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Aqueous Outflow Channels and its Lymphatic Association: A Review

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Abstract

The human eye has a unique immune architecture and behavior. While the conjunctiva is known to have a well-defined lymphatic drainage system, the cornea, sclera, and uveal tissues were historically considered "alymphatic" and thought to be immune privileged. The very fact that the aqueous outflow channels carry a clear fluid (aqueous humor) along the outflow pathway makes it hard to ignore its lymphatic-like characteristics. The development of novel lymphatic lineage markers and expression of these markers in aqueous outflow channels and improved imaging capabilities has sparked a renewed interest in the study of ocular lymphatics. Ophthalmic lymphatic research has had a directional shift over the last decade, offering an exciting new physiological platform that needs further in-depth understanding. The evidence of a presence of distinct lymphatic channels in the human ciliary body is gaining significant traction. The uveolymphatic pathway is an alternative new route for aqueous outflow and adds a new dimension to pathophysiology and management of glaucoma. Developing novel animal models, markers, and

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non-invasive imaging tools to delineate the core anatomical structure and physiological functions may help pave some crucial pathways to understand disease pathophysiology and help develop novel targeted therapeutic approaches for glaucoma.

Keywords

Aqueous humor; glaucoma; lymphatics; uveolymphatic pathway; Schlemm's canal; lymphangiogenesis; lymphatic markers

1. Introduction & historical perspectives

The human eye has a unique immune architecture and behavior. It is well known and accepted that the conjunctiva possesses a well-defined lymphatic drainage system whereas the cornea, sclera and uveal tissues historically were considered "alymphatic" and often defined to be immune-privileged.[34, 84, 117, 134] Mascagni was the first to report the presence of lymphatic vessels in the eyelids of cadavers.[82] Confirmation of the findings in his seminal work was confirmed a century later [86], with Schreger and Teichmann further demonstrating lymphatics in the conjunctiva[104, 125] via the conventional approach using cadaveric eyes and a complicated injection technique to demonstrate the presence of such vasculature.[125] Based on Schwalbe's work, there was alternative school of thought that considered the anterior chamber as a large lymphatic space and the Schlemm canal a lymphatic channel that facilitated in the drainage of the aqueous.[107] In 1873, Leber showed that when a solution consisting of two dyes of different particle size is injected in the anterior chamber, only the smaller dye particles filled the episcleral and conjunctival veins around the corneal margin. He postulated that the *Canalis Schlemmii* in humans is a venous circular vessel and not a lymphatic vessel.[77] Over the next century, considerable deliberations persisted on whether these initial reported demonstrations were actual lymphatics or only artefacts due to the injection technique. The ensuing work from Eisler and Bartels in this regard is especially important as this led to the belief that the eye has no lymphatic drainage and all previous demonstrations were in fact artefacts.[9, 30] Bartels highlighted the sources of error in the injection methods, while Eisler pointed out that no physiological drainage had been reported from the clefts assumed in endothelium-lined lymphatics. It was only after observations by Foldi and coworkers and Casley-Smith that there was a renewed interest in ocular lymphatics.[16, 17, 31] They observed dilation of perivascular spaces in tissues like the retina, iris and the optic nerve in animal models with cervical lymphatic blockage, indicating pre-lymphatic transportation pathways.[17] Interestingly, the aqueous was regarded as stagnant until the discovery of aqueous veins by Ascher in 1942.[6, 7] This landmark work triggered off research that led to a better understanding of the aqueous humour dynamics. The lack of biological markers and limitation in imaging technology to study the lymphatics dampened interest, and the science related to it was neglected. The very fact that the aqueous outflow channels carry a clear fluid to a certain extent along the outflow pathway makes it hard to ignore its lymphatic-like characteristics. Over the last few decades, development of novel lymphatic lineage markers and expression of these markers in aqueous outflow channels and improved imaging capabilities, has sparked a renewed interest in the study of ocular lymphatics;[69, 72, 74, 121, 146] however, the challenges and controversies of being able

to delineate a "classical lymphatic system" in the inner ocular structures persist. Schroedl and coauthors point out that one of the major reasons for this is the inability to detect lymphatic vessels with adequate sensitivity and specificity by routine histology. They also suggest that factors like reliance on positive identification of blood vessels strategy rather than an exclusive marker for lymphatics and the presence of atypical lymphatic cells such as the endothelial cells of Schlemm's canal further contribute to lack of consensus in defining true tissue lymphatics.[106,114] To standardize and strengthen this platform of research they recommend use of more than one lymphatic endothelial marker or a marker panel for immunohistochemistry in the eye except for regions where the existence of lymphatics is already well established. They also suggest the use of markers in ultrastructural analysis and the use of appropriate control tissue to substantiate findings.

The two large areas of focus for the scientific community currently are to segregate the true lymphatic drainage of ocular structures and the lymphatic-like behaviour of the aqueous outflow channels.

2.   Lymphatic markers and their expression in ocular tissues

Lymphatic vessel development begins at the cardinal vein in the embryo, when endothelial cells from the anterior cardiac vein commit to the lymphatic lineage, sprout, and migrate to form the primary lymph sacs in the jugular region. Endothelial sprouting from these primary lymph sacs into the surrounding tissues and organs results in the centrifugal spread of lymphatic vessels and the formation of local lymphatic capillaries.

There are several factors that drive the development of the lymphatic system -- including growth factors, cell surface proteins, and transcription factors. The identification of proteins that are specifically expressed in lymphatic endothelial cells (LECs) have allowed them to be used as markers of lymphatic vessels and for monitoring of lymphangiogenesis.[135, 136] The important proteins that mediate lymphangiogenesis are transmembrane receptor tyrosine kinase vascular endothelial growth factor receptor (VEGFR) –3, mucin-type transmembrane glycoprotein podoplanin (PDPN), cell surface lymphatic vessel endothelial receptor 1(LYVE-1)and transcription factor prospero homeobox protein 1(PROX1).[59, 65, 81] While these markers are useful, it is important to note that none of them are exclusively expressed in all lymphatic tissues. It is therefore recommended that the detection of a combination of these markers should be used for the reliable identification of lymphatic vessels.[113]

Lymphatic specific transcription factor PROX1 is a lymphatic lineage marker. PROX1 plays a major role in the differentiation of LECs from the embryonic veins,[136] and it is also necessary for maintenance of the sprouting of the venous endothelial cells and differentiation towards the lymphatic vasculature phenotype.[56, 91] The initiation of LECs specification into a lymphatic lineage involves activation of the expression of PROX1 by Sox-18 (Sex Determining Region Y-Box 18) transcription factor.[32] Hong and colleagues showed that PROX1 expression resulted in the upregulation of PDPN and VEGFR-3.[48] PDPN is a cell-surface glycoprotein that is highly expressed in LECs, kidney podocytes and type-1 alveolar cells.[14, 83] As it is expressed in lymphatic endothelium and not on

vascular endothelium, it is frequently used as a marker for LECs. It has been shown that PDPN is not required for the early steps of lymphatic development, but rather in the later stages where it plays a crucial role in separating lymphatic endothelial cells from the blood vasculature.[11, 129] LYVE-1 is a transmembrane receptor for hyaluronan, involved in the transport of hyaluronan across lymphatic endothelium, from interstitium to lymph.[95] It is present on both the luminal and abluminal sides of lymphatic capillaries and is used as a marker for lymphoid tissues and/or lymphangiogenesis.[88] Birke and coworkers, in their work demonstrated the intense staining of trabecular cells on immunofluorescence for PDPN throughout all layers of the TM and the anterior surface of iris.[12] They reported LYVE-1 staining seen in the form single dendriform cells distributed throughout the entire anterior segment. Dendriform cells within the iris stroma were stained for LYVE-1 or PDPN, and, to a small extent, for both markers. Whilst they further confirmed expression of LYVE-1 or PDPN and Prox-1 mRNA by polymerase chain reaction (PCR) in TM and iris tissue, VEGF-R3 expression was not observed, either by immunofluorescence or by PCR.

