

RESEARCH ARTICLE

Resident immune cells of the avascular lens: Mediators of the injury and fibrotic response of the lens

A. Sue Menko^{1,2} | JodiRae DeDreu¹ | Caitlin M. Logan¹ | Heather Paulson¹ |
 Alex V. Levin^{2,3} | Janice L. Walker^{1,2}

¹Department of Pathology, Anatomy and Cell Biology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA

²Department of Ophthalmology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA

³Wills Eye Hospital, Philadelphia, PA, USA

Correspondence

Janice L. Walker, Department of Pathology, Anatomy and Cell Biology, Sidney Kimmel Medical College, Thomas Jefferson University, Room 571 JAH, Philadelphia, PA 19107, USA.

Email: Janice.Walker@jefferson.edu

Present address

Caitlin M. Logan, Mary M. and Sash A. Spencer Center for Vision Research, Byers Eye Institute, Stanford University, Palo Alto, CA, USA

Alex V. Levin, Flaum Eye Institute and Golisano Children's Hospital, University of Rochester Medical Center, Rochester, NY, USA

Funding information

HHS | NIH | National Eye Institute (NEI), Grant/Award Number: EY02615 and EY021784

Abstract

Tissues typically harbor subpopulations of resident immune cells that function as rapid responders to injury and whose activation leads to induction of an adaptive immune response, playing important roles in repair and protection. Since the lens is an avascular tissue, it was presumed that it was absent of resident immune cells. Our studies now show that resident immune cells are a shared feature of the human, mouse, and chicken lens epithelium. These resident immune cells function as immediate responders to injury and rapidly populate the wound edge following mock cataract surgery to function as leader cells. Many of these resident immune cells also express MHCII providing them with antigen presenting ability to engage an adaptive immune response. We provide evidence that during development immune cells migrate on the ciliary zonules and localize among the equatorial epithelial cells of the lens adjacent to where the ciliary zonules associate with the lens capsule. These findings suggest that the vasculature-rich ciliary body is a source of lens resident immune cells. We identified a major role for these cells as rapid responders to wounding, quickly populating each wound where they can function as leaders of lens tissue repair. Our findings also show that lens resident immune cells are progenitors of myofibroblasts, which characteristically appear in response to lens cataract surgery injury, and therefore, are likely agents of lens pathologies to impair vision like fibrosis.

KEYWORDS

fibrosis, injury, lens, resident immune cells, wound healing

1 | INTRODUCTION

The lens is a transparent eye tissue with an essential role of focusing images onto the retina, making it a critical component of the visual process. While resident immune cells are integrated

among the endogenous cells of almost all tissues of the body, the fact that the lens is an avascular tissue led to the presumption that it would not be populated by resident immune cells. Resident immune cells are a first line of defense, surveilling tissues in response to infectious and noninfectious insults to

Abbreviations: ARVO, Association for Research in Vision and Ophthalmology; IACUC, Institutional Animal Care and Use Committee; MAGP1, microfibril-associated glycoprotein 1; MHC ClassII, major histocompatibility complex class II; PCO, posterior capsule opacification; α SMA, alpha smooth muscle actin.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *The FASEB Journal* published by Wiley Periodicals LLC on behalf of Federation of American Societies for Experimental Biology

maintain homeostasis.¹⁻⁴ Tissue resident immune cells such as macrophages and dendritic cells are considered professional phagocytes critical to removing cell debris, apoptotic cells, and pathogens.^{5,6} Resident immune cells also have nonimmune-related functions within a tissue. For instance, tissue resident macrophages are involved in long range signaling during tissue remodeling in the adult zebrafish⁷ and remodeling of ducts in mammary glands during pregnancy and lactation.⁸ Additional immune-related duties for these phagocytes include the ability to present antigens to engage an adaptive immune response and as recruiters and regulators of other leukocyte cells.⁹⁻¹¹ The earliest stages of lens development as it pinches off from the overlying surface ectoderm to form the lens vesicle are associated with the presence of macrophages in both the mouse and chicken.^{12,13} Macrophages were observed between the ectoderm and the developing lens and within the lens vesicle and lens epithelium.^{12,13} These early studies were focused on identifying phagocytic cells that can remove cellular debris and apoptotic cells as the lens develops.^{12,13} In these early studies, it was not considered whether these immune cell populations take up residence in the lens. In the mouse lens study, the macrophage marker F4/80 was not detected later in lens development.¹³ It is now understood that when an immune cell takes residence within a tissue, a distinct transcriptional signature is typically acquired along with changes in their metabolism.^{4,14,15} These changes are critical to establish distinct resident immune cell identity within a specific tissue and to ensure that resident immune cells live in sync with their local tissue environment. Each tissue has its own unique complement of immune cells with distinct micro-niche locations within the tissue.^{4,15,16} The local niche microenvironment helps to shape the identity of the tissue resident immune cells.^{15,17} Whether the avascular lens contains resident immune cells is an important unanswered question for lens biology and the visual sciences that could impact our understanding of lens development, homeostasis, and disease. Another important consideration is how and when immune cells are delivered to the lens, a tissue of the body that is noninnervated, stroma- and vascular-free.

While the lens was long believed to be immune privileged, a property considered essential to limit a damaging inflammatory response that could result in loss of visual function,^{18,19} it is now appreciated that the avascular lens can engage an immune response as a result of dysgenesis of the lens,²⁰ corneal wounding,²¹ and cataract surgery.²² In N-cadherin lens-conditional knockout mice, the lenses become dysgenic and develop opacities. It was discovered that immune cells are recruited to these dysgenic lenses, first macrophages, followed by B- and T-cells, both associated with the adaptive immune response.²⁰ We then found that immune cells are induced to surveil the lens in response to corneal wounding traveling to and along the surface of the lens on the ciliary zonules that link this tissue to the vasculature-rich ciliary body. These immune cells are then able to migrate from the superficial surface of the lens across the lens basement membrane capsule.²¹ Studies also show that

circulating neutrophils and macrophages are recruited to the mouse lens post-cataract surgery wounding following expression of pro-inflammatory cytokines by the lens epithelium.²² Immune cells such as macrophages and giant cells are also found associated with implanted intraocular lenses in rabbits, post-cataract surgery.²³⁻²⁵ It remains unknown whether there is a population of lens resident immune cells that provide a first line of defense for this tissue with the ability to initiate recruitment of circulating innate and adaptive immune cells.

Resident immune cells can alter their phenotype and transcriptional programs in response to local signals within the microenvironment revealing their highly plastic nature.^{17,26} The tradeoff of this powerful trait is that an altered tissue environment can lead to tissue resident immune cell dysfunction. Therefore, not surprisingly, resident immune cells which serve important protective roles in tissues are also known to cause chronic inflammation and pathogenesis.²⁷⁻³⁰ There is a major interest in understanding how this dysfunction occurs with the aim to prevent disease outcomes by resident immune cells. Lens injury induced by cataract surgery often leads to the lens fibrotic disease, Posterior Capsule Opacification (PCO) in age-related cataract, with epidemiological reports stating the incidence ranges from 20% to 50%, while in pediatric cataract patients this fibrotic injury response is reported to approach 100% prevalence.^{31,32} Elucidating if the lens contains populations of resident immune cells that could be dysregulated by an altered injury environment and contribute to lens pathologies such as fibrosis is an important consideration.

Here, we provide evidence that resident immune cells populate human, mouse, and chick lenses. We show that they locate among the cells of the lens epithelium. Our findings demonstrate that these immune cells associate with the avascular lens during lens development and support the conclusion that the vascular-rich ciliary body is a source of lens resident immune cells, with these cells traveling to the lens along the ciliary zonules. The resident immune cells of the lens are the first responders to damage, including the injury that occurs upon cataract surgery. When activated in response to a sterile lens tissue injury, this immune cell population rapidly emerges from its niches and migrates to the wound edge. We found that subsets of lens resident immune cells have antigen presenting ability, consistent with the ability to engage an adaptive immune response. In addition, lens resident immune cells activated by lens cataract surgery are highly susceptible to acquiring a myofibroblast phenotype, indicating their potential to become agents of lens fibrosis.

2 | METHODS

2.1 | Animals

All animal studies performed were approved by the Thomas Jefferson University Institutional Animal Care and Use

Committee (IACUC) and comply with all relevant guidelines. These studies also comply with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research (<https://www.arvo.org/About/policies/statement-for-the-use-of-animals-in-ophthalmic-and-vision-research>). Mice were analyzed at embryonic day (E) 18.5 and as adults (2 years or older). Chick embryo studies were performed with embryonated eggs (Poultry Futures, Lititz, PA, USA and Charles River, Wilmington, MA, USA), removed from their shells at the developmental times indicated and decapitated. For immunolabeling of cryosections of whole eyes, the eyes were removed, fixed directly in 4% paraformaldehyde for 24 hours, cryoprotected in 30% sucrose, and then, placed in OCT freezing media (Polyscience, Niles, Illinois, USA). To prepare mouse lens explants, lenses were first isolated from adult mouse eyes and the posterior lens capsule was removed using a pointed tip forceps, exposing the fiber cell mass, which were pulled from inside the lens with the forceps. This approach results in isolation of the remaining mouse lens capsule together with its closely associated epithelial and immune cells. This tissue fraction was pinned as an explant, cell side up, to the culture dish using the pointed tips of the forceps. The lens epithelial explants were fixed directly in 4% paraformaldehyde. For preparation of ex vivo mock cataract surgery explant cultures lenses are removed from isolated chick embryo eyes, mock cataract surgery performed and placed in culture as previously described.³³ The clinically relevant mock cataract surgery wound-repair cultures were developed to parallel human cataract surgery and investigate the mechanisms promoting both tissue regeneration and fibrosis in response to lens injury with large numbers of age-matched tissue. For these studies, microsurgery is performed ex vivo on isolated E15 chick embryo lenses. A large region of the anterior capsule is removed with sharp-tipped forceps providing access to the lens fiber cell mass, which is removed by hydroelution. This approach removes these differentiated fiber cells from their attachment sites on the posterior lens basement membrane capsule resulting in what is referred to as a capsular bag. The wounded lens epithelium remains as an intact sheet attached along the equatorial and remaining anterior surfaces of the capsule together with their closely associated resident immune cells. The cataract surgery wound-edge is located where the fiber cells had bordered the lens epithelium. Then, cuts are made perpendicular to the lens equator with sharp-tipped forceps, beginning at the edge of the site where the anterior capsule had been removed, which introduces additional injury sites on the anterior facing edge of the equatorial epithelium. This technique makes it possible to flatten the wounded epithelial cells and their closely associated resident immune cells on the culture platform, creating a star-shaped explant with the lens epithelial cells surrounding the fiber cell-denuded native basement membrane (Figure S1). These

ex vivo mock cataract surgery explants were cultured in complete media containing Media 199 (ThermoFisher Scientific, Waltham, MA, USA) that included 1% penicillin/streptomycin (Mediatech-Cellgro, Manassas, VA, USA), and 1% l-glutamine (Mediatech-Cellgro, Manassas, VA, USA) with 10% fetal calf serum (ThermoFisher Scientific, Waltham, MA, USA) and fixed at indicated times. Cultures were maintained in a humidified incubator at 37°C in 5% CO₂.

2.2 | Human samples

Anterior lens capsule with attached anterior lens epithelial cell samples from pediatric cataract patients undergoing manual anterior curvilinear capsulorrhexis (all ≥ 2 years old) were collected after obtaining informed consent according to the protocol approved by the Institutional Review Board of Wills Eye Hospital (Philadelphia, PA, USA) and Thomas Jefferson University (Philadelphia, PA, USA). Anterior lens capsule explants were attached to the tissue culture dish using Corning Cell-Tak Cell and Tissue Adhesive (Millipore Sigma, St. Louis, MO, USA), fixed directly or cultured with complete media containing Media 199 (ThermoFisher Scientific, Waltham, MA, USA), 1% penicillin/streptomycin (Mediatech-Cellgro, Manassas, VA, USA), 1% l-glutamine (Mediatech-Cellgro, Manassas, VA, USA) with 10% fetal calf serum (ThermoFisher Scientific, Waltham, MA, USA) and fixed at indicated times. Cultures were maintained in a humidified incubator at 37°C in 5% CO₂.

2.3 | Immunofluorescence

For immunolabeling studies of mouse eyes, whole mouse eyes were removed immediately after euthanasia fixed overnight at 4°C in 3.7% paraformaldehyde, washed in PBS, cryoprotected in 30% sucrose, and frozen in OCT. 20 μm thick cryosections were cut. Sections were permeabilized (0.5% Triton X-100 in PBS (Mediatech-Cellgro, Manassas, VA, USA), for 1 hour, incubated in block buffer (5% goat/donkey serum, 3% bovine serum albumin (BSA) in PBS, 0.25% Triton X-100) for 2 hours, incubated in primary antibody diluted in block buffer overnight at 37°C, washed, and then, incubated for 1 hour in secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) diluted in block buffer. Samples were counterstained with fluorescent conjugated Phalloidin (ThermoFisher Scientific, Waltham, MA, USA) to detect F-actin and/or DAPI (Biolegend, San Diego, CA) to detect nuclei. Primary antibodies used included MAGP1 (gift, R. Mecham, Washington University, St. Louis) and CD45 (Biolegend, San Diego, CA, USA).

