

Immune regulation in the retina

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Abstract Immune reactivity in the retina can be critically important in inflammation and infections, but regulation of this response is essential. The retinal pigment epithelial (RPE), a unique retinal cell, displays a number of essential functions to support the health of the retina. In this review, we highlight how the RPE cell plays a pivotal role in immune defense. The RPE cell orchestrates both innate and adaptive immunity since it expresses TLRs, complement components, MHC class I and II molecules, and serves as an antigen presenting cell. Moreover, both of these immune responses result in the production of a plethora of cytokines, mainly proinflammatory. In order to counteract these inflammatory factors and silence unwanted immune reactivity, the RPE cell also generates suppressive molecules. Recently, chronic immune reactivity has been implicated in a number of retinal diseases, such as age-related macular degeneration (AMD). Current evidence suggests that the generation of excessive retinal inflammation may be the consequence of a loss of RPE immunosuppressive factors. Herein, we summarize the varied interactions of the RPE cell with the immune response and highlight how the RPE cell survives and participates in this dynamic environment.

Keywords RPE · IFN- β · AMD · Retina · ECOR

Introduction

The retina is a highly specialized tissue that serves a central role in the visual cycle. Damage to any of the intricate structures of the retina can lead to visual impairment and blindness. There are approximately 6 million people in the United States diagnosed with

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retinal diseases. Although retinopathies consist of a diverse group of disorders, immunity can often be a part of the pathogenesis in many of these diseases. For example, ocular infectious diseases and autoimmune disorders clearly have an immune component. More recently, immune reactivity has been implicated in retinal diseases, such as AMD, diabetic retinopathy, and retinitis pigmentosa (RP) [1, 2]. In fact, compelling evidence now suggests that inflammatory responses may be an underlying factor in the initiation and progression of these retinal diseases.

Recent discoveries have highlighted the important role of the immune response in the eye and our understanding about suppressive factors that regulate immunity in the ocular microenvironment. New information about innate immunity and the involvement of complement, TLRs, cytokines, and the RPE cell in regulating immune-mediated inflammation offers exciting new avenues for intervention. This report will begin with a brief overview of an inimitable cell in the retina, the RPE cell. How this cell participates in both innate and adaptive immune responses within the retina as well as its new role as an immunosuppressive factor in the eye will be reviewed. Finally, an animal model of a virus-triggered retinal degenerative disease that recapitulates key elements of human retinal degenerative diseases will be discussed.

The RPE cell

The retinal pigment epithelium consists of a single layer of cells of neural ectoderm origin which lie between the photoreceptors of the neural retina and the blood-rich choroid. Along with the photoreceptor layer, the RPE is an indispensable component of the visual process. Physiologically, the RPE cell displays a number of essential functions to support the neural retina. These vitally important cells phagocytize the shed discs from the photoreceptor outer segments, transport nutrients from the choroid into the retina and transport waste in the reverse direction. This cell also absorbs light and provides adhesive properties for the retina [3]. Immunologically, the RPE cell plays a pivotal role in immune responses. The RPE cell expresses Toll-like receptors, complement components, Fc-gamma receptors, responds to IFN- γ treatment and up-regulates the expression of both MHC Class I and II molecules, and serves as a resident APC in the retina [4–8]. In addition, this cell is a rich source of cytokines, chemokines, and growth factors that may contribute to or limit a variety of pathologic processes [9–12]. Finally, the RPE cell serves as a primary site for retinal damage or alterations that may have important consequences resulting in ocular pathology. For example, inherited retinal degenerative diseases can be associated with mutations of RPE cellular genes [13, 14]. Also, AMD and diabetic retinopathy can be associated with early damage and atrophy of the RPE cell [15, 16]. In addition, numerous *in vivo* and *in vitro* studies have identified this cell as an ideal target for infectious agents, such as cytomegalovirus in CMV retinitis, *Toxoplasma gondii* in ocular Toxoplasmosis, and more recently, coronavirus in experimental coronavirus retinopathy [9, 17–20]. Despite the upsurge of interest in the RPE cell and its critically important role in retinal health and disease, the exact mechanisms of how the RPE cell participates in regulation remain largely unclear.