The key molecular regulator of lymphangiogenesis is VEGFR-3. This protein is expressed primarily on the surface of LECs and is activated by growth factors VEGF-C and VEGF-D.[123] Activation of VEGFR-3 signalling results in the proliferation, migration and survival of LECs. Blocking of the VEGFR-3 pathway has been shown to inhibit both inflammation and cancer-induced lymphangiogenesis. VEGFR-3 is expressed on the tip cells of sprouting lymphatic capillaries; the tip cells are critical for the growth of new lymphatic vessels.[137] VEGFR-2 is also expressed on LECs and is involved in the regulation of lymphangiogenesis. Proteolytic processing of VEGF-C and VEGF-D allows them to activate both VEGFR-2 and VEGFR-3. The activation of VEGFR-2 and VEGFR-3 on the LECs have different effects; VEGFR-2 induces lymphatic hyperplasia or vessel enlargement, whereas VEGFR-3 signalling results in the generation or initiation of new vessels by sprouting lymphangiogenesis.[137]

Kaser-Eichberger and coworkers, in their experiments, processed for immunohistochemistry and evaluated for multiple marker combinations using confocal microscopy and reported dense PDPN-immunoreactivity at the iris tip, anteriorly of the sphincter muscle while the muscle itself was lacking PDPN.[60] Double immunohistochemistry with PDPN and LYVE-1 revealed numerous LYVE-1–positive cells located in the sphincter muscle as well as in the PDPN-positive areas. Blood vessels, iris smooth muscles, and individual cells were VEGFR3+. While PDPN+ cells were rarely detected posteriorly of the iris root, many LYVE-1+ cells were present within the ciliary body muscle and villi.

Organogenesis and distribution of lymphatics in the anterior eye

The lymphatic system includes a wide network of blind-ended, thin-walled lymphatic capillaries and larger collecting vessels lined by a continuous layer of endothelial cells. They permeate most organs and tissues, and transports lymph back to the venous circulation. This lymphatic drainage of the head and neck region originates at the base of the skull. It then proceeds to the jugular chain along the internal jugular vein. It then moves to the spinal accessory chain along spinal accessory nerve, and then to the supraclavicular chain. These lymphatics then drain on the left side, either directly into

the vasculature via the jugulo-subclavian venous confluence or directly into the thoracic duct. Lymph flows directly into the lymphatic duct, on the right side.[73] Structurally, lymphatic microvasculature (~50μm) differ from blood capillaries as they have a more irregular and larger lumen, attenuated endothelium, and large overlapping intercellular gaps without tight junctions. The basal lamina is also absent or poorly developed and lacks pericytes and smooth muscle cells.[92] The larger collecting lymphatic channels (80–200 μm) have lymphatic muscle cells, continuous interendothelial junctions and a basement membrane.[52] Lymphatic flow is unidirectional and requires a complex interplay between different intrinsic (preload, afterload, transmural pressure, shear stress and neural/humoral signals) and extrinsic (skeletal muscle contraction, motion of surrounding organs and arterial pulsations) mechanisms.[80] The direction of flow is regulated by valves which are found across initial, pre collecting and collecting lymphatics. While the endothelium in the initial lymphatics serves as a primary valve mechanism, the collecting lymphatics have secondary (usually bicuspid) valves.[80, 127] The precollecting channels serve as a transition zone and may have both primary and secondary valves. The part of the collecting lymphatics between two valves is known as a lymphangion and can perform rhythmic contractions. These units thus exhibit both a Starling response (the extent of contraction increasing with the degree of lymphangion filling), and an Anrep effect (increase in contraction strength with afterload) which help in regulation of lymphatic flow.[52, 85, 124] Though lymphangion units have not been specifically studied in the ocular region, ultrastructure studies indicate similar hierarchy in animal and human eyes.[73, 100, 146] It is also imperative to mention that blood flow via veins is also vital in regulating the lymphatic flow. Though most veins are considered to be valveless in the head and neck region, recent evidence shows that veins like the facial and superior ophthalmic vein also have valves.[89, 150] These anatomical and physiological differences are however poorly understood and further studies in the ocular region can help unravel mechanisms that may have role in modulating processes like inflammation, infection, cancer spread, intraocular pressure (IOP) regulation and post-surgical outcomes (especially lid edema, flap healing and filtration surgery) around the eye.

In the eye, the conjunctiva is normally endowed with lymphatic vessels, whereas in other tissues, such as the cornea and retina, their presence is controversial. Recent studies, however, have shown limbal lymphatic vessels to be unevenly distributed around the limbus in both normal and inflamed conditions. Using lymphatic reporter transgenic animals (mouse and rat), Wu and coworkers investigated the development and distribution of the ocular lymphatics, and Schlemm's canal (Figure 1) [138] Substantial morphological and sequential differences were also observed between lymphangiogenesis and Schlemm's canal development. The limbal and conjunctival lymphatics were found to be relatively thin, branched, and valved, whereas the Schlemm's canal was thicker, unbranched and without valves. Furthermore, limbal lymphatics were situated on the outer surface of the limbus and were directly connected to the conjunctival lymphatics along the corneal edge. In contrast, Schlemm's canal is located at the inner side of the limbus, close to the iris base. In terms of distribution, the ocular lymphatics are more densely distributed on the nasal than on the temporal side.[138]

Further experiments have reported the development of the lymphatics network to be derived from a primary lymphatic vessel on the nasal side of the developing eye; followed by

sprouting and bifurcation to encircle the cornea. The denser distribution of lymphatics on the nasal side were believed to be due to the entrance pattern of the major lymphatic trunk from the medial canthus region and supported by the presence of more lymphatic sprouts nasally. Efficient fluid drainage (using fluorescent tracer) was observed in the nasal compared to the temporal side and was consistent with the reported higher lymphatic density at the nasal conjunctiva. Data from cadaveric human eyes using anti-LYVE-1 staining further supports the nasal predilection of lymphatic vessel distribution.[138]

3. Lymphatic architecture of the human eye: clinical implications

Unlike many lymphatic-developed tissues in the body, the lymphatic distribution in normal ocular tissues is largely heterogeneous, with the conjunctiva showing dense lymphatics while the cornea and retina having some lymphatic lineage cells. The identification of several lymphatic-specific markers has led to the discovery of lymphatic vessels in other ocular tissues.

Cornea:

While the cornea is normally devoid of any vasculature, pathological conditions including infection, inflammation and chemical injuries have been shown to induce lymphangiogenesis.[19, 22, 24, 53, 57] Corneal lymphatics play a crucial role in determining the survival of corneal transplants. The presence of lymphatic vessels promotes transportation of antigens and antigen-presenting cells, thereby facilitating corneal inflammation and transplant rejection.[19, 23, 24, 50]

Conjunctiva:

The normal conjunctiva is drained with a dense network of lymphatic vessels. Previous studies have elucidated the characteristics and distribution of limbal/corneal lymphatics.[18, 28, 29, 49, 99, 106, 149] Ecoiffier and colleagues had previously demonstrated the differential distribution of limbal lymphatics in normal as well as in inflamed corneal conditions.[28, 29] More recently, Wu and coworkers showed greater distribution in the nasal compared to the temporal side.[138] It has been postulated that the conjunctival lymphatics play a key role in fluid clearance in bleb-forming glaucoma surgeries resulting in better IOP reduction.

Iris, Ciliary Body and Choroid:

Using immunofluorescence with specific LECs markers, namely D2–40 antibody for PDPN and LYVE-1, Yucel and colleagues identified the presence of lymphatic channels in the human ciliary body. These channels have a distinct lumen and were negative for blood vessel endothelial cell marker CD34.[146] Fluorescent nanospheres injected into the anterior chamber of sheep eyes were detected in LYVE-1 positive channels of the ciliary body. Furthermore, following the intracameral injection of I125 radiolabelled human serum albumin into sheep eyes, the authors noted preferential drainage into head and neck lymph nodes, including the cervical, retropharyngeal, submandibular, and preauricular nodes. These findings support the presence of a 'uveolymphatic' pathway for drainage of fluid from the anterior chamber of the eye.

In contrast, Heindl and coworkers reported the absence of LYVE-1–positive/PDPN-positive lymphatic vessels with an erythrocyte-free lumen in the ciliary body region of human control eyes and demonstrated their presence only in the LYVE-1–positive extraocular tumor component of ciliary body melanomas with extraocular extension.[45, 46] This is in contrast with the findings of Khan AM and coworkers who reported presence of lymphatics in all ciliary body melanoma cases studied with or without extraocular extension.[66] Kaser-Eichberger and colleagues explored the possibility of a topographic distribution of lymphatics in the iris and ciliary body as a prime reason for the conflicting reports from various interest groups.[60] They processed cross sections of tissue blocks at 12/3/6/9 o 'clock position for immunohistochemistry of LYVE-1, PDPN, PROX1, FOXC2, VEGFR3, and CCL21, and when necessary, combined the lymphatic markers with CD31, α-smooth muscle-actin, CD68, and 4′,6-diamidino-2 phenylindole dihydrochloride (DAPI). They reported locating numerous PDPN+ cells at the anterior border of the iris while LYVE-1+ cells were distributed throughout the nonpigmented part. Blood vessels, iris smooth muscles, and individual cells were VEGFR3+. LYVE-1 positive cells were present within the ciliary body muscle with occasional PDPN positive vessel-like structures, but these were never colocalized with LYVE-1. They eventually concluded various structures in the anterior uvea were immunoreactive for several lymphatic markers, but lacked a classical lymphatic system or a tissue specific topography.