For immunolabeling of sections of chick embryo eyes, whole eyes were removed after decapitation, fixed as stated

above, cryoprotected in 30% sucrose, frozen in OCT, and then, cut into 20 μm thick sections. Sections were then permeabilized (0.5% Triton X-100 in PBS) for 1 hour, incubated in block buffer (5% goat/donkey serum, 3% bovine serum albumin in PBS, and 0.5% Triton X-100) for 2 hours, incubated in primary antibody diluted in block buffer overnight at 37°C, washed and incubated for 1 hour in secondary antibody diluted in block buffer. Primary antibodies used included VE-Cadherin (Abcam, Cambridge, MA, USA), Fibrillin-2 (JB3, DSHB, Iowa City, Iowa, USA), Tenascin-C (Millipore Sigma, Burlington, MA, USA), KUL01 (Bio-Rad, Hercules, CA, USA), and MHC-II (Bio-Rad, Hercules, CA, USA). For VE-cadherin staining sections were blocked overnight at room temperature following permeabilization, incubated with antibody to VE-cadherin for 4 hours at 37°C, then, washed overnight at 37°C followed by incubation with secondary antibody for 1 hour as described above. Sections were counterstained with fluorescent conjugated Phalloidin (ThermoFisher Scientific, Waltham, MA, USA) to detect F-actin and/or DAPI (Biolegend, Sand Diego, CA) to detect nuclei.

For immunolabeling of ex vivo mock cataract surgery explants, human lens explants and mouse lens explants were fixed as stated above, permeabilized (0.25% Triton X-100 in PBS) for 10 minutes, incubated in block buffer (5% goat/donkey serum in PBS) for 30 minutes, and then, incubated in primary antibody diluted in 0.1% Tween20 in DPBS for 30 minutes at 37°C, followed by 30 minutes incubation in secondary antibody diluted in 0.1% Tween20. Primary antibodies for the chick lens study used included: MHC-II (Bio-Rad, Hercules, CA, USA), CD45 (BioRad, Hercules, CA, USA), CD14 (BioRad, Hercules, CA, USA), Vimentin (Abcam, Cambridge, MA, USA) Vimentin (gift, P. Fitzgerald, University of California, Davis, CA), β 2-integrin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and CD45 (Abcam, Cambridge, MA, USA). The CD45 (Biolegend, San Diego, CA, USA) primary antibody was used for the mouse explant study. Primary antibodies for the human explants study included: CD44 (H4C4, DSHB, Iowa City, Iowa, USA), CD45 (Novus Biologicals, Centennial, CO, USA), and α SMA (Abcam, Cambridge, MA, USA). Cultures were counterstained with fluorescent conjugated Phalloidin (ThermoFisher Scientific, Waltham, MA, USA) to detect F-actin and/or DAPI (Biolegend, Sand Diego, CA) to detect nuclei.

2.4 | Image analysis

Images of immunolabeled samples were examined using a Zeiss LSM500 or LSM800 confocal microscope. Confocal Z-stacks with 0.33 or 0.49 μm optical sections were collected

and analyzed using Zeiss Zen software and 3D images were created using Imaris software (Version 9.5).

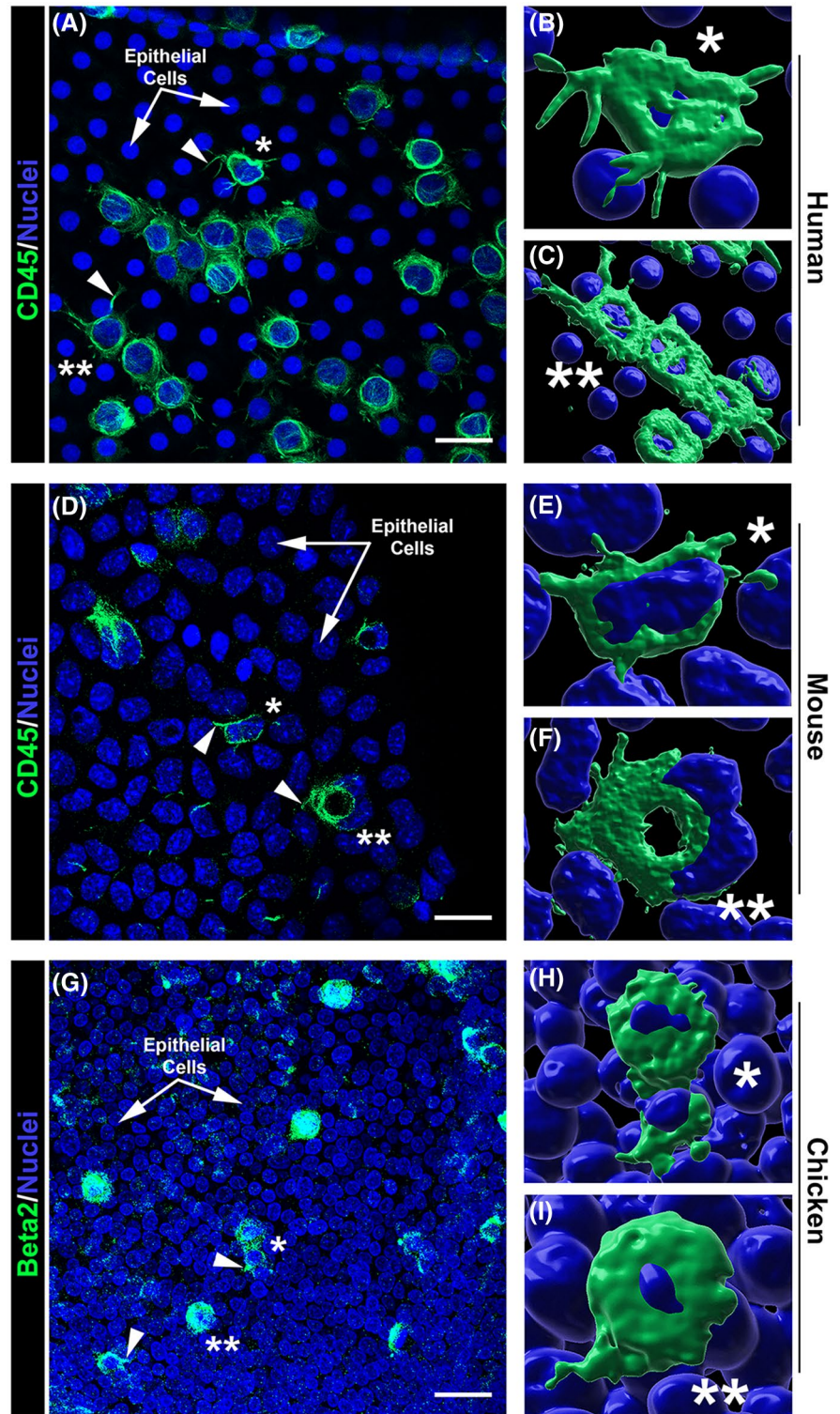
3 | RESULTS

3.1 | Resident immune cells locate among the epithelial cells of human, mouse, and chicken lenses

Most tissues are populated by resident immune cells whose purpose it is to function as immediate responders to injury or a pathological insult.^{1-4,34-36} The fact that the lens is avascular was previously believed to preclude the possibility that the lens harbored this self-protective cell type. Given their likely importance to lens homeostasis, we examined whether resident immune cells populate the lens in studies with lens epithelia across species. For these studies, we prepared epithelial explants from pediatric human, adult mouse, and embryonic D15 chick lenses, which consist of both the lens epithelium and its endogenous basement membrane, the lens capsule, and pinned them to a culture dish capsule side down. The explants were fixed immediately for immunolocalization analysis with antibody to either the leukocyte common antigen CD45 (Figure 1A-F) or the leukocyte integrin β 2 (CD18) (Figure 1G-I) and co-labeled with DAPI to detect nuclei. Confocal imaging showed for the first time that resident immune cells are localized among the cells of the normal epithelium of human (Figure 1A), mouse (Figure 1D), and chick (Figure 1G) lenses. Three-dimensional (3D) surface structure renderings created with Imaris software from the confocal z-stacks from which Figure 1A,D,G are single optical planes that highlight the morphology of the resident immune cells and demonstrate that many of them extend dendritic-like processes that reach around and between individual lens epithelial cells (Figure 1B,C,E,F,H,I).

The localization of lens resident immune cells in the lens was also examined by immunolabeling cryosections of E18.5 mouse eyes for the common immune cell surface protein CD45. These sections were co-labeled with fluorescent-conjugated phalloidin which detects F-actin and reveals lens cell cytoarchitecture, DAPI for nuclei and microfibril-associated glycoprotein 1 (MAGP1) to detect the ciliary zonules along the surface of the lens. Confocal microscopy image analysis shows that resident immune cells become localized among the cells of the lens epithelium during lens development, shown here in the region of the lens equator (Figure 2B). 3D surface structure renderings of the confocal z-stack of which a single optical plane is shown in Figure 2B reveal the immune cells are integrated between the lens epithelial cells, their cell bodies predominant along the basal surfaces of the lens epithelium

FIGURE 1 Resident immune cells are present among the human, mouse, and chicken lens epithelium. CD45 and $\beta 2$ integrin⁺ resident immune cells (green) are found localized among the epithelial cells (indicated by double arrows) in explants from (A-C) human, (D-F) mouse, and (G-I) chick lens tissue. Lens tissue was counterstained with DAPI to identify nuclei (blue). Within the lens tissue from each species, many of these CD45⁺ and $\beta 2$ integrin⁺ immune cells have dendritic morphologies indicated by the arrowheads. The dendritic-like processes of resident immune cells indicated by asterisks in A, D, G are highlighted by 3D surface structures created by Imaris software from confocal z-stack images and shown in (B, C, E, F, H, and I). In 3D structure images, the dendritic processes of immune cells are seen reaching between the lens epithelial cells. Magnification bars = 20 μ m. Studies are representative of at least three individual lenses



defined here by the basal actin cytoskeleton (Figure 2C,D). Immune cells are also detected within the MAGP1-rich zonules that extend anteriorly along the equatorial surface of the embryonic mouse lens basement membrane capsule (Figure 2B,C). The localization of these immune cells traveling just outside the lens suggests they may be a source of the immune cells that populate this tissue during development.

3.2 | The ciliary zonules provide a conduit for immune cells traveling to the lens during development

Our previous studies show that, in response to corneal wounding, immune cells surveil the adult lens after traveling along the ciliary zonules that link the ciliary body to the lens. These immune cells continue to migrate along the

