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Review

Biological Immune Mechanism of Retina

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Abstract

The blood-retinal barrier (BRB) is a well-recognized mechanism that underlies the retina's immunological privilege. The BRB is formed locally by inhibitory molecules that bind to cell membranes, as well as by the suppression of systemic immune responses. Recent studies have revealed that microglial cells are essential for maintaining immunological privilege within the retina by regulating the immune response. They achieve this by enhancing or reducing ocular inflammation. Furthermore, retinal pigment epithelium (RPE) regulates the behavior of immune cells within the retina, which can lead microglial cells to reduce inflammation and promote immunological tolerance. With the aim of better understanding the biology of immunological processes within the retina, this article reviews the BRB and discusses the factors, systemic immune responses, microglia, RPE, and their associated enzymes that enable the BRB.

Keywords: retina; immune mechanisms; BRB; microglia; retinal pigment epithelium

1. Introduction

The mechanisms that establish the well-known immune-privilege within the retina include the blood retinal barrier (BRB), cell membrane bound proteins, and suppression of systemic immune responses. These three elements restrict invasion of the immune system into the retina. In addition to these three mechanisms, studies have shown that immunological control of microglia within the retina is important because microglia are essential for neuronal development and synaptic connection [1]. This has led several investigators to link retinal microglia with the presence and survival of particular retinal cell subtypes, such as optic nerve subtypes [2]. Additionally, microglia have frequently been shown to play an immunological role in retinal disorders [3]. Therefore, it is important to understand how the immune system controls microglia in both healthy and diseased retinas.

In addition to the discovery of the microglia, researchers have also found that retinal pigment epithelium (RPE) has a prominent role in immunological regulation within the retina. The RPE is a physical barrier that supports both the neural retina and choroid, and is positioned within the outermost layer of the retina. To maintain the stability of the retina's immunological environment, the highly immunomodulatory RPE secretes cytokines and other immunosuppressive molecules. Amongst these molecules, microRNAs (miRNAs) are involved in coordinating general tissue maintenance and chronic inflammation [4]. Exosome-mediated miRNA transfer is also an important RPE signaling mechanism that regulates microglia [5,6]. Moreover, several enzymes linked to the RPE play a decisive role in the immune regulation of RPE.

2. Immune Privilege

The BRB can be divided into an inner and an outer barrier. Retinal capillary endothelial cells possess tight junctions that connect these cells to form the inner endothelial barrier. The outer barrier is comprised of RPE and supporting structures. The RPE is bounded by the basal zonula adheres, lateral tight junctions, and apical gap junctions [7].

Based on cell structure, the permeability of the BRB can be divided into inward permeability, where substances enter the retina through the barrier, and outward permeability, where substances within the retina reach the retinal capillary or choroid through the barrier. Under normal conditions, inward permeability is significantly lower than outward permeability. Infrequently, immune molecules can enter the retina and trigger an immune response while isolating themselves from the rest of the body due to the asymmetric permeability formed by the inward and outward barriers. This mechanism prevents pathogens and systemic inflammatory disturbances, as well as preserving the stability of the intraocular environment.

2.1 Maintenance of BRB Integrity is Crucial for Preventing a Variety of Retinal Disorders

Studies employing both *in vivo* and *in vitro* models have demonstrated that hyperhomocysteinemia (HHcy) modulates the BRB. Specifically, it has been shown that HHcy negatively affects the function of both the inner and outer barriers, as well as altering the retinal vasculature. Stimulation of the glutamate receptor, N-methyl-d-aspartate receptor (NMDAR), has been implicated as the molecular mechanism for HHcy-induced BRB dysfunction following increased concentrations of Hcy in the blood [8].

Vascular leakage may also stem from dysfunction of tank-binding kinase 1 (TBK1) in endothelial cells, since reduced TBK-1 function interferes with the development of retinal vascular tissue. The phosphorylation of CXCR4 at Ser355 by TBK1 has also been found to play an essential role in the maintenance of endothelial junctions [9].

