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REVIEW

Hyalocyte functions and immunology

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ABSTRACT

Introduction: Vitreous hyalocyte functions have only recently been explored in-depth. These macrophage-like cells play critical roles in immunologic surveillance and physiology of the developing and adult eye.

Areas covered: Hyaloid vasculature involution during embryogenesis, synthesis and degradation of vitreous components during development and aging, and maintenance of vitreous transparency will be discussed. This article also reviews immunologic features during development and in the adult.

Expert opinion: Recent transcriptional analyses have demonstrated that despite similarity to other myeloid cell populations such as microglia and monocyte-derived macrophages, hyalocytes possess a distinct expression profile and molecular signature. Hyalocytes are important in hyaloid vasculature involution during development, ocular immune privilege and immune surveillance, synthesis and degradation of vitreous components, as well as migration and phagocytic activity during adulthood.

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vitreous; hyalocytes; macrophages; embryology; hyaloid vessel regression; innate immunity; vitreous body-associated immune deviation (VBAID)

1. Introduction

Hyalocytes were first described by the Danish anatomist Hannover in 1840 [1] as a distinct macrophage-like cell population residing within the vitreous body. Since that time, we have come to recognize hyalocytes as specialized and adapted for the tissue-specific needs of the human vitreous. In recent years, there has been expansion of research into understanding the role of immune cells in the developing, healthy, aging, and diseased vitreous and retina. While considerable efforts have been made to unravel the role of retinal microglia during development and disease, little is known about the function of hyalocytes. Article 1 in this series of expert reviews on hyalocytes focused on the origin and turnover of hyalocytes, cell morphology, and imaging [2], while article 3 summarizes our knowledge on the role of hyalocytes in the pathophysiology of proliferative vitreo-retinal diseases [3]. In the present article, we exploit technical advances in molecular biology to elucidate the functions of hyalocytes during development and in adult homeostasis via immune surveillance and privilege, as well as to assess their biological function in inflammatory and neurodegenerative disorders. New insights into the universe of hyalocytes are likely to have significant implications for the treatment of sight-threatening eye disease and may help to design new strategies to promote restoration of tissue homeostasis in the vitreous body and retina.

Hyalocytes are mononuclear phagocytic cells distributed within the vitreous cortex. They display a stellate, sometimes bipolar shape with long protrusions [4] (Figure 1) and their nature has fascinated and challenged scientists since their discovery nearly two centuries ago. Over the years, numerous theories regarding the origin and functions of hyalocytes have emerged, but it is only recently that distinct features of hyalocytes are being unraveled in detail. State-of-the-art imaging techniques (see article 1 in this series [2]) that include optical coherence tomography (OCT) imaging [2,5], adaptive optics (AO) OCT imaging [6], and AO-scanning light ophthalmoscopy (AOSLO) [7] coupled with high-throughput transcriptional analyses [8], have not only revived and underpinned established notions, for instance, regarding the migration potential and the phagocytic activity of hyalocytes, but have also unveiled novel facts about the immunomodulatory nature of these cells. It is now established that, although hyalocytes bear certain similarities with other tissue-specific macrophages and microglial cells, they represent a distinct population adapted for the needs of the vitreous body, particularly the maintenance of a clear optical axis. The deleterious effects of activated hyalocytes in proliferative vitreo-retinal diseases will be described in the third article of this series of expert reviews

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Article 2 of 3 articles on hyalocytes. View additional articles here:
Article 1: Hyalocyte origin, structure, and imaging [https://doi.org/10.1080/17469899.2022.2100762]
Article 3: Hyalocytes in proliferative vitreo-retinal diseases [https://doi.org/10.1080/17469899.2022.2100764]

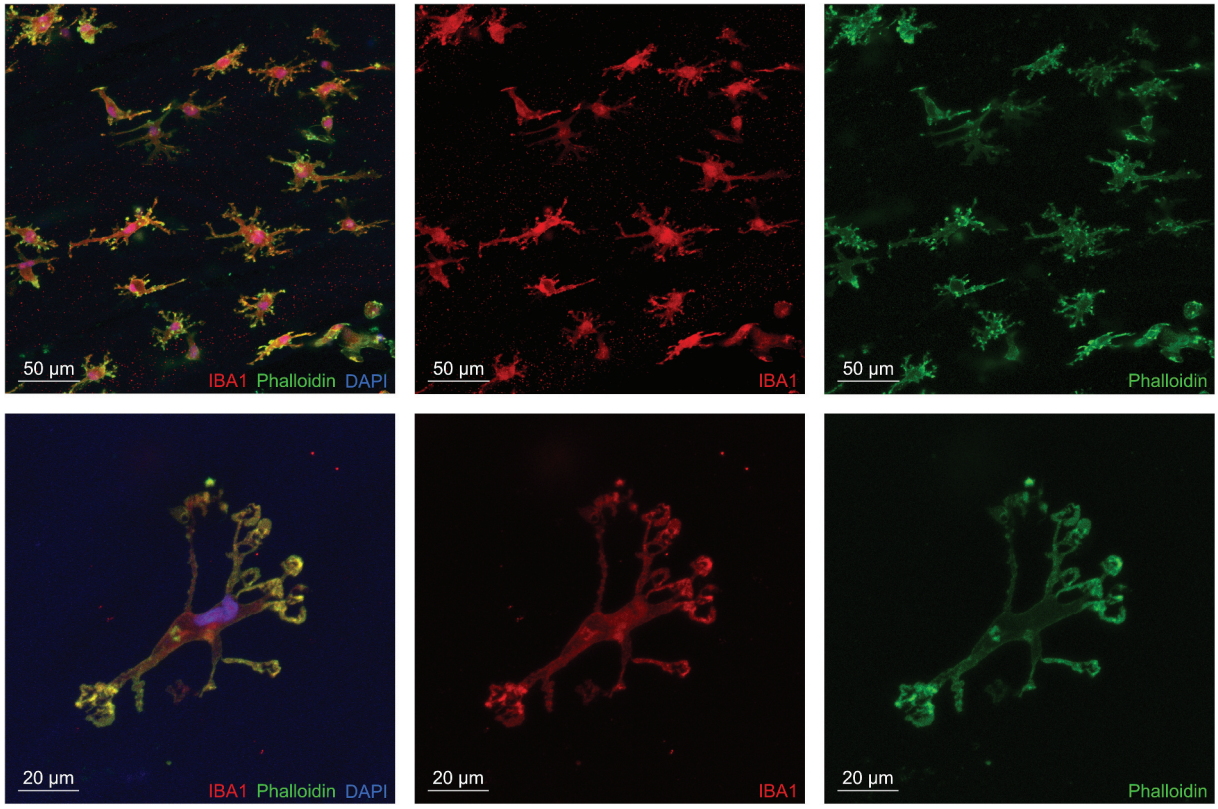

Figure 1. **Morphologic characterization of human hyalocytes.** Human hyalocytes were obtained from the vitreous body of a donor eye (enucleated due to ciliary body melanoma) and characterized using immunohistochemical staining against IBA1, ionized calcium-binding molecule 1 (red), phalloidin (green), including an overview of several hyalocytes (upper panel) and a more detailed view of one hyalocyte (lower panel). Nuclei are counterstained with DAPI (4',6-Diamidin-2-phenylindol). Scale bars correspond to 50 µm (upper panel) and 20 µm (lower panel). Reproduced from [4], licensed under CC-BY 4.0 (http://creativecommons.org/licenses/by/4.0).

on hyalocytes [3], as their close resemblance with microglia cells suggests both pernicious and beneficial qualities in this population [9]. Might hyalocytes represent a double-edged sword in the course of life? Which mechanisms mediate the salubrious balance of benign over malign properties of hyalocytes, and vice versa? Do hyalocytes act as a friend or as a foe in the pathophysiology of vitreo-retinal disease? Could there be more than one hyalocyte population, perhaps an active one and one that usually rests, then experiences a deleterious twist in pathologic situations? These questions and more will be broached in the following, but definitive answers may require years of continued scientific and clinical research.