Innate immune responses in the retina

Toll-like receptors, TLRs, are a family of evolutionary conserved innate immune recognition molecules that sense molecular patterns associated with microbial pathogens. TLR

recognition of these specific microbial patterns leads to a signal transduction cascade that generates a rapid and robust inflammatory response marked by cellular activation and the production of a variety of cytokines, including proinflammatory cytokines, cytokines that promote T cell differentiation, type 1 interferons, and chemokines. The TLR molecules, which serve as the first line of defense, are now thought to be present on numerous cell types within the body [21]. Just recently, these sentinel molecules were described on the RPE cell. It is not surprising that the RPE cell is endowed with many of the TLRs, since it is strategically situated and can provide a rapid defense system for the retina [22]. Using real time PCR analysis of TLR gene expression, we described the presence of TLR1 through 7, 9, and 10 on human RPE cells. Interestingly, TLR3, an important TLR for defense against virus infection, is highly expressed on these cells. TLR3 recognizes a dsRNA motif, an intermediate product of virus replication or Poly I:C, an analog of dsRNA. When we analyzed signaling through TLR3, it was discovered that the RPE cells secreted several proinflammatory cytokines, chemokines, and adhesion molecules, including, IL-6, IL-8, MCP-1, and sICAM-1. One of the cytokines that was highly expressed by RPE cells was IFN- β . It is well known that the IFNs are involved in numerous immune interactions and induction of type 1 IFN gene expression is an essential component of innate immunity [21]. Additionally, IFN- β is a potent anti-viral cytokine as well as an immunoregulatory and anti-proliferative cytokine [23]. Nevertheless, its role in the retina had not yet been described. Additional kinetic studies revealed that IFN- β levels continued to increase over a 48-h period and this continuous IFN- β production by RPE cells was associated with the up-regulation of IRF7 gene expression, a known positive feedback molecule for IFN- β production [24]. Since IFN- β is a potent immunosuppressive cytokine that is effective in treating patients with MS, we have continued to explore this property of IFN- β in the retina. These findings enhance the cytokine repertoire of RPE cells and underscore that this cell produces not only inflammatory cytokines important for immune responses but also immunosuppressive cytokines that serve to down-regulate immune reactivity within the posterior segment, a vital mechanism for protecting retinal tissue from excessive or unwanted damage.

Interest in TLR molecules in the retina has continued at a rapid pace, and recent work has demonstrated a potential involvement of TLRs in a variety of retinal diseases. For example, TLR polymorphisms have been associated with AMD [25, 26]. Investigations on the utility of siRNA treatment for AMD identified that siRNA was signaling through TLR3. This signaling process results in the inhibition of VEGF expression [27]. Future experiments are needed to better define the role of TLRs in human ocular diseases and to offer additional strategies to limit or boost TLR function with the hope of generating new therapies.

Adaptive immune responses in the retina

Perhaps, one of the most productive research areas during the last couple of decades has been devoted to antigen presentation and the factors that define this central component of adaptive immunity. Our laboratories provided the initial description of how the RPE cell functions as an APC [4–6, 28]. First, we noted that when retinal tissue from patients with uveitis, RP, or sympathetic ophthalmia were evaluated, the RPE cell expressed MHC class I and II molecules and the infiltrating T cells contained IL-2 and IFN- γ . Next, our early in vitro studies demonstrated that IFN- γ treatment up-regulated both class I and class II molecules on RPE cells and hence equipped this cell to participate as a resident APC in the

retina. Both retinal and non-retinal (BCG) antigens can be processed and presented by the RPE cell [5]. These findings were an essential first step leading to further investigations underscoring the important role of the RPE cell in inflammatory, infectious, and degenerative processes. In order to further elucidate fundamental mechanisms of disease progression, we choose an animal model. Experimental autoimmune uveitis, EAU, is a T cell-mediated disease that mimics human uveitis. Using this model system, we showed that anti-MHC class II antibody treatment delayed the onset of EAU and diminished the severity of disease [29]. Furthermore, this treatment down-regulated expression of MHC class II molecules on RPE cells and limited infiltration of inflammatory cells into the retina. Over the years, EAU has continued to serve as an excellent model in which to delineate the various components of retinal inflammation and to explore addition therapies that may modify this disease process.