Endothelial Annexin-A1 (ANXA1) interacts with the actin cytoskeleton in endothelium to maintain vessel integrity, and is crucial for maintaining vascular homeostasis and stability during development. In addition, the overexpression of endothelial cytochrome P450 epoxygenase 2J2 (CYP2J2) can reverse the loss of ANXA1 induced by oxidative stress injury, mitigate retinal ganglion cell loss, and maintain BRB integrity following ischemia-reperfusion injury. In response to oxidative stress, CYP2J2 upregulates the expression of methyltransferase-like 3 (METTL3), which promotes ANXA1 translation by modifying ANXA1 m6A methylation within the endothelium. Therefore, the CYP2J2-METTL3-ANXA1 pathway represents a potential therapeutic target for treating BRB impairment [10].

2.2 Membrane-Associated Molecules Inhibit and Suppress Systemic Immune Responses

The major histocompatibility complex (MHC), membrane complement regulatory molecules, and Fas ligand (FasL) are cell surface molecules linked to ocular immune control.

Fas+ cells undergo apoptosis when they interact with cells expressing FasL on their surface. This is the case with inflammatory T lymphocytes that encounter endothelial cells and retinal epithelial pigment cells. Fas+ inflammatory T cells that enter ocular tissue die as a result of the baseline expression of FasL in these tissues [11,12], thereby preventing ocular tissue from being attacked and destroyed by activated T cells. This mechanism was recently harnessed in the cancer field by developing an adenovirus that expresses a truncated *FasL* gene. Specifically, leukemia cells that come into contact with cells expressing this recombinant FasL undergo apoptosis more efficiently. This strategy could therefore be used as a potential treatment for cancer, as well as for autoimmune conditions such as autoimmune retinopathy [13].

3. Microglial Cells

Should lymphocytes breach the initial two lines of defense, the inner and outer barriers, the systemic suppression of immune responses becomes necessary to limit injury to the retina. This is achieved through the chemotaxis of intraocular suppressive cytokines and retinal pigment epithelial (RPE) cells. Such responses trigger the induction of regulatory T cells and suppressive antigen-presenting cells.

3.1 Microglia Play a Crucial Role in Maintaining Ocular Immune Privilege

Microglial cells are found throughout the retina and play a crucial role in maintaining ocular immune privilege (OIP). These cells have the ability to modify the immune response by enhancing, or reducing, ocular inflammation. Microglia play an important role in maintaining the structural integrity of the retina, particularly with regard to retinal neovascularization and the BRB. Microglia migrate throughout the developing retina, and can also migrate or undergo replication to replenish damaged sections of the retina [14]. Studies have demonstrated that microglia are the only phagocytes capable of migrating to the subretinal space and protecting the integrity of RPE structures in models of photoreceptor degeneration [15]. In addition to their phagocytic activities, microglia interact with the retinal vasculature by binding to these structures and secreting trophic and angiogenic factors. These factors in turn regulate the apoptosis of pericytes and endothelial cells, facilitate the rapid removal of excess vascular debris, and control vessel diameter and blood flow velocity [16–18].

The shrinkage of retinal choroidal vasculature and RPE dysfunction stemming from the depletion of microglia by PLX5622 provide strong support for the necessity of microglia in maintaining retinal homeostasis [19]. Retinal neovascularization may be treated by suppressing microglia, as there is an intriguing link between the quantity of microglia and the retinal vasculature that occurs throughout development [20]. Current evidence suggests that dramatic ablation of microglia/macrophages reduces the production of retinal neovascular tufts and enhances neuronal activity, as measured by electroretinography [21]. These results suggest that therapeutic management of such disorders may involve addressing microglial activation.