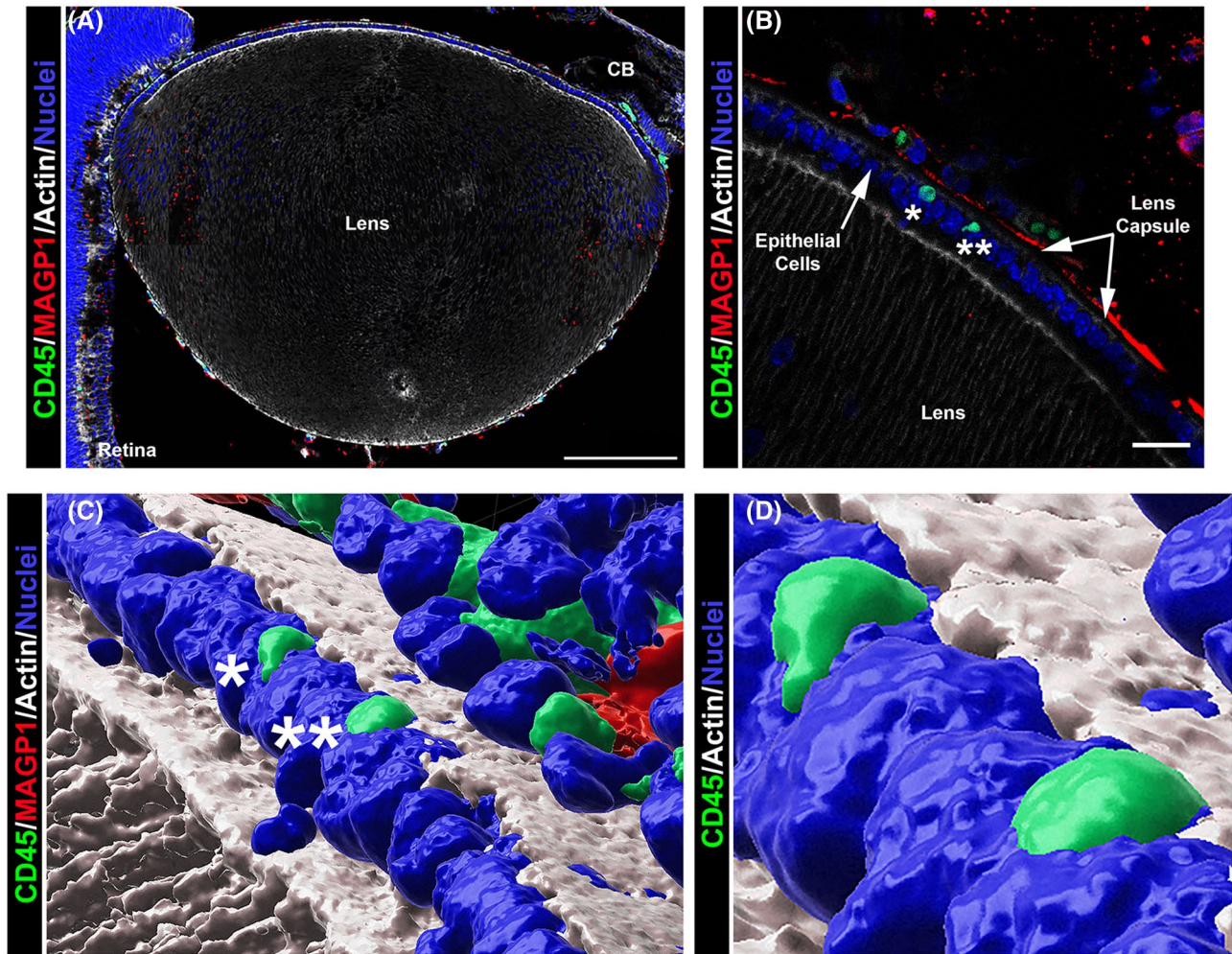


FIGURE 2 Resident immune cells associate with the zonule fibers closely linked to the lens capsule and locate to the mouse lens during development. A, Overview confocal image of a cryosection of E18.5 mouse embryo eye co-immunolabeled for the ciliary zonule protein MAGP1 (red) and the immune cell protein CD45 (green), co-labeled for F-actin (white) and nuclei (blue) at high magnification in B. B, Single optical plane from a confocal z-stack of an E18.5 mouse embryo eye cryosection in the region of the lens adjacent to the ciliary zonules at high magnification following co-labeling for MAGP1 (red), CD45 (green), F-actin (white), and nuclei (blue). CD45+ cells are present along ciliary zonule fibers associated with both the anterior and equatorial surfaces of the lens, and as resident cells of the equatorial epithelium. The lens epithelium is indicated by the arrow and the lens capsule by a double arrow. C, Imaris 3D surface structure rendering from the confocal z-stack of which panel B is a single optical plane highlights the position of CD45+ (green) immune cells associated with MAGP1 enriched zonule fibers (red) and resident immune cells located among the lens epithelium. D, Zoomed in view of the 3D structural image in C showing the position of CD45+ lens resident immune cells integrated among the lens epithelium. Single and double asterisks denote the CD45+ lens resident immune cells within each panel (B-D). Magnification bars A = 200 μm , B = 20 μm

zonules extended along the lens surface and migrate across the lens capsule.²¹ The immunolabeling studies of E18.5 mouse embryos above suggested that there may be a similar path that leads to resident immune cells populating the lens during development. However, in the mouse embryo the lens is surrounded by a fetal vasculature comprised of the anastomosed anterior pupillary membrane and the tunica vasculosa that could also be a source of the resident immune cells that populate the mouse embryo lens.³⁷ The presence of the fetal vasculature so near the mouse embryo lens makes this a complicated model for investigating the potential role of the ciliary zonules in delivering resident immune cells to

the lens. Unlike mammals, the lenses of most non-mammalian vertebrates like chickens develop in the absence of an associated fetal vasculature.³⁷ We confirmed the lack of a fetal vasculature in association with the chick embryo lens by co-immunolabeling cryosections of E9 chick embryo eyes for the vascular-specific endothelial cell protein VE-cadherin and the ciliary zonule protein fibrillin-2, together with DAPI labeling to detect nuclei, and imaging by confocal microscopy (Figure 3A). These studies show the high level of vascularization of the ciliary body and the absence of a vasculature along ciliary zonules that link the ciliary body and the lens or in association with the surface of the chick embryo

lens (Figure 3A). The movement of immune cells between the ciliary zonules and the lens during development would, therefore, occur in the absence of a vasculature.

The molecular components of the ciliary zonules, which include fibrillins, MAGP1 and matricellular proteins like thrombospondin-1,^{21,38-40} provide a matrix environment that is consistent with one permissive for cell migration. We investigated whether immune cells travel to the embryonic chick lens along the ciliary zonules during development. For these studies, cryosections of E15 chick embryo eyes were co-immunolabeled for tenascin-C, which we show to be a component of the ciliary zonules in the chick and KUL01, an antibody that is specific to chick monocytes/macrophages.⁴¹ The sections were co-labeled with DAPI to detect nuclei and fluorescent-conjugated phalloidin which binds F-actin and denotes lens cytoarchitecture. Confocal image analysis revealed the presence of KUL01+ immune cells with processes extended suggestive of a migratory phenotype along the tenascin-C+ ciliary zonules that link the lens to the ciliary body (Figure 3B, higher magnification in inset). To better understand the relationship between these immune cells and the ciliary zonules, we created 3D surface renderings from the confocal z-stack of which Figure 3B is a single optical plane (Figure 4A-D). For this analysis, we either kept all channels opaque which precludes fluorescence overlay in this imaging modality (Figure 4A) or rendered tenascin-C transparent (Figure 4B-D). Under conditions where all fluorescent channels are kept opaque, the movement of monocytes/

macrophages within the zonule fibers is obscured by any overlaying zonule fibrils (Figure 4A). In contrast, when tenascin-C was made transparent the KUL01+ immune cells traveling within the ciliary zonule fibrils that link the lens to the ciliary body are revealed (Figure 4B,C). The inclusion of F-actin labeling in the structural rendering highlights the position of the lens cells in relation to both the migrating immune cells and the zonule fibrils (Figure 4B). This image also shows that tenascin-C+ zonules become coincident with the lens capsule. This structural analytic approach also provided evidence of KUL01+ immune cells moving within tenascin-C-rich region of the lens capsule (Figure 4D). These findings suggest that resident immune cells are sourced from the vascular-rich ciliary body during development and show their movement along ciliary zonule fibrils to the lens from where they can transit the lens capsule to take up residence in the lens.

3.3 | Response of lens resident immune cells to cataract surgery wounding

We investigated the response of the lens resident immune cell population to a sterile tissue injury. For these studies, we performed ex vivo mock cataract surgery on E15 chick embryo lenses. In this microsurgery procedure, the differentiated fiber cells that comprise the mass of lens tissue are removed from their attachment sites on the posterior

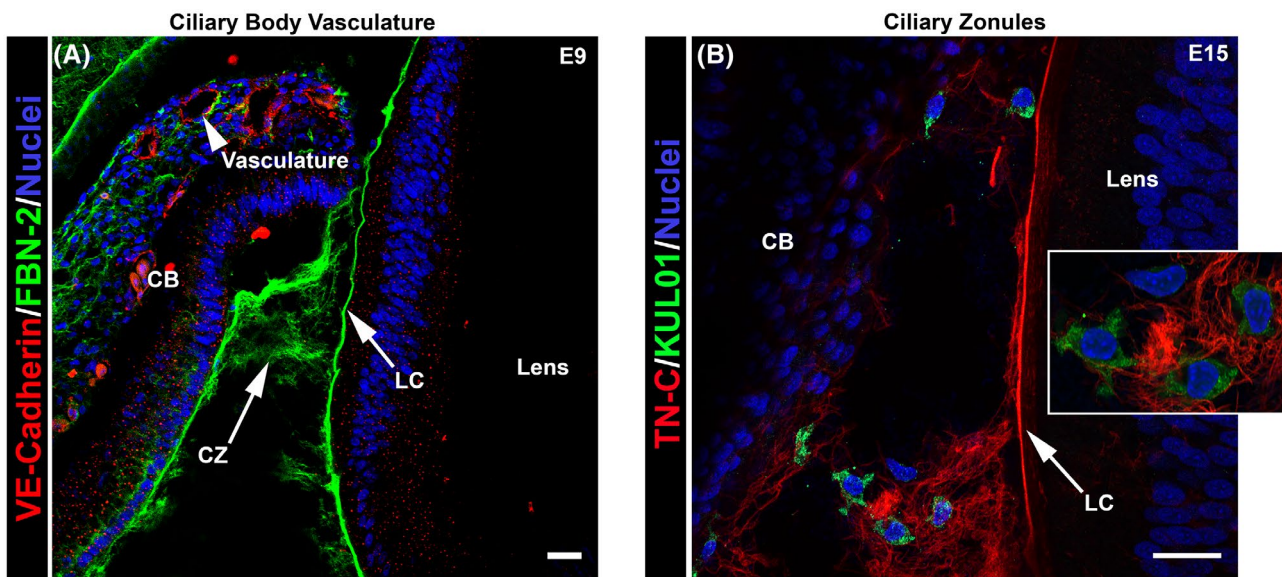


FIGURE 3 The ciliary zonules provide a path for immune cells to travel from the local vascular-rich ciliary body to take entry into the lens during development. A, Co-immunolabeling of E9 chick embryo eye cryosections for fibrillin-2 (green), a basic structural component of the ciliary zonules, the vasculature specific cell-cell adhesion protein VE-cadherin (red), and nuclei (blue) shows the rich vasculature of the ciliary body (CB, arrowhead) and confirms the absence of a vasculature along the ciliary zonules (CZ, arrow) and in association with the lens capsule (LC, arrow). B, Immunolabeling of E15 chick embryo eye cryosections for KUL01 (green), which is specific to chick monocytes and macrophages, with the ciliary zonule protein tenascin-C (red) and nuclei (blue) reveals that immune cells travel along the ciliary zonules during development; inset high magnification of KUL01+ cells migrating along the TN-C rich zonules. Magnification bars = 20 μ m

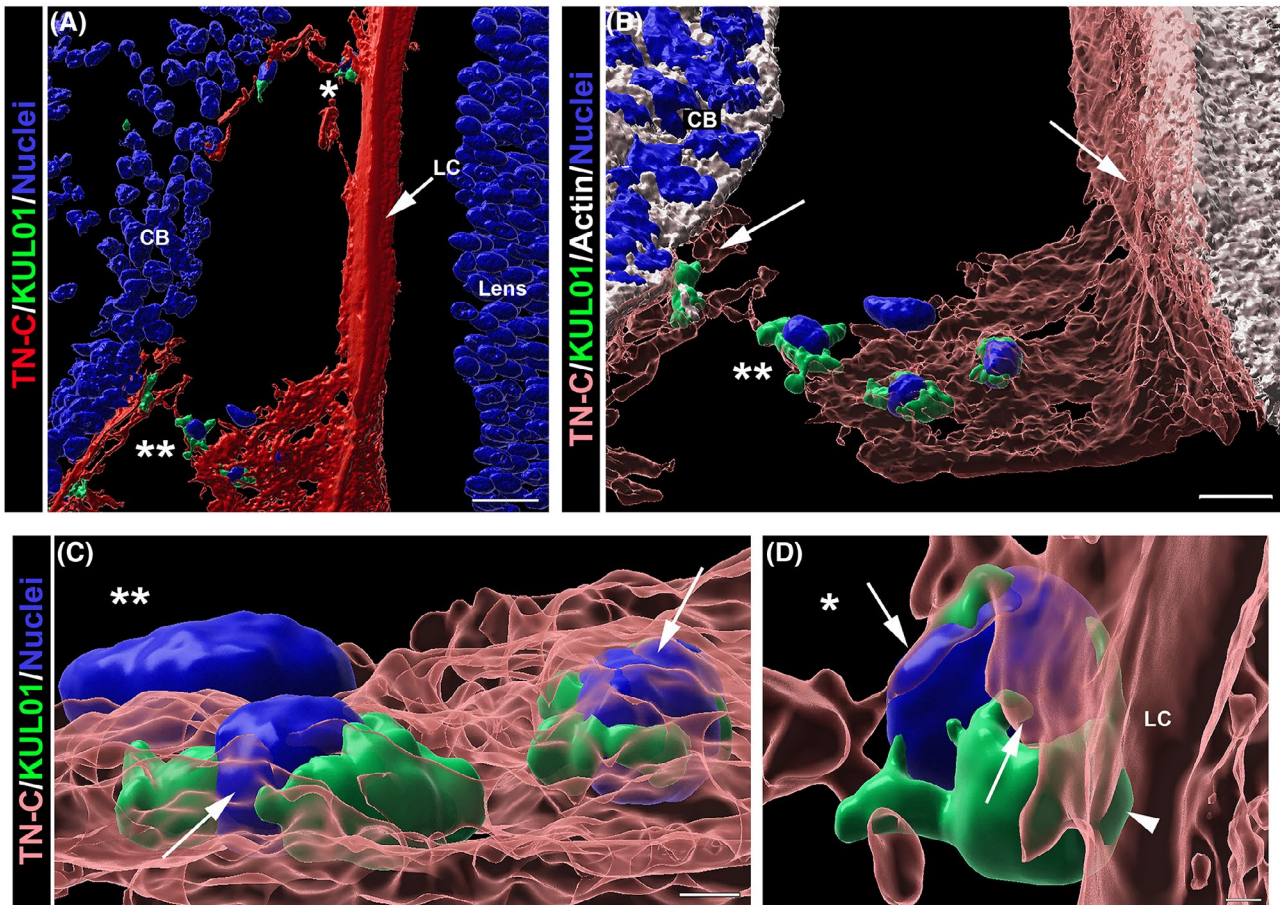


FIGURE 4 Structural imaging of resident immune cells moving along the ciliary zonules and inserting into the lens capsule. A-D, 3D surface structures were created with Imaris software from confocal z-stack images of E15 chick embryo eye cryosection presented as a single optical section in Figure 3B that were immunolabeled for the monocyte/macrophage marker KUL01 (green), the ciliary zonule protein tenascin-C (red), and nuclei (blue). A, 3D structural image in which the fluorescent channels are kept opaque shows KUL01+ cells located along the ciliary zonules. B-D, To better visualize the position of the KUL01+ cells moving along the TN-C- rich ciliary zonules, the fluorescent channel for TN-C was made transparent using the Imaris software. Arrows in B indicate where the TN-C rich-ciliary zonules attach to the ciliary body (CB) and associate with lens capsule (LC). C,D, 3D structural views at higher magnification showing KUL01+ cells (arrows) moving within the TN-C-rich zonules and KUL01+ cell immune cell associated with both the TN-C rich zonules (arrow) entering the TN-C enriched lens capsule region (LC, arrowhead). A single or double asterisk indicates the position of the same KUL01+ cell in each panel. Magnification bars A = 20 μ m, B = 10 μ m, C = 2 μ m, D = 1 μ m