RPE cell-associated cytokines and immunoregulation

The concept that natural regulatory mechanisms exist in the ocular microenvironment to restrain immune-mediated inflammation and exaggerated immune reactivity is not new. Over 60 years ago, Sir Peter Medawar, the Nobel laureate and pioneer of transplantation immunology, identified the concept of immune privilege in the brain, the eye, and the pregnant uterus [30]. In the anterior chamber of the eye, he eloquently demonstrated that transplanted skin allografts survived without being rejected. He attributed the survival to immunologic ignorance. Today, we recognize that ocular immune privilege extends to the retina as well and is operational due to an extensive array of regulatory mechanisms acting in concert to prevent immunologic inflammation and maintain tissue integrity. Some of these mechanisms include, the unique anatomic features of the blood-retinal barrier, a lack of lymphatic drainage pathways, immunomodulatory cytokines, and regulatory T cells. This section will identify how the RPE cell survives and participates in this dynamic environment.

One of the major functions of the RPE cell in regulating immunity is the production of cytokines. Many of the cytokines produced, such as IL-6, IL-8, and MCP-1 are mainly proinflammatory. Nevertheless, the eye has also evolved suppressive mechanisms to down-regulate the immune response. For example, the RPE cell participates by releasing a variety of suppressive factors, including TGF- β , IL-11, and IFN- β , and hence, the immune response can be silenced. These three molecules will be addressed briefly [10, 24].

TGF- β

TGF- β is a multifunctional cytokine that regulates a variety of cellular functions including cell growth, immune reactions, differentiation, and extracellular matrix synthesis and remodeling. A number of studies have identified that TGF- β plays an active role as an immunosuppressive factor in the eye [31]. For example, investigators have shown that TGF- β contributes to ocular immune privilege by down-regulating APC function as well as inhibiting selected T-cell functions. Moreover, TGF- β is associated with retinal disorders such as retinal detachments, proliferative vitreoretinopathy, and choroidal neovascularization. We have investigated the nature and mechanism of TGF- β production by human RPE cells. These studies have revealed that the RPE cell is a major source of TGF- β in the retina and TGF- β itself can activate RPE cells to produce VEGF, PDGF, and heme oxygenase [10, 11, 32].

IL-11

The second immunosuppressive cytokine is IL-11. IL-11, a 19-kDa secreted protein, is a pleiotropic cytokine with anti-inflammatory, cytoprotective, and hematopoietic actions [33]. We recently demonstrated the production of IL-11 by human RPE cells [34]. These cells do not constitutively produce IL-11. However, treatment with TGF- β , IL-1, and TNF- α results in the up-regulation of IL-11 gene expression and protein production. Moreover, we observed that IFN- γ significantly inhibits IL-11 expression in human RPE cells. This is the first description of IL-11 in ocular tissues and will require additional studies to explore the potential role of this immunosuppressive cytokine in retinal diseases.

IFN- β

Although the IFNs were originally identified as potent anti-viral molecules, we now recognize that their influence extends to a diverse array of functions, including immunoregulatory and anti-proliferative. We hypothesize that IFN- β produced by RPE cells primarily through TLR 3 signaling and secondarily by autostimulation (IRF7) is a major regulator limiting immunopathologic damage in the retina [23].