2. Role of hyalocytes during development

Around the fourth week of gestation during development of the eye, mesodermal cells enter the forming eye cup via the optic fissure, and, together with surrounding fibroblasts, constitute the primordial vitreous. Some of the mesodermal cells exhibit the morphological features of cells destined to be hyalocytes, expressing myeloid cell markers, such as major histocompatibility complex (MHC) and cluster of differentiation 45 (CD45) antigens, and can therefore be considered hyalocyte precursor cells [10,11]. The mesodermal cells further contribute to the anlage of the hyaloid vascular system, which is composed of the vasa hyaloidea propria, tunica vasculosa lentis, the pupillary membrane, and the hyaloid artery (Figure 2). The vasa hyaloidea propria anastomoses with the tunica vasculosa lentis and pupillary membrane to nourish the developing anterior segment. After reaching its zenith at approximately the 10th week of gestation, regression of the hyaloid vasculature is observed [12]. This process is vital for a clear optical axis and failure can result in crucial impairment of vitreo-retinal and ocular development. Changes in the proteomic profile of vitreous from human embryos aged 14 to 20 weeks gestation have suggested cytokine events that may be important in hyaloid vasculature regression [13].

Although the exact involution mechanisms of the hyaloid vasculature are not yet understood in detail, it is known that migration of hyalocytes into the adventitia of vitreous blood vessels and cytolysis of endothelial cells adjacent to hyalocytes is involved [12]. Numerous experimental studies suggest that macrophage impairment, for instance, by intraocular application of toxic liposomes [16,17] or by genetic manipulation, e.g. in *PU.1*-deficient mice [18], is associated with persistence of the normally transient hyaloid vessels and pupillary membrane, suggesting that hyaloid vessels are the target of macrophage-mediated programmed tissue remodeling [17]. The underlying mechanisms are currently poorly understood but may depend on the expression of *Wnt7b* in murine hyaloid macrophages, which activates Wnt signaling by binding to the frizzled-4 (FZD4)/low-density lipoprotein receptor-related protein 5 (LRP5) receptor in adjacent endothelial cells to initiate cell apoptosis, eventually leading to the regression of hyaloid vessels [18] (Figure 3). This notion is supported by the findings that

Article highlights

- Hyalocytes play a role in hyaloid vessel involution during embryogenesis.
- In adults, hyalocytes play a role in the synthesis and degradation of vitreous macromolecules.
- Hyalocytes demonstrate migration and phagocytic activity during adulthood, protecting the eye from infection and other exogenous threats.
- There is an important role for hyalocytes in ocular immune privilege and immune surveillance.
- Hyalocytes may be important in autoimmune disorders, neurodegenerative diseases, and possibly age-related macular degeneration.

Ndp-, *Lrp5-*, and *Fzd4*-deficient mice all display persistent hyaloid vasculature, implying a critical regulatory role of Wnt signaling in hyaloid vessels [19]. Other factors that may also contribute to hyaloid regression and modulate the function of hyalocytes in eliciting target cell death in the developing vitreous include the von Hippel-Lindau protein and the hypoxia-inducible factor 1 alpha (HIF-1α) [20,21], vascular endothelial growth factor (VEGF) [22], collagen XVIII [23], the alternative reading frame (Arf) tumor suppressor protein [24], angiopoietin-2 [25], and the bone morphogenetic protein-4 [26,27]. In addition, vitreous hyalocytes have been reported to be the only cell type in the eye expressing all four forms of transforming growth factor beta (TGF-β) [28], the production of which may contribute to apoptosis during regression of the hyaloid vasculature. Indeed, localization of TGF-β2 in the human vitreous during early stages of development correlates with receding hyaloid vessels [29].

To summarize, hyalocyte precursor cells present in the primordial vitreous have been implicated in regression of the hyaloid vasculature, which is crucial for a clear optical axis. A large number of preclinical studies with various murine models suggest that dysfunction of macrophages such as hyalocytes is involved in the persistence of embryonic hyaloid vessels, resulting in developmental failure. Thus, further research focusing on the molecular mechanisms in fetal hyalocytes that promulgate hyaloid vessel regression could provide new avenues to pursue novel therapeutic strategies to treat pathologic neovascularization. Finally, study of the divergence(s) of fetal hyalocytes compared to adult hyalocytes is warranted, since this might be very revealing in elucidating the exact role of hyalocytes in the developing vitreous.

3. Roles of hyalocytes in the adult

3.1. Synthesis and degradation of vitreous components

The human vitreous body consists of 98% water and 2% structural proteins (primarily collagen and hyaluronan), as well as components of the extracellular matrix (ECM), such as chondroitin sulfate [30] (Figure 3). As early as the 19th century it was assumed by Schoeler (1848) and Virchow (1852), and later by Szirmai and Balazs (1958) that hyalocytes take part in the anabolism of vitreous, most probably via the production of hyaluronan [31,32]. Consistent with this hypothesis, Boneva and colleagues, who studied the RNA expression profile of hyalocytes in people aged about 70 years, found that several ECM components, including *COL5A1* and *COL9A2*, are abundantly expressed in vitreous hyalocytes, suggesting that hyalocytes synthesize structural proteins in the vitreous body during aging [8]. Balazs had previously described that the total vitreous collagen concentration in patients aged 70–90 years was significantly increased compared to ages 15–20 years (0.1 mg/mL versus 0.05 mg/mL). While this increase in collagen concentration was explained by an age-related decrease in the volume of the vitreous gel and a simultaneously stable total collagen amount during aging [10], it is feasible that collagen synthesis by hyalocytes contributes to the increased collagen content in senescent vitreous. This notion is supported by the identification of a collagen precursor, the type II procollagen, in the adult vitreous [33]. Contrary to the general assumption that hyalocytes contribute to hyaluronan synthesis and despite the fact that hyalocytes are located in the region with the highest hyaluronan concentration in the vitreous [10], Boneva and colleagues found a relatively low expression of the key factors for hyaluronan production in hyalocytes, namely hyaluronan synthase 1–3 (Table 1), suggesting that, at least in adults, hyaluronan is not produced by hyalocytes [8]. However, it is

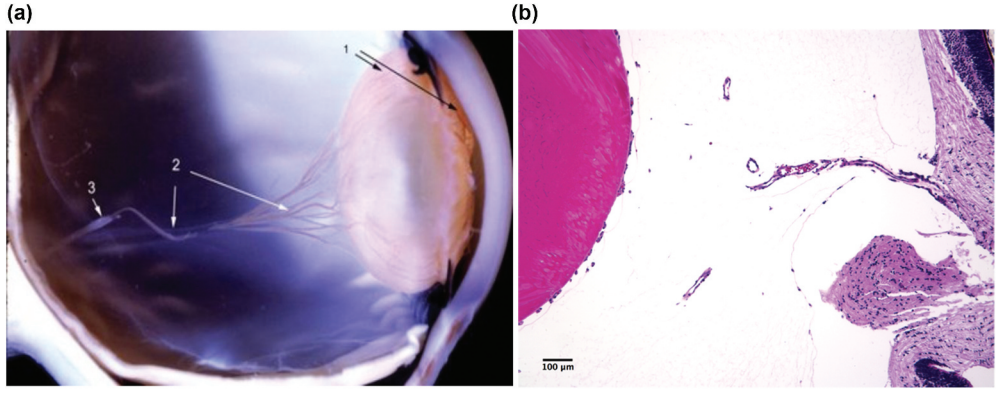

Figure 2. **Human fetal hyaloid vasculature.** (a) The hyaloid vasculature in the human embryo consists of the hyaloid artery (3), the vasa hyaloidea propia (2), and the tunica vasculosa lentis (1), which on the anterior surface of the lens constitutes the pupillary membrane. Reproduced with permission from [15] © 2014 Springer Science+Business Media New York. (b) Light microscopy of embryonic hyaloid vasculature in the human (Hematoxylin & Eosin stain; bar = 100 uM). Reproduced with permission from [14] © 2020 Elsevier Ltd.