lens basement membrane capsule by hydroelution.^{33,42} The wounded lens epithelium remains attached along the anterior and equatorial surfaces of the lens capsular bag; the wound-edge located where the fiber cells had bordered the epithelium. Cuts are made in the anterior aspects of the capsular bag to flatten the wounded tissue on the culture platform, creating a star-shaped explant with the lens epithelial cells surrounding the fiber cell-denuded native basement membrane (Figure 5A, Figure S1A). We show previously that regenerative repair of the mock cataract surgery wound leads to re-epithelialization of the wounded area within 3 days postinjury,^{42,43} which is accompanied by wrinkling of the posterior capsule (Figure S1) typical of PCO. As the lenses are isolated and the cataract surgery performed ex vivo, this model is ideal for identifying the response of lens resident immune cells to injury

in the absence of any contribution from immune cells that respond to this injury from outside the lens.

The immediate response of resident immune cells to this injury was examined by fixing the ex vivo cataract surgery explants at 15 minutes post-wounding. The resident immune cell population was detected by immunolabeling the postsurgery explants for the leukocyte common antigen CD45 and co-immunolabeling for vimentin (Figure 5B-H). Co-labeling of F-actin with fluorescent-conjugated phalloidin delineated the cells of the wounded lens epithelium. Confocal image analysis showed that the CD45+/vimentin+ cells were present in expanded multicellular niches located among the wounded lens epithelium (Figure 5B,C). 3D surface rendering of the confocal z-stack from which Figure 5B is a single optical plane reveals the morphology of the activated resident immune cells in these niches, which appears to

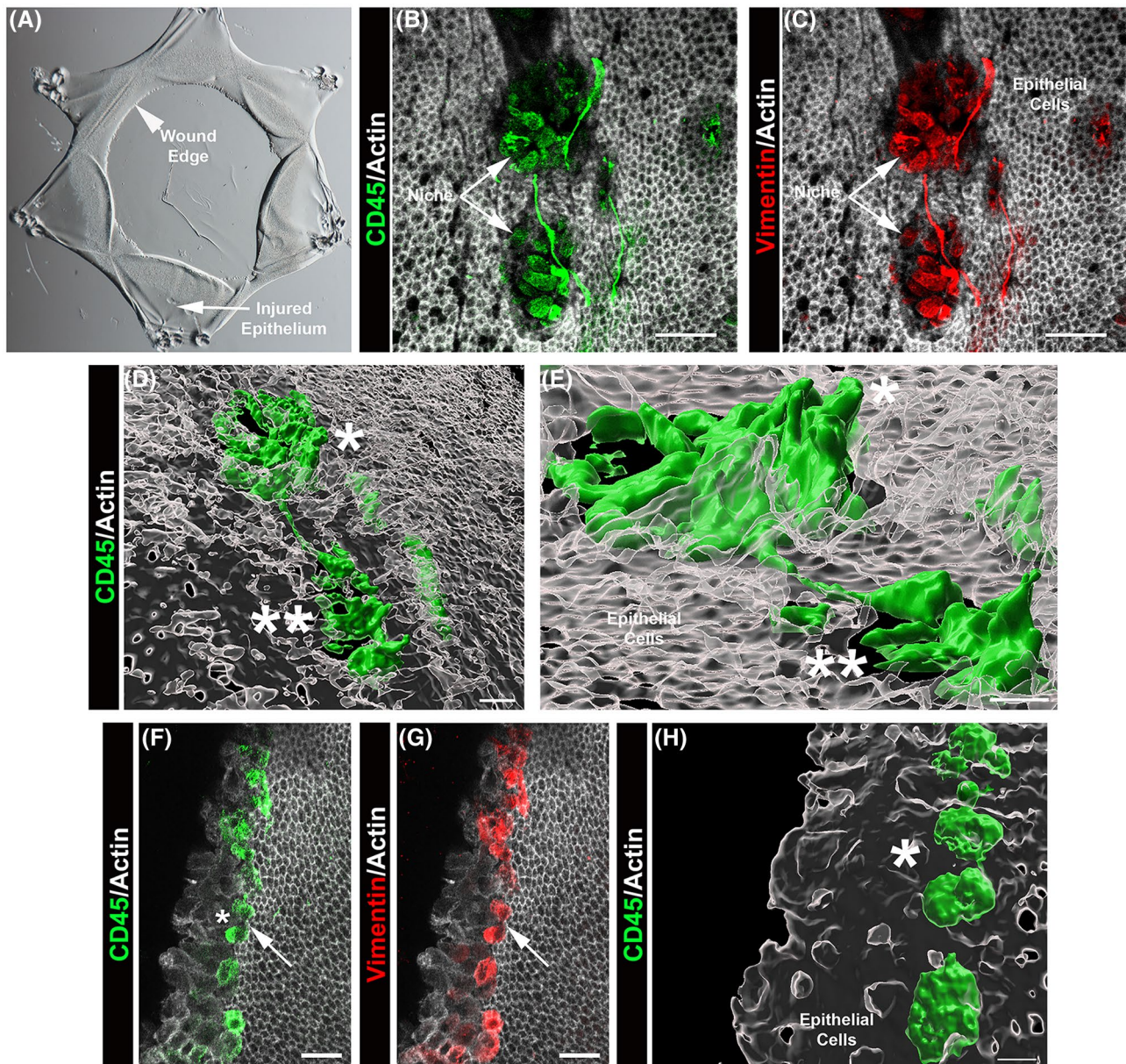


FIGURE 5 Activation of resident immune cells occurs in response to mock cataract surgery wounding. A, Ex vivo mock cataract surgery culture created by performing mock cataract surgery on isolated E15 chick embryo lenses. The microsurgery procedure removes the fiber cells from their association with the posterior lens capsule basement membrane. The fiber cell-denuded posterior basement membrane creates the central wound area. The wounded lens epithelial cells (arrow) are located in the points of the star-shaped culture that surround the wound area. The wound edge is (arrowhead) is located where the fiber cells once bordered the epithelial cells. B-H, Ex vivo mock cataract surgery cultures at 15 minutes post-wounding were labeled for the leukocyte antigen CD45 (green), vimentin (red), and F-actin (white) to delineate the lens epithelium. B,C, CD45/vimentin-rich+ cells are present in multicellular niches among the epithelium (white arrows). D,E, 3D surface structure images of CD45+ cells in niches (marked by single and double asterisk) within the lens epithelium (actin, white) were created from z-stacks of image of the single confocal images presented in B. 3D structural renderings show activated CD45+ resident immune cells emerging from their niches located among the lens epithelium in response to injury. F-G, CD45/vimentin-rich+ cells (arrow) migrating toward the wound edge. H, 3D structural image from confocal z-stacks presented as a single optical plane in (F) showing CD45+ cells migrating over the lens epithelium toward the wound edge. Asterisk indicates the location of the same CD45+ cells shown in (F). Magnification bars B,C,F,G = 20 μm , D,E,H = 5 μm . Studies are representative of at least three independent experiments

show these cells emerging from their niches (Figure 5D,E). Confocal imaging at the cataract surgery wound edge revealed that the activated CD45+/vimentin+ resident immune cells have migrated to positions adjacent to the wound edge

within 15 minutes postinjury (Figure 5F,G), also viewed as a 3D structural rendering (Figure 5H). The behavior of the CD45+/vimentin+ immune cells population in response to lens injury parallels our previous studies that showed the

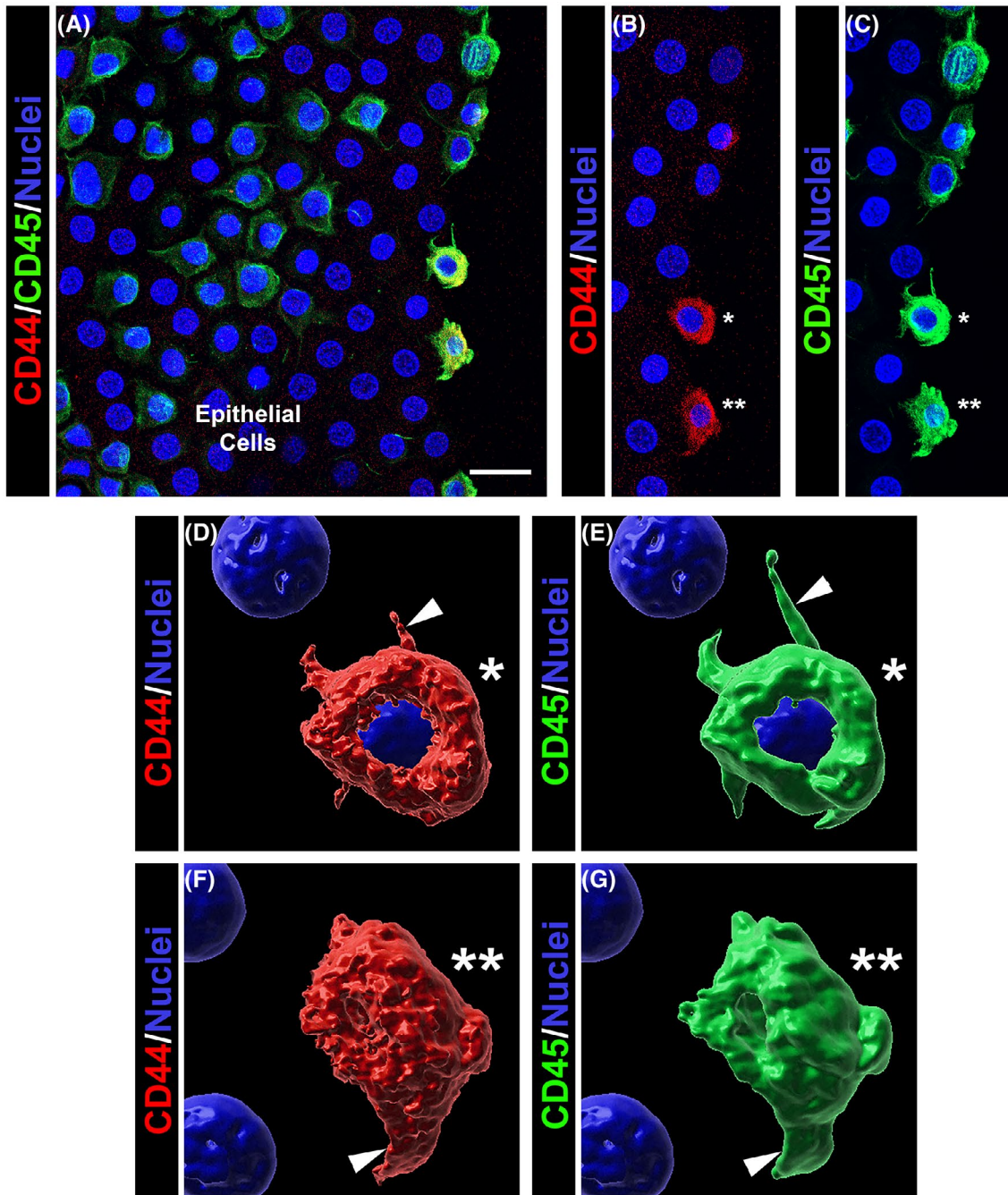


FIGURE 6 CD45 resident immune cells co-express CD44 at the wound edge within the human pediatric lens explants. A-C, Human pediatric explants cultured post-cataract surgery wounding were co-immunolabeled for CD45 (green) and the hyaluronic receptor CD44 (red) with nuclei (blue). CD45+ cells are interspersed among the lens epithelium and recruited to the wound edge. At the wound edge, most of the CD45+ cells co-express the CD44 receptor. D-G, 3D surface reconstructions from z-stacks of CD44/CD45+ rich cells presented as a single confocal plane in (B,C) show protrusions being extended from CD45 (E,G) and CD44 (D,F) cells at the wound edge (marked by single and double asterisk). Magnification bar = 20 μ m

presence of a resident vimentin-rich cell population in niches among the lens epithelial cells of the unwounded embryonic chick lens that is activated in response to cataract surgery, and rapidly populates the wound edge,⁴⁴ where they function as leader cells that direct the wound repair process.^{43,45}