3.2 Polarized Microglia Phenotypes Impact the Retina

Microglia are crucial for preserving the structural integrity of the retina. However, microglia can also harm retinal barriers when activated. The two polarized phenotypes of microglia are M1 and M2, with M1 microglia secreting inflammatory factors such as tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), and reactive oxygen species (ROS) [22]. Anti-inflammatory factors such as transforming growth factor-β1 (TGF-β1) and IL-4 can alter the M1 microglial phenotype to the M2 phenotype [23]. The BRB is harmed when MI microglia predominate due to the activation of various stimuli, including disruption of the endothelial tight junction proteins zona occludens-1 (ZO-1) and Claudin-5 [24]. Additionally, pro-inflammatory microglia activate the TLR4/MyD88/NF-κB p65 signaling axis, which damages BRB and aggravates the loss of retinal integrity [25]. The targeting of activated microglia is key to repairing damaged BRB. For example, one strategy is to prevent M1 microglia from becoming activated, while another approach is to promote the conversion of M1 to

M2 microglia. A recent *in vivo* and *in vitro* study demonstrated that asiatic acid reduced breakdown of the BRB and enhanced M2 polarization in the retina by attenuating TLR4/MyD88/NF-B p65 pathway activity [25]. Melatonin may also assist the conversion of M1 to M2 macrophages, thereby promoting tissue repair in response to increased infiltration of regulatory T cells (Tregs). Cellular protection through the engagement of Tregs is an important aspect of aging-associated microglia polarization, as well as inflammatory responses involved in neuroprotection.

4. Retinal Pigment Epithelium

4.1 Physical Barrier Function of the Retinal Pigment Epithelium

The BRB limits entry of blood components into the retina. It is made up of a single layer of cells termed the RPE and located between the neuronal retina and the choroid [26]. RPE cells are believed to play a significant role in the immune response and contribute to the maintenance of ocular immune privilege. In addition to protecting the neuroretina from blood-borne toxins through the function of their tight junctions (TJs), RPE provide ocular immune privilege for immune and inflammatory responses in several critical ways. These include the production of membrane-bound negative regulators and soluble immunomodulators that induce regulatory T cells, and which can directly or indirectly suppress effector T cells [27].

The TJs located between neighboring RPE cells establish a microenvironment that inhibits the entry of immune system components into the retina [28]. TJs are composed of transmembrane proteins and cytoplasmic plaque proteins that interact to form a complex network [29]. Transmembrane proteins such as claudins, occludin, and junctional adhesion molecules (JAMs) all contribute to the integrity of this network. However, the cytoplasmic plaque protein zona occludens-1 (ZO-1) remains the most extensively studied component of TJs [30].

Claudins regulate the selectivity and permeability of paracellular transport between epithelial cells, help to establish apical-basal cell polarity and adhesion, and are essential for the establishment of TJs [31]. Several studies suggest that preservation of claudin-5 and claudin-1 integrity in TJs could be a promising therapeutic strategy to prevent BRB in diabetic retinopathy [32]. A recent study demonstrated that ripasudil administration, a selective rho-associated coiled-coil containingkinases (ROCK) inhibitor, effectively attenuated retinal inflammation and prevented the redistribution of claudin-5. These findings imply that the development of anti vascular endothelial growth factor (VEGF) resistance in diabetic macular edema is significantly influenced by the inflammation-induced, ROCK-mediated translocation of claudin-5 [33].

JAMs belong to the immunoglobulin superfamily of cell adhesion receptors and have various functions, such as facilitating cell/cell adhesion and maintaining barrier function [34]. Laser treatment promotes the production of JAM-C in an animal model of choroidal neovascularization (CNV), and inhibition of JAM-C prevents the development of CNV, blood vessel leakage, and macrophage infiltration in response to laser exposure [35]. The dynamic translocation of JAM-C in response to vasoactive molecules could be responsible for BRB dysfunction in endothelial cells. Inhibition of JAM-C or protein kinase C (PKC) in retinal capillary endothelial cells (RCECs) could potentially help to maintain normal BRB function in endothelial cells. Moreover, elevated levels of soluble serum JAM-C may serve as a molecular indicator of wet age-related macular degeneration (wAMD), and modulation of JAM-C activity could have therapeutic benefits for ocular diseases related to BRB dysfunction [36]. Overall, these results suggest that JAM-C targeting could be a promising therapeutic strategy for such disorders.