Schroedl and colleagues reported that normal human choroid contained a significant number of LYVE-1+ macrophages, and the presence of net-like structures with a pseudo-vessel appearance. [105] Koina et al. provided evidence for the presence of lymphatics in developing and adult human choroid by using immunohistochemistry of multiple lymphatics and vascular-specific markers and transmission electron microscopy of human fetal and adult choroid.[72] They describe the presence of a system of lymphatic-like channels, including blind-ended lymph sacs just external to the choriocapillaris as well as the presence of infrequent pre-collector and collector lymphatic channels. They suggested that choroidal lymphatics may play an important role in ocular growth and in the aetiology of refractive errors.

Retina:

The normal retinal tissue is rich in blood vessels, but devoid of lymphatics. Like the cornea, the retina is also considered as an immune-privileged tissue; however, LYVE1+ cells have been detected in retina, but their function is yet to be determined.[140] These contrasting reports despite the development of specific markers and advanced immunohistochemistry continues the perplex the scientific community and emphasizes on the complexity of ocular lymphatics.

4. Aqueous outflow and its lymphatic association

Aqueous humour is produced by the ciliary body epithelium in the posterior chamber and flows into the anterior chamber. It is composed of electrolytes, organic solutes, growth factors, and other proteins that supply nutrients to the nonvascularized tissues of the anterior chamber, i.e., trabecular meshwork, lens, and corneal endothelium.

The aqueous exits the eye either through

1. The 'conventional pathway' through the trabecular meshwork and Schlemm's canal (Figure 2)

2. The 'unconventional pathway' through the ciliary muscle and other downstream tissues (Figure 3)

Proper fluid homeostasis in the eye is critical for the maintenance of ocular health. In general, homeostasis of such nature is achieved in other parts of the human body via a well-structured lymphatic network and regional lymph nodes. The eye achieves this via the complex and intricate trabecular and uveoscleral outflow pathways. However, scientists are still perplexed as to how the eye achieves this complex homeostatic balance without well-defined lymphatics. This is where it is hard to ignore, the strong similarities between the lymphatic vessels and the Schlemm's canal. The Schlemm's canal is a specialized vascular structure is lined by a single layer of endothelial cells that displays features of both blood vascular endothelial cells (BECs) and LECs and some unique features of their own. Ramos and coworkers, in their detailed review, describe the intricate and intrinsic cellular characteristics that highlight the similarities and differences between the, Schlemm's canal endothelial cells (SCECs), BECs and LECs.[97] Both the LECs and SCECs have a continuous endothelial monolayer, a discontinuous basement membrane and lack encompassing pericytes. This contrasts with BEC that have a continuous basement membrane with embedded pericytes. The inter-endothelial junctions of the BVECs and SCECs is similar and so is the physiological functionality in terms of the fluid flow. Both the structures demonstrate a basal to apical fluid flow direction i.e., from interstitium to lumen unlike the BECs where the flow is apical to basal. Developmentally there is a fundamental difference where in the BECs primarily originate from the mesoderm which contrasts with the LECs and the SCECs that have a secondary origin differentiating from the endothelial cells of the veins and from the deep intrascleral venous plexus respectively. Like BECs and LECs, SCECs express several endothelial cell markers, such as CD31, cadherin 5, VEGF receptor 2, and vWF.[137] Schlemm's canal identity has cross expression of markers related to lymphatic and blood vessels as indicated by the expression of the lymphatic markers Prox1, VEGFR3, and integrin α9 (no LYVE-1 and PDPN) and blood markers such as TIE-2.[8, 70, 91, 126] Although originating from and directly connected to the blood vascular system, under normal physiological conditions, the Schlemm's canal is devoid of blood-borne cells, such as red blood cells. Furthermore, the junction between Schlemm's canal endothelial cells and trabecular meshwork is composed of a specialized tethering structure, which is responsible for providing an appropriate pressure gradient across the inner wall of Schlemm's canal to maintain the integrity of Schlemm's canal endothelial cells, thereby providing a tissue pressure-sensing/responding mechanism, such as anchoring filaments for lymphatic vessels. Grierson et al, demonstrated presence of macula adherens (desmosomes) and macular gap junctions between cells of the trabecular meshwork.[40] Subsequently Schmelz and coworkers demonstrated desmosome-like structures (complexus adhaerentes) made of plakoglobin and desmoplakin in LECs.[103] These structures are important as they have been found to be absent on vascular endothelial cells and can help differentiate LECs and BECs.[25] However specialised veins like the umbilical vein have also demonstrated desmoplakin.[131]

LECs are differentiated from BECs during early development, and the homeodomain transcription factor, PROX1 controls the BECs-to-LECs differentiation process. Truong was the first to demonstrate a high level of expression of the PROX1 lymphatic transcription factor in the canal's endothelial cells, thus showing the similarity to lymphatic endothelial cells.[32] In a seminal piece of work, Khizatil and coworkers establish that Schlemm's canal has a unique molecular phenotype and develops by a previously unknown sequence of vascular development that they name "canalogenesis".[70] They further established that both the developing and mature Schlemm's canal express the lymphatic master controller PROX1, which is likely of critical importance for inducing and maintaining key features of Schlemm's canal's functional specialization. They divided the development of Schlemm's canal into four stages, starting from differentiation of the canal's precursor cells, proliferation and migration of frontal cells, formation of the canal's lumen, and separation from the venous vascular system. PROX1 and VEGFR-3 expression is required for division of frontal cells and shaping them into the canal. Functional inhibition of VEGFR-2, a critical receptor in initiating angiogenesis, shows that this receptor is required during canalogenesis. TIE-2 (*tunica interna endothelial cell kinase*) is expressed before Prox1 in SCEC and is maintained at a high level to critically regulate Schlemm's canal integrity during adulthood. TIE-2 is a member of receptor tyrosine kinase family with a high degree of homology with TIE-1.[108] They are differentially required during vascular development. TIE-2 mediated signals are crucial for the endothelial cell survival, maturation, and maintenance of blood vessel integrity;[27, 79, 102] however, TIE-2 expression is inhibited in LECs with high expression of PROX1.[68, 94] In contrast, TIE-1 is co-expressed with PROX1 by LECs.[96]

Further, Park and coworkers demonstrated using lymphatic and blood vasculature reporter mice, that Schlemm's canal, which originates from blood vessels during the postnatal period, acquires lymphatic identity through upregulation of PROX1, the master regulator of lymphatic development.[91] Schlemm's canal expressed lymphatic valve markers FOXC2 and integrin α9 and exhibited at continuous vascular endothelial– cadherin (VE-cadherin) junctions and basement membrane, like collecting lymphatics. Schlemm's canal, on the other hand notably lacked luminal valves and expression of the lymphatic endothelial cell markers PDPN and lymphatic vessel endothelial hyaluronan receptor 1 (LYVE- 1). Using an ocular puncture model, they determined that reduced AHO altered the fate of Schlemm's canal both during development and under pathologic conditions; however, alteration of VEGF-C/VEGFR3 signalling did not modulate Schlemm's canal integrity and identity. PROX1 expression levels linearly correlated with Schlemm's canal function. Collectively, their data indicated that PROX1 is an accurate and reliable biosensor of Schlemm's canal integrity and identity. Schlemm's canal endothelial cells originate from choroidal veins and undergo partial lymphatic reprogramming during postnatal development, giving rise to endothelial cells, which display both phenotypes.

Aspelund et al. demonstrated the expression of lymphatic endothelial cell markers by the Schlemm's canal in murine and zebrafish models as well as in human eye tissue. [8] Using gene deletion and function-blocking antibodies in mice, they determined that the lymphangiogenic growth factor VEGF-C and its receptor, VEGFR-3, are essential for Schlemm's canal development. Delivery of VEGF-C into the adult eye resulted in

sprouting, proliferation, and growth of Schlemm's canal endothelial cells, whereas VEGF-A obliterated the aqueous outflow system. Furthermore, a single injection of recombinant VEGF-C induced Schlemm's canal growth and was associated with a trend toward a sustained decrease in IOP in adult mice. VEGFR-3 expression was restricted to Schlemm's canal endothelial cells and episcleral lymphatic vessels. He also demonstrated that precursor cells are vascular endothelial cells with VEGFR-2 and TIE-2 (*tunica interna endothelial cell kinase*) expression. Precursor cells then gain PROX1 expression to create and form the canal's lumen and VEGFR-3 for later maturing of the canal's cells. Both aqueous humor and VEGF-C are required for proper Schlemm's canal development. A reduction in aqueous humour in mice resulted in the loss of elements of canal cells' lymphatic identity. Lineage tracing studies by Aspelund et al.[8] validated that, like lymphatic vessels, the Schlemm's canal has a blood vascular origin and does not originate from pre-existing lymphatic vasculature. Instead, the Schlemm's canal endothelial cells acquire lymph-like properties as development progresses. Similarly, Park and coworkers performed serial intra-vital imaging of endothelial cells of in PROX1-GFP mice that uncovered a lymphatic reprogramming during postnatal development whereby expression of blood vessel markers decreases as expression of certain lymphatic markers increases.[91] The strong evidence accumulated from these studies stand testament to the intricate lymphatic like characteristics of the conventional aqueous outflow pathways. Table 1. gives an overview of the complex embryological, histological, and physiological overlap between the two major aqueous outflow pathways.

5. Uveal lymphatics and aqueous outflow: the "uveolymphatic pathway"

Prior efforts to delineate the lymphatic role in aqueous outflow has been conflicting. Gruntzig and coworkers, in their initial work on rabbit eyes, injected eye colloid solutions (198Au-colloid and 99mTc-sulfur-c) into the right anterior chamber and traced the radioactivity both in-vivo and in-vitro.[41] They were able to demonstrate drainage into the cervical node in 50% of their cases. Significant activity could be found in blood, liver, kidney, and bone tissue and a concentration of radioactivity significantly surpassing the concentration in blood was measured in the right retrobulbar space. Bradbury and coworkers injected radio-iodinated albumin into the anterior chamber of rabbits and cats and could not demonstrate significant radioactivity in deep cervical lymph of the rabbit or in deep and superficial cervical lymph of the cat over a period of 6 hours after injection.[13] A higher 5f-nucleotidase (5f-NT) and lower alkaline phosphatase (ALP) activity in the walls of lymphatic vessels relative to the activity in blood vessels has been documented in the literature.[62, 128, 132] Krohn and coworkers, in an attempt to delineate the lymphatics associated with aqueous outflow, explored this in their study and found no expression of 5f-nucleotidase in any part of the aqueous outflow pathway.[74] This pattern of conflicting evidence did dampen the research on lymphatic involvement in aqueous outflow. However, the development of novel lymphatic markers and their strong expression by the structures of aqueous outflow pathway over has spiralled a renewed enthusiasm in this area. Camelo and coworkers in their experiments with Lewis mice used epifluorescence and confocal microscopy to demonstrate cells bearing fluorescent antigens in the regional lymph nodes following injection of lysine fixable fluorescein (FITC)and cascade blue-dextran (CB-Dx)

into the anterior chamber of the eye.[15] They proposed that the antigens reached the lymphoid organs mainly in a soluble form via both the blood and lymph. After bilateral intracameral injections, individual cells bearing both fluorescent antigens were identified in CD169 subcapsular sinus macrophages of the ipsilateral submandibular, deep, and superficial cervical, and facial LNs.

6.1 Uveal lymphatics and prostaglandins

Yucel and colleagues examined human cadaver eyes and used a used a multipronged approach of immunofluorescence and confocal microscopy, and electron microscopy to establish the presence of uveal lymphatics. They were able to identify the D2–40-immunoreactive lymphatic channels among the smooth muscle fibre bundles labelled with SMA, and these D2–40-positive channels were distinct from CD34-positive blood vessels.[146] Further experiments with LYVE-1 as a lymphatic marker performed on frozen sections confirmed findings of distinct lymphatic channels in both the conjunctiva and ciliary body along with collagen IV-positive blood vessels. Ultrastructural analysis of the ciliary body showed the presence of D2–40 immunogold stained lymphatic endothelial cells forming a lumen and devoid of continuous basement membrane, unlike D2–40-negative blood vessel containing red blood cells and invested in continuous basement membrane. They further conducted experiments in live sheep using fluorescent nanospheres injected into the anterior chamber of the sheep eye. Sheep are a well-established model in lymphatic research with large lymphatic channels compared to other species, and drainage of intracamerally injected radiolabelled human serum albumin was examined. As tracer concentration reached a plateau in plasma four hour after injection preferential drainage of tracer in the head and neck region to the cervical, retropharyngeal, preauricular and submandibular lymph nodes was noted compared to lymph nodes draining other regions in the body such as reference popliteal nodes. These findings suggest that aqueous fluid leaving the anterior chamber is partly drained into lymph nodes that drain the head and neck region, rather than through a direct communication with the bloodstream. Based on these findings, the authors concluded that evidence strongly supported the presence of a tertiary "uveolymphatic pathway" for drainage of fluid from the anterior chamber of the eye. The same group of authors in a further publication reported the ability to visualize and quantify lymphatic drainage of aqueous humour from the eye to cervical lymph nodes in the dynamic state.[145] A near infrared tracer was injected into the right eye anterior chamber of 10 mice under general anaesthesia. Following right eye intracameral injection of tracer, an exponential decrease in tracer signal was observed from 20 minutes to 6 hours in all mice. Simultaneously, increasing tracer signal was observed in the right neck node from 20 minutes to 6 hours. Active lymphatic drainage of aqueous from the eye to cervical lymph nodes was measured non-invasively by photoacoustic imaging of near-infrared nanoparticles. In a follow up study by the same investigators, lymphatic drainage in mice was assessed in vivo, in 11 latanoprost-treated and 11 control animals using hyperspectral imaging at multiple times following quantum dot (QD) injection into the eye.[122] This was done to determine whether latanoprost, a prostaglandin F2 alpha analogue commonly used to lower IOP to treat glaucoma, increases lymphatic drainage from the eye. They found that in the latanoprost-treated group, lymphatic drainage rate into the submandibular lymph node was increased compared with controls. This is the first evidence that latanoprost increases

lymphatic drainage from the eye. Ciliary muscle relaxation, by prostaglandin action on Prostaglandin F (FP) receptors may contribute indirectly to increased ocular lymphatic drainage. The potential explanation is that prostaglandins act on lymphatic endothelial and contractile cells surrounding lymphatic channels and aid the peristaltic movement of lymph. It is however imperative to understand that lymphatic flow depends on several intrinsic and extrinsic factors that require more research before a definitive mechanism is suggested for these observations.

Kim and coworkers explored the evaluated whether defects in cervical lymphatic drainage influence the IOP lowering effect of latanoprost in human patients with primary open-angle glaucoma who have undergone unilateral radical neck dissection (uRND)[69] They enrolled (1) POAG patients who had started (bilateral) latanoprost 0.005% monotherapy prior to their uRND and (2) treatment naive, bilateral glaucoma suspects (GSs) who had undergone the same surgery. They compared the eyes ipsilateral to the uRND with their fellow eyes in terms of the changes in IOP between the baseline (prior to the uRND) and the follow-up visits (1, 3, and 6 months after the uRND). They reported a significant increase in IOP at all follow up visits in the POAG eyes ipsilateral to the uRND compared to their fellow eyes. An IOP increase by 15% or more in 82% (9/11) eyes was documented. The GS group patients (Ipsilateral and contralateral eyes) did not demonstrate any significant changes in their IOP profile over the 6 months follow up period. Based on their study, the authors hypothesized that a defective cervical lymphatic drainage affected the efficacy of latanoprost. These observations support the hypothesis that lymphatics do have a role in aqueous outflow and PG analogues appear to enhance outflow via this channel of "uveolymphatic "outflow.

6. Conjunctival lymphatics and aqueous outflow

As discussed earlier, aqueous humor outflow is divided into two distinct outflow pathways in the eye, conventional (trabecular) and unconventional (uveoscleral).[54, 78] Both pathways begin with aqueous production from the ciliary processes in the sulcus space. After aqueous moves into the anterior chamber, the conventional outflow pathway begins at the trabecular meshwork, and the unconventional outflow pathway begins at the ciliary body band. For conventional outflow, after passing the trabecular meshwork, aqueous moves into the Schlemm's canal, collector channels, an intrascleral venous plexus, aqueous veins and then episcleral veins before returning aqueous back to the systemic blood circulation.[54,78] For unconventional outflow, aqueous enters the extracellular space of the ciliary muscle at the junction of the meshwork and ciliary muscle tendon, then it passes via the supraciliary and suprachoroidal spaces into multiple potential routes, including intraocular lymphatics, into and through the sclera and its vasculature, or into vortex veins.[54, 55, 78, 146] Described in these terms, the pathways appear to be independent.

Aqueous humor outflow is interconnected throughout the eye. First, this must be the case as no pathway in the body (be it blood vessels or lymphatics) is completely impermeable to water. Though larger vessels have relatively thicker walls and bulk fluid moves rapidly with minimal leakage, smaller capillaries have large gaps in their walls and leak continuously. For ophthalmologists and vision scientists, this is the clearest when observing intravenous retinal angiography. During intravenous fluorescein angiography, fluorescein is a small tracer that

is initially seen within blood vessels, but eventually leaks out during the late stages of imaging.[64] When a larger tracer is used (such as with indocyanine green [ICG]that is not only larger than fluorescein, but also protein bound), less leakage is seen.[64] Intraluminal retention during angiography is maintained. This is best evidenced by the ability of ICG to image leaky and fenestrated choroidal vessels when fluorescein cannot.[63] Similarly, aqueous does not leave the eye through completely independent routes. Instead, aqueous exits via preferred pathways of least resistance (conventional and unconventional) which can leak, likely resulting in points where the two pathways can merge. For example, in and around the sclera both pathways are present, and this serves as a potential mixing point.

What happens to fluid that leaks out of aqueous humor outflow pathways? As vasculature leaks aqueous, fluid collects in the extracellular space and can take residence in otherwise potential spaces. The most easily accessible "potential" space in the anterior segment is the subconjunctival space.

The subconjunctival space is well-known to be expandable. Chemosis is defined by the collection of fluid in the subconjunctival space that can either arise by particularly leaky vessels (due to ocular surface infection or inflammation) or during periods of fluid overload. An example of the latter includes, intensive care patients in positive fluid balance after receiving intravenous medicines and fluids.[44] What keeps the subconjunctival space a potential space is subconjunctival lymphatics.[2, 42, 138, 144] An ocular analogy more familiar to ophthalmologists or vision scientists is how the retinal pigment epithelial (RPE) cells keep the sub-RPE space "potential" as well.[115] Therefore, in the context of physiologic aqueous humor outflow, aqueous may dynamically leak out of both the conventional and unconventional pathways particularly in and around the sclera, collect in extracellular spaces with the potential to expand the subconjunctival space, and then be drained by subconjunctival lymphatics. These pathways can be seen directly with injection of fluorescent tracer under the conjunctival (Figure 4). Subconjunctival lymphatics are thus likely to be intricately involved in physiologic aqueous humor outflow while not necessarily providing native outflow resistance nor the pathologic outflow resistance seen in ocular hypertension. Further studies to evaluate these aspects could be immensely informative.

Nonphysiologically, the subconjunctival space can be accessed as well to influence ocular disease treatment. Drug delivery can involve the injection of medicines into the subconjunctival or subtenon space to avoid use of topical medicine application associated with problems of poor patient adherence and persistence.[87] These injections result in drug-delivery blebs which disappear hours later, proving the presence of subconjunctival outflow and consistent with the presence of subconjunctival lymphatics (Figure 5). In this case, the subconjunctival lymphatics are detrimental to the therapeutic process. Strategies designed to limit lymphatics or target lymphatic poor areas (such as on the temporal side of the eye[2, 138)] may have advantages. In contrast, bleb-forming glaucoma surgeries (trabeculectomies, glaucoma drainage devices, and subconjunctival minimally invasive glaucoma surgeries) aim to permanently open the subconjunctival space. This is done to provide a low-resistance outflow pathway out of the eye, bypassing natively high-resistance diseased outflow pathways tissue in ocular hypertension to lower IOP. In this case, promoting subconjunctival lymphatic presence may benefit aqueous humour outflow out of these artificially created

low-resistance pathways and blebs. Thus, for treatment of eye diseases, both limiting or promoting subconjunctival lymphatics and subconjunctival outflow can be potentially useful.

7. Imaging the ocular lymphatics

Non-invasive imaging of lymph vessels is difficult because of the morphologic similarities with blood vessels. Most of the techniques employ contrast agents to specifically visualize the lymphatic system. Le and co-workers have for instance administered intrastromal fluorescein to visualize both corneal blood and lymphatic vessels.[76] Using lymph endothelial-specific antibodies, allows for unequivocal identification of lymph vessels and was widely used among different organs including the eye, but cannot be used in the clinical setting. For an in-depth review of *in vivo* live imaging in mice using different fluorescent reporter transgenic mice the reader is referred to a recent in-depth review.[26]

A wide variety of techniques were used to image the outflow pathways of the eye. The morphology of the trabecular meshwork, Schlemm canal, the collector channels and the episcleral venous system *ex vivo* were visualized using electron microscopy[130], two-photon autofluorescence microscopy[37], and three-dimensional micro-computed tomography.[43] A recent approach used endoscopic OCT to image collector channels in ex vivo human eyes.[98, 139]

Visualizing the aqueous outflow system of the eye using anterior segment OCT remains difficult despite continuous improvements in technology.[5] Problems include light scattering from surrounding tissues including the sclera and eye movement that cannot easily be corrected by eye trackers because of the lack of landmark structures. Swept source OCT systems may be preferable over spectral domain OCT systems in visualizing the aqueous outflow pathways, because of the longer wavelengths used and the reduced sensitivity roll-off. Several studies have provided quantitative values for Schlemm canal diameter and cross-sectional area, but the agreement between the reported results is poor.[58, 109, 142] We have recently described a full 360-degree reconstruction of the Schlemm canal and the collector channels using OCT. [141] Whereas the introduction of OCT angiography has largely improved the visualization of blood vessels[21, 61], lymph vessels are hardly visible because of a lack of scatterers such as red blood cells. In *ex vivo* studies gold nanorods[133] and lipid emulsions were used to enhance the signal.[38]

Recently techniques were developed to visualize the outflow system during surgery using either indocyanine green (ICG) channelography[148], episcleral venous fluid wave using the operating microscope[75], or aqueous angiography.[51] Whereas these techniques have provided insights into the regulation of the aqueous outflow system, their invasive nature prevents wide-spread clinical use. Hemoglobin video imaging is a slit-lamp technique that images red blood cells in the conjunctival and episcleral vessels based on the haemoglobin absorption spectrum.[67]

Approaches to specifically image ocular lymphatics in the aqueous outflow system are sparse. Tam AL and co-workers administered quantum dots and assessed lymphatic drainage

via hyperspectral imaging.[122] Lymphatic drainage in vivo has also been studied by using quantitative photo- acoustic tomography.[145] For this purpose, a near infrared tracer was injected into eye, and imaging of ipsilateral cervical lymph nodes was performed using photoacoustics.

As mentioned above lymph does not scatter light and lymphatic vessels therefore appear dark in OCT images. These low intensity regions can be segmented using Hessian filters, which provides better results than classical intensity-based threshold methods.[143] The technique has, however, not yet been applied to the eye because movement artifacts make the segmentation particularly challenging. Gong and coworkers used OCT scans at 1300 nm and 785 nm to identify the lymphatic vessels via their optical transparency in ex vivo porcine eyes.[36] Whereas yet no *in vivo* results have been published, the authors mention that translation of the technique to the clinical application is a goal of the project. Along this line of thought corneal lymphatic vessels can also be imaged using an OCT system with an axial resolution as high as 1 μm.[49]

8. Clinical implications and directions

IOP manipulation continues to be the mainstay of glaucoma management. Medical management typically revolves around suppressing aqueous inflow or enhancing aqueous outflow. The current spectrum of IOP reducing drugs has played a significant role in disease management, but may be inadequate either because of aggressive disease, ineffectiveness, or may be contraindicated. It would be a welcome opportunity to exploit a novel target or novel aqueous outflow pathway for glaucoma management. The discovery of uveolymphatic pathway has opened an exciting avenue to target and exploit it to manipulate IOP. Lymphatic drainage in the human body is an active process and involves peristalsis.[35, 147] The lymphatic vessels have been documented to have contractile abilities and some molecules such as PGF2 alpha have been shown to have this effect on them.[4, 33, 110] This knowledge furthers our fundamental understanding of the mechanism of IOP reduction associated with the PG class of drugs and offers a platform to explore drugs with similar physiological effect on lymphatic channels. Tam's work on the mouse model using non-invasive in vivo multispectral imaging conclusively demonstrates that topical PGA analogue therapy increases lymphatic drainage from the eye.[121] The indirect evidence of this plausibility in humans has been deliberated upon from the study by Kim and coworkers.[69] Furthermore, this pathway now presents a potential alternative of exploring lymphangiogenesis as a therapeutic strategy for enhancing uveolymphatic outflow. Aspelund and colleagues provided promising data that a single, low-dose injection of intraocular recombinant VEGF-C increased sprouting and proliferation of Schlemm's canal endothelial cells and, importantly, showed a trend toward normalized IOP in adult mice.[8] These lymphangiogenic effects are likely to be seen further downstream including the uveolymphatic, episcleral and conjunctival components of the outflow channels offering a potential novel treatment target to be exploited. There is however some evidence from murine corneal suture models that 0.5% timolol maleate can lead to decrease in lymphangiogenesis.[20] It is, however, unclear that whether this pathological response to a trigger would be similar in physiological conditions. This effect is mediated by a multitude of pro angiogenesis, lymphangiogenesis and inflammatory molecules like VEGF-A, VEGF-C, TNF-alpha, IL-6, VEGFR-2, and

VEGFR-3 and may affect the uveolymphatic outflow.[20] These observations indicate that when prostaglandins are combined with timolol they may be potentially antagonistic to each other in terms of ocular lymphatic drainage. However how these interactions affect IOP reduction needs to be further evaluated. [120]

The significance of the lymphangiogenic growth factors, VEGF-C and VEGF-D, is well established in animal models of metastasis, and a strong correlation exists between an increase in expression of VEGF-C and VEGF-D, and metastatic spread in various solid human cancers.[3, 93, 116, 118] Based on this knowledge, therapeutic strategies aimed at controlling lymphangiogenesis are being explored. The strategy of both promoting and blocking lymphangiogenesis have relevant therapeutic applications.[101] Lymphangiogenesis could play a major role in management of lymphedema in disorders such as filariasis and post breast cancer surgery. Promising lymphedema treatment has been achieved in preclinical models using viral gene transfer vectors that induce lymphangiogenic factors.[101] In a rodent model of surgically-induced lymphatic obstruction, the exogenous administration of human recombinant VEGF-C restored lymphatic flow.[119] These principles could be potentially applied to enhance bleb function via a stimulated peribleb lymphatic network. Currently, bleb function and longevity has been addressed using strong antifibrotic agents such as mitomycin-C and 5-fluorouracil.[10, 39, 47, 71, 90, 112] These drugs have been reported to be detrimental to lymphatics.[2] As a result these agents have long-term sequelae and vision threatening complications such as leaks, and infections and glaucoma surgeons have been searching for alternative wound modulation strategies. Understanding and manipulating lymphatics to enhance bleb outflow may open a much-needed new therapeutic avenue for glaucoma management. The potential for blocking lymphangiogenesis as a therapeutic strategy has been explored as an option to prevent cancer metastasis. Scientists have reported that inhibition of VEGF-C/VEGF-D/VEGFR-3 axis in animal models inhibit tumour lymphangiogenesis and lymph node metastases.[1, 111] The subconjunctival space is lymph rich and a potential reservoir for drug delivery. This reservoir effect can be prolonged by inhibiting lymphatics and thereby prolonging the effect of a desired therapeutic agent.

9. Conclusion

In summary, ophthalmic lymphatic research has had a directional shift over the last decade, offering an exciting new physiological platform that needs further in-depth understanding. Developing novel animal models, markers, and non-invasive imaging tools to delineate the core anatomical structure and physiological functions may help open some crucial pathways to understand disease pathophysiology and help develop novel targeted therapeutic approaches.

10. Method of Literature Search

The review was based on a comprehensive search in the PubMed and Google Scholar database. The main search was performed using the keywords 'eye', 'lymphatics' and 'aqueous channels'. An additional search was carried out with the keyword's 'eye', 'lymph', 'lymphangiogenesis', 'channels', 'Schlemm's canal 'and lymphatic markers. To select articles with a topic that was within the scope of the current review, the titles and abstracts

of the articles were read. In this primary selection, the entire article was also read where necessary to judge its relevance according to the aim of the review. In cases where the topic of the article was considered relevant, the article was read in full text. Additionally, some publications on basic and historical research were also included. The review covers mainly articles published after 2000. As ocular lymphatics is a controversial topic, some articles published before 2000 were included. The reference lists in the selected PubMed/ Google Scholar articles were considered for this historical perspective. None of the compiled articles were excluded based on language. In the initial evaluation of non-English articles, English abstracts were mainly used. Full-text translations were performed if necessary.

Acknowledgment

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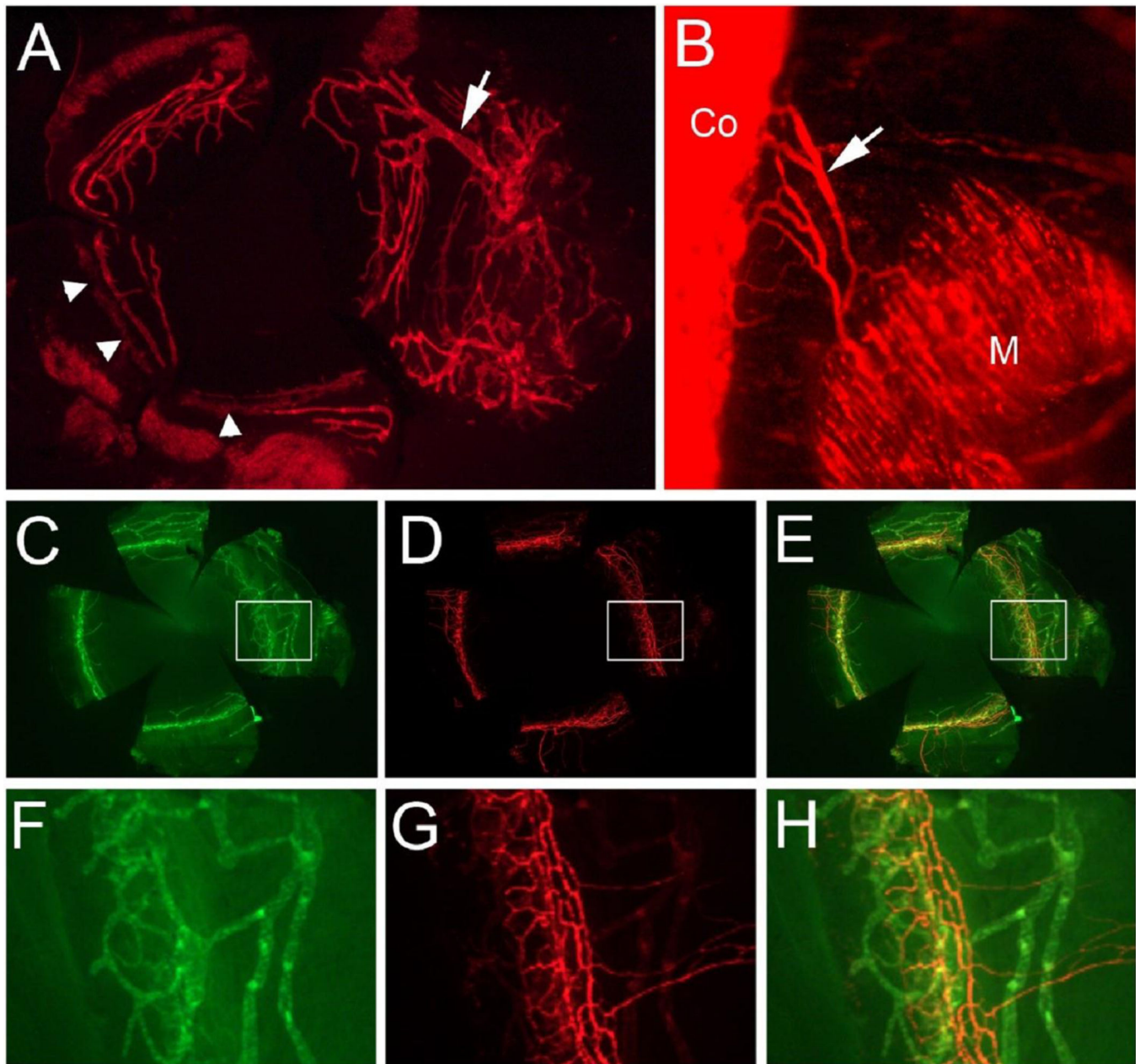
Figure 1. Visualization of the ocular lymphatics using transgenic mouse models.
(**A**) Corneal flat mount from the eye of Prox1-tdTomato bacterial artificial chromosome
(BAC) transgenic mouse (postnatal day 5). The entire ocular lymphatic network is rooted
to one or two collecting lymphatics extended from the nasal side of the eye (arrow).
Schlemm's canal is being formed at this stage (arrowheads). (**B**) Connection between the
limbal lymphatics and the conjunctival lymphatics (arrow) in the eye of Prox1-tdTomato
adult mouse. The cornea (Co) and muscle (M) are also shown in red due to the expression of
Prox1 in the lens fibre cells and muscle cells. (**C-H**) Simultaneous visualization of the ocular
lymphatic (green) and blood (red) vessels in the eye of a triple transgenic mouse (Prox1-
EGFP/Cdh5-CreER[T2]/R26-LSL-tdTomato). The Prox1-EGFP allele labels the lymphatics

in green (C, F), while the pan endothelial inducible Cre allele (Cdh5-CreER^{T2}) induces the tdTomato protein expression from the R26-LSL-tdTomato allele upon Tamoxifen administration (D, G). Merged images (E, H). The eye was collected from the adult triple transgenic mouse after 5 days of Tamoxifen intraperitoneal injection. The boxed areas are enlarged (F, G, H).

CILIARY BODY EPITHELIUM

AQUEOUS HUMOR

TRABECULAR MESHWORK

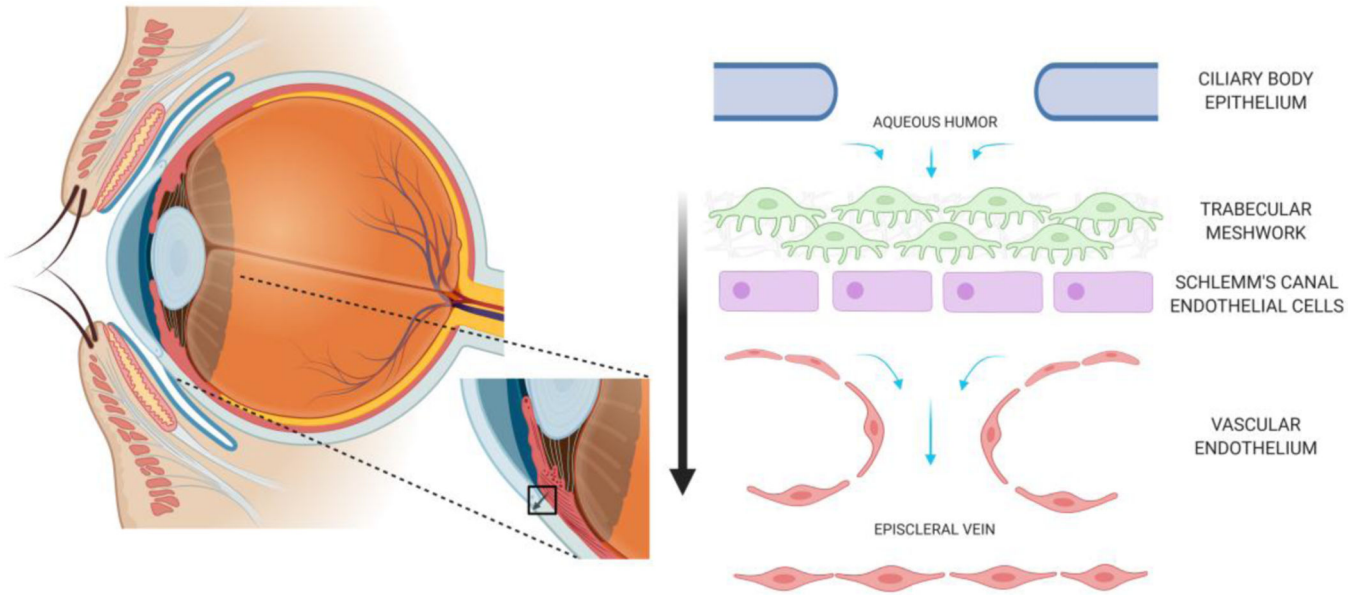SCHLEMM'S CANAL ENDOTHELIAL CELLS

VASCULAR ENDOTHELIUM

EPISCLERAL VEIN

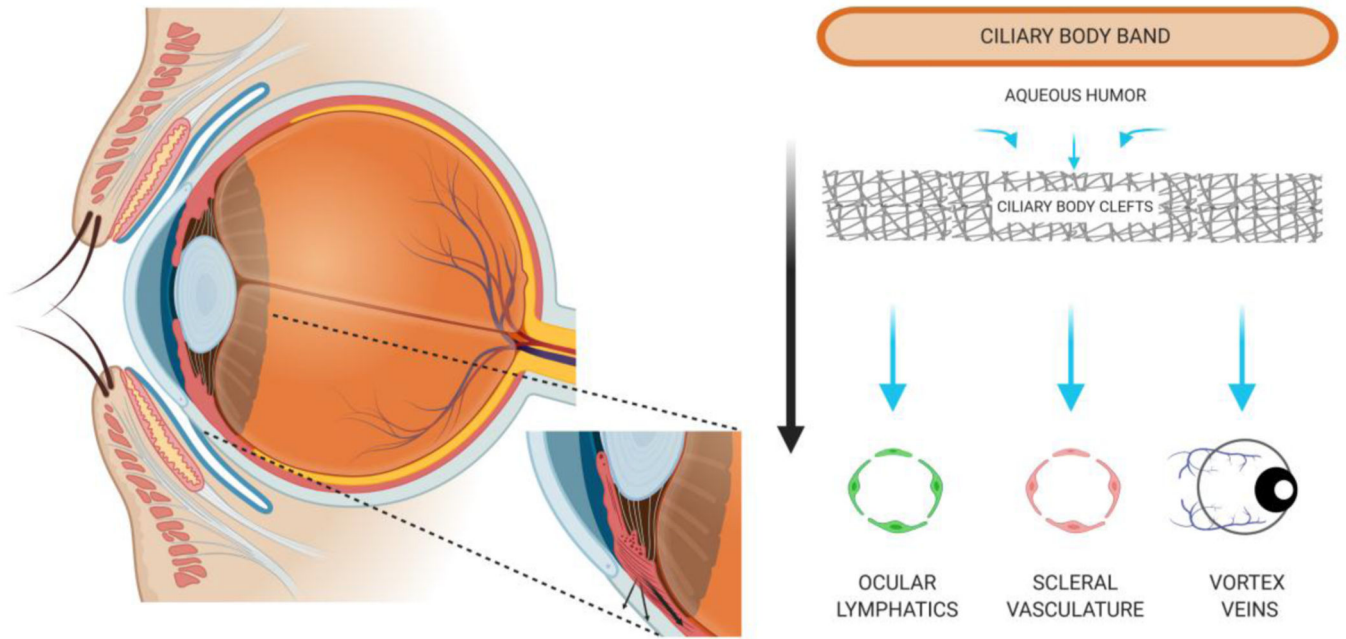**Figure 2. Conventional aqueous drainage pathway of the eye**

Figure 3. Alternative or non-conventional aqueous drainage pathways for aqueous humor
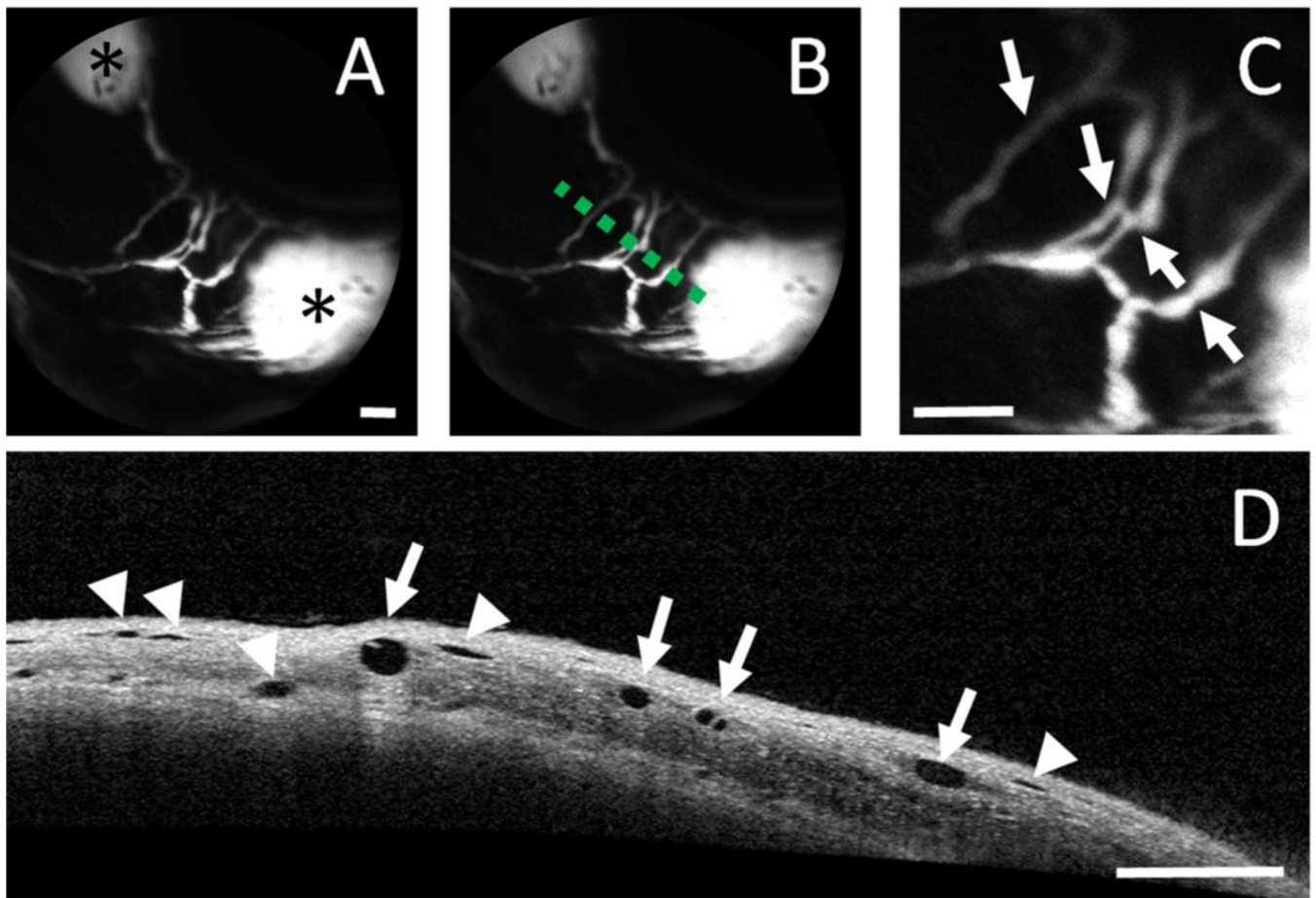
Figure 4. Subconjunctival Outflow Pathways and Structural Imaging.
A) Two blebs (black asterisks) were created by injecting fixable fluorescent dextrans under the conjunctiva of a post-mortem porcine eye. Outflow pathways between the blebs were visualized using a Spectralis HRA+OCT (Heidelberg Engineering). OCT was performed (B, green dotted line shows B-scan placement; D, OCT B-scan). Outflow pathways in the angiographic image (C; 4 white arrows) corresponded to lumens on the OCT (D; same 4 white arrows) using the Spectralis cross-modality comparison function. D) Lumens not associated with subconjunctival outflow pathways (5 white arrowheads) were also seen. D) Note that they have smaller cross-sectional areas (white arrowheads), consistent with a lack of pressure in these pathways that can instead be found in the bleb-related outflow pathways (4 white arrows) owing to the injection pressure during bleb creation. These smaller pathways may represent blood vessels. Scale bars: all 1mm.
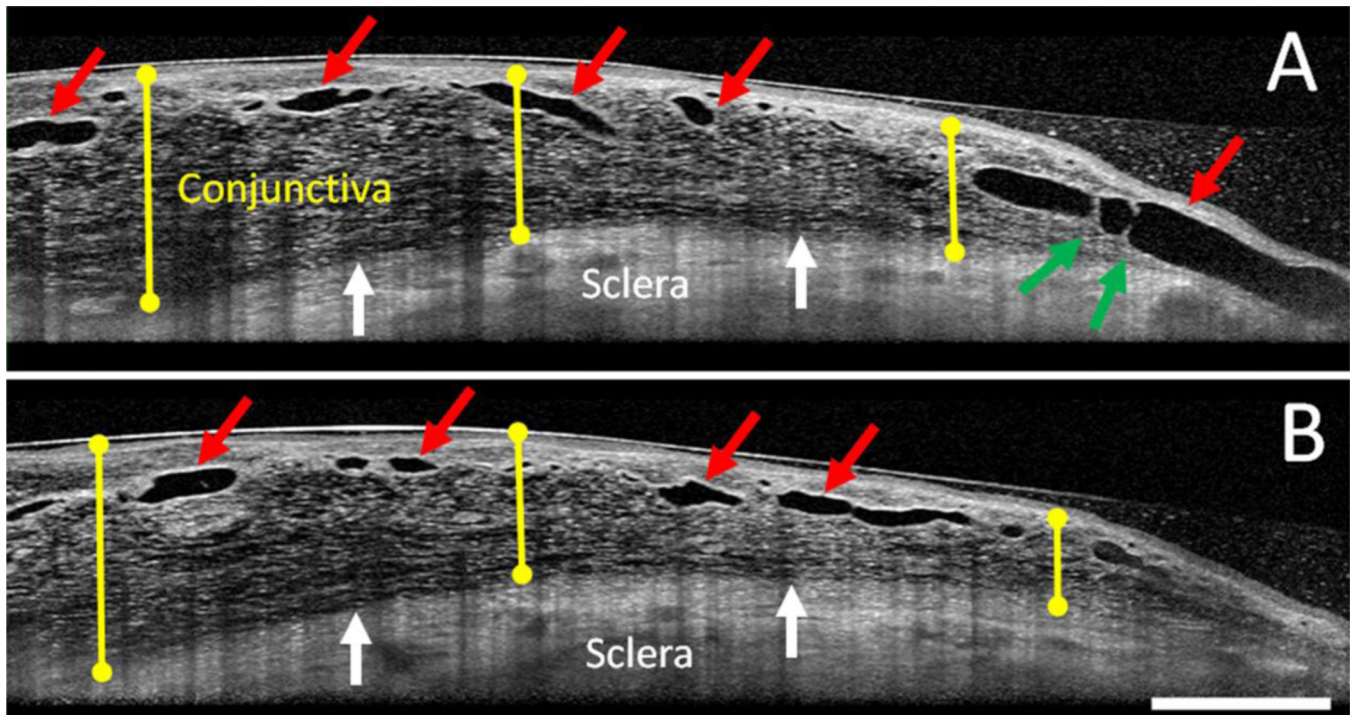
Figure 5. Subconjunctival Fluid and Outflow Pathways.
A/B) Two anterior segment OCT B-scans were obtained from the right eye of a 56-year old Hispanic male who was diagnosed with a branch retinal vein occlusion. Subconjunctival lidocaine was given for anesthesia prior to intravitreal bevacizumab injection. After the subconjunctival lidocaine injection, OCT demonstrated edematous and thickened conjunctiva (yellow vertical bars) above the conjunctival: scleral border (denoted by white arrows). Dilated lumens (red arrows) appeared as potential outflow pathways for the subconjunctival drug injection with intraluminal septae and valve-like structures seen (green arrows). Scale bar: 1 mm.

Table 1 -

ABCD.

Feature	Conventional Aqueous Outflow Pathway	Uveolymphatic Pathway
Pathway	AC>TM>SC>CC>1SVP>AV>EV	AC>TM>CMT>SCS>IL>LN
Fluid source	Aqueous humor	Aqueous humor + leaked fluid
Fluid composition	Aqueous combines with blood	Aqueous combines with lymph
Embryological functional components	BEC+LEC+SCEC	LEC
Flow pattern	BEC: apical to basal LEC+SCEC: basal to apical	LEC: basal to apical
Molecular markers	BEC+LEC+SCEC: CD31, cadherin 5, VECFR-2, vWF BEC: CD34, CD 105/endoglin, TIE-2 LEC: PROX1, LYVE-1, VECFR-3, podoplanin, TIE-1 SCEC: PROX1, VECFR-3, integrin α9 and TIE-2	LEC: PROX1, LYVE-1, VECFR-3, podoplanin, TIE-1
Desmosomes	BEC: No LEC/SCEC: Yes	LEC: Yes
Primary Propulsion	TM movement in response to ocular pulse, blinking and eye movement	Lymphangion contraction
Pressure system	High pressure, circular	Low pressure, diffuse
Systemic entry	Surface of eye	Thoracic duct and lymphatic duct
Clinical significance	Physiological drainage of aqueous humor, outflow pump failure occurs in glaucoma	Novel pathway: potential role in prostaglandin action, MICS, glaucoma filtration surgery

AC: anterior chamber, TM: trabecular meshwork, SC: Schlemm's canal, CC: collector channels, ISVP: intrascleral venous plexus, AV: aqueous veins, EV: episcleral veins, CMT: ciliary muscle tendon, SCS: supraciliary and suprachoroidal spaces, IL: intraocular lymphatics, LN: lymph nodes, BEC: blood endothelial cells, LEC: lymphatic endothelial cells, SCEC: Schlemm's canal endothelial cells