There is a similar response in human post-cataract surgery wounded explants. CD45+ cells were observed

migrating toward and taking residence at the edge of the wounded explant (Figure 6A,C,E,G). Our previous studies showed the vimentin-rich cells that populate the leading edge of the chick mock cataract surgery wound express the transmembrane glycoprotein CD44,^{45,46} a hyaluronic receptor expressed by immune cells.^{47,48} Here, we co-immunolabeled the resident immune cells activated posthuman

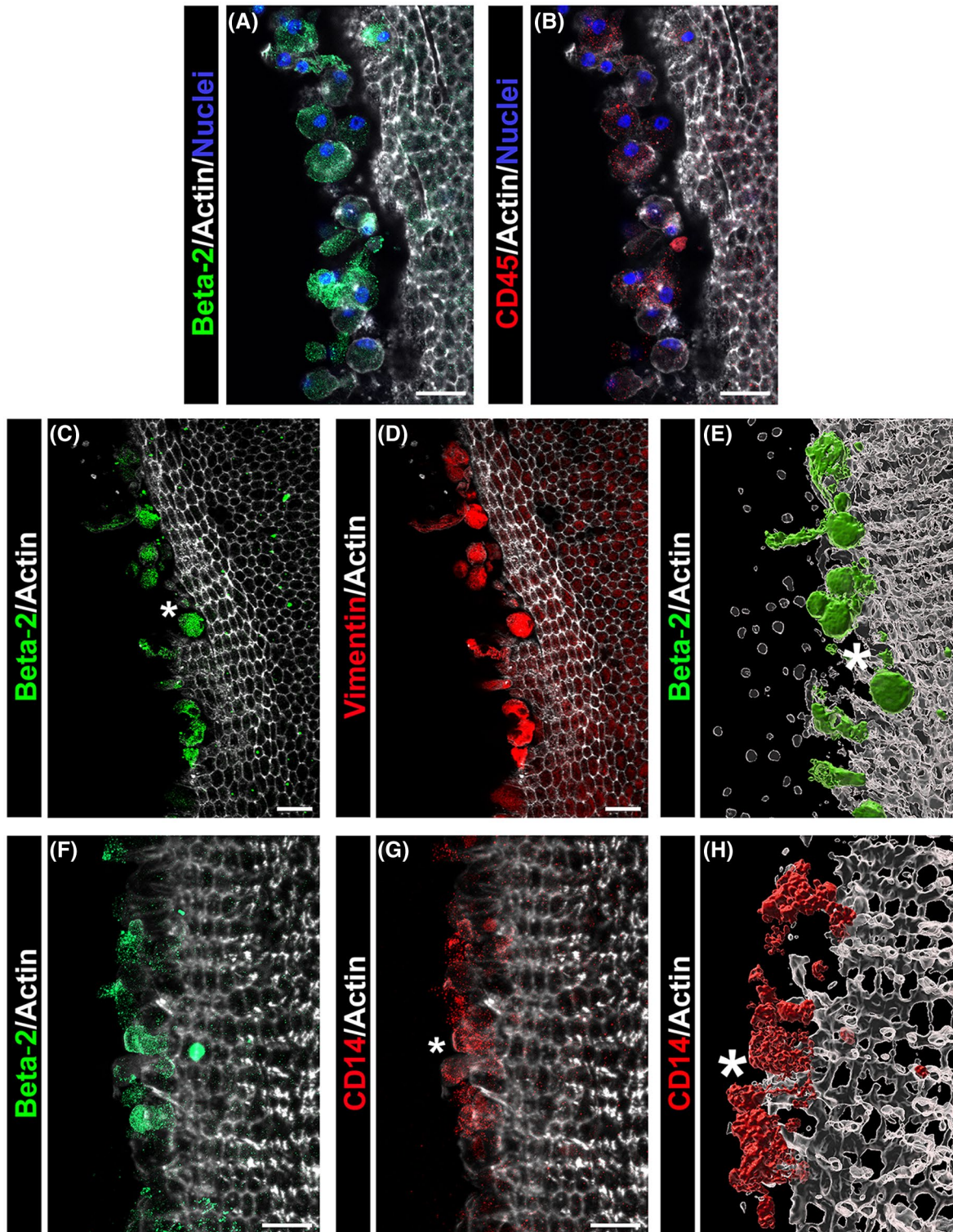


FIGURE 7 Resident immune cells migrate to the wound edge in response to mock cataract surgery wounding. A-H, Ex vivo mock cataract surgery chick explants 1 hour postinjury were labeled for the leukocyte specific integrin $\beta 2$ (CD18) (green, A, C, E, and F) and co-labeled for the leukocyte antigen CD45 (red, B), vimentin (red, D) or the monocyte/macrophage lineage marker CD14 (red G, H). Explants were labeled for F-actin (white, A-H) to delineate the lens epithelial cells. CD45/ $\beta 2$ + cells were also counterstained with DAPI to identify nuclei (A,B). $\beta 2$ integrin/CD45+ resident leukocytes migrated to the wound edge in response to mock cataract surgery. $\beta 2$ integrin+ immune cells at the wound edge were enriched for vimentin (C,D) and subpopulations of $\beta 2$ integrin+ cells expressed CD14 (F,G), identifying that subsets of resident immune cells are from the monocyte/macrophage lineage. E,H, 3D surface images from z-stack confocal images of images presented as single optical planes in C and G highlight the morphology of the resident immune cells extending protrusions at the wound edge. Asterisks in (E,H) indicate the position of the same cells shown in (C,G), respectively. Magnification bars = 20 μ m. Studies are representative of at least three independent experiments

pediatric cataract-surgery wounding for CD45 and CD44 and found that the injury activated CD45+ resident immune cells that had migrated to the wounded edge co-expressed CD45 and CD44 (Figure 6A,B). 3D surface structure rendering of these CD44+/CD45+ resident immune cells highlight the filopodial protrusions extended by these immune cells (Figure 6D-G). Since CD44 expression was not detected on the CD45+ cells that remain localized among the lens epithelial cells postinjury (Figure 6A), it appears that expression of CD44 is acquired after these immune cells populate the wound edge.

In studies with the chick embryo *ex vivo* cataract surgery model, we examined the resident immune cell response at 1-hour post-wounding by confocal microscopy imaging at the wound edge following co-immunolocalization with antibodies to CD45 and $\beta 2$ integrin and co-labeling for F-actin (Figure 7A,B). Both CD45 and $\beta 2$ integrin are leukocyte-specific antigens; $\beta 2$ integrin a receptor that mediates both the attachment and migration of leukocytes.⁴⁹ CD45+/ $\beta 2$ integrin+ leukocytes populate the leading edge of the wounded lens epithelium at 1-hour postsurgery (Figure 7A,B). Co-immunolabeling for $\beta 2$ integrin and vimentin showed that the $\beta 2$ integrin+ immune cell population is enriched for vimentin (Figure 7C,D); similar to what was observed for CD45+ cells (see Figure 5). The 3D surface structure for $\beta 2$ integrin created from the confocal z-stack of which a Figure 7A is a single optical plane is shown in Figure 7E. Further characterization revealed that the $\beta 2$ integrin+ cells at the leading-edge post-wounding express monocyte/macrophage marker CD14 (Figure 7F,G; 3D surface structure of the CD14+ cells is provided in Figure 7H). Together, these findings show that resident immune cells are activated by cataract surgery wounding and rapidly migrate to the wound edge and implicate this wound-activated resident immune cell population as mediators of the lens injury response.

3.4 | Antigen presenting immune cells are resident to the lens and among the first responders to injury

Resident immune cells are typically antigen presenting immune cell types such as dendritic cells and macrophages that mediate the immune response by processing and presenting antigens.⁹ Major histocompatibility complex class II (MHC Class II) is a molecule expressed by professional antigen presenting cells, their presentation on the cell surface integral to the mechanism that activates an adaptive immune response.⁵⁰ Therefore, we examined if MHCII expressing resident immune cells are found within the developing chicken embryonic lens. Cryosections of chick embryo eyes at both E5, a very early stage of lens development, and E15, a stage of

development by which time the lens has matured, were labeled with an antibody to MHC class II protein (Figure 8). E5 sections were co-labeled for tenascin-C (Figure 8A), and both E5 and E15 sections were co-labeled for F-actin and nuclei (Figure 8). At E5, MHCII+ cells were found associated with the tenascin-C+ zonules between the closely apposed ciliary body and lens; however, at this early stage of development, MHCII+ cells were not detected within the lens (Figure 8A). 3D surface structure image analysis of the z-stack containing the MHCII+ cell in Figure 8A in which tenascin-C was rendered transparent demonstrated the presence of this immune cell within the fibrils of the ciliary zonules at this stage of development (Figure 8B,C). In contrast, a single optical plane and a 3D surface structure rendering of a confocal z-stack of an E15 lens section co-labeled for MHCII, F-actin, and nuclei revealed that by this time of development MHCII+ immune cells had come to reside in the lens and were located between the cells of the equatorial epithelium (Figure 8D,E). These studies support the conclusion that the resident immune cells that populate the lens during development originate in the vasculature of the ciliary body and travel to the lens along the ciliary zonules.

The response of the MHCII positive resident immune cells present in E15 chick lenses to *ex vivo* cataract surgery wounding was examined by co-immunolabeling cataract surgery-wounded lens epithelial explants at 1 hour postinjury for MHCII and vimentin (Figure 9A-D). Confocal imaging showed that the wound-activated MHCII+ cells within the lens epithelium exhibit a dendritic morphology, their MHCII-rich dendritic processes inserted between the lens equatorial epithelial cells (Figure 9A). 3D surface structure image rendering revealed the morphology of these wound-activated dendritic cells and their close relationship to the cells of the lens equatorial epithelium, with the immune cells' dendritic processes interdigitated with the lens epithelial cells (Figure 9B). This imaging study also revealed the predominance of MHCII in the dendritic tails and vimentin in the cell body of these activated immune cells (Figure 9A,B). Within 1 hour postinjury there are also wound-activated MHCII+ cells that have migrated to the cataract surgery wound edge (Figure 9C). 3D structural rendering of these MHCII+ cells show that their extension of dendritic processes reaching around neighboring lens epithelial cells is also a property of these cells at the wound edge (Figure 9D). Co-immunolabeling of the *ex vivo* post-cataract surgery wounded explants for MHCII and $\beta 2$ integrin at 1 hour post-injury confirmed the immune-cell identity of the MHCII+ antigen presenting cells (Figure 9E,F). The morphology of these $\beta 2$ integrin+ cells was created from the z-stack of which Figure 9F is a single optical plane using 3D surface rendering analytics (Figure 9G). The expression of MHCII by these activated resident immune cells suggests that they play a role in engaging an adaptive immune response.