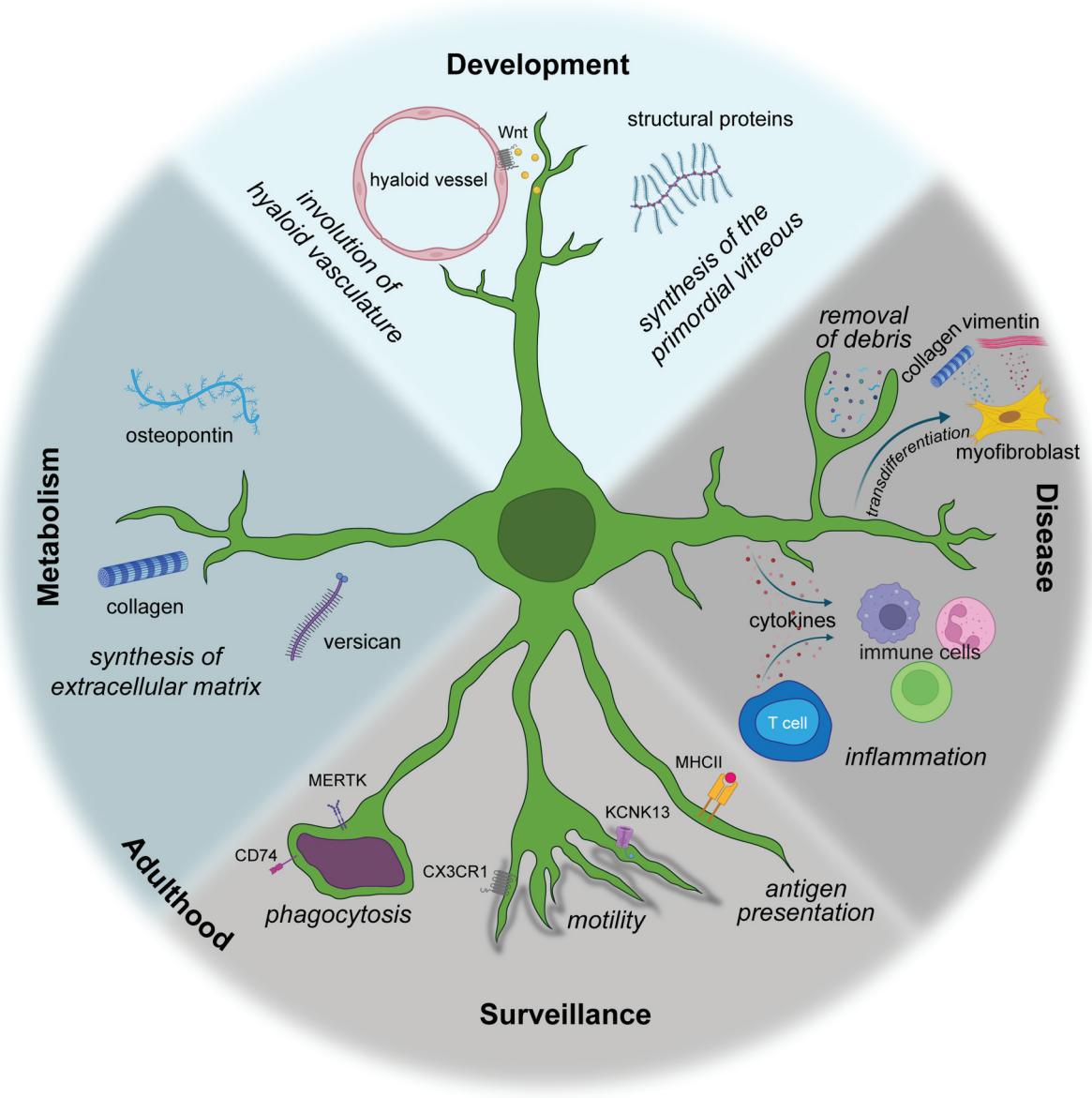

Figure 3. **Functions of hyalocytes during development, adulthood, and disease.** Hyalocytes exhibit a diversity of functions according to the developmental stage and health status: In the course of Wnt-mediated pre-programmed tissue-remodeling, hyalocytes play a role in the physiologic regression of the hyaloid vasculature during development of the eye, essential for a clear optical axis. Furthermore, hyalocytes might contribute hyaluronan and other structural molecules during formation of the secondary vitreous. Under steady state postnatal conditions, hyalocytes play a role in vitreous metabolism and homeostasis by producing extracellular matrix components, such as secreted phosphoprotein 1 (SPP1, osteopontin), different collagen types, and versican. As part of their surveillance function, hyalocytes play a role in phagocytosis, as evidenced by the expression of MERTK (MER proto-oncogene, tyrosine kinase) and CD74 (cluster of differentiation 74). Furthermore, hyalocytes are capable of scanning their environment by extending and retracting their projections, in line with the surface expression of important cell motility markers, such as KCNK13 (potassium channel, subfamily K, member 13) and CX3CR1 (CX3C chemokine receptor 1). In the course of inflammation, hyalocytes abundantly express MHCII (major histocompatibility complex class II) molecules, participate in removal of debris, and present antigens to T cells, which themselves contribute to the chemotaxis of other immune cells by cytokine expression. In proliferative vitreo-retinal diseases, hyalocytes can transdifferentiate into α-SMA (alpha smooth muscle actin)-positive myofibroblasts, capable of producing collagen and vimentin and thus contribute to the fibrotic nature of these diseases, as well as induce pathologic membrane contraction (see article 3 in this series of expert reviews on hyalocytes [3]). Portions of this figure were created using Biorender.com.

presently unclear if hyalocytes produce hyaluronan during development of the secondary vitreous and thereby contribute to vitreous formation. Therefore, further studies focusing on the molecular biology of fetal hyalocytes and their differences from adult hyalocytes are needed to clarify the precise role of hyalocytes in vitreous anabolism during embryonic development. The data from Boneva and colleagues further indicate that aged hyalocytes strongly express versican [8], which, in addition to collagen type IX, is the second chondroitin sulfate proteoglycan in the vitreous body forming complexes with hyaluronan, fibulin-1, and fibulin-2, thus playing a critical role in maintaining the molecular morphology of vitreous [34].

Changes in versican and minor glycosaminoglycans were hypothesized to promote vitreous liquefaction during aging [35], which may point toward a role of hyalocytes in age-

Table 1. Most prominent extracellular matrix (ECM) and immune privilege (IP) factors in hyalocytes sorted by transcripts per million (TPM). Expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is listed for reference.