ZO-1 plays a crucial role as both a component and regulator of RPE TJs [37]. Studies conducted on diabetic retinopathy have demonstrated that inflammatory cytokines, including tumor necrosis factor-alpha (TNFα) and interleukin 6 (IL-6), activate the transcription factor NF-κB, leading to reduced ZO-1 expression in RPE cells [38]. Furthermore, inflammatory cytokines can disrupt the distribution of ZO-1 in ARPE-19 cells, decrease transepithelial electrical resistance (TEER), and damage the BRB through the JAK/STAT pathway. Targeting this pathway may be a promising strategy for treating inflammation-induced macular edema [39]. Increased permeability can worsen inflammation due to reduced production of TJ proteins such ZO-1 and occludin in RPE cells, and disruption of the outer BRB.

4.2 Immunologic Barrier Function of the Retinal Pigment Epithelium

In addition to its physical barrier function, the RPE monolayer also serves as an immunologic barrier by releasing soluble mediators and expressing cell surface molecules that are involved in regulating immune privilege [40].

The RPE monolayer has the ability to secrete a plethora of immunosuppressive factors, including interleukin 10 (IL-10) [41], transforming growth factor β (TGF-β), pigment epithelium-derived factor (PEDF), prostaglandin E2 (PGE2), somatostatin (SST), and alpha-melanocyte-stimulating hormone (α-MSH) [42]. Additionally, RPE cells can express membrane-bound negative regulators, such as Fas ligand (FasL) [43] and programmed death 1 ligands (PDLs), which are involved in inhibiting the activation of intraocular immune cells.

Programmed cell death 1 (PD-1) belongs to the cluster of differentiation 28 (CD28) family of inhibitory receptors and is predominantly expressed on immune cells. PD-1 binds to negative regulators like programmed death ligand 1 (PDL1), which is expressed by the RPE [44]. Inflammatory T cells such as helper T cell 1 (Th1), helper T cell 17

(Th17), helper T cell 22 (Th22), and naive T cells can all be directly suppressed by PDL1. Elevated PDL1 expression on RPE cells can increase their suppressive effect on T cells through a relationship with PD1, hence reducing T cell proliferation. The harnessing of this mechanism has been suggested as a way to promote the healing of uveitis [45].

The distribution and integrity of RPE cells in the retina has a significant impact on microglia. Cultured RPE eyecups from laser-damaged eyes were found to show considerably lower amounts of α-MSH in their conditioned media [42]. Macrophages and granulocytes were also reported to gather alongside microglia near the laser burn site in the retina [46]. Altered expression levels of MHC class II [47] and of co-stimulatory factors such as CD40 and CD86 on these activated microglia further supports the critical role that an intact RPE monolayer has on the regulation of immune cells. Furthermore, RPE injury may cause retinal microglia to secrete proinflammatory cytokines, which may then exacerbate neuronal damage. This emphasizes the role of physical damage to the RPE monolayer on immune cell infiltration and inflammation, with implications for both laser wounding and age-related macular degeneration [48].