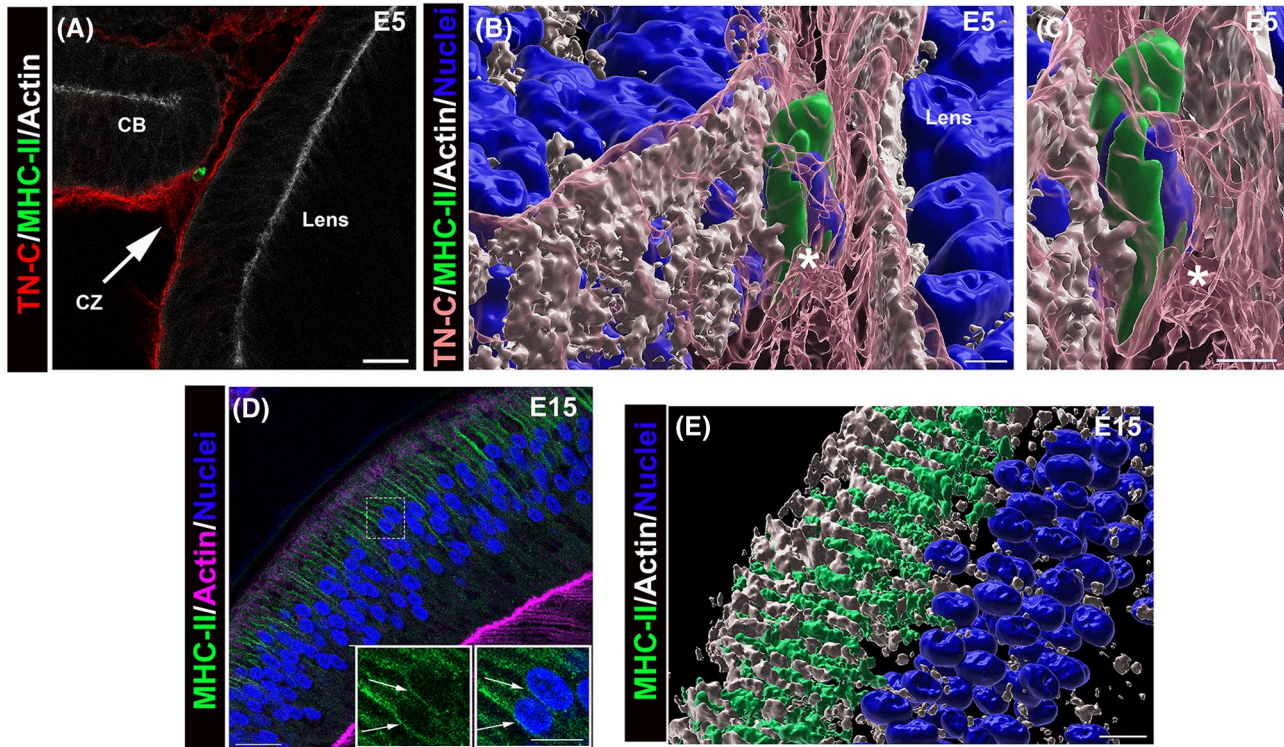


FIGURE 8 Antigen presenting cells located along ciliary zonules and resident within the lens equatorial epithelium of the E15 lens. A, Single optical plane of a confocal z-stack of a cryosection of an E5 developing chick eye labeled for MHC-II (green), ciliary zonule marker TN-C (red), and F-actin (white). At this early time during development, the ciliary zonules (CZ) are closely apposed to the lens and MCH-II+ cells are seen associated with the zonules near the lens capsule. B,C, 3D surface structure renderings of the confocal z-stack at E5 from which a single optical plane is shown in A in which the fluorescent channel for TN-C (pink) was made transparent to view the localization of an MHC-II+ cell (green, asterisk) within the TN-C- rich zonules near the lens (F-actin (white), nuclei (blue)), zoomed in at increasing levels of magnification. D, Single optical plane of a confocal z-stack of a cryosection of an E15 developing chick lens labeled for MHC-II (green), F-actin (magenta), and nuclei (blue); boxed area shown at higher magnification in the insets with and without nuclei. E, 3D surface structure rendering the of the E15 chick embryo lens cryosection labeled for MHC II (green) with F-actin (white) and nuclei (blue) of which a single optical plane is shown in D. These images show that MHCII+ immune cells are inserted among the cells of the equatorial epithelium by E15 (arrows). CB-ciliary body, CZ-ciliary zonules, Magnification bars A = 20 μm , D = 20 μm , D insets = 10 μm , E = 7 μm , B,C = 3 μm

3.5 | Resident immune cells are progenitors of $\alpha\text{SMA}+$ myofibroblasts

While resident immune cells surveil tissues and maintain homeostasis, they also can become agents of pathogenesis.^{1-3,27-30} Lens fibrotic disease, like fibrotic disease in most tissues, is caused by alpha smooth muscle actin (αSMA)+ myofibroblasts.⁵¹⁻⁵³ Our previous studies with the ex vivo mock cataract surgery chick model showed that in fibrosis-inducing environments the lens resident CD44+/vimentin-rich cells we have now identified as resident immune cells are induced to become $\alpha\text{SMA}+$ myofibroblasts.⁴⁵ We now show that CD45+ resident immune cells present in human pediatric post-cataract surgery explants can acquire a myofibroblast phenotype (Figures 10 and 11). For these studies, tissue from the same pediatric cataract surgery patient was divided in two and placed in different culture dishes. These post-cataract surgery explant cultures were fixed at either day 0 (Figure 10A-C) or day 6 (Figures 10D-I and 11) and

co-immunolabeled for αSMA and CD45. At day 0, there was no expression of αSMA by either the CD45+ resident immune cells localized among the injured lens epithelium or those at the leading edge of the wound (Figure 10A-C). In contrast, by day 6 CD45+ cells located among the lens epithelium express $\alpha\text{SMA}+$ (Figure 10 D-F), also shown as 3D surface structure renderings (Figure 10G-I). In the same patient sample at the 6 day post-wounding time period, CD45+ immune cells that had moved off the edge of the wounded human post-cataract surgery explant onto the rigid tissue culture substrate also expressed αSMA , here, shown organized into stress fibers typical of myofibroblasts (Figure 11). These findings demonstrate that resident immune cells have the potential to serve as agents of lens fibrotic disease progression and show that myofibroblast emergence and persistence is much greater in the rigid environment of the tissue culture plastic than on the basement membrane capsule. This finding is consistent with the general fibrosis literature showing that rigid environments promote myofibroblast differentiation

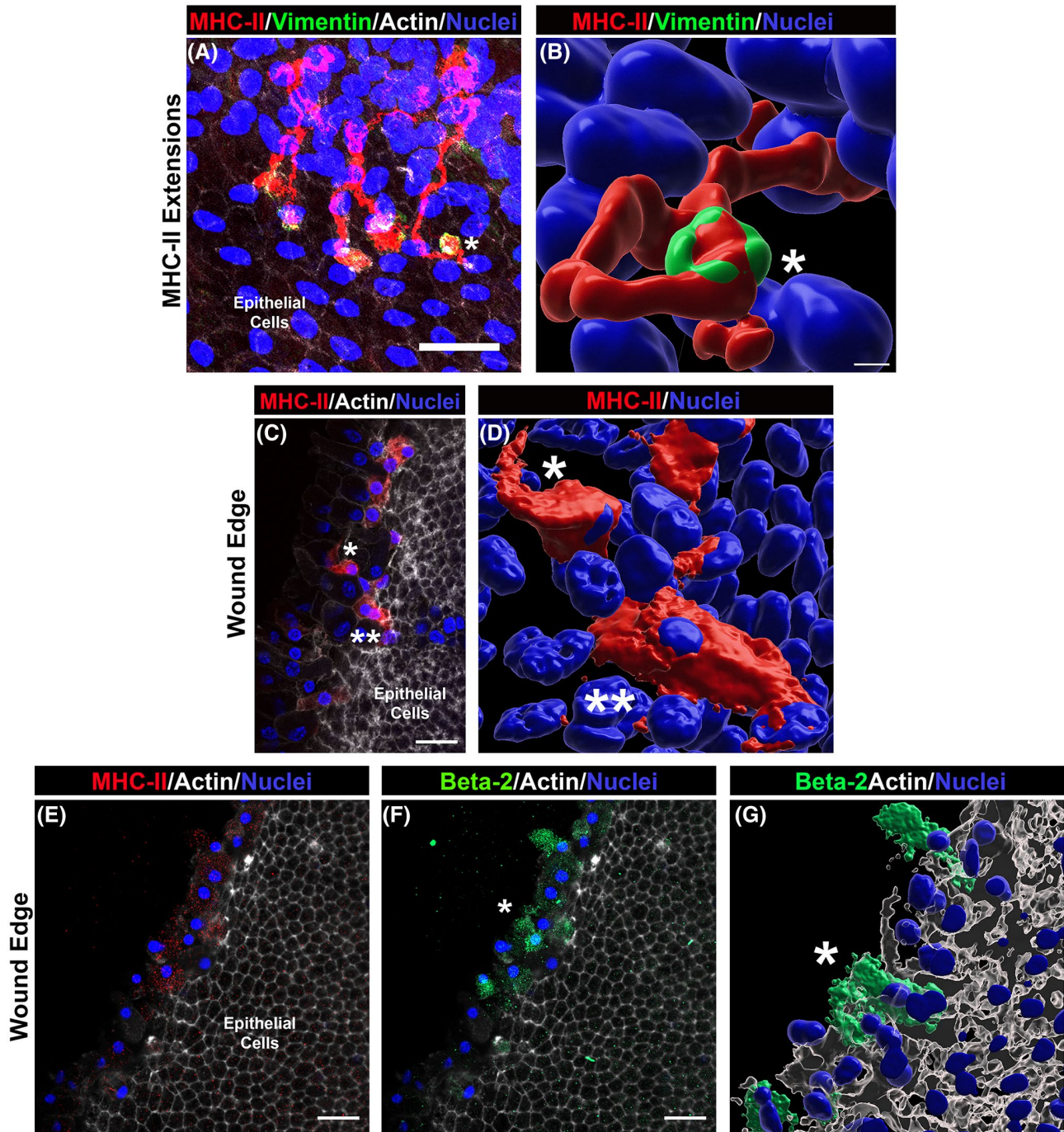


FIGURE 9 Resident immune cells of the lens with antigen presenting ability are among the first to respond to cataract surgery wounding. A-D, Ex vivo mock cataract surgery chick cultures were labeled 1 hour postinjury for MHCII (red), a marker associated with professional antigen presenting cells, vimentin (green), F-actin (white), and nuclei (blue). A, MHCII+/vimentin+ cells are shown with dendritic tails that insert between the lens epithelial cells. B, 3D structural view of cells from the confocal z-stack of the image presented in A as a single optical section. 3D view shows a resident immune cell (asterisk) with a vimentin-rich cell body and MHCII enriched dendritic tail wrapped around the neighboring lens epithelial cells. C, Imaging at the wound edge shows the presence of MHC II+/vimentin+ cells and the lens epithelial follower cells demarcated by F-actin (white). D, 3D structural view of the wound edge shows MHCII+/vimentin+ cells (indicated by asterisks in C) near the leading edge where they are intertwined among the lens epithelial cells. E-F, Co-immunolabeling of ex vivo mock cataract surgery cultures 1 hour postinjury for MHC-II (red) and β 2 (green), counterstained for F-actin (white) and nuclei (blue) shows MHCII+ cells express β 2 confirming their immune identity. G, 3D structural view of β 2+ cells at the wound edge from the confocal z-stack of the image presented in F. Asterisk denotes the location of the same β 2+ cells at the wound edge in F and G. Studies are representative of at least three independent experiments. Magnification bars A,C,E,F = 20 μ m, B = 2 μ m

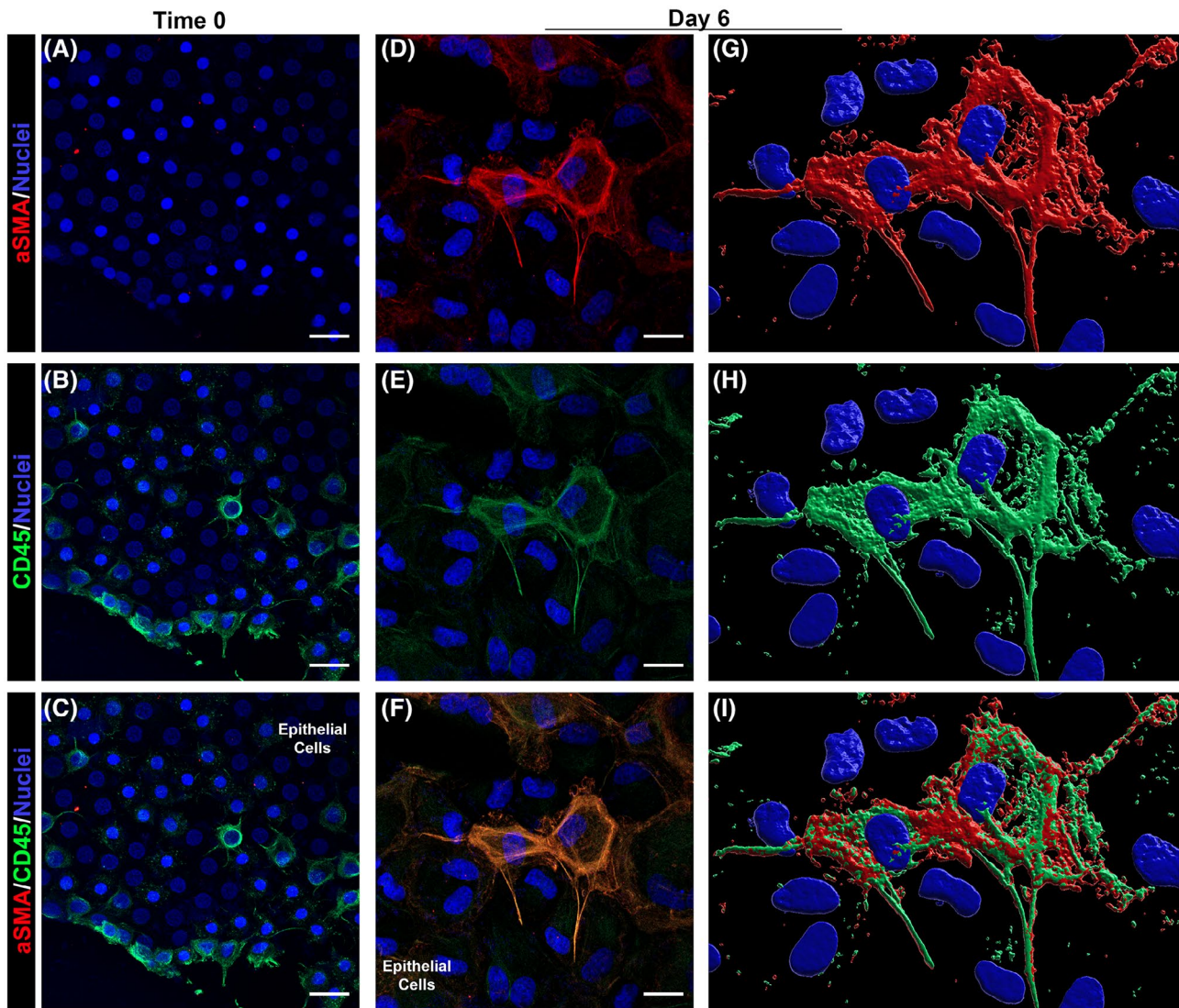


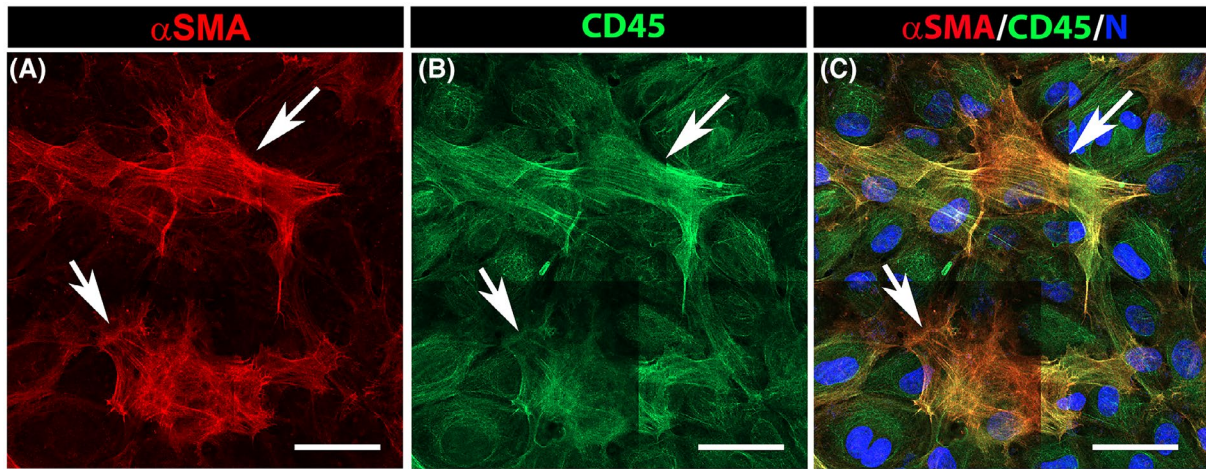
FIGURE 10 CD45⁺ resident immune cells serve as α SMA⁺ myofibroblast progenitors. A-I, A pediatric cataract surgery lens explant was divided into two pieces and fixed on day 0 or day 6 postinjury. Cultures were co-labeled for CD45 (green) and α SMA (red) and counterstained with DAPI to identify nuclei (blue). A-C, On day 0, CD45⁺ resident immune cells were present among the lens epithelium, however, no labeling for α SMA expression was detected. D-F, By day 6, α SMA⁺ myofibroblasts emerged that co-localized with CD45⁺ cells on the lens capsule indicating that these cells are the source of the myofibroblasts. G-I, 3D structural view of CD45⁺/ α SMA⁺ cells located among the lens epithelial cells from a confocal z-stack of the images shown as single optical planes in D-F. Magnification bars = 20 μ m

and proliferation,^{54,55} and suggests that in the environment of the lens basement membrane capsule the growth of myofibroblasts that leads to a fibrotic outcome would be slower than the process when the cells immediately encounter a rigid substrate.

4 | DISCUSSION

Almost all tissues of the body rely on resident immune cell populations to serve as sentinels to protect the tissue. They act as first line of defense to injury and infectious insults by producing a local rapid immune response.¹⁻⁴ Yet, resident immune cells were not previously considered in the lens, a

tissue of the eye whose transparency and function are essential to vision. For the first time, we show that resident immune cells populate the epithelium of chicken, mouse, and human lenses demonstrating this is a shared feature of the lens across species. Our studies followed the resident immune response to sterile injury to the lens, which showed their rapid migration from single and multicellular niches to the wound edge. Resident immune cells with antigen presenting ability (MHC Class II⁺) were identified within the avascular lens, equipping these cells with the ability to engage an adaptive immune response. Our studies also revealed that lens resident immune cells can serve as precursors of myofibroblasts, the cell type associated with the lens fibrotic disease PCO, thereby altering



Day 6 post-cataract surgery on the tissue culture substrate

FIGURE 11 Resident immune cells transition into myofibroblasts with α SMA organized into stress fibers when they move off of the lens capsule onto the rigid tissue culture substrate. A-C, Day 6 postinjury a pediatric cataract surgery lens explant was co-labeled for CD45 (green) and α SMA (red) and counterstained with DAPI to identify nuclei (blue). B, Confocal tile image of CD45+ resident immune cells that have moved off of the lens capsule onto the rigid tissue culture substrate. Within this rigid environment CD45+ cells are observed differentiating into myofibroblasts with α SMA organized into stress fibers (A,C, white arrows). Magnification bars = 50 μ m

our understanding about the etiology of lens pathogenesis. Furthermore, we propose that the avascular lens provides an ideal reductionist model system in which to study resident immune cell function as the inherent properties of the lens include that it is avascular, noninnervated, and stroma free. The unique ability to perform surgery on isolated lenses to create an *ex vivo* mock cataract surgery culture model makes it possible to investigate the resident immune cell response to sterile injury in the absence of the involvement of circulating immune cells.

In our studies with the developing chick embryo eye, which unlike mammalian embryo eyes has no lens-associated tunica vasculosa that could serve as a vascular source of lens resident immune cells, we show that the ciliary zonules between the ciliary body and the lens are the likely path by which immune cells populate the lens during development. Since we previously show that immune cells that surveil the lens in response to corneal wounding are able to migrate across the lens basement membrane capsule,²¹ we expect that immune cells use this same path to take residence in the lens during development. We also suggest that this is the pathway by which resident immune cells repopulate the lens in the adult as the vasculature of the tunica vasculosa of mammals is removed by apoptosis prior to birth.⁵⁶ In addition, it is a general characteristic of immune cells that they alter their shape to squeeze through small spaces, and are able to move through basement membranes using both protease dependent- and independent-pathways.^{57,58} Together, these findings support a role for the ciliary zonules as a conduit for immune cells to travel between the vascular-rich ciliary body and the lens during development, and then, migrate across the lens capsule to take residence among the cells of the lens epithelium.

In studies focused on examining the phagocytic cells responsible for cleaning up debris and apoptotic cell remnants during early stages of lens development, it was discovered that macrophages were associated with the lens primordia.^{12,13} Prior to the development of the ciliary zonules, at the lens vesicle stage, macrophages also were identified within both the lens epithelium and the lens vesicle.^{12,13} Using embryonic chick/quail chimeras, Caudros et al showed that lens-associated macrophages are derived from a hemangioblastic lineage originating from the yolk sac.¹² The yolk-sac derived macrophage populations are thought to arrive at the lens primordia by migrating through the mesenchyme that surrounds the developing eye structures.¹² While these studies were not focused on identifying resident immune cells, they provide insight into how a first wave of macrophages can take residence in the lens during the earliest stages of lens development. Finding that these early lens-associated macrophages are derived from the yolk sac is significant since the ontology of resident immune cells can affect their phenotype and function within a tissue.^{3,17} Resident tissue macrophages can self-renew within a tissue and derive from distinct precursors that differ in origin, which include the yolk-sac erythro-myeloid progenitors, fetal liver monocytes, or bone-marrow derived monocytes.^{3,17} Therefore, tissues can be populated by resident tissue macrophages that are derived from distinct origins and become established at different times.^{3,17} Yolk-sac derived macrophages that seed tissues of the embryo are in large part replaced by macrophages derived from fetal-liver monocytes.¹⁷ Moreover, after birth, bone-marrow derived monocytes can replace and contribute to resident tissue macrophage populations.¹⁷ Future studies are necessary

to determine the ontology of lens resident immune cells. We propose that as the lens continues to develop, the ciliary zonules offer a likely path for subsequent waves of early immune precursors to take residence in the lens. Furthermore, in adult animals, the ciliary zonules provide a mechanism to replenish depleted sources of resident immune cell populations that are recruited from local vascular sources in the eye.

Since resident immune cells are a first line of defense to insults in most tissues, we also explored the response of lens resident immune to the sterile, clinically relevant injury caused by cataract surgery. Our studies showed that many of the lens resident immune cells that are activated in response to sterile injury and populate the wound edge express the TLR4 co-receptor CD14, identifying them as monocytes/macrophages and/or dendritic cells.⁵⁹⁻⁶² Expression of the CD14 receptor by resident immune cells links them to a role in activation of the innate immune system since it functions together with TLR4 to detect pathogen-associated molecular pattern LPS and protect a tissue against pathogen infection.⁶³⁻⁶⁵ Part of the response of tissue resident leukocytes to an insult is their ability to call in back up by producing factors that induce the recruitment of circulating immune cells. In response to cataract surgery, the lens epithelium was found to produce a number of pro-inflammatory factors and chemokines such as CCL-2 (MCP-1) known to drive recruitment of monocytes.²² Whether injury activated resident immune cells integrated among the lens epithelium are responsible for contributing to the production of pro-inflammatory factors and chemokines is not yet known. Lens resident immune cells provide a potential mechanism to recruit circulating innate immune cells to the lens as has been shown to occur in response to lens dysgenesis caused by conditional deletion of N-cadherin, corneal wounding, and cataract surgery.²⁰⁻²²

While resident immune cells play critical roles in protecting a tissue from insult, they can become dysregulated to cause chronic inflammation and disease.²⁷⁻³⁰ We now show that the injury-activated lens resident immune cells in human pediatric cataract surgery explants are progenitors of α SMA+ myofibroblasts. These new findings support our previous studies with the *ex vivo* mock cataract surgery chick model where we showed that a subpopulation of CD44+/vimentin+ cells we now identify as resident immune cells are myofibroblast progenitors and associated with lens fibrotic PCO.^{44,45} Fibrosis often is the result of an aberrant wound healing response characterized by myofibroblast persistence and the excessive production of extracellular matrix proteins like collagen I leading to the loss of tissue structure and function.^{66,67} In the lens, the fibrotic disease PCO is a major complication of cataract surgery and is health burden with no effective treatment strategies.^{68,69} Our finding that lens resident immune cells serve as myofibroblast progenitors

strongly suggests that these cells are mediators of PCO. In addition, autoimmune pathogenesis has long been considered a factor in age-related cataracts, as well as in the high incidence of cataracts in autoimmune diseases such as diabetes and uveitis.⁷⁰⁻⁷⁵ Our discovery of resident immune cells in the lens would be consistent with a role in cataractogenesis. Resident immune cells provide a potential new therapeutic target to consider for both PCO and cataract. How lens resident immune cells become aberrantly regulated to serve as agents of fibrotic lens disease progression remains an important unanswered question.

Subsets of lens resident immune cells were positive for MHC-II, a molecule constitutively expressed on antigen presenting cells including dendritic cells and macrophages, providing these cells with the ability to engage an adaptive immune response. In chick embryo lenses, MHC II+ cells are integrated between the epithelial cells in the lens equatorial zone adjacent to the site that ciliary zonules insert into the lens, wrapping their dendritic tails around lens epithelial cells. When activated upon injury these cells are induced to migrate to the wound edge, yet, it is not clear how the lens resident antigen presenting cells function in mounting an adaptive immune response to activate naïve T-cells. Typically, antigen presenting cells travel through lymphatic vessels to reach lymph nodes to present antigens to naïve T-cells. The lymphatics of the anterior eye,⁷⁶ provide a potential path for the antigen presenting cells of the lens to the lymph nodes.

Overall, findings from our studies show for the first time that resident immune cells stationed within the lens epithelium are the initial responders to sterile injury that can serve as the liaisons between the innate and adaptive immune response in this tissue. Our studies open up new avenues for consideration in vision biology including (1) the role of resident immune cells in shaping the immune response of the lens, (2) maintaining lens homeostasis and restoring lens function after insult, (3) how lens resident immune cell function gets dysregulated, and (4) whether dysregulation of lens resident immune cells can contribute to the development of other lens pathologies such as the formation of cataracts.

ACKNOWLEDGMENTS

We thank Robert Mecham for providing us with his MAGP1 antibody and Paul Fitzgerald for his vimentin antibody for our studies. The JB3 antibody was deposited by Little, CD (DSHB Hybridoma Product JB3) and H4C4 was deposited to the DSHB by August, JT / Hildreth, JEK (DSHB Hybridoma Product H4C4). Both these antibodies were obtained from the Developmental Studies Hybridoma Bank, created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242. This work was funded by National Institute of Health grant EY021784 to ASM and MAS and EY02615 to JLW and AM

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

J. DeDreu conducted experiments and created the figures for the paper. C.M. Logan and H. Paulson conducted experiments for the paper. A.V. Levin provided human lens tissue. J.L. Walker and A.S. Menko conceived the idea for the project, designed, coordinated, and analyzed the studies and wrote the paper. All authors approved the final manuscript.

REFERENCES

- Masopust D, Soerens AG. Tissue-resident T cells and other resident leukocytes. *Annu Rev Immunol*. 2019;37:521-546.
- Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol*. 2013;14:986-995.
- Ginhoux F, Guillems M. Tissue-resident macrophage ontogeny and homeostasis. *Immunity*. 2016;44:439-449.
- Caputa G, Castoldi A, Pearce EJ. Metabolic adaptations of tissue-resident immune cells. *Nat Immunol*. 2019;20:793-801.
- Lim JJ, Grinstein S, Roth Z. Diversity and versatility of phagocytosis: roles in innate immunity, tissue remodeling, and homeostasis. *Front Cell Infect Microbiol*. 2017;7:191.
- Arandjelovic S, Ravichandran KS. Phagocytosis of apoptotic cells in homeostasis. *Nat Immunol*. 2015;16:907-917.
- Eom DS, Parichy DM. A macrophage relay for long-distance signaling during postembryonic tissue remodeling. *Science*. 2017;355:1317-1320.
- Dawson CA, Pal B, Vaillant F, et al. Tissue-resident ductal macrophages survey the mammary epithelium and facilitate tissue remodeling. *Nat Cell Biol*. 2020;22:546-558.
- Jakubczik CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol*. 2017;17:349-362.
- Kim ND, Luster AD. The role of tissue resident cells in neutrophil recruitment. *Trends Immunol*. 2015;36:547-555.
- Oishi Y, Manabe I. Macrophages in inflammation, repair and regeneration. *Int Immunol*. 2018;30:511-528.
- Caudros MA, Martin C, Rios A, Martin-Partido G, Navascues J. Macrophages of hemangioblastic lineage invade the lens vesicle-ectoderm interspace during closure and detachment of the avian embryonic lens. *Cell Tissue Res*. 1991;266:117-127.
- Nishitani K, Sasaki K. Macrophage localization in the developing lens primordium of the mouse embryo—an immunohistochemical study. *Exp Eye Res*. 2006;83:223-228.
- T'Jonck W, Guillems M, Bonnardel J. Niche signals and transcription factors involved in tissue-resident macrophage development. *Cell Immunol*. 2018;330:43-53.
- Guillems M, Thierry GR, Bonnardel J, Bajenoff M. Establishment and maintenance of the macrophage niche. *Immunity*. 2020;52:434-451.
- Liu F, Dai S, Feng D, et al. Distinct fate, dynamics and niches of renal macrophages of bone marrow or embryonic origins. *Nat Commun*. 2020;11:2280.
- Bleriot C, Chakarov S, Ginhoux F. Determinants of resident tissue macrophage identity and function. *Immunity*. 2020;52:957-970.
- Niederhorn JY. Immune privilege and immune regulation in the eye. *Adv Immunol*. 1990;48:191-226.
- Lang RA. Apoptosis in mammalian eye development: lens morphogenesis, vascular regression and immune privilege. *Cell Death Differ*. 1997;4:12-20.
- Logan CM, Bowen CJ, Menko AS. Induction of immune surveillance of the dysmorphogenic lens. *Sci Rep*. 2017;7:16235.
- DeDreu J, Bowen CJ, Logan CM, et al. An immune response to the avascular lens following wounding of the cornea involves ciliary zonule fibrils. *FASEB J*. 2020;34(7):9316-9336.
- Jiang J, Shihan MH, Wang Y, Duncan MK. Lens epithelial cells initiate an inflammatory response following cataract surgery. *Invest Ophthalmol Vis Sci*. 2018;59:4986-4997.
- Uenoyama K, Tamura M, Kinoshita C, et al. Experimental intraocular lens implantation in the rabbit eye and in the mouse peritoneal space. Part V: phagocytosis and nuclear patterns of giant cells observed on the implanted lens surface. *J Cataract Refract Surg*. 1990;16:465-470.
- Saika S, Uenoyama S, Kanagawa R, Tamura M, Uenoyama K. Phagocytosis and fibronectin of cells observed on intraocular lenses. *Jpn J Ophthalmol*. 1992;36:184-191.
- Saika S, Ohmi S, Tanaka S, Ohnishi Y, Yamanaka A, Ooshima A. Light and scanning electron microscopy of rabbit lens capsules with intraocular lenses. *J Cataract Refract Surg*. 1997;23:787-794.
- Lavin Y, Mortha A, Rahman A, Merad M. Regulation of macrophage development and function in peripheral tissues. *Nat Rev Immunol*. 2015;15:731-744.
- Lu J, Zhao J, Meng H, Zhang X. Adipose tissue-resident immune cells in obesity and type 2 diabetes. *Front Immunol*. 2019;10:1173.
- Richmond JM, Harris JE. Immunology and skin in health and disease. *Cold Spring Harb Perspect Med*. 2014;4:a015339.
- Ardain A, Marakalala MJ, Leslie A. Tissue-resident innate immunity in the lung. *Immunology*. 2020;159:245-256.
- Boyman O, Conrad C, Tonel G, Gilliet M, Nestle FO. The pathogenic role of tissue-resident immune cells in psoriasis. *Trends Immunol*. 2007;28:51-57.
- Jensen AA, Basti S, Greenwald MJ, Mets MB. When may the posterior capsule be preserved in pediatric intraocular lens surgery? *Ophthalmology*. 2002;109:324-327; discussion 328.
- Raj SM, Vasavada AR, Johar SR, Vasavada VA, Vasavada VA. Post-operative capsular opacification: a review. *Int J Biomed Sci*. 2007;3:237-250.
- Walker JL, Bleaken BM, Wolff IM, Menko AS. Establishment of a clinically relevant ex vivo mock cataract surgery model for investigating epithelial wound repair in a native microenvironment. *J Vis Exp*. 2015;100:e52886.
- Minutti CM, Knipper JA, Allen JE, Zaiss DM. Tissue-specific contribution of macrophages to wound healing. *Semin Cell Dev Biol*. 2017;61:3-11.
- Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity*. 2016;44:450-462.
- Hu W, Pasare C. Location, location, location: tissue-specific regulation of immune responses. *J Leukoc Biol*. 2013;94:409-421.
- Beebe DC. Maintaining transparency: a review of the developmental physiology and pathophysiology of two avascular tissues. *Semin Cell Dev Biol*. 2008;19:125-133.
- Sakai LY, Keene DR, Engvall E. Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. *J Cell Biol*. 1986;103:2499-2509.
- Gibson MA, Hughes JL, Fanning JC, Cleary EG. The major antigen of elastin-associated microfibrils is a 31-kDa glycoprotein. *J Biol Chem*. 1986;261:11429-11436.

40. De Maria A, Wilmarth PA, David LL, Bassnett S. Proteomic analysis of the bovine and human ciliary zonule. *Invest Ophthalmol Vis Sci.* 2017;58:573-585.
41. Mast J, Goddeeris BM, Peeters K, Vandesande F, Berghman LR. Characterisation of chicken monocytes, macrophages and interdigitating cells by the monoclonal antibody KUL01. *Vet Immunol Immunopathol.* 1998;61:343-357.
42. Walker JL, Wolff IM, Zhang L, Menko AS. Activation of SRC kinases signals induction of posterior capsule opacification. *Invest Ophthalmol Vis Sci.* 2007;48:2214-2223.
43. Menko AS, Bleaken BM, Libowitz AA, Zhang L, Stepp MA, Walker JL. A central role for vimentin in regulating repair function during healing of the lens epithelium. *Mol Biol Cell.* 2014;25:776-790.
44. Walker JL, Zhai N, Zhang L, et al. Unique precursors for the mesenchymal cells involved in injury response and fibrosis. *Proc Natl Acad Sci U S A.* 2010;107:13730-13735.
45. Walker JL, Bleaken BM, Romisher AR, Alnwibit AA, Menko AS. In wound repair vimentin mediates the transition of mesenchymal leader cells to a myofibroblast phenotype. *Mol Biol Cell.* 2018;29:1555-1570.
46. Bleaken BM, Menko AS, Walker JL. Cells activated for wound repair have the potential to direct collective invasion of an epithelium. *Mol Biol Cell.* 2016;27:451-465.
47. Haynes BF, Telen MJ, Hale LP, Denning SM. CD44—a molecule involved in leukocyte adherence and T-cell activation. *Immunol Today.* 1989;10:423-428.
48. Pure E, Cuff CA. A crucial role for CD44 in inflammation. *Trends Mol Med.* 2001;7:213-221.
49. Evans R, Patzak I, Svensson L, et al. Integrins in immunity. *J Cell Sci.* 2009;122:215-225.
50. Roche PA, Furuta K. The ins and outs of MHC class II-mediated antigen processing and presentation. *Nat Rev Immunol.* 2015;15:203-216.
51. Shu DY, Lovicu FJ. Myofibroblast transdifferentiation: the dark force in ocular wound healing and fibrosis. *Prog Retin Eye Res.* 2017;60:44-65.
52. Hinz B, Lagares D. Evasion of apoptosis by myofibroblasts: a hallmark of fibrotic diseases. *Nat Rev Rheumatol.* 2020;16:11-31.
53. Rocher M, Robert PY, Desmouliere A. The myofibroblast, biological activities and roles in eye repair and fibrosis. A focus on healing mechanisms in avascular cornea. *Eye.* 2020;34:232-240.
54. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol.* 2002;3:349-363.
55. Hinz B. The myofibroblast: paradigm for a mechanically active cell. *J Biomech.* 2010;43:146-155.
56. McLeod DS, Hasegawa T, Baba T, et al. From blood islands to blood vessels: morphologic observations and expression of key molecules during hyaloid vascular system development. *Invest Ophthalmol Vis Sci.* 2012;53:7912-7927.
57. Chang J, Chaudhuri O. Beyond proteases: basement membrane mechanics and cancer invasion. *J Cell Biol.* 2019;218:2456-2469.
58. Kelley LC, Lohmer LL, Hagedorn EJ, Sherwood DR. Traversing the basement membrane in vivo: a diversity of strategies. *J Cell Biol.* 2014;204:291-302.
59. Todd RF 3rd, Nadler LM, Schlossman SF. Antigens on human monocytes identified by monoclonal antibodies. *J Immunol.* 1981;126:1435-1442.
60. Griffin JD, Ritz J, Nadler LM, Schlossman SF. Expression of myeloid differentiation antigens on normal and malignant myeloid cells. *J Clin Invest.* 1981;68:932-941.
61. Ancuta P, Weiss L, Haeflner-Cavaillon N. CD14+CD16++ cells derived in vitro from peripheral blood monocytes exhibit phenotypic and functional dendritic cell-like characteristics. *Eur J Immunol.* 2000;30:1872-1883.
62. Klechevsky E, Morita R, Liu M, et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity.* 2008;29:497-510.
63. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science.* 1990;249:1431-1433.
64. Triantafilou M, Triantafilou K. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends Immunol.* 2002;23:301-304.
65. Jiang Q, Akashi S, Miyake K, Petty HR. Lipopolysaccharide induces physical proximity between CD14 and toll-like receptor 4 (TLR4) prior to nuclear translocation of NF-kappa B. *J Immunol.* 2000;165:3541-3544.
66. Hinz B, Gabbiani G. Fibrosis: recent advances in myofibroblast biology and new therapeutic perspectives. *F1000 Biol Rep.* 2010;2:78.
67. Pakshir P, Noskovicova N, Lodyga M, et al. The myofibroblast at a glance. *J Cell Sci.* 2020;133:jcs227900.
68. Fisu AD, Findl O. Capsular fibrosis: a review of prevention methods and management. *Eye.* 2020;34:256-262.
69. Ursell PG, Dhariwal M, O'Boyle D, Khan J, Venerus A. 5 year incidence of YAG capsulotomy and PCO after cataract surgery with single-piece monofocal intraocular lenses: a real-world evidence study of 20,763 eyes. *Eye.* 2020;34:960-968.
70. Jancevski M, Foster CS. Cataracts and uveitis. *Discov Med.* 2010;9:51-54.
71. Blum-Hareuveni T, Seguin-Greenstein S, Kramer M, et al. Risk factors for the development of cataract in children with uveitis. *Am J Ophthalmol.* 2017;177:139-143.
72. Llop SM, Papaliodis GN. Cataract surgery complications in uveitis patients: a review article. *Semin Ophthalmol.* 2018;33:64-69.
73. Negahban K, Chern K. Cataracts associated with systemic disorders and syndromes. *Curr Opin Ophthalmol.* 2002;13:419-422.
74. Obrosova IG, Chung SS, Kador PF. Diabetic cataracts: mechanisms and management. *Diabetes Metab Res Rev.* 2010;26:172-180.
75. Lu WL, Shen PC, Lee CH, Su YT, Chen LM. High risk of early cataracts in young type 1 diabetes group: a nationwide cohort study. *Int J Endocrinol.* 2020;2020:8160256.
76. Wu Y, Seong YJ, Li K, et al. Organogenesis and distribution of the ocular lymphatic vessels in the anterior eye. *JCI Insight.* 2020;5(13):e135121.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

How to cite this article: Menko AS, DeDreu J, Logan CM, Paulson H, Levin AV, Walker JL. Resident immune cells of the avascular lens: Mediators of the injury and fibrotic response of the lens. *The FASEB Journal.* 2021;35:e21341. <https://doi.org/10.1096/fj.202002200R>