ECM factors	TPM	IP factors	TPM
GAPDH	2990.4	*HLA-DRB1*	11,849.6
FN1	649.6	*HLA-DRA*	10611.4
VCAN	54.9	*HLA-DRB5*	4007.5
EFEMP1	30.4	*GAPDH*	2990.4
HYAL2	22.1	*C3*	2712.7
COL5A1	16.6	*CD86*	940.1
COL9A2	14.2	*HLA-DPA1*	793
OPTC	14.1	*HLA-DQB1*	588.4
FBLN2	1.8	*LGALS1*	580
FBLN1	1.8	*HLA-DQA1*	547.4
HYAL3	1.7	*HLA-E*	546.2
FBN2	0.5	*HLA-DPB1*	458.4
FBN1	0.3	*HLA-B*	453.3
HAS1	0.3	*HLA-DMA*	378.3
COL9A3	0.3	*HMOX1*	238.1
HAPLN3	0.2	*HLA-DMB*	220.4
HYAL1	0.1	*CD46*	185
HAPLN1	0.1	*LGALS3*	172.8
COL2A1	0.1	*HLA-A*	163.6
COL11A1	0	*SERPINF1*	123.1
HAPLN2	0	*TGFB1*	121.9
COL5A2	0	*HLA-C*	114.1
COL11A2	0	*CD55*	107.3
HAS2	0	*CXCL2*	107.3
HAS3	0	*TNFSF10*	96.1
HAPLN4	0	*CD40*	71.6
		IL1RN	66.9
		PDCD1LG2	63.9
		HEBP1	57.3
		IL10	49.9
		CD1D	45.8
		HLA-G	43.3
		CD59	36.1
		HLA-DQA2	20.8
		HLA-DOA	20.6
		SERPINE1	20.4
		POMC	13.5
		FAS	10.8
		THBS1	10.3
		ARG2	8.1
		HLA-F	6.4
		HLA-DQB2	6.3
		CD274	6
		TGFB2	3.6
		HLA-DOB	1.2
		FASLG	0.8
		GATA3	0.6
		IL6	0.3
		CR1L	0.1
		CALCB	0.1
		IL12A	0
		SST	0
		IL12B	0
		VIP	0
		CALCA	0
		MIF	0

dependent vitreous liquefaction. Similarly, mutations that alter splicing of the chondroitin sulfate-bearing domains in versican have been associated with conditions of excessive vitreous liquefaction, such as Wagner syndrome [36], suggesting a possible role for hyalocytes in vitreo-retinal dystrophies. In line with this hypothesis, hyalocytes were shown to express high levels of enzymes engaged in hyaluronan degradation, such as hyaluronidases [8], and they might thus contribute to age-dependent vitreous gel liquefaction [37]. While the role of human hyalocytes in synthesis of vitreous components during development remains unclear due to the lack of appropriate experimental setups, current evidence suggests that that adult hyalocytes do indeed produce different components of the extracellular matrix, such as different types of collagen and other proteoglycans, but not hyaluronan. Any potential contribution of hyalocytes to the hyaluronan fraction in the developing vitreous remains to be elucidated.

3.2. Hyalocyte physiologic surveillance

3.2.1. Hyalocyte migration

Already in the 19th century Iwanoff (1865) and Potiechin (1879) postulated that hyalocytes are able to move in an ameboid manner. Consequently, Schwalbe classified hyalocytes in the group of the wandering cells (‘*Wanderzellen*’), a term, which at that time referred to circulating and migrating macrophages and lymphocytes [38]. Recently, Toco Chui and Rich Rosen identified movement of human hyalocytes with OCT imaging [5] and subsequently employed AOSLO [7] to image and map the movement of hyalocytes *in situ* (see article 1 in this series of expert reviews on hyalocytes for video display of these features [2]). They elegantly examined key characteristics of macrophage-like cells above the inner limiting membrane (ILM), which are likely to be hyalocytes, in healthy and diseased (see article 3 in this series [3]) human eyes. This was possible in part because these cells can be visualized directly due to their location anterior to the ILM. They found that human hyalocytes are distributed distinctively, age differently, and have clear dynamic characteristics. As such, hyalocytes constantly explore their local environment by extending and retracting their projections within a timeframe of a few minutes, which is consistent with the hypotheses of Iwanoff and Potiechin from years ago [5].

These findings on cell motility are consistent with studies showing a high expression of the potassium channel *KCNK13* (potassium channel, subfamily K, member 13) and *CX3CR1* (CX3C chemokine receptor 1) in human hyalocytes [8], known to be important for microglia cell motility and immune surveillance [39] (Figure 3). In addition, hyalocytes were found to express membrane receptors that enable the cell to probe its environment, such as the adenosine A3 receptor [8], ‘sensing’ ADP (adenosine diphosphate) released by e.g. dying neurons and thus contributing to cell process extension [40]. These activities most probably do not represent a continuous process, as hyalocytes are likely to remain in a resting state until stimulated by various triggering events or circumstances. Similar properties have been described for resting microglia in the brain and in the inner and outer plexiform layer of the retina [41]. Within minutes following acute injury, microglial processes converge toward the site of damage, and after hours to days, the reactive microglia retract their processes, form new motile protrusions, and transform into an amoeboid shape, thus migrating to the lesion [42]. Chemotaxis of retinal microglia to sites of tissue damage, such as retinal neovascularization or retinal angiomatous proliferation [43], depends on activation of P2YR12 (purinergic receptor P2Y12) receptors on microglia that bind ATP adenosine triphosphate) or ADP released from neural cells [44]. Since hyalocytes express

P2RY12 to a similar extent as microglia [8], it is very likely that hyalocytes are capable of a similar reaction to tissue injury in diseases of the vitreo-retinal interface, such as proliferative diabetic vitreo-retinopathy (PDVR) or proliferative vitreo-retinopathy (PVR), and may migrate to sites of retinal inflammation or degeneration. This hypothesis would be in line with recent studies showing an increase of macrophage-like cells anterior to the ILM in patients with PDVR and PVR, which at least in part may be assembled by migrating and/or proliferating hyalocytes [45,46]. Indeed, the studies of Chui and Rosen have shown evidence of hyalocyte changes in various vitreo-retinal diseases (see article 3 in this series of expert reviews on hyalocytes [3]).

3.2.2. Hyalocyte phagocytosis

Long ago, Hamburg assumed that hyalocytes contribute to the clearance of metabolic products and that they may take part in the maintenance of the 'hemato-ocular' barrier [31]. The phagocytic capacity of hyalocytes has been demonstrated *in vivo* in rabbits [47] and *in vitro* [48], clearly indicating that hyalocytes belong to the mononuclear phagocyte system. Hyalocytes have been shown to constantly scan their environment with their extensions [5], anticipating danger signals. As soon as injury or foreign matter is detected, hyalocytes are very likely to migrate to the harmful substance(s) and phagocytose them, just like their related cells, microglia of the central nervous system (includes the neural retina). In line with this postulate, recent evidence shows that hyalocytes express factors that are important for phagocytosis, including *MERTK* (MER proto-oncogene, tyrosine kinase), *CD74* (cluster of differentiation 74) and *MHCII* (major histocompatibility complex class II) [8] (Figure 3). As such, hyalocytes may contribute to erythrophagocytosis and vitreous hemorrhage clearance in patients with PDVR or vitreous bleeding following anomalous posterior vitreous detachment [49–51].

However, other studies suggest that hyalocytes can have a deleterious effect and exacerbate vitreo-retinal diseases. In 1965 Hamburg already suggested that hyalocytes contribute to intraocular fibrosis based on the observation that hyalocytes increase in numbers in patients with Coats disease, which can present with a fibrous mass behind the lens. He even postulated that hyalocytes can transform into fibroblasts, thus furthering fibrosis. Similarly, he suggested that hyalocytes might contribute to PVR formation [31]. Indeed, as a result of vitreoschisis [52,53], the peripheral vitreous in patients with rhegmatogenous retinal detachment can harbor pathologic membranes with hyalocytes able to recruit monocytes from the circulation and glial cells from the retina, as well as retinal pigment epithelium (RPE) cells in developing PVR. Consequently, the studies of van Overdam have shown that meticulous chromodissection [54] of the peripheral vitreous reduces the incidence of post-operative PVR [55,56] (see article 3 in this series of expert reveiws on hyalocytes [3]). Recent evidence shows that hyalocytes can transdifferentiate into alpha-smooth muscle actin (α-SMA)-positive cells during retinal neovascularization in patients with PDVR and PVR [46,57], again highlighting the potential of hyalocytes involvement in scar formation in vitreo-retinal interface disease.