Studies have demonstrated that microglia residing within the retina are influenced by the ocular microenvironment to promote Treg cell activation. Microglia have been shown to significantly suppress immune activity, as evidenced by their co-expression of Nitric Oxide Synthase 2 (NOS2) and Arginase 1 [42]. These have been implicated in suppressing the immune attack on tumors [49]. Interestingly, co-expression of NOS2 and Arginase 1 can be induced in microglia treated with RPE soluble factors such as α-MSH [50], and NPY which can alter phagocytosis and enhance apoptosis in activated effector T cells. Neuropeptide α-MSH could be used as a therapeutic strategy, since α-MSH2 possesses immune regulating/anti-inflammatory capabilities, as well as contributing to the mechanism of ocular immune privilege. In a recent study, α-MSH was shown to mitigate the severity of ischemia/reperfusion-induced retinal damage under hyperglycemic conditions [51]. These beneficial effects of α-MSH may have important therapeutic implications for ischemia/reperfusion in hyperglycemic individuals.

5. Relevant Epi-Transcriptomic Mechanisms in RPE

Methyltransferase-like 14 (METTL14) is a critical component of the N6-methyladenosine (m6A) methyltransferase complex, which catalyzes the methylation of m6A in mRNA or non-coding RNA transcripts. This directly regulates m6A levels and the expression of microtubule-associated protein-2 (MAP2). Studies have shown that METTL14 silencing in RPE cells reduces phagocytosis and proliferation while increasing apoptosis. Similarly, MAP2 overexpression has the same effects as METTL14 knockdown in RPE cells, supporting the notion that METTL14 regulates the expression of MAP2 through m6A modification. Notably, the expression of METTL14 is significantly reduced in patients with retinitis pigmentosa (RP), while MAP2 is highly expressed in the retina of AMD patients [52]. These findings suggest that therapeutic strategies targeting the m6A modification of MAP2, or the METTL14/YTHDF2/MAP2/NEUROD1 signaling axis, may prove effective in treating RPE-associated ocular diseases.

miR-125 levels may play a relevant role in the metabolic activity of RPE cells, since this microRNA can regulate the expression of the most crucial glycolytic enzyme, hexokinase 2. Overexpression of miR-125b was found to significantly attenuate hyperglycemia-induced RPE cell death [53]. These findings suggest a novel mechanism for miRNA-mediated cellular protection against RPE cell death and represent a promising approach for treating diabetic retinopathy.

Another well studied miRNA in RPE is miR-192. This miRNA is downregulated by high glucose levels, while its overexpression can counteract pyroptosis induced by hyperglycemia. The mechanisms underlying miR-192 activity appear to be associated with FTO (fat mass and obesity-associated), a demethylase RNA enzyme. FTO in turn controls the expression of NOD-like receptor thermal protein domain associated protein 3 (NLRP3), a member of the inflammasome complex and pyroptosis pathway [54]. A novel miRNA in RPE, termed miR-191-5p, influences a transcriptional regulatory mechanism that controls NLRP3. Specifically, the decrease in endogenous miR-191-5p expression induced by Amyloid β causes damage to the RPE. This results in upregulation of its target gene, C/EBPβ, which acts as a positive transcription factor for NLRP3 and also upregulates the downstream inflammatory factors Caspase-1 and IL-1β. These findings suggest an anti-inflammatory effect of miR-191-5p in Aβ_{1-40}-induced RPE impairment. They also shed light on novel preventive or therapeutic approaches for AMD-associated RPE impairment. Taken together, these data suggest that epi-transcriptomic mechanisms play important roles in RPE cells and could be potential targets for novel therapeutic approaches.

6. Conclusions

Recent research on retina-related diseases has demonstrated that biological and immune factors within the retina play a prominent role in the pathogenesis of various diseases, including age-related degenerative diseases and inflammation-related diseases. The principal finding of this systematic review is the existence of moderate-quality evidence supporting a biological immune mechanism within the retina, suggesting potentially novel treatment options. Therefore, a greater understanding of this subject may lead to the development of additional treatment strategies for patients with retinal diseases, both prophylactic and additive,

to minimize progressive irreversible vision loss. However, additional therapeutic targets need to be identified, together with the clinical viability of these new treatment alternatives.

Author Contributions

XJZ and JXH provided help and advice on the research direction. XYZ contributed to the conception and design of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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