Because chemokines and adhesion molecules orchestrate immune cell migration into the retina during an immune response, we evaluated the effect of IFN- β on these molecules. The chemokine, CXCL9 (MIG), is an IFN- γ inducible chemokine that attracts T cells and NK cells to the site of inflammation. Both cell types contain the receptor, CXCR3, on their surface. Several studies have identified these molecules as key components of host responses in autoimmune diseases, cancer, and virus infections. We have also shown that CXCL9 and CXCL10 are highly expressed within the retina and, as we discuss in another section, may modulate innate immune responses in experimental coronavirus retinopathy [12].

Recently in a series of *in vitro* studies, we demonstrated that cytokine activation of RPE cells resulted in the production of CXCL9, CXCL10, and CXCL11 as well as sICAM-1. However, pretreatment of RPE cells with IFN- β resulted in the inhibition of sICAM-1 production and the elimination of CXCL9 production. This treatment did not alter CXCL10 or CXCL11 production. Furthermore, anti-IFN- β antibody blocked the inhibitory action of IFN- β . Real-time PCR analysis revealed that IFN- β treatment inhibited the gene expression of ICAM-1 and CXCL9. These results indicate a critical role for RPE-cell-derived IFN- β that inhibits CXCL9 and ICAM-1 expression in the retina and suggest that this inhibition is an immuno-suppressive mechanism that protects the retina from excessive inflammation and damage [23].

Retinal diseases

Many investigators are now exploring the various ways in which the immune response participates in retinal pathology. Our analysis of pathogenic processes in retinal diseases has also incorporated studies on RPE65 in inherited retinopathies, an animal model of a virus-triggered retinal disease and translational research studies related to retinal vasculitis and autoimmune retinopathy [35, 36]. We will discuss the first two studies in this report.

RPE65

Initial studies were performed to characterize the human RPE cell by developing murine monoclonal antibodies to identify epitopes that were unique to the RPE cell [37]. One unique epitope from 1,000 clones was identified that only reacted with RPE cells. This epitope was evolutionarily conserved, reacting with a variety of species and was termed RPE65 [13, 37, 38]. Investigators from a variety of laboratories have utilized this information to identify the role of RPE65 in human retinal disease. RPE65 is required for the production of a retina-specific form of vitamin A. Mutations in RPE65 gene have been identified in a retinal degeneration called Leber congenital amaurosis, and recently, RPE65 gene therapy was used successfully in three patients with this disease [27, 39].

Experimental coronavirus retinopathy [23]

Experimental coronavirus retinopathy (ECOR) is an animal model system that we generated in the 1990s to demonstrate that a virus could trigger a progressive retinal degenerative disease. Studies during the past 19 years have identified that this retinal degenerative disease is composed of three basic components; a virus component, a genetic component, and an immunologic component [40, 41]. In our system, we selected a naturally occurring neurotrophic strain (JHM) of a mouse hepatitis virus.

Initial studies in the ECOR system showed that inoculation of this JHM strain into the vitreous or anterior chamber of BALB/c mice resulted in retinal tissue damage [40, 41]. Infectious virus could be detected within the retina between 1 and 6 days post inoculation (PI), reaching a peak level of $10^{4.5}$ pfu/ml at day 3 [42]. Virus antigen was also identified within the retina between day 2 and 6 PI [40]. Virus antigen was first detected within the RPE cell and the ciliary body epithelial cell at day 2. After day 7, infectious virus and viral antigen could not be detected. However, *in situ* hybridization studies identified that the viral RNA persisted within the retina until 60 days PI [43]. Anti-virus neutralizing antibodies were first noted at day 7 PI and coincided with the disappearance of infectious virus and viral antigen.

After virus inoculation, two distinct patterns of retinal pathology were noted [40]. The early phase of the disease, day 1–8, was characterized by retinal vasculitis and perivasculitis. The late phase of the disease, after day 10, was characterized by retinal degenerative changes. The retinal layers revealed disorganization with large areas of outer and inner segment loss. In addition, the RPE cells were morphologically abnormal with focal RPE cell swelling, proliferation, and RPE cell atrophy. Analysis of retinal cell function also revealed dramatic changes [44, 45]. There was a significant decrease or complete loss of electroretinogram (ERG) patterns and the disappearance of an important transport protein in the retina, the interphotoreceptor retinoid-binding protein (IRBP). It is worth noting that these distinctive ocular pathologies have been described in human retinal degenerative diseases [35].

The host immune response to this virus infection was evaluated by tracking the cellular infiltrate and identifying the cytokine profile within the retina [46]. The most prominent infiltrating cell was the macrophage followed by the T cell. CD4 T cells were present at days 3 and 6, while CD8 T cells were observed at day 6 and 10 PI. On day 4, retinal gene expression and serum protein expression revealed the presence of IL-6, IFN- γ , and TNF- α . The presence of retinal mRNA for IFN- γ was also associated with the up-regulation of MHC Class I and II molecules within the RPE cell. Interestingly, the RPE cell is also the

first cell to express new viral antigens during the infection *in vivo* and is persistently infected *in vitro* [47].

The genetic constitution of the host can be a critical factor in determining the outcome of a virus infection [48]. We therefore evaluated the possible role of host genetics in ECOR and noted that different strains of mice behave differently after virus infection. Two strains of mice, BALB/c and CD-1, were extensively studied after coronavirus infection. During the early phase of the disease (day 1–8), the virus infects and replicates within the retina of both BALB/c and CD-1 mouse strains [48]. However, on days 10–140, only the BALB/c mice experience a late phase of the disease which is marked by a retinal degeneration. The CD-1 mouse does not undergo the retinal degenerative phase, but rather, the retina reverts to a normal architecture within 20 days.

We next examined very early cytokine and chemokine profiles as a measure of intensity of immune reactivity in coronavirus-infected mice. These studies identified a distinct difference in the early innate immune response between the two mouse strains [12]. These differences are noted in the production of IFN- γ , and the two chemokines triggered by IFN- γ , CXCL9, and CXCL10. At day 2 and 3 PI, BALB/c mice have high levels of IFN- γ , CXCL9, and CXCL10 in their sera. At the same time, significantly lower levels of these molecules are detected in sera from CD-1 mice. Moreover, real-time PCR analysis of retinas identified that CXCL9 and CXCL10 gene expression is significantly greater in retinas from BALB/c mice in comparison with CD-1 mice. CXCL9 and CXCL10 interact with CXCR3 present on activated T cells and NK cells, and they direct the migration of these cells to specific targets, such as the retina [12]. These studies are truly exciting and identify possible mechanisms that allow the BALB/c mouse to have a robust immune response that could trigger an autoimmune component.

In ECOR, the late phase of the disease was associated with the lack of direct evidence for viral replication within the retina. Inasmuch as viruses are known to trigger an autoimmune phenomena and some human retinopathies may be associated with autoantibody formation, we studied the possible production of antiretinal autoantibodies [18]. We found that the retinal degenerative process in BALB/c mice was associated with the presence of antiretinal autoantibodies that were directed against the neural retina and the RPE. These autoantibodies were not found in sera from normal or mock-injected mice. Likewise, none of the CD-1 mice developed antiretinal autoantibodies. Thus, the mice that failed to develop antiretinal autoantibodies also failed to develop a retinal degeneration. These findings suggest a role for autoimmunity in the pathogenesis of ECOR.

Summary: future directions

In this report, we summarize the regulatory actions of the RPE cell in innate and adaptive immune responses. The RPE cell is a rich source of both proinflammatory and immunosuppressive cytokines. Studies exploring the factors that trigger IL-11 and IFN- β production in human RPE cells and mechanisms by which these cytokines exert immunoregulation are in progress. Thus, a better understanding of the immunoregulatory actions of IL-11 and IFN- β may provide insight into novel therapeutic modalities and interventional strategies. Moreover, studies are on going to determine whether defects in the RPE immune suppressive functions are associated with chronic inflammation in retinal diseases, such as AMD [49].

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