3.2.3. Antigen presentation

Hyalocyte phagocytosis of noxious agents, such as foreign bodies or microorganisms, may be important in order to present antigens on their surface and trigger an immune response. For instance, by expressing MHCII-related genes, such as *HLA-DR* (human leukocyte antigen – DR isotype), hyalocytes may be capable of presenting antigens to cluster of differentiation (CD4)-positive T-lymphocytes [58]. Whether hyalocytes thus provoke an adaptive immune response or suppress T cell function, as it was assumed for microglia in experimental autoimmune encephalitis [59], is currently a subject of debate. Recent studies suggest that immunosuppressive properties in hyalocytes outweigh pro-inflammatory activity in order to limit damage by self-reactive T cells in the eye, thus preventing irreparable neurodegeneration and maintaining a clear optical axis [8].

In summary, consistent with the 150-year-old notion of migrating hyalocytes, latest studies visualize the ameboid movements of vitreous cells and demonstrate expression of factors important for retraction and protrusion of cell processes and thus for cell migration. Further, as cells of the myeloid lineage, hyalocytes are capable of phagocytosis, constantly reacting to danger signals and noxious threats. These features of hyalocytes might be crucial for their alleged role in proliferative diseases of the vitreo-retinal interface. Unfortunately, pernicious effects of hyalocytes in the setting of excessive scarring are likely, and are the subjects of current research.

4. Hyalocytes and immunology

4.1. Comparisons to other myeloid cell types

Although hyalocytes were assumed by Schwalbe to have macrophage characteristics as early as 1874 [38], it took more than 100 years for these cells to be designated as 'macrophage-like' cells with phagocytic properties and IgG receptors on their surface [48]. Resemblance to epiplexus cells (innate immune cells in the brain ventricles) was observed by Hamburg, who considered these cells histiocytes and assumed an origin from the adventitial cells of the blood vessels of the optic disc, retina, and/or the ciliary body [31]. Kolmer, on the other hand, referred to the vitreous cells as microglia [60], although a 2005 study identified the origin of hyalocytes in the bone marrow by the use of lethally irradiated bone marrow chimeras, a model that is since known to be associated with severe irradiation-induced tissue damage and promotion of leukocyte infiltration from the blood [61]. Controversy surrounding cell origin aside, it is certain that hyalocytes derive from the monocyte/macrophage lineage (also known as myeloid cells), as evidenced by immunohistochemical and electron microscopic studies of the last decades [62,63]. Although they seem to originate from the same progenitor cells, retinal microglia and vitreous hyalocytes appear to differ from each other due to their different localization in the eye and the associated different physical and metabolic characteristics [4,8,64]. Recent evidence indicates a close relationship between human hyalocytes and brain microglia on the transcriptional level, including a similar expression of the most prominent genes *SPP1* (secreted phosphoprotein 1),

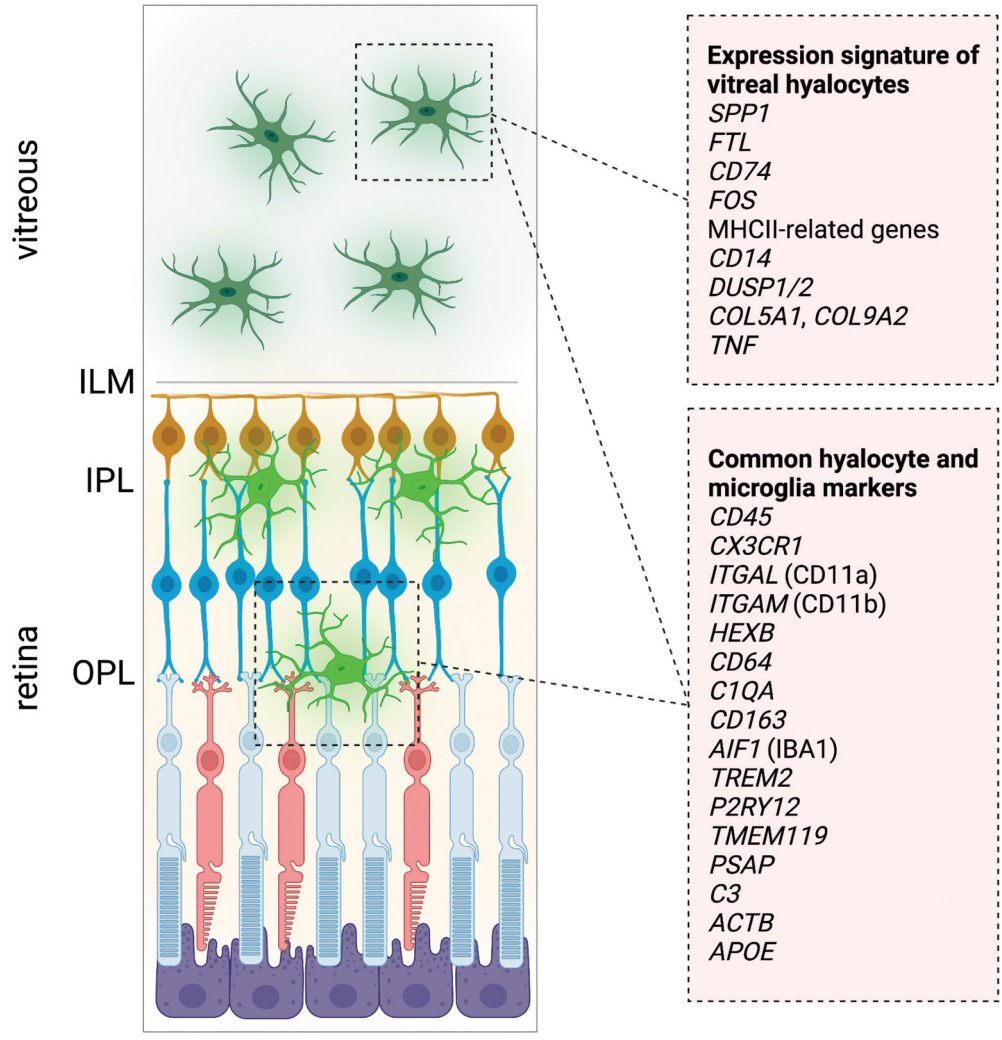

Figure 4. The microenvironment and gene signature of human vitreous hyalocytes and retinal microglia cells. Hyalocytes reside primarily in the posterior vitreous cortex, adjacent to the inner limiting membrane (ILM), whereas retinal microglia cells are located in the inner (IPL) and outer plexiform layers (OPL). Hyalocytes are distinguished from microglia not only by the affinity to a defined niche, but also by the expression of a number of specific factors, such as *SPP1*, secreted phosphoprotein 1, *FTL*, ferritin light chain, MHCII-related genes, etc. However, common leukocyte markers have been found to be expressed to a similar extent in both vitreous hyalocytes and retinal microglia.

Associated transcripts are summarized based on data from [4,8,65]. *CD74*, cluster of differentiation 74. *FOS*, Fos proto-oncogene. MHCII, major histocompatibility complex class II. *CD14*, cluster of differentiation 14. *DUSP1/2*, dual specificity protein phosphatase 1/2. *COL5A1*, collagen alpha-1(V) chain. *COL9A2*, collagen alpha-2(IX) chain. *TNF*, tumor necrosis factor. *CD45*, cluster of differentiation 45. *CX3CR1*, CX3C chemokine receptor 1. *ITGAL*, integrin, alpha L (*CD11a*, cluster of differentiation 11a). *ITGAM*, integrin alpha *M* (*CD11b*, cluster of differentiation 11b). *HEXB*, beta-hexosaminidase subunit beta. *CD64*, cluster of differentiation 64. *C1QA*, complement C1q subcomponent subunit A. *CD163*, cluster of differentiation 163. *AIF1*, allograft inflammatory factor 1 (*IBA1*, ionized calcium-binding molecule 1). *TREM2*, triggering receptor expressed on myeloid cells 2. *P2RY12*, purinergic receptor P2Y12. *TMEM119*, transmembrane protein 119. *PSAP*, prostatic specific acid phosphatase. *C3*, complement component 3. *ACTB*, beta-actin. *APOE*, apolipoprotein E. Figure created with Biorender.com.

