

Review Article

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Targeting Toll-like receptor signaling as a novel approach to prevent ocular infectious diseases

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Toll-like receptors (TLRs) play a key role in the innate immune response to invading pathogens. Thus, their discovery has opened up a wide range of therapeutic possibilities for various infectious and inflammatory diseases. In the last several years, extensive research efforts have provided a considerable wealth of information on the expression and function of TLRs in the eye, with significant implications for better understanding of pathogenesis of infectious eye diseases affecting the cornea, uvea, and the retina. In this review, by using bacterial keratitis and endophthalmitis as examples, we discuss the possibilities of targeting TLR signaling for the prevention or treatment of ocular infectious diseases.

Key words Inflammation - NF-kB - ocular immune privilege - ocular surface - proinflammatory cytokines - TLR signaling - Toll-like receptors

Introduction

Pathogenic infections of the eye are common and account for a number of sight-threatening diseases, such as herpes simplex virus (HSV) keratitis and keratouveitis, *Pseudomonas* keratitis, ocular onchocerciasis, bacterial endophthalmitis, toxoplasmic retinochoroiditis, and cytomegalovirus (CMV) retinitis, *etc.* This is despite the fact that the ocular surface, which is the first line of defence in the eye against environmental microbes, is well-equipped with an array of specific- and non-specific defense mechanisms to head off potential pathogens, including an intact and continuous epithelium that forms a physical barrier

from the external environment (commensal bacteria, pathogens, toxic chemicals, irritants, allergens, *etc.*) and a repertoire of secretory proteins and enzymes in the tear film that have potent microbicidal activity¹⁻⁶. However, at times these defences may be gravely compromised through multiple breaches by the invading microbes⁷. Moreover, such infections may be critically important in the aetiology and severity of a number of immune-mediated non-infectious inflammatory diseases. Infection or inflammation results when the ocular surface is altered or its consistency is disgraced, such as in contact lens wear, dry eye disease, systemic disease, trauma or surgery^{8,9}. More specifically, the infection is introduced to the eye either directly through

trauma or surgery or through infected adjacent tissues, or indirectly by haematogenous dissemination to the eye¹⁰.

In response to infection, an inflammatory reaction is triggered by the host. The extent as well as the outcome of this retaliation is directly related to innate and adaptive immune responses¹¹. Innate immunity represents the immediate and rapid host defence against microbial challenge and involves several resident and immune cells and their products *i.e.*, cytokines and chemokines whereas adaptive immunity is a delayed response and takes a few days to develop, involves T- and B-lymphocytes and is antigen-specific. One of the cardinal steps in initiating an innate immune response is pathogen recognition by specific receptors of the host¹². With the help of these receptors, the innate immune system manages to alert the host of the pathogens and to instruct to deal with them in a pathogen-specific manner. The major class of receptors which the host employs is a family of recently characterized innate immune receptors, called the Toll-like receptors (TLRs)¹³. TLRs recognise and respond to microbes by identifying highly conserved biochemical structures called pathogen associated molecular patterns (PAMPs). Apart from facilitating self-nonsel self discrimination (as mammals lack these PAMPs altogether), TLRs also provide considerable specificity for the different classes of pathogens as a given molecular signature is invariably present in the microbes of a given class¹⁴. The interaction of microbes or their ligands with TLRs may be of critical importance not only in the view of infectious ocular diseases but also in better understanding the pathogenesis of non-infectious immune mediated ocular diseases, where pathogenic infections act as triggers^{15,16}.

The ocular innate immunity

The eye is a very sensitive organ that is in constant communication with the external environment. The continuous exposure to the outer world places the ocular surface at risk for infestation and warrants for strong defences. The ocular interface functionally comprises the eyelid, lacrimal glands, tear-film, cornea and conjunctiva and is equipped with a number of non-specific defenses¹⁷⁻¹⁹. For instance, tears constantly flush the ocular surface to sweep foreign particles and carry a number of potent anti-microbial proteins such as lysozyme, β -lysin, lactoferrin, lipocalin, *etc.* and immunoglobulins (Igs) such as IgA and IgG which bind to bacteria and viruses and prevent their adherence to

the ocular surface^{20,21}. The corneal and conjunctival epithelia are the cellular interface with the external environment and represent an active physical barrier which is not only capable of recognizing pathogens but is armed to generating fairly robust immune responses against them. Although the detection of microbes is arguably the most important task of the immune system, a hyperbolized epithelial host defense may initiate a perpetual inflammatory mucosal response^{22,23}. Generic inflammation by its very own nature is tissue distorting; although essential for immunity against invading pathogens, it brings as a rule a variable burden of non-specific tissue destruction²⁴. Maintenance of the delicate microanatomy of the eye is essential for the precision and the nicety of the functions it does. Even small distorting lesions, unlike most other organs (with exception of the brain) can be devastating to the eye. It is due to the uttermost sensitivity of this high fidelity apparatus to even the slightest microanatomical distortions along the visual axis. Moreover, vision in mammals must have been a strong selection pressure and the individuals with loss of vision or impaired vision would be subjected to negative survival fitness. Conceivably, during the course of evolution development of "immune privilege" must have taken place in the eye as an adaptation to circumvent the sight destroying consequences of ocular inflammation. As a result, although the ocular surface epithelium is in constant contact with microbes and their products, the healthy ocular surface is only seldom in an inflammatory state. The eye harbours unique innate immune mechanisms to regulate inflammation induced by microbes¹¹.

The ocular immune privilege

Immune privilege in the eye is achieved through modifications in both the afferent and efferent limbs of immunity, *i.e.* both the induction and expression of immunity against foreign antigens. It is characterized by a state of immune-unresponsiveness, immune-ignorance or immune-tolerance²⁵. There are anatomical and biochemical features which are crucial for the establishment and maintenance of ocular privilege. The features that are most important for the existence of this privilege include (i) the integrity of the blood-ocular barrier (comprising blood-retinal barrier and blood-aqueous humour barrier) which prevents the incursion of blood-borne molecules and cells into the eye, (ii) the virtual absence of lymphatic drainage from within the ocular globe, including the cornea which acts as afferent block to immune responses²⁶, and (iii)

an immunosuppressive intraocular microenvironment. Ocular fluids contain a variety of cytokines, neuropeptides and growth factors, such as transforming growth factor (TGF)- β , soluble Fas ligand, vasoactive intestinal peptide, calcitonin gene-related peptide, α -melanocyte stimulating hormone (α -MSH), *etc.* that suppress inflammation either directly, *e.g.* α -MSH annuls TLR4-mediated inflammation by triggering interleukin-1 receptor associated kinase M (IRAK-M)²⁷, or by causing impaired activation of primed and alloreactive T-lymphocytes, suppressing functional activation of macrophages, and by modifying antigen processing and presentation by professional antigen presenting cells (APCs). Eye-derived antigens, which escape when local APCs migrate via the blood route to the spleen, selectively activate regulatory T-lymphocytes (T_{reg}) which directly act on the efferent limb to downregulate the development of antigen-specific delayed hypersensitivity²⁸⁻³⁰. This phenomenon is also known as anterior chamber associated immune deviation (ACAID). Although a lot has been studied about the inflammation-controlling mechanisms in the anterior segment, relatively less is known about the posterior segment. The extent to which inflammation in response to infection is cut short in the posterior segment of the eye is less clear. The anatomic barrier provided by an intact posterior lens capsule is important for limiting the spread of bacteria to the vulnerable posterior segment³¹. The lens capsule is held by zonules that are porous and likely allow for communication between anterior and posterior chamber fluids, especially when pressure in the anterior chamber rises such as during a cataract surgery.

Breakdown of the ocular immune privilege

Maintenance of ocular immune privilege is required for preserving a clear visual axis free of inflammation; however, it also leaves the eye vulnerable to infection. As experiments suggest, bacteria in the vitreous appear to benefit from immune privilege, however, most bacteria appear to trigger vigorous inflammation.

Despite the fact that several defense mechanisms are functional, *e.g.* induction of defensins, expression of α -B crystalline by the retinal pigment epithelium and the neural retina³², activation of the complement, *etc.* these do not appear to be the binding elements of the immunity in the posterior segment of the eye³²⁻³⁴. The most potent form of innate immunity in the eye comes from TLRs which would be reasonably wise to believe, as the findings demonstrate that TLRs are expressed

in most eye tissues³⁵. TLRs are known to activate both innate and adaptive arms of immunity³⁶. The sequent immune responses may protect the eye from infection; however, the sequelae from the inflammatory response may result in ocular assault over and above that from the primary infection. Despite the multiple mechanisms to prevent potentially harmful ocular inflammation, activation of multiple TLR pathways may cause the state of immune privilege to break down as during instances of heavy infection, resulting in sight threatening inflammatory eye diseases such as uveitis and keratitis. Both infectious and immune mechanisms are important in triggering the breakdown in ocular immune privilege and in the development of various forms of inflammatory eye diseases. Thus over-activation of the innate immune system through TLRs is believed to play an important role in triggering infection-induced inflammatory eye diseases.

Toll-like receptor signaling

TLRs are type I transmembrane glycoprotein receptors which comprise an extracellular domain containing 19-25 tandem repeats of leucine-rich motifs, a transmembrane domain and a conserved cytoplasmic domain of around 200 amino acids that is referred to as the Toll/IL-1 receptor (TIR) domain due to its homology to the signaling domain of the interleukin (IL)-1 receptor³⁷. The cytoplasmic domain mediates activation of intracellular signaling pathways by ligating to the TIR-containing adaptor proteins: MyD88, TIR domain-containing adaptor protein (TIRAP)-also known as MyD88-adaptor-like (MAL), TIR domain-containing adaptor protein inducing IFN β (TRIF) and TRIF-related adaptor molecule (TRAM)^{38,39}. Once activated, TLRs signal through different intracellular signaling cascades, generally resulting in the activation of nuclear factor-kB (NF-kB) and activator protein-1 (AP-1) in MyD88-dependent pathways and/or type I interferons (IFNs) in TRIF-dependent antiviral pathways, leading to the induction of cytokines, chemokines and cell adhesion molecules⁴⁰. To date, 13 mammalian TLRs have been identified, of which 10 functional TLRs are known to be expressed in humans and 12 functional TLRs in mice with TLRs1-9 being conserved in both. TLR10 and TLRs11-13 are unique to humans and mice, respectively. TLR1, 2, 4, 5, 6, and 10 are typically displayed on the cell surface, where these interact with their corresponding ligands. TLR3, 7, 8, and 9 are typically located intracellularly on endosomal membranes as their natural ligands might only be found in the acidic compartments of the

cell⁴¹. TLRs associate with other proteins to form a heteromeric receptor complex (Fig. 1). These accessory proteins might be one of the TLRs that form either homo- or heterodimeric complexes or proteins which do not belong to the TLR family. This dimerization induces conformational changes which are essential for the recruitment of adaptor molecules to the cytosolic domain of TLRs. TLR2 forms heterodimers with TLR6 and with TLR1 and is a sentinel for the recognition of Gram-negative bacteria through diacyl and triacyl lipopeptides, respectively^{42,43}. TLR4 forms a complex with lipopolysaccharide (LPS) binding protein (LBP), MD-2 and CD14 and recognizes LPS from Gram-negative bacteria^{44,45}. TLR5 recognizes flagellin, a component of bacterial flagella⁴⁶. The ligand

for TLR10 is yet to be discovered, though it is known to dimerize with TLR1 and TLR2⁴⁷. TLR3 recognizes double stranded RNA from viruses⁴⁸ whereas TLR7 and 8 recognize viral single stranded RNA^{49,50}. TLR9 spots the presence of unmethylated cytosine-phosphateguanosine dinucleotide (CpG) motifs of both bacterial and viral DNA^{51,52}.

Most of the inflammatory responses downstream of TLRs are dependent on a common signaling pathway mediated by the adaptor molecule MyD88. On stimulation, MyD88 associates with TIRAP to form a complex which recruits several isoforms of IL-1R-associated kinase (IRAK), IRAK4 being particularly important as it is indispensable for the responses to

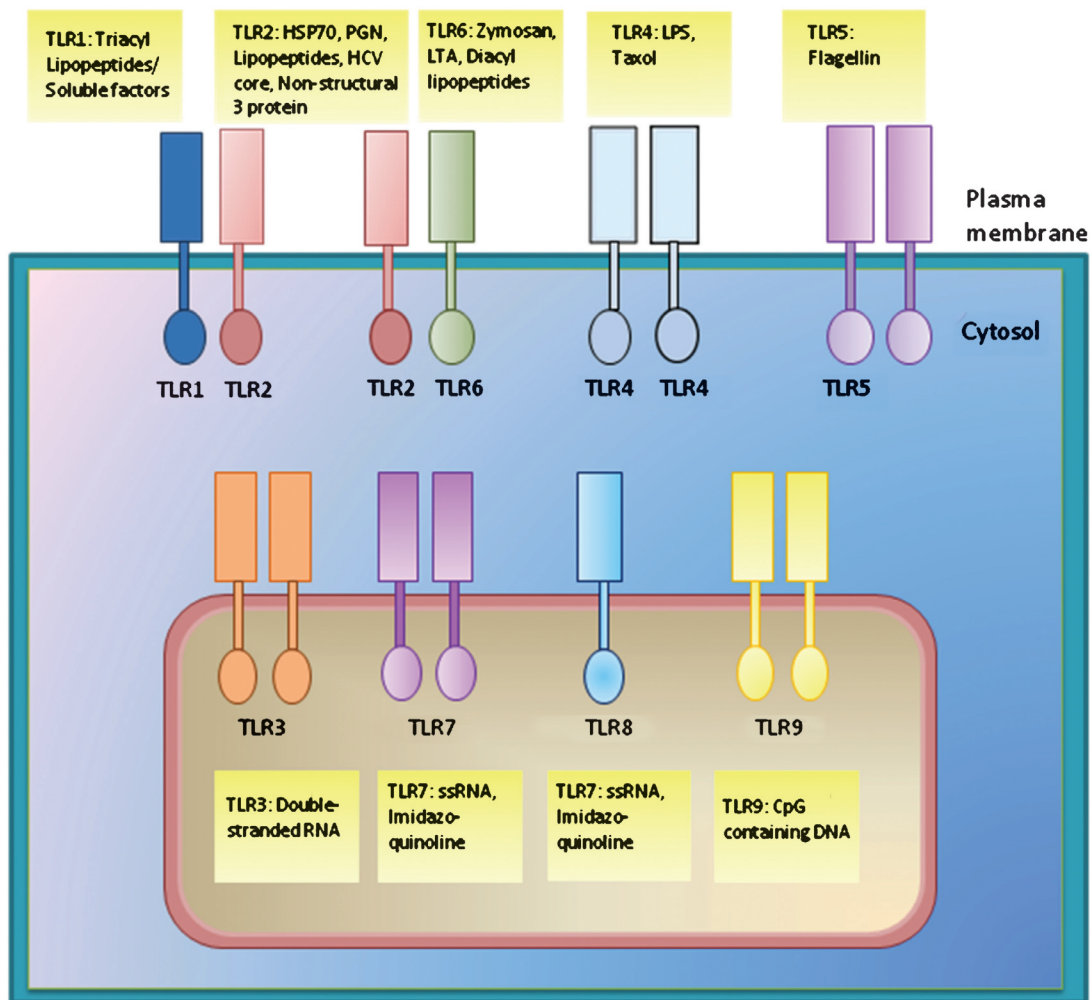


Fig. 1. Cellular distribution of Toll-like receptors (TLRs): TLRs 1, 2, 4, 5 and 6 are expressed on the cell surface, while TLRs 3, 7, 8 and 9 are expressed intracellularly on endosomal membranes. After binding to their respective ligands, TLRs 3, 4, 5, 7 and 9 are thought to signal through their homodimers; TLR2 may heterodimerize with TLR1 or TLR6 depending upon the ligand in question. TLR4 requires MD2 in addition to its ligand for signal transduction. HSP70, heat shock protein 70; HCV core, hepatitis C virus; LTA, lipoteichoic acid.

several TLR ligands. Activated IRAK subsequently associates with tumour-necrosis factor receptor-associated factor (TRAF)-6 leading to the activation of c-Jun N-terminal protein kinase (JNK) - and NFκB- dependent pathways, which in turn regulate the expression of several genes involved in orchestrating the inflammatory response. Alternatively, TLR3 and TLR4 can recruit TRIF to activate IFN responses, where MyD88 requirement is not observed. Recruitment of TRAM/TRIF is known to be critical for type I interferon

production and the maturation of dendritic cells, while MyD88 is essential for the production of Th1 supporting inflammatory responses (Fig. 2). Depending upon the ligands in question, different TLRs induce distinct patterns of cytokine responses resulting in a Th1/Th2 polarization that is most suitable for the pathogen. For instance, while activation of TLR4 in dendritic cells (DCs) induces production of IL-12, thereby skewing Th differentiation towards the Th1 type, indirect activation of DCs by inflammatory mediators alone does not

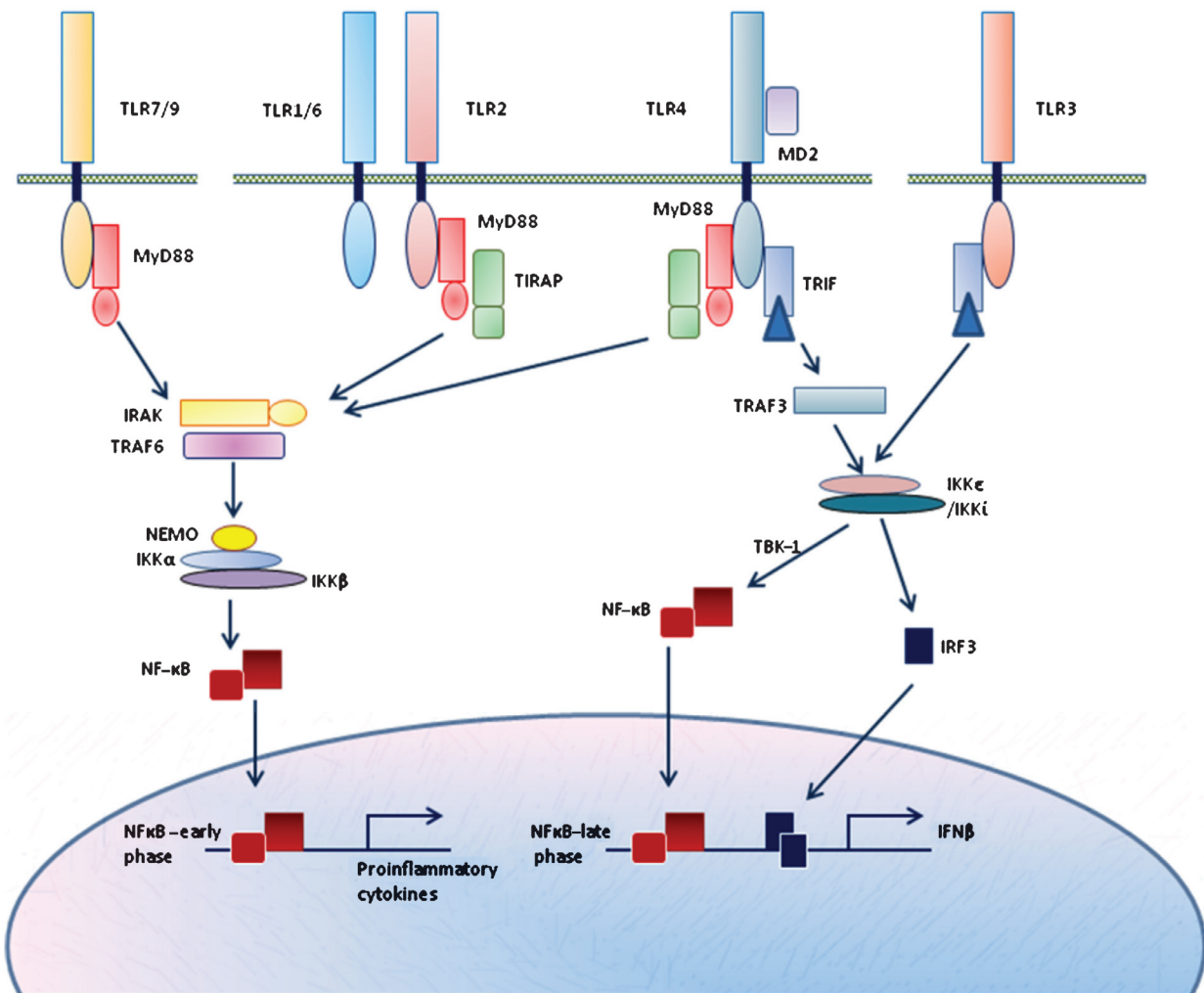


Fig. 2. TLR signalling pathways: TLRs signal through myeloid differentiation primary response gene 88 (MyD88) or/and TIR-domain-containing adaptor-inducing interferon-β (TRIF)-dependent pathways. TLRs 1, 2, 5, 7, 9 require the adaptor MyD88 for their action, whereas TLR3 signals through TRIF-dependent pathway. TLR4 on the other hand activates both MyD88 and TRIF dependent pathways and may induce pro-inflammatory cytokines as well as IFNβ. MyD88 recruits TNF receptor associated factor-6 (TRAF6) and members of the interleukin-1 receptor-associated kinase (IRAK) family, which in turn causes phosphorylation of IκB, after proteasomal degradation of which NFκB dimers are translocated into the nucleus where they cause induction of proinflammatory cytokines. TRIF recruits TRAF3, which through its interaction with serine/threonine-protein kinase 1 (TBK1) and IKKι causes phosphorylation of interferon regulatory factor 3 (IRF3). Phosphorylated IRF3 dimerizes and translocates into the nucleus where it causes induction of interferon β (IFNβ). TIRAP, TIR domain-containing adaptor protein; NEMO, NF-kappa-B essential modulator.

promote Th cell differentiation in spite of T-cell clonal proliferation⁵³. Hence, individual TLRs are important in both triggering and modulating the activation of the adaptive immune response⁵⁴.

TLR expression in the eye

TLRs have been reported to be expressed by each ocular tissue, though cells from different ocular tissues may differ in their expression of one or more individual TLRs. For example, while cornea and conjunctiva express most of the TLRs, TLR4 is the lone TLR known to be expressed by uvea and sclera. Similarly, there are differences in the expression of TLRs at transcript and protein levels from different tissues, *i.e.* some cells express only the transcripts while others produce functional TLRs (Table)⁵⁵. Detailed study of this variability in the expression of individual TLRs in different parts of the eye reveals some sort of strategic evolution which also seems to have contributed to the privileged state of the eye. For instance, corneal epithelial cells which are in constant communication with bacteria and their products from the external environment express TLR4 only intracellularly and not on the cell surface, and thus are incapable of functionally responding to LPS from Gram-positive bacteria⁵⁶. Furthermore, the corneal epithelial cells also do not express MD2 which is an essential component of the LPS-TLR4 signalling complex⁵⁷. As a consequence, these cells are in a state of unresponsiveness to PGN and LPS. On the other hand, these cells express functional TLR3 which recognises dsDNA from viruses⁵⁸. Similarly, TLR5 is expressed at the basal and wing cell layers but not at the apical layers of the corneal epithelium⁵⁹ illustrating another

identical mechanism by which the corneal epithelium remains non-responsive to non-pathogenic bacteria at the apical surface, but may generate TLR mediated innate immune responses once the epithelium has been breached. It would be worth emphasizing here that this functional immune-silencing at the level of individual TLRs is a protective adaptive mechanism that confers protection from the damaging effects of TLR-mediated inflammation against the normal bacterial flora unlike the cases with LPS-mediated *Pseudomonas* keratitis and *Staphylococcus aureus* mediated bacterial keratitis.

TLRs in the pathogenesis of ocular diseases

During ocular infections, damage occurs not only due to the toxins produced by the pathogens but also due to the bystander damage resulting from the heavy influx of inflammatory cells into the posterior segment. A number of pathologies arise due to immune-driven inflammation around the site of infection. TLRs being the principal machinery through which infection is sensed, TLR signalling has been implicated and observed to be the culprit in many of the immunogenic inflammatory diseases^{60,61}. One of the ways in which it may happen is through production of proinflammatory cytokines like TNF- α as a direct consequence of the activation of TLR signalling. Normally, the anterior and vitreous chambers, retina, and subretinal space are sequestered from the systemic circulation by the blood ocular barrier⁶²⁻⁶⁴. The blood ocular barrier limits the influx of macromolecules into the aqueous, vitreous, and the subretinal spaces. TNF- α is secreted by macrophages and neutrophils in response to infection and may lead to breakdown of the blood-retinal barrier⁶⁵. TNF- α causes upregulation of cell adhesion molecules, particularly selectins, on

Table. Expression of TLRs in the eye

TLR	Cornea	Conjunctiva	Uvea	Retina	Sclera
TLR1	mRNA	mRNA+Protein		mRNA	
TLR2	mRNA+Protein	mRNA+Protein		mRNA+Protein	
TLR3	mRNA+Protein	mRNA+Protein		mRNA+Protein	
TLR4	mRNA+Protein	mRNA+Protein	mRNA +Protein	mRNA+Protein	mRNA
TLR5	mRNA+Protein	mRNA+Protein		mRNA	
TLR6	mRNA	mRNA+Protein		mRNA	
TLR7	mRNA+Protein	mRNA		mRNA	
TLR8	mRNA	mRNA		mRNA	
TLR9	mRNA+Protein	mRNA+Protein		mRNA	
TLR10	mRNA	mRNA		mRNA	

Source: Ref. 55

vascular endothelial cells and thus increases vascular permeability⁶⁶⁻⁶⁸. Moreover, TNF- α further induces secretion of cytokines such as IL-6 which in turn induce expression of chemokines with strong chemotactic properties like macrophage inflammatory protein 1 alpha (MIP-1 α) and MIP2^{69,70}. Such a strong chemotactic drift causes rapid extravasation of neutrophils through the reduced blood-retinal barrier into the vitreous and the sub-retinal space, which through secretion of inflammatory mediators further amplify the extent of inflammation⁷¹. Disruption of the blood-retinal barrier has been associated with almost all retinal diseases. A strong correlation has been reported between the levels of expression of inflammatory mediators like TNF- α and the severity of bacterial endophthalmitis⁷². The escalated inflammation may be lethal for the retinal architecture due to damage to glial cells, retinal pigmented cells and the neurosensory retina resulting in straight loss of vision.

Retinal-neurogenesis is an early stage process during vertebrate development, which gives rise to neurons and Muller glial cells in the retina. Although this process ends early during postnatal period, a small number of quiescent retinal progenitor cells persist at the margin of the mature retina near the junction of the ciliary epithelium. Lately, TLR4 activity has been associated with the loss of proliferative potential among retinal progenitor cells⁷³. Recent studies have shown that Muller glial cells actively participate in the innate immune response during bacterial infections and undergo activation (as measured by cellular hypertrophy and enhanced expression of glial fibrillary acidic protein, GFAP) in a TLR2-dependent manner. TLR2 has been associated with the aetiology of atopic keratoconjunctivitis⁷⁴, whereas TLR9 has been generally associated with the pathogenesis of allergic conjunctivitis⁷⁵. Genetic studies have shown that certain polymorphisms of TLR2 increase the susceptibility toward oculomycosis⁷⁶. Endotoxin induced keratitis is another serious ocular pathology which is characterized by extensive neutrophil extravasation into the corneal stroma. Activation of TLR4 has been shown to be the crucial step in the aetiology of endotoxin induced keratitis. TLR4 induces secretion of the neutrophil chemoattractant MIP-2 in the corneal stroma and the expression of platelet endothelial cell adhesion molecule (PECAM)-1 on the surface of endothelial cells⁷⁷. TLR4 mediated inflammation has also been associated with the aetiology of ocular onchocerciasis (popularly known as river blindness) which is a case of corneal inflammation with potential loss of vision^{78,79}.

TLR4 has been implicated in the pathogenesis of several other ocular diseases, including non-infectious immune-mediated diseases such as acute anterior uveitis, which is probably the most common form of immune-mediated uveitis^{80,81}.

TLR based therapeutic approaches

Notwithstanding their role as the first-line defenders against microbial infection, TLRs have been implicated in the aetiology of several ocular pathologies whether arising due to an infectious agent or self-antigens, or other immune mediated mechanisms where TLRs are directly or indirectly involved in the breakdown of immune tolerance. This fact also makes them a suitable target for therapeