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Müller Glia in Retinal Innate Immunity: A perspective on their roles in endophthalmitis

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Abstract

Müller cells are the predominant glial cell type in the retina and have a unique anatomy, with processes that span the entire retinal thickness. Although extensive morphological and physiological studies of Müller glia have been performed, much less is known about their role in retinal innate immunity, specifically in infectious endophthalmitis. They were found to express Toll-like receptors (TLRs), a major family of pattern recognition receptors (PRRs) that mediate innate responses and provide an important mechanism by which Müller glia are able to sense both pathogen- and host-derived ligands in the vitreous and the retina. An increasing body of evidence suggests that TLR-signaling mediates beneficial effects in the retina via production of pro-inflammatory cytokines/chemokines, antimicrobial peptides and neuroprotective growth factors to restore tissue homeostasis. In this review, we discussed retinal innate immunity in general with emphasis on the role of Müller glia in initiating retinal innate defense.

Keywords

Retinal innate immunity; Müller glia; Toll-like receptor; Endophthalmitis; Inflammation; Neuroprotection

Introduction

Endophthalmitis is a vision-threatening condition that requires prompt diagnosis and treatment to prevent vision loss.¹ Bacterial endophthalmitis, with an estimated incidence between 0.07% and 0.3%, is among the most severe complications of cataract surgery, one of the most common surgical procedures performed in the aged population worldwide.² Moreover, the incidence of endophthalmitis is likely to grow in the coming years due to increasing use of multiple intravitreal injections for the treatment of age related macular degeneration and diabetic retinopathy.³ The incidence of endophthalmitis after ocular surgery or injection is 0.016% to 0.46%,³⁻⁸ and after trauma is 0.9% to 17%.^{3, 9-11} Among the bacterial pathogens, most postoperative endophthalmitis cases are caused by Gram-positive staphylococci, with *Staphylococcus epidermidis* and *Staphylococcus aureus* accounting for 70% and 10% of all cases, respectively.¹ Compared to *S. epidermidis*, *S. aureus* is a more pathogenic bacterium and causes severe inflammation and destruction of the retina, resulting in worse visual prognosis even with treatment.¹² As such, *S. aureus* infections are a major public health concern due to the bacterium's ubiquitous presence and

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increasing resistance to antimicrobial agents.^{13, 14} In the past, methicillin resistant *S. aureus* (MRSA) was confined to hospital-related infections, but now it has been identified with increasing incidence in community-acquired infections.¹⁵ Therefore, the overall incidence of MRSA infections has increased in recent years. Infections with community-associated MRSA subtypes can lead to aggressive infections in the eye even in patients with no previous hospital exposure.¹⁶ Since the publication of the Endophthalmitis Vitrectomy Study in 1996, several recent studies have reported an increased incidence of MRSA endophthalmitis after cataract surgery.^{17, 18} This serious infection may not be preventable by the use of preoperative fluoroquinolone antibiotics.¹⁷ Although this class of powerful antibiotics covers a broad spectrum of organisms, they seem to be less effective against MRSA. To date, vancomycin has been regarded as the ultimate drug for the treatment of endophthalmitis caused by MRSA. However, in the past few years the appearance of vancomycin-resistant *S. aureus* strains in hospital settings, food and within the environment has become an important public health concern.^{19, 20}

In this context, one long-term goal would be to understand the regulation of retinal innate immunity in endophthalmitis. The knowledge derived from such studies would conceivably serve as a cornerstone for the identification of target candidates for intervention. Recognition of microbial infection and initiation of the host defense responses are controlled by multiple mechanisms. The host innate immune system uses a series of pattern recognition receptors (PRRs) to detect the presence of pathogens, thus allowing rapid host defense responses toward invading microbes.²¹ A major class of such receptors is a family of proteins, famously known as “Toll-like receptors” (TLRs).²² Previous studies from our laboratory and others have revealed an important role of TLRs in mediating innate immune responses at the ocular surface.^{13, 23-27} Although the expression and function of TLRs in many tissues have been studied extensively, studies have just begun to investigate their role in the retina and various retinal diseases. Earlier retinal pigment epithelial (RPE) cells have been shown to express TLR2, TLR3, TLR4 and TLR9.²⁸ However, many questions fundamental to the understanding of retinal innate immunity remain elusive. For example: which cell types in the retinal tissue are involved in the recognition of invading pathogens in endophthalmitis? Another pertinent question has been if the TLR innate immune system functionally exists and operates in the retina? If yes, which TLRs are involved and how do they orchestrate among themselves? How might retinal cells, most importantly microglia and Müller cells, respond to bacteria or TLR ligand(s) challenge?

Several mouse models of bacterial endophthalmitis and cultured retinal cells have been utilized to understand host-pathogen interaction in endophthalmitis and to address some of these questions. In this review, we shall discuss the myriad roles Müller cells may play in different immunological backgrounds, with reference to bacterial endophthalmitis.

Pathogenesis of Bacterial Endophthalmitis

Bacterial endophthalmitis occurs when bacteria, primarily from the ocular surface, enter through direct external invasion following an intraocular injection, trauma or surgery, or hematogenously by crossing the blood-retinal barrier. As discussed in previous reports, the disease severity of bacterial endophthalmitis is directly related to the virulence potential of the infecting bacteria.²⁹ For example, retinal damage caused in cases of *S. epidermidis* endophthalmitis is relatively low compared to those caused by highly virulent pathogens such as *Bacillus cereus*, *Enterococcus faecalis* and *Staphylococcus aureus*. Figure 1 shows a working model of the pathogenesis of bacterial endophthalmitis and involvement of retinal residential cells in initiating innate responses.

Retinal Innate Immunity

Components of Retinal Innate Immunity

The retina is considered an immune-privileged tissue. Therefore, a multitude of mechanisms operate in the eye to limit the harmful levels of inflammation arising due to foreign substances. At the same time, diverse means are at play to defend the eye from invading pathogens. Ocular cell surface and soluble factors maintain the eye's immunosuppressive nature.^{1, 30} Ocular fluids contain a variety of cytokines, neuropeptides and growth factors, such as: transforming growth factor, soluble Fas ligand, vasoactive intestinal peptide, calcitonin gene-related peptide, α -melanocyte stimulating hormone, etc. These signaling molecules either suppress inflammation directly e.g. α -melanocyte stimulating hormone attenuates TLR4-mediated inflammation by triggering interleukin-1 receptor-associated kinase-M,³¹ or indirectly by impairing activation of primed and alloreactive T-lymphocytes, suppressing functional activation of macrophages and modifying antigen processing and presentation by professional antigen presenting cells. Eye-derived antigens, which escape when local antigen presenting cells migrate through the blood to the spleen, selectively activate regulatory T-lymphocytes, which in turn downregulate the development of the antigen-specific delayed response.^{32, 33} Fas ligand, an ocular cell surface factor, enhances defense by activating resident glia or neutrophils.^{3, 34} Defensins are produced in the vitreous; however, defensin levels seem too low to be antimicrobial.^{3, 35} Alpha-B crystalline is expressed in the retinal neurons and pigment epithelium,³ which prevents activation of caspase 3, rescuing retinal cells from apoptosis.^{3, 36} Although the immunological environment in the vitreous, which directly flushes the retina, is not well defined, it still appears to be under strict surveillance and tight control.

Role of Glial and Other Retinal Cells in Retinal Innate Defense

Retinal glial cells are presumed to play a major role in the recognition and subsequent clearance of pathogens during infection and are believed to constitute the first line of defense in the retina. There are three types of glia found in the retina: Müller glia, astroglia (or astrocytes), and microglia. As the bystander effect of infiltrating cells accounts for the major damage in the later stages of infection or injury, the role of another cell type, namely retinal pigment epithelial (RPE) cells becomes crucial. Although this review revolves mostly around Müller glia, it is important to briefly discuss RPE cells and the other two glial cell types due to their continuous interplay with Müller glia.

Microglia—Microglia are the macrophage equivalent for the central nervous system and function as immune sentinels. However, unlike Müller glia and astroglia, they supposedly are not a typical neuroglia strictly owing to their mesodermal origin. They span the entire central nervous system as a network of resting immunocompetent cells and constantly patrol the intraocular space for changes in morphology, migration and proliferation.³⁷⁻³⁹ They exhibit characteristic phenotypes, i.e. they appear ramified in their mature resting state, whereas on activation they withdraw their filopodic processes to acquire an oval shape.⁴⁰ Once activated, microglia travel to areas of damage and phagocytize pathogens and cellular debris. Similar to typical resident macrophages in the first line of host defense, microglia play a pivotal role as actively phagocytic antigen presenting cells acting in concert with infiltrating immune cells. They also produce growth factors and aid in the remodeling of retinal neurons.^{38, 41-43} Two recent reports have demonstrated an important contribution of microglia in providing retinal innate defense in staphylococcal endophthalmitis.^{23, 44}

Astrocytes—These are support cells of the central nervous system and have characteristic processes radiating out of a flattened cell body. They share many functions with Müller glia. Their distribution is almost entirely restricted to the nerve fiber layer of the retina.⁴⁵

Astrocytes are arranged over the surface of the axon bundles of ganglion cells as they course into the optic nerve head, forming almost a tube through which the axons run. The blood vessels running in and traversing through the ganglion cell bundles are also ensheathed by both the processes and occasional cell bodies of astrocytes. Functioning as axonal and vascular sheaths, astrocytes become a part of the blood-retinal barrier. Being abundant sources of glycogen, they also provide energy to neurons. Apart from regulating the ionic homeostasis, they also maintain the intraocular compartments through the secretion of trophic factors and antioxidants.^{41, 46-48} It is interesting to note that retinal astrocytes were also found to recognize and respond to TLR ligands.⁴⁹ Thus, it can be postulated that retinal astrocytes might also be involved in defending the retina from infectious agents.

Müller glia—These cells represent the most abundant glia in the retina. Apart from their supporting role, they provide structural stabilization to the retina. They are the principle neuroglia in the retina in the sense that they share a common progenitor with the retinal neurons,⁵⁰ and live in a symbiotic relationship with them. This is in conjunction with the fact that Müller glia are the only macroglial cells which span the entire thickness of the retina and contact virtually all retinal neurons.⁵¹ They have a radial morphology,⁵² with cell processes extending from the soma in the inner nuclear layer (INL) to envelop neuronal cell bodies, processes and synapses,⁵² from the outer limiting membrane to the inner limiting membrane.⁴¹ Müller cells perform many crucial functions including the maintenance of ion homeostasis, ammonia levels and the blood-retinal barrier.⁵³⁻⁵⁹ Müller glia protect the retina from glutamate excitotoxicity via the glutamate-aspartate transporter.^{60, 61} Malfunction of this transporter may contribute to neuronal dysfunction and apoptosis.^{53, 62} They also protect photoreceptors and retinal neurons from cell death through the release of neurotrophic factors.⁶³ Müller cells are capable of dedifferentiating to cells resembling pluripotent stem cells.⁶³ As stem cells, they can replenish damaged neurons and create more Müller cells if necessary.^{53, 64} Recent studies have shown that Müller glia actively participate in retinal innate immunity, specifically in infectious conditions.

RPE cells—RPE cells are located between the light-sensitive outer segments of the photoreceptors and the blood supply of the choroid. RPE helps with light absorption and improves the quality of vision by absorbing scattered light. RPE also helps maintain the ion homeostasis in the subretinal space by epithelial transport of ions. RPE cells are also efficient phagocytes and clear the shed tips of photo-oxidized photoreceptor outer segments. RPE also secretes a good number of factors and signaling molecules such as: ATP, Fas-L, fibroblast growth factors, TGF- β , insulin-like growth factor-1, ciliary neurotrophic factor (CNTF), platelet-derived growth factor, vascular endothelial growth factor, lens epithelium-derived growth factor, etc.⁶⁵ But during infection or other instances of ocular injury, blood-retinal barrier maintenance becomes the most critical function of the RPE. In addition, they have been found to express TLRs,²⁸ and may even initiate proinflammatory responses themselves. Rat and human RPE have been shown to be capable of antigen presentation.⁶⁶ Recent findings have demonstrated that RPE plays a crucial role during infection by secreting cytokines like IL-6 and controlling the blood-retinal barrier.⁶⁷ As they constitute the outer blood-retinal barrier, they may be providing the first line of defense in endogenous endophthalmitis, where the pathogens are entering the retina via the choroid. Although, the incidence of endogenous endophthalmitis is relatively low, it is very common in immune-compromised individuals like diabetics.⁶⁸

Müller glia and retinal innate immunity

Müller cells actively participate in retinal innate immunity through several mechanisms including their Toll-like receptors, phagocytic function and secretion of cytokines and chemokines.^{53, 60, 69, 70} Müller cells also affect the adaptive arm of immunity by inhibiting

the proliferation of T-cells via membrane-bound molecules. The consequence of Müller cell activation is synthesis and release of pro-inflammatory cytokines and other potent mediators of inflammation. Inflammation is a non-specific event and therefore, can't distinguish between pathogen and host cells. Although essential for innate immunity against the pathogen, inflammation also brings a variable burden of non-specific tissue destruction. Owing to this 'non-specific' nature, this machinery, which has originally evolved as a protective response against non-self molecules, would become the sole cause of self-destruction in the absence of regulatory or protective mechanisms. As mentioned earlier, the retina is an immune-privileged tissue and is extremely sensitive to inflammatory damage. Therefore, protective mechanisms are of paramount importance for the preservation of the optical axis and function of the eye. Recent findings have demonstrated that Müller cells also act as saviors and through a multitude of mechanisms mediate neuroprotection in the retina. In this context, Müller cells appear to play a dual role and in the following sections, we will discretely discuss these two modalities of Müller cell function.

1) Activation of Müller Glia—In response to infection and in certain inflammatory scenarios, Müller cells show reactive gliosis. Müller gliosis is a generalized, more unspecific, but seemingly very complex response of the Müller glia toward injury or infection. Müller cells become activated by nearly every pathological challenge in the retina.^{53, 63} This activation results in Müller cell down-regulation of proteins involved in its homeostatic functions:⁵³ hypertrophy, proliferation and up-regulation of the intermediate filaments, glial fibrillary acid protein, nestin and vimentin.^{63, 70, 71} In fact, induction of glial fibrillary acid protein in the Müller cells is an early and very sensitive indicator of retinal pathology. Gliosis can be beneficial or detrimental to the retina depending on the timing and the immunological context. Acute gliosis is usually beneficial, as it tends to be conservative or non-proliferative.⁴¹ This type of gliosis is neuroprotective and limits further tissue damage. It involves the up-regulation of intermediate filaments, expression of neuroprotective factors and Müller cell hypertrophy.⁴¹ Proliferative gliosis is generally associated with chronic gliosis and is usually detrimental. In such a state, the integrity of the blood-retinal barrier is lost. As a result, the serum components leak into the perivascular space, causing proliferation of the Müller cells which involves the re-entry of Müller cells into the cell cycle.^{41, 63} Proliferation often results in glial scar formation, which prevents tissue repair. Gliosis can also harm the retina through Müller cell production of pro-inflammatory cytokines and nitric oxide (NO).⁶³ Activation of Müller glia causes down-regulation of the transcription factor Lhx2, which, under resting conditions checks the autonomous induction of reactive gliosis in Müller cells,⁷² and is also important for the secretion of neurotrophins (NTs).

2) Secretion of Pro-inflammatory Cytokines—Cytokines are proteins that regulate the immune system. There are pro- and anti-inflammatory cytokines. Chemokines are chemotactic cytokines. They specialize in the recruitment of leukocytes to an area of injury or infection. For instance, IL-8 attracts neutrophils.⁷³ Cytokines like IL-1, TNF- α and the chemokines promote inflammation by attracting leukocytes to the area, triggering signaling cascades and inducing endothelial cells to express adhesion molecules so the migrating leukocytes can adhere to the endothelium before infiltrating the damaged or infected tissue.⁷⁴ As a consequence of this adhesion-based facilitation, the infected site will quickly be teeming with a repertoire of immunocompetent cells. TNF- α also induces neutrophil secretion of IL-1 and IL-6, both mediators of chemotaxis.³ Anti-inflammatory cytokines like IL-4 and IL-10 block these signaling cascades.⁷⁴ Cytokines from the IL-6 family, like CNTF, are capable of inducing gliosis in Müller cells.⁷⁵ Müller glia are responsive to different pathogens by secreting pro-inflammatory cytokines and chemokines.⁵⁵ Müller glial exposure to TLR2 ligand, Pam3Cys, a triacylated lipopeptide, and Pam2Cys, a diacylated

lipopeptide, induces the expression of IL-6, IL-8, TNF- α and IL-1 β .⁵⁵ Infection of Müller glia with *Pseudomonas aeruginosa*, a Gram-negative bacterium, and *Candida albicans*, a fungal pathogen, result in increased levels of TNF- α and IL-6 secretion.

3) Direct Anti-microbial Activity of Müller Glia—Phagocytosis is a key component of the immune response. Macrophages, neutrophils and microglia play a role in the phagocytosis of infected retinal cells, pathogens and cellular debris during an inflammatory response. Müller glia have not been shown to phagocytize pathogens directly. However, Müller cells phagocytize foreign molecules that are injected into the eye.⁷⁶ Friedenwald and Chan observed Müller glial engulfment of injected pigment granules.⁶⁹ Similarly, Mano et al demonstrated phagocytic activity of cultured Müller glia towards fluorescein-labeled latex beads.⁷⁷ Based on these findings, we can postulate that Müller cells may contribute to host defense in endophthalmitis by directly engulfing bacteria. Müller glia can directly kill bacteria through production of antimicrobial peptides, reactive oxygen species and reactive nitrogen species. We recently showed that Müller glia produce the antimicrobial peptide LL-37 in response to TLR2 activation.⁷⁰ During an inflammatory response, they also produce TNF- α which causes the induction of NO and superoxide, O₂⁻.⁷⁸ These species combine to form peroxynitrite, which might also contribute to the antimicrobial activity of Müller glia.⁷⁸ Together, the combination of reactive nitrogen species and reactive oxygen species not only provides a potent bactericide, but also offers protection for retinal cells from free radical damage through NO's antioxidant properties.⁷⁹ *S. aureus* is extremely sensitive to this pair of molecules, but only when exposed first to reactive oxygen species followed by immediate exposure to reactive nitrogen species.⁷⁹

4) Expression of Innate Immune Receptors on Müller Glia—To date, 13 mammalian TLRs have been identified.⁵⁵ TLRs 1-9 have been identified in humans and mice, TLR10 is unique to humans and TLRs 11-13 are unique to mice.^{55, 80} Pathogen-associated molecular patterns (PAMPS) have been assigned to many TLRs. PAMPs are ideal targets for the innate immune system because they are molecularly conserved moieties, and they are unique to different classes of microbes.^{25, 81} TLRs can be categorized by their ability to recognize characteristic PAMPs present on the cell wall or in the genetic material (i.e. DNA or RNA),²⁵ of the microbes. TLRs 1, 2 and 6 recognize Gram-positive bacteria, whereas TLR4 binds to LPS on the surface of Gram-negative bacteria. TLR5 recognizes the flagellin of motile bacteria. TLRs 3, 7 and 9 recognize components of pathogenic nucleic acid.⁵⁵ We recently reported the expression of TLRs 1-5, 7 and 9 by Müller cells, suggesting their ability to provide innate defense against Gram-positive and Gram-negative bacteria and fungal pathogens.⁵⁵ Moreover, our studies demonstrated an important role of TLR2 in initiating Müller glia innate response to *S. aureus*. In response to bacterial challenge, Müller glia exhibited the activation of NF- κ B and p38 MAPK signaling pathways, increased secretion of pro-inflammatory cytokines and chemokines, as well as secretion of the antimicrobial peptide LL-37.⁷⁰ In addition to TLRs, Müller glia also express receptors for advanced glycation end products. These receptors help modulate the retinal innate immune response by triggering several pro-inflammatory signaling cascades: MAPKs including p38, Janus kinase/signal transducers and NF- κ B.⁸²⁻⁸⁶ Müller cells also regulate the innate response through NOD-like receptors. These receptors are capable of up-regulating and down-regulating the inflammatory response. NOD-like receptors can activate or inhibit NF- κ B and inflammasome signaling pathways,⁸⁷ inhibit autophagic processes or up-regulate pro-inflammatory cytokines.

Role of Müller Glia in Neuroprotection

In response to pathogens, Müller glia secrete NTs and growth factors in concert with pro-inflammatory cytokines and chemokines to protect retinal ganglion cells, bipolar neurons

and photoreceptors from the damaging effects of inflammation. Müller cells produce a variety of neurotrophic factors including: brain-derived neurotrophic factor (BDNF), CNTF, basic fibroblast growth factor, insulin-like growth factor, nerve growth factor, NT-3 and NT-4, glial cell line-derived neurotrophic factor, leukemia inhibitory factor and pigment epithelial-derived factor.⁶³ Müller glia have demonstrated a close relationship with microglia. Microglia are initially activated by pathogens. They then modify photoreceptor survival by controlling Müller cell production of NTs.⁶³ Müller cells that have been exposed to activated microglia express higher mRNA levels of glial cell -derived neurotrophic factor and leukemia inhibitory factor.⁸⁸ These results suggest that microglial/Müller cell crosstalk amplifies the neuroprotective abilities of Müller glia.⁸⁸ A study by Arroba et al. found that reducing the microglial population using clodronate-encapsulated liposomes greatly attenuated the anti-apoptotic effect of insulin-like growth factor on photoreceptors.³⁷ This indicates a necessary relationship between activated microglia and Müller cells for neuroprotection. It will be interesting to note here that during Müller gliosis there is concomitant activation of microglia, which further elucidates the close functional association of Müller cells and microglia.

1) Reception of Neuronal Cues by Müller Glia—Müller cells may respond to multiple cues from neurons including K^+ ions, metabolites such as CO_2 and neurotransmitters. These factors may actually cause differential changes in the state of Müller cells and important signaling functions may be ascribed to these changes. For instance, increases in the extracellular concentration of K^+ ions may cause a rapid depolarization of Müller cells, which in turn may result in glycogenolysis and increased intracellular Ca^{2+} inside Müller cells,^{89, 90} Similarly, extracellular release of CO_2 by activated retinal neurons may cause rapid intracellular acidification of Müller cells resulting in altered pH-dependent functions such as glutamate uptake.⁹¹ Müller cells express receptors for a wide variety of neurotransmitters, including the ones for amino acids, catecholamines, growth factors, hormones, etc. Binding of these ligands to their corresponding receptors may directly cause depolarization of Müller cells.⁹²⁻⁹⁴ Thus, there is a myriad of ways through which neurons may indirectly and directly signal to Müller cells.

2) Neuroprotective Functions of Müller Glia—Müller cells protect the retinal neurons in a number of ways. Selective destruction of Müller glia results in retinal dysplasia and photoreceptor apoptosis.⁹⁵ They limit fluctuations in the neuronal excitability through different layers of the retina by maintaining even levels of extracellular K^+ ion concentrations through a spatial buffering mechanism which involves active, as well as passive influx, temporary storage and release of K^+ ions. As mentioned previously, Müller cells protect the retina from glutamate excitotoxicity. Glutamate-aspartate transporters are expressed on the surface of Müller cells, which take up glutamate, abolishing its post-synaptic action. Müller cells also possess efficient machinery for the uptake of other amino-acid neurotransmitters such as glycine and γ -amino butyric acid. Müller cells express high-affinity Na^+/Cl^- -dependent γ -amino butyric acid transporters, and are the only cells responsible for γ -amino butyric acid uptake in the outer layers of retina. Neurotrophin levels show a dose-dependent increase when exposed to glutamate. BDNF levels in particular, are especially sensitive to rising concentrations of glutamate.⁹⁶ Müller cell-mediated neuroprotection was observed *in vitro* by culturing Müller cells with glutamate-sensitive neurons. These glutamate-sensitive neurons showed resistance to glutamate in the presence of Müller cells, demonstrating the neuroprotective function of Müller glia. There are two main receptors involved in neurotrophin-mediated neuronal survival, tropomyosin receptor kinase and the $p75^{NTR}$ receptor.^{60, 63} Tropomyosin receptor kinase receptors have a high affinity for pro-survival NTs, while $p75^{NTR}$ have a low affinity for anti-survival NTs.^{63, 97} In mice retina, the B receptor for BDNF is found in the pigment epithelium, Müller cells and

retinal ganglion cells, but not on the photoreceptors.^{63, 98} BDNF increases the survival of bipolar neurons by activating p75^{NTR} on Müller cells and increasing their secretion of basic fibroblast growth factor, which acts directly on bipolar neurons.^{63, 99} Many NTs are able to act directly on photoreceptors and/or inner retinal neurons. For instance, photoreceptors possess receptors for neurotrophin-3 and basic fibroblast growth factor, providing a direct survival-promoting effect. Photoreceptors and inner retinal neurons also have receptors for BDNF, glial cell line-derived neurotrophic factor, neurturin, CNTF, pigment epithelium-derived factor and fibroblast growth factor.^{63, 100-115} Müller cells' ability to secrete NTs and growth factors provides a key form of protection for the retina. We recently showed bacterial-challenged Müller glia produced NTs (ARVO poster, 2012 # 2769). Thus we hypothesize that in addition to production of inflammatory mediators, TLR-signaling also regulates the expression of NTs, which in turn play a pivotal role in protecting the retinal neurons from damage incited by infectious agents in endophthalmitis (Fig. 3). Studies are in progress to understand the underlying mechanism for production of NTs in endophthalmitis. A recent report has implicated the role of Müller cells in directly controlling inflammation. Under the influence of endocannabinoids, activated but not resting Müller cells may inhibit production of pro-inflammatory cytokines, at the same time upregulating anti-inflammatory molecules, thus favoring a more balanced and pro-survival milieu for retinal neurons.¹¹⁶

Signaling Mechanisms Involved in Müller Glia Activation

Activating signals for Müller cells may be derived from two sources: The first signal may come from the pathogenic infection, either directly through recognition of PAMPs displayed by microbes or indirectly via already activated microglia. The second class of signals come through neurons under stress, which may well be SOS calls. For instance, once the pathogenic ligand is bound to the TLRs expressed by Müller cells, the NF- κ B signal transduction pathway is initiated.^{25, 55} This pathway is shared by all retinal TLRs. From this signal transduction pathway, TLRs can then initiate other MAPKs such as p38 and ERK.⁵⁵ These pathways trigger the production and secretion of pro-inflammatory cytokines and chemokines, and are essential in initiating an inflammatory response. Through the secretion of NTs, like CNTF, Müller cells can also activate the JAK-STAT pathway.⁷⁵ Neurotrophins can also mediate their effects through Müller cells in an autocrine, as well as paracrine fashion as Müller cells themselves express NT receptors on their surfaces. CNTF is up-regulated during gliosis and quickly activates glial fibrillary acidic protein production in Müller glia.^{75, 117-120} Müller cells also activate the Wnt signaling pathway to provide retinal neurons with protection during instances of inflammation and injury.¹²¹⁻¹²⁶ Müller glia can modulate this neuroprotection through TLR4, which suppresses the Wnt pathway.¹²¹ In addition to signaling cascades initiated through TLRs or other possible but unexplored PAMP receptors, Müller cells may also exhibit signaling through second messenger systems such as cAMP, Ca²⁺ ions or IP3, e.g., higher extracellular K⁺ ion concentration causes Müller cell depolarization resulting in Ca²⁺ influx through voltage-gated Ca²⁺ channels.⁹⁰ Similarly, glutamate also elicits Ca²⁺ influx through non-NMDA receptors.¹²⁷ Activating signals may cause entry of Ca²⁺ into the cytosol even in the absence of extracellular Ca²⁺ ions through the release of Ca²⁺ ions from intracellular Ca²⁺ stores.⁹⁰ Signaling through intracellular secondary messengers is one of the oldest known mechanisms during physiological stress, most notably infection. Although recently, some focus has been generated in studying the ligand receptor-based mechanisms of immune regulation by Müller cells, specifically in the model of bacterial endophthalmitis, there still remains a big lacuna in our understanding of direct activation of secondary messenger systems in an infection scenario, such as bacterial endophthalmitis.

Conclusions and Future Perspective

The current treatment options for endophthalmitis involve intravitreal injections of antibiotics, which, while destroying the bacteria, also release lipopolysaccharides and peptidoglycan from their cell walls, thereby enhancing the inflammatory response leading to more tissue damage. Moreover, the effects (beneficial or detrimental) of corticosteroids, which are the only available treatment in ophthalmic solutions to suppress the ongoing inflammatory response after bacterial infection, are still debatable.^{128, 129} Therefore, the controversial role of corticosteroids and the emerging resistance of bacteria to antibiotics warrant the development of new adjunctive therapeutic modalities for treating eye infections. In coming years, we need to clearly define the role played by various bacterial products, host innate receptors and the mechanisms of signal transduction in regulating inflammatory responses in the retina. Furthermore, the complexity of the regulatory processes, which has been the rule of thumb in the cytokine network, need to be investigated more thoroughly in order to better understand the associated pathophysiology in different scenarios. Being an immune-privileged organ, the eye may follow a very different approach to any pathogenic insult, which is very confounding. Recently, a lot of interest has been observed in studying the immune response that individual cell types in the eye generate, and the ways in which they interact with each other to influence the final outcome. Many recent studies investigated microglial response under various pathologic conditions as these cells are the macrophage equivalent of the retina. Some of these studies discovered a close functional association of microglia with Müller cells, and it was found that a number of microglial responses are actually mediated through Müller cells. The discovery of TLR expression in Müller cells has generated further interest in studying the role of TLRs in retinal infection. Müller glial function as PAMP sensors in the retina and act as high-fidelity listeners to microglial cues. They respond to pathogenic stimuli by mounting a potent inflammatory response in the form of cytokines and chemokines, secrete antimicrobial factors and take care of neurons in their vicinity by clearing out toxic compounds around them and providing them with trophic factors. Müller cells carry out vital physiological processes in the retina, in addition to playing a major immune cell role. Their contributions may be immense in the etiology of retinal pathologies. Preconditioning retinal innate responses offers promising opportunities for treating infectious endophthalmitis. For instance, Yi et al. found that Müller glial TLR4 through the pro-inflammatory cytokine, TNF- α , suppressed the Müller glial Wnt pathway, which is important in photoreceptor survival after oxidative injury. However, activating TLR4 prior to retinal injury decreased the secretion of TNF- α , thus increasing the neuroprotective effects of the Wnt pathway.¹²¹ Our studies have shown that preconditioning with TLR2 ligand, Pam3Cys, muted the TLR2-mediated signaling pathways, which attenuated the inflammatory response from microglia, but at the same time increased their phagocytic activity.⁴⁴ Using Toll-like receptor ligands as prophylactic treatments for patients undergoing ocular surgery may be a useful pharmacologic approach to regulate inflammation while preserving innate immunity and neuroprotection. Understanding host-pathogen interactions in endophthalmitis can direct potential therapies to better protect the delicate retina and preserve vision. Conceivably, novel therapeutic strategies may utilize Müller cells as an effective and viable intervention, not only in bacterial endophthalmitis, but also in a spectrum of retinal or even neuronal pathologies. Given the explosive interest in this field, we hope that it will be possible in the near future to devise adequate therapeutic approaches to counter retinal pathologies by targeting Müller cells.

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Abbreviations

TLRs	Toll-like receptors
PRRs	pattern recognition receptors
RPE	Retinal Pigment Epithelium
PAMPs	Pathogen-associated molecular patterns
BDNF	Brain-derived neurotrophic factor
NTs	neurotrophins
CNTF	ciliary neurotrophic factor

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Pathogenesis of bacterial endophthalmitis

Fig 1. Pathogenesis of bacterial endophthalmitis

The entry of bacteria into the eye occurs due to breakdown of the ocular barriers during intraocular surgery or postsurgical complications. Once inside the vitreous cavity, retinal residential cells i.e. microglia, Muller, astrocytes and RPE recognize the invading pathogen via pathogen recognizing receptors (PRRs, e.g. TLRs). These TLR-activated retinal cells initiate the cascade of innate response involving the production of proinflammatory cytokines/chemokines. The infiltrating monocytes and neutrophils further add to the pro-inflammatory signal by secreting more cytokines into the vitreous which culminates into irreversible retinal damage and subsequent loss of vision.

Fig.2. Pleiotropic roles of Muller glia in retinal innate defense

Following microbial invasion, Muller cells get activated via TLRs or other innate immune receptor-signaling leading to the production of pro-inflammatory cytokines chemokines and antimicrobial peptides⁵⁵. These inflammatory mediators drive the recruitment of PMNs and other innate immune cells to the retina. These early innate responses incited by infection plays an important role in bacterial clearance; however, its dysregulation can lead to bystander damage to retinal neurons. To counter these effects Muller also produces neurotrophins (NTs) which provided neuroprotection via binding their receptors expressed on the retinal neurons, neuron e.g. ganglion cells or photoreceptors.