Fig. 4D). Quantitative analysis revealed a significant increase in the numbers of preretinal hyalocytes after a single injection of LPS (33.1 ± 5.2 cells/mm²) (P < 0.001) (Fig. 4E). In mice examined 2 weeks after the last of three LPS injections, there was also a significant increase in hyalocyte numbers compared with controls (PBS IP injections) (24.4 ± 1.4 cells/mm²) (P < 0.001) (Fig. 4E).

**Response of Hyalocytes to Topical Unmethylated CpG-ODN Exposure**

We have recently described the occurrence of retinal inflammation in a model of murine keratitis after topical exposure of the debrided corneal surface to unmethylated CpG-ODN. One of the defining features of this TLR9-induced posterior segment inflammation was an acute neutrophilic vitritis at 24 hours after treatment. In the present study, we sought to determine whether hyalocytes were responsive to this inflammatory stimulus. Retinal wholemounts from C57BL/6 mice were, therefore, examined 1 week after corneal exposure to CpG-ODN. Examination and quantitation of Iba1-stained tissues revealed a significant increase in hyalocyte numbers (Figs. 5A–C), with densities of approximately 79.6 ± 6 cells/mm². Control eyes (corneal epithelium debridement and exposure to control ODN) revealed a slightly higher density (20.9 ± 2.1 cells/mm²) than naive eyes (11.2 ± 1.1 cells/mm²).

**DISCUSSION**

Although vitreal macrophages, or hyalocytes, have been implicated in various vitreoretinal abnormalities, they are poorly characterized in both normal and pathologic conditions compared with other myeloid cells in the eye, such as retinal microglia. The present study characterized the in situ immunophenotype of murine hyalocytes under varied physiologic and pathologic conditions and revealed that these cells are responsive to aging, hyperglycemia, locally produced VEGF, and both systemic and ocular-derived TLR ligands.

The morphology, distribution, and localization of anterior hyalocytes and preretinal hyalocytes in the normal murine eye corresponded to previous descriptions of hyalocytes in other species. They were scattered in an apparently random fashion in the cortical vitreous over the retinal surface when viewed en face in immunostained retinal wholemounts and on the inner surfaces of the ciliary processes. In strict anatomic terms, many of these myeloid cells on the surfaces of the ciliary processes were situated in the posterior chamber because the apices of the ciliary processes are located anterior to the true anterior hyaloid face. However, given that the anterior vitreous abuts the posterior aspect of the ciliary processes, these cells are likely identical to the more classically accepted preretinal hyalocytes, and they form a continuum with these more posteriorly located macrophages. The present study confirmed previous observations that the immunophenotype of preretinal hyalocytes in the mouse eye resembled other ocular macrophage populations in the rodent uveal tract in that they were Iba1+ CD14+ CD45− Cx3cr1b+ but differed from retinal microglia in that they were CD11bI F4/80I and CD169+. Our data support previous descriptions and confirm that hyalocytes are morphologically and immunophenotypically distinct from the microglia embedded in the neural retina.
they represent mobile scavenging macrophages in the preretinal space.8,10–14

It has recently been proposed that advanced age represents a condition of para-inflammatory,44,45 and, in light of the interest in the putative role of the immune system in the pathogenesis of age-related macular degeneration (AMD),46 we postulated that in the aging mouse eye there would be an increase in hyalocytes in parallel with the recently characterized age-related increase in subretinal macrophages.47,48 Indeed, our data confirmed a fivefold increase in hyalocyte density in animals older than 2 years. It is largely unknown whether similar changes occur in the human eye either as part of aging or in the early stages of AMD, but clearly, in light of the ability of activated macrophages to act as a source of VEGF, this would be highly worthy of further investigation.

Until now there has been no information on the distribution, density, or phenotype of hyalocytes in experimental background diabetic retinopathy in mice. Unlike toxin-induced models of diabetes, Ins2Akita mice develop diabetes spontaneously, thus avoiding the confounding effects of alloxan or streptozotocin.50 Despite only having been in a hyperglycemic state for 3 to 4 weeks, and in the absence of any other overt retinal abnormality, there was a significant increase in hyalocyte density in Ins2Akita mice, suggesting that these cells were responsive to hyperglycemic changes in blood glucose. Although previous studies have indicated that vitreal macrophages are responsive to acute14,16 and chronic intraocular pathologic conditions,51,52 the present data are the first to indicate, to our knowledge, that changes in these cells appear to act as a first indication of a perturbation in retinal physiology. We also investigated the possibility that hyalocytes were increased in overt retinal pathologic conditions in Kimba mice. Previously, it had been shown that the overexpression of human VEGF165 (hVEGF) in the photoreceptors of these mice resulted in mild...

FIGURE 4. The effect of either single or repeated systemic LPS exposure on the distribution and morphology of preretinal hyalocytes. In control (PBS-treated) mice (A), normal populations of F4/80+ macrophages are present in the crypts and on the apical surfaces of the ciliary processes. After a single LPS injection, clusters of F4/80+ macrophages are observed at the peripheral retina-ciliary body junction (B). In the eyes of mice subjected to repeated systemic LPS injections, large MHC II+ Iba1+ hyalocytes exhibited a bloated appearance in the retina 2 weeks after the final injection (C). Higher magnification of these cells shown in (D). Quantitative analysis (E) of Iba1+ hyalocytes in eyes subjected to either a single injection 24-hour postinjection, or repeated LPS injections (n = 7) revealed increased density 1 week after injection compared with PBS-treated controls. n = 8.

FIGURE 5. Increased number of Iba1+ hyalocytes in the retina 1 week after topical application of CpG-ODN to the abraded mouse cornea. Normal pleomorphic Iba1+ hyalocytes (A, arrows) displaying characteristic stout processes in control treated eyes. One week after corneal treatment with CpG-ODN, large bloated Iba1+ hyalocytes are present in the retina (B, arrows). Quantification of the density of Iba1+ hyalocytes reveal a statistically significant increase after CpG-ODN exposure (C). n = 6 mice/group. Error bars represent SEM.
to moderate retinopathy, similar to that in human patients with nonproliferative diabetic retinopathy or very early stages of proliferative diabetic retinopathy. In these mice, we observed a very marked increase in preretinal round Iba-1+ myeloid cells, many of which coexpressed MHC class II+, indicative of local activation. The eightfold increase in density of hyalocytes or myeloid cells likely indicates either recent recruitment from the retinal vasculature or from the existing pool of resident microglia. We propose that the latter may be an unrecognized source of vitreal macrophages and may be similar to the migration of microglia toward the vitreous and subretinal space proposed by Santos et al. in response to retinal light damage.

The experimental model of endotoxin-induced uveitis in rodents is considered to reproduce some elements of human anterior uveitis. The induction of experimental endotoxicemia in mice and rats with the TLR4 ligand LPS results in breakdown of the blood-aqueous barrier and the influx of neutrophils into the anterior chamber, posterior chamber, and anterior vitreous. This is typically accompanied by a mild monocytic infiltrate with an overall increase in MHC class II-bearing cells in the ciliary body. Thus, the twofold to threefold increase in density in Iba-1+ myeloid cells in the vitreous noted in the present study either 1 day or 2 weeks after systemic LPS injection, while not completely unexpected, did illustrate that the effect was not completely localized to the anterior portion of the eye.

Data from the present LPS and CpG-ODN experiments indicate that either systemic or local exposure to these dangerous molecules or pathogen-associated molecular patterns may initiate an influx of myeloid cells into the vitreous. The present study revealed that 1 week after application of CpG-ODN to the debrided cornea, there was a marked increase in hyalocyte density. This change raises the intriguing possibility that infections at the corneal surface may result in changes in resident macrophage populations in the posterior segment of the eye. These data lead us to conclude that the increased activation and elevation in hyalocyte numbers may be an indication of potential viral or bacterial infection either at the corneal surface or systemically, and they reinforce the notion of the eye as susceptible to infective organisms outside the blood-ocular barrier. It is also noteworthy that hyalocyte density in eyes 1 week after corneal abrasion and application of control ODN treatment are slightly increased compared with naive mice of the same age. This is not surprising given that the trauma alone produces a mild degree of corneal inflammation and supports our main hypothesis that hyalocytes are sensitive early indicators of inflammatory changes in the eye.

In conclusion, we verified that the healthy mouse eye contains two distinct subpopulations of vitreal macrophages, preretinal and ciliary body hyalocytes, which are highly sensitive to physiological perturbations both locally and systemically. We have shown that hyalocytes are responsive to aging, hyperglycemia, and local VEGF production. In addition, they are sensitive to acute systemic and corneal exposure to TLR ligands, as evident by the increases in density, alterations in morphology, and changes to their immunophenotype. Examination of the ciliary body in our models of inflammation to systemic and ocular exposure to TLR4 and TLR9 ligands, respectively, also revealed that the ciliary body was likely a potent source of inflammatory cells that possibly replenished vitreal macrophages in the mouse eye. In light of widespread interest in the role of the ocular macrophages in eye diseases such as AMD, hyalocytes could act as a valuable indicator of prodromal changes in various ocular and systemic diseases.

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