CD74 and *FTL* (ferritin light chain) in both populations [4,8,65] (Figure 4, Table 2).

However, a detailed comparison of hyalocytes to brain microglia, blood-derived monocytes, and monocyte-derived macrophages shows that the transcriptional profile of hyalocytes is distinct from microglia and most similar to macrophages. When compared to the three other myeloid cell populations, hyalocytes differed significantly in the expression of 91 genes, among them MHCII-related genes such as *HLA-DRA*, a classic antigen-presenting cell marker (Figure 4). This finding was confirmed on the protein level, as pronounced immunoreactivity for HLA-DRA was observed in hyalocytes, but not in microglia cells (Figure 5) [8]. This study underscores the notion of a myeloid cell identity of hyalocytes, but at the same time highlights them as a unique immune cell population with tissue-specific immunogenic properties. Despite the resemblances that hyalocytes bear with other cells of the myeloid cell lineage (such as microglia), hyalocytes exhibit a unique molecular signature presumably adapted to the tissue-specific needs of the human vitreous.

4.2. Immune privilege and vitreous body-associated immune deviation

Similar to the brain, the eye has long been regarded as an immune privileged site [66]. Medawar interpreted this phenomenon as immunological ignorance due to the lack of lymphatic drainage and the presence of blood-tissue barriers. Subsequently, an altered form of systemic immunologic

Table 2. Most prominent genes in hyalocytes sorted by transcripts per million (TPM). Expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is listed for reference.

Top expressed factors	TPM
SPP1	44,917
FTL	37850.2
CD74	25,094.1
ACTB	23141.3
PSAP	17229.7
FOS	11907
HLA-DRB1	11,849.6
HLA-DRA	10611.4
TMSB10	10,385.6
CD14	9555.2
C1QB	9535.4
DUSP1	8317
C1QA	7241.5
CST3	6156.4
FCER1G	6078.9
SRGN	5436
LYZ	5294.6
APOE	4883.9
TMSB4X	4597.3
TREM2	4412.9
HLA-DRB5	4007.5
S100A11	3900.5
ALOX5AP	3780.6
TYROBP	3526.9
CXCL8	3500.9
CCL2	3202.4
CSF1R	3131.4
CTSB	3040.8
GAPDH	2990.4
FCGR3A	2902.9

response, so-called immune deviation, was defined as a response following antigen inoculation into a privileged site [67]. In the case of the eye, the term anterior chamber-associated immune deviation (ACAID) was coined [68] and later expanded to vitreous cavity-associated immune deviation (VCAID) [69]. However, since vitreous is a structure and not a cavity or empty space, in this article and in the future we are refining the initially described VCAID as vitreous body-associated immune deviation (VBAID). While ACAID is partly mediated by bone marrow-derived antigen-presenting cells expressing F4/80 and carrying ACAID-inducing signals to the spleen [70], VBAID has been suggested to be mediated by antigen-presenting hyalocytes [60]. These hypotheses are based upon experiments performed by Sonoda and colleagues showing that immune responses were significantly delayed after injection of antigens, such as ovalbumin or allogeneic splenocytes, into the vitreous body [69]. When mice were sensitized subcutaneously with these antigens, and then challenged one week later with antigen-pulsed peritoneal exudate cells in the ear pinnae, animals that had previously received inoculations with antigen in the vitreous showed significantly reduced ear swelling compared with control animals that had not been inoculated with antigens. These observations suggest that immune deviation could be elicited in the vitreous body, similar to the anterior chamber, resulting in systemic tolerance. Since F4/80-positive hyalocytes were found to be the only cells present in the vitreous body, it

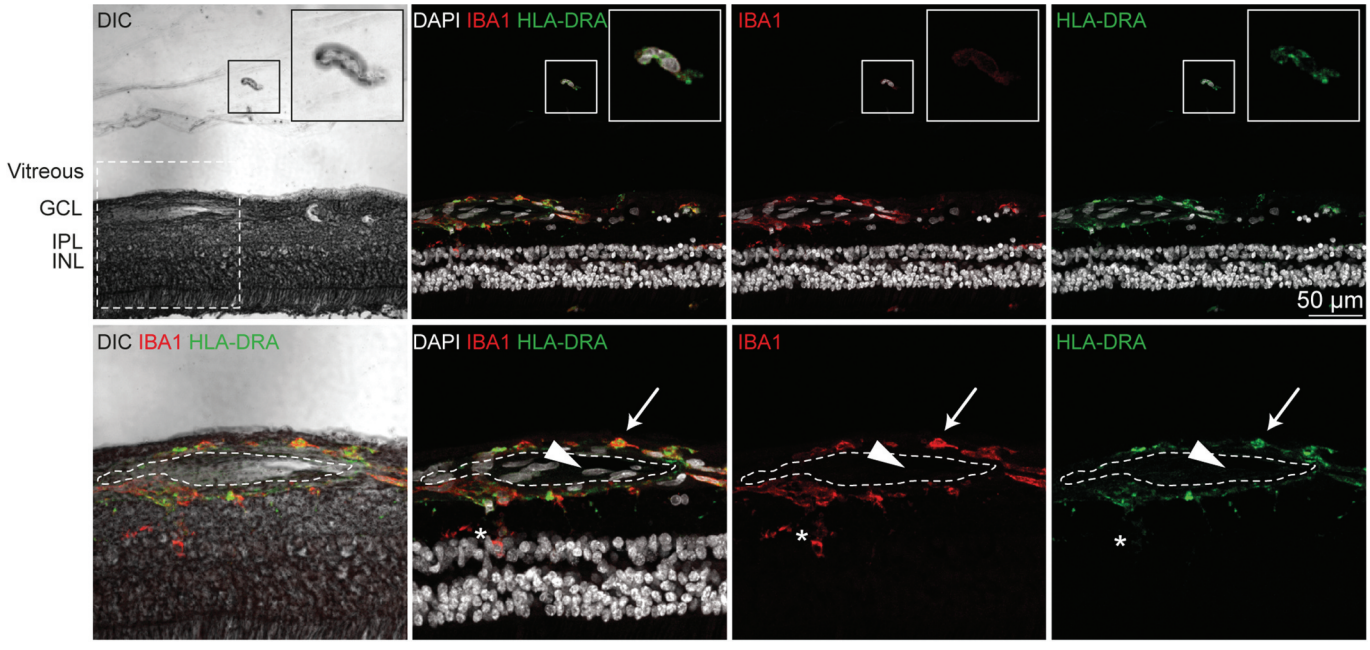

Figure 5. **Immunohistochemical staining of vitreo-retinal myeloid cells.** Immunohistochemical staining for HLA-DRA, human leukocyte antigen – DR isotype alpha, and IBA1, ionized calcium-binding molecule 1. A vitreous cell is presented in higher magnification in the upper right corner. Higher magnification of the section within the dashed white square in the lower panel. The lumen of an intraretinal vessel is traced with the white dashed line. The arrows point to a perivascular macrophage, the arrowheads to an intraluminal leukocyte. The asterisk is positioned between two microglial cells (positive for IBA1). Nuclei are counterstained with DAPI (4′,6-Diamidin-2-phenylindol). DIC, differential interference contrast; GCL, ganglion cell layer; IPL, internal plexiform layer; INL, inner nuclear layer. Scale bar corresponds to 50 µm. Reproduced from [8], licensed under CC-BY 4.0 (http://creativecommons.org/licenses/by/4.0/).

was suggested that hyalocytes are the antigen-presenting cells responsible for mediating VBAID. As in ACAID, antigens inoculated into the vitreous body are thought to be captured by hyalocytes and carried via the bloodstream to the spleen where they stimulate natural killer T cells to produce immunosuppressive factors, such as IL-10 (interleukin 10) and TGF-β, and generate antigen-specific regulatory T cells thus shaping the adaptive immune response [69]. Therefore, it is currently believed that both the anterior and posterior segments of the eye are capable of inducing systemic tolerance, reducing excessive inflammation in the eye, and maintaining immune privilege.

In addition to these indirect immunosupressive effects, hyalocytes are thought to reduce intraocular inflammation by contributing directly to an immunosuppressive milieu in the vitreous body in order to maintain vitreous transparency and visual function. Recent evidence suggests that hyalocytes express a variety of factors known to be key players in immune privilege of the eye, for instance, alpha-melanocyte-stimulating hormone (*α-MSH*), cluster of differentiation 86 (*CD86*), cluster of differentiation 46 (*CD46*), and *TGF-β2* (Table 1). It is known from animal studies that *TGF-β2* [71,72] and *α-MSH* [73] participate in the immune privilege by suppressing inflammatory responses of T helper cells [72] and inducing regulatory T cells [74]. Furthermore, *in vitro* studies have demonstrated that CD86-expressing iris pigmented epithelial cells are capable of preventing cell proliferation and cytokine secretion of T cells [75]. *CD46*, which functions as a regulator of the alternative complement pathway, is responsible for the suppression of adaptive T helper cells immune responses, thus protecting tissues from the autologous complement system [76]. The abundant expression of *CD46* by hyalocytes (Table 1) and its presence in the human vitreous, may be yet another factor contributing to the immune-privileged microenvironment. Further research is warranted to elucidate the exact mechanisms underlying the immunosuppressive properties of hyalocytes in health, and potential pro-inflammatory properties in disease.

In summary, in the immune-privileged eye the induction of systemic tolerance by the inoculation of antigens into the vitreous body, is considered an indirect immunosuppressive effect of hyalocytes. Moreover, hyalocytes contribute directly to the control of intraocular inflammation by expressing a plethora of immunomodulatory factors. However, further research is needed in order to understand the exact mechanisms, underlying the immunosuppressive properties of hyalocytes in the steady state and their interplay with the high expression of pro-inflammatory factors in disease.

5. Hyalocytes in non-proliferative diseases

5.1. Autoimmune disorders

As cells of the innate immune system, hyalocytes are likely to participate in and modulate inflammatory diseases of the posterior segment, such as uveitis. A role for hyalocytes as inhibitors of intraocular inflammation within the context of ocular immune privilege has been previously suggested [8,77]. As mentioned above, it can be assumed that under physiologic conditions the immunosuppressive properties of hyalocytes overweigh pro-inflammatory antigen-presenting properties, in order to maintain vitreous transparency. In uveitis, however, this balance appears to be disturbed, and there is a local inflammatory response and an accumulation of vitreous cells which manifest clinically as vitreous opacities. Preclinical evidence examining the endotoxin induced murine uveitis (EIU) model [78] and equine recurrent uveitis [79] showed that MHCII-positive hyalocytes increase in numbers in particular on the apical processes of the ciliary body adjacent to the anterior vitreous membrane. Recently, Joseph and colleagues used a custom AOSLO to image immune cells in the retina *in vivo* from the onset to resolution of EIU in mice [80]. They elegantly showed that several immune cell types accumulate in the inflamed retina, among them CD68-positive, Cx3cr1-positive tissue-resident myeloid cells such as retinal microglia or hyalocytes, which disappeared in the course of disease development. However, it is to date unclear if the increase in myeloid cells in uveitis is explained by a recruitment of blood-derived macrophages or by a clonal expansion of hyalocytes. The latter may be plausible in the light of *in vitro* studies showing that tumor necrosis factor alpha (TNF-α), which is an established cytokine that induces uveitis and is mainly expressed by macrophages [81], promotes proliferation, migration, and gel contraction by porcine hyalocytes [82]. Since reports on the role of hyalocytes in uveitis are scarce, it can only be speculated that the obstruction of fundus details is due to proliferating hyalocytes, additional infiltrating blood-derived immune cells, and/or protein exudation from these cells. As primary vitrectomy is not indicated for the treatment of uveitis, direct examination of hyalocytes in human uveitis is limited. Thus, new diagnostic methods such as adaptive optics imaging approaches are needed to expand knowledge [7]. Furthermore, it is still unclear whether an increased number of hyalocytes in the vitreous body of patients with uveitis is intended to suppress the inflammatory reaction and reduce tissue damage, or if it promotes inflammation. Considering the close relationship between hyalocytes and microglia and both the deleterious and beneficial roles of microglia in autoimmune and neurodegenerative disorders [9,83], it is further tempting to postulate the existence of different hyalocyte subpopulation and their respective functions in diseases such as multiple sclerosis, which manifest with an intermediate uveitis in the eye.

5.2. Neurodegenerative diseases

Since the retina is an extension of the central nervous system, it is considered a window to brain for both research [84] and clinical care. Several central neurodegenerative diseases manifest in the eye and recent developments in noninvasive retinal imaging technologies have contributed to the study of neuronal and vascular alterations in these disorders. For instance, Alzheimer's disease (AD) patients often exhibit a reduction in the number of optic nerve head axons and a decrease in the thickness of the nerve fiber layer [85]. Extracellular deposits of beta-amyloid (Aβ) and intraneuronal accumulation of hyperphosphorylated tau protein, a hallmark of the pathophysiology of AD in the brain, have also been detected in the retina and the optic nerve [86,87] and may contribute to the observed neural retinal degeneration. Furthermore, significantly enhanced inflammation, as evidenced by retinal microglial activation, has been shown in an AD mouse model and correlated with the presence of Aβ plaques [88], while

in the TgF344-AD rat model Aβ deposition has been associated with microglial recruitment and loss of visual function [89]. To date, it remains controversial whether microglial activation is involved in the elimination of Aβ deposition and thus in disease deceleration, or in AD progression via enhanced neuroinflammation. While the contribution of microglia to neuroinflammation in the retina of neurodegenerative diseases has been studied extensively, little is known about the related population of immune cells in the vitreous body, hyalocytes. On the transcriptional level it has been demonstrated that hyalocytes abundantly express *CD74* [8], which is implicated in a number of inflammatory processes in neurodegenerative afflictions, such as AD [90]. State-of-the-art noninvasive imaging, (see article 1 in this series of expert reviews on hyalocytes [2]) may be of utility in the elucidation of the role(s) of hyalocytes as a further source of neuroinflammatory activity at the vitreo-retinal interface in neurodegenerative diseases.

5.3. Age-related macular degeneration

Age-related macular degeneration (AMD) is a progressive, degenerative disease of the retinal pigment epithelium and neural retina characterized in its most aggressive neovascular form (nAMD) by infiltrating immune cells, myofibroblasts and various extracellular matrix factors [91]. The composition of human choroidal neovascularization (CNV) lesions has been studied extensively and our knowledge has recently been complemented by RNA sequencing and *in silico* analysis of membranes surgically excised at the time before anti-VEGF-therapy was available [92]. Inflammatory cells (such as microglia and blood-derived macrophages) were found to be involved in the formation of CNV membranes [93,94], presumably by sensing danger-associated signals and migrating to sites of CNV. According to the stage of disease progression, microglia cells may play both beneficial and pernicious roles in the course of disease. For instance, myeloid cells are involved in tissue damage, secondary to immune cell recruitment, and resultant scarring in AMD. Nevertheless, they can also be anti-inflammatory by promoting tissue repair [95]. Support for this derives from studies of human and murine CNV, which were found to express the matricellular protein osteopontin (OPN), thus forming a gliotic membrane [92,96]. Hyalocytes have not been studied in detail in AMD, but it is tempting to speculate whether hyalocytes modulate AMD by secreting cytokines that diffuse into the outer retina or by migrating to the site of lesion in the outer retina, as has been reported for retinal microglia [97–99].

It has been previously reported, that vitreo-macular adhesion is associated with a higher risk of CNV in AMD [100] and that subretinal neovascularization is less common in patients with complete posterior vitreous detachment (PVD) [101]. Furthermore, eyes with exudative AMD and no PVD are less responsive to anti-VGEF injections than eyes with PVD [102]. Theories concerning the mechanism(s) underlying this observation include the prevention of macular oxygenation from the ciliary body by the attached posterior vitreous cortex, the sequestration of pro-angiogenic cytokines in the macula by the attached posterior vitreous cortex [103], and pro-inflammatory effects of anomalous PVD with persistent vitreo-macular traction [102]. Given that an attached posterior vitreous has hyalocytes near the site of neovascularization [104,105], it is tempting to postulate a role for hyalocytes in this disease process. Although the exact mechanisms by which vitreous influences the progression of nAMD have not yet been elucidated in detail, it is nonetheless intriguing to speculate whether hyalocytes might sustain an inflammatory stimulus to promote CNV in AMD eyes with attached posterior vitreous and thus stimulate CNV development and/or persistence.

6. Conclusion

Hyalocytes represent the resident myeloid cell population of the vitreous body exhibiting various properties during development, in health, and in disease [106]. They appear to be important during embryogenesis by inducing regression of the fetal vitreous vasculature, as well as in the adult for macromolecule metabolism, immune privilege and surveillance, and phagocytic activity that protects the eye from infection and other exogenous threats. Hyalocyte participation in inflammation may play an important role in autoimmune, neurodegenerative, and proliferative diseases (see article 3 in this series [3]). While the exact mechanisms by which hyalocytes mediate beneficial effects then switch to deleterious effects are currently unknown, recent research strongly indicates that this cell population is capable of being both promoters of healthy homeostasis and participants in disease.

7. Expert opinion

Hyalocytes are mononuclear phagocytes that reside within the vitreous body of many species, including humans. Although hyalocytes are found throughout the vitreous body, their location within the posterior vitreous cortex just anterior to the retina appears to be very important. There, the number of hyalocytes is estimated to be 150 cells per mm^2, which is relatively small compared with other cell populations, such as those in the neural retina. While vitreous hyalocyte scarcity has hampered detailed scientific investigation in the past, recent sophisticated methods of cell isolation coupled with transcriptional analysis of even small cell populations have answered various questions concerning vitreous hyalocytes, including their origin (see article 1 in this series [2]), as well as identification of their important functions. This review article explores the important roles of vitreous hyalocytes in the developing eye and in maintaining homeostasis in the adult. In the former case, it appears that hyalocytes participate in the involution of the hyaloid vasculature in the fetus. In the adult, hyalocytes play a role in the synthesis and degradation of vitreous macromolecules. Hyalocytes might also be key players in ocular immune privilege and immune surveillance, while their phagocytic activity protects the eye from infection and other exogenous threats.

Despite the recent identification of hyalocytes as a unique immune cell population with tissue-specific immunogenic properties, an unambiguous marker that distinguishes vitreous hyalocytes from retinal microglia cells and perivascular macrophages has yet to be identified. Importantly, its absence currently thwarts the possibility of creating a hyalocyte-specific knockout mouse model. This is a weakness in the various arguments proposing hyalocytes as a unique cell population

and the different postulates of their role(s) in ocular physiology and pathology. Nevertheless, applying current research techniques for further study of hyalocytes is feasible. In this sense, the identification of disease-relevant factors via RNA sequencing and emerging drug screening approaches should enable preliminary drug discovery on a bioinformatic level. The application of resulting new treatment options in mouse models of vitreo-retinal disease could be a promising step toward developing better clinical care in the future. This would be particularly helpful in improving our understanding of the role of hyalocytes in proliferative vitreo-retinal disorders, a group of diseases with devastating effects upon the eye and vision (see article 3 in this series [3]).

At the present time, the acquisition of tissue from the vitreo-retinal interface during vitrectomy provides specimens for research and continues to be a useful approach to further our understanding of hyalocytes. Ever-evolving state-of-the-art techniques (such as single-cell transcriptional and proteomic analyses [94,107]) will be applied to answer the intriguing question about the alleged dual role of hyalocytes and the potential deleterious twist wherein they switch from promoters of homeostasis to participants in pathophysiology. However, since vitrectomy is generally not indicated for the treatment of uveitis or neurodegenerative disorders, the optimization of non-invasive imaging methods would need to fill this void (see article 1 in this series [2]). The application of this knowledge to future diagnosis and treatment of disease is eagerly anticipated, as all evidence suggests that we will probably witness the revival of centuries-old notions and the establishment of novel ideas. What is certain is that this thrilling journey promises to keep us in suspense for years to come.

Abbreviations

ACAID	anterior chamber-associated immune deviation
ACTB	beta-actin
AD	Alzheimer's disease
ADP	adenosine diphosphate
AIF1	allograft inflammatory factor 1
AMD	age-related macular degeneration
APOE	apolipoprotein E
ATP	adenosine triphosphate
AO	adaptive optics
AOSLO	AO-scanning light ophthalmoscopy
Arf	alternative reading frame
Aβ	beta-amyloid
α-SMA	alpha-smooth muscle actin
α-MSH	alpha-melanocyte-stimulating hormone
CD4	cluster of differentiation 4
CD14	cluster of differentiation 14
CD45	cluster of differentiation 45
CD46	cluster of differentiation 46
CD64	cluster of differentiation 64
CD74	cluster of differentiation 74
CD86	cluster of differentiation 86
CD163	cluster of differentiation 163
CNV	choroidal neovascularization
COL5A1	collagen alpha-1(V) chain
COL9A2	collagen alpha-2(IX) chain
CX3CR1	CX3C chemokine receptor 1
C1QA	complement C1q subcomponent subunit A
C3	complement component 3
DAPI	4′,6-Diamidin-2-phenylindol
DIC	differential interference contrast
DUSP1/2	dual specificity protein phosphatase 1/2
ECM	extracellular matrix
EIU	endotoxin induced uveitis
FOS	Fos proto-oncogene
FTL	ferritin light chain
FZD4	frizzled-4
GCL	ganglion cell layer
HEXB	beta-hexosaminidase subunit beta
HIF-α	hypoxia-inducible factor alpha
HLA-DR	human leukocyte antigen – DR isotype
IBA1	ionized calcium-binding molecule 1
ILM	inner limiting membrane
IL-10	interleukin 10
INL	inner nuclear layer
IPL	inner plexiform layer
ITGAL	integrin, alpha L (CD11a, cluster of differentiation 11a)
ITGAM	integrin alpha M (CD11b, cluster of differentiation 11b)
KCNK13	potassium channel, subfamily K, member 13
LRP5	low-density lipoprotein receptor-related protein 5
MERTK	MER proto-oncogene, tyrosine kinase
MHC	major histocompatibility complex
nAMD	neovascular age-related macular degeneration
Ndp	Norrie disease protein
OCT	optical coherence tomography
OPL	outer plexiform layer
OPN	osteopontin
PSAP	prostatic specific acid phosphatase
PVD	posterior vitreous detachment
PVR	proliferative vitreo-retinopathy
P2YR12	purinergic receptor P2Y12
RPE	retinal pigment epithelium
SPP1	secreted phosphoprotein 1
TGF-β	transforming growth factor beta
TNF-α	tumor necrosis factor alpha
TMEM119	transmembrane protein 119
TREM2	triggering receptor expressed on myeloid cells 2
VBAID	vitreous body-associated immune deviation
VCAID	vitreous cavity-associated immune deviation
VEGF	vascular endothelial growth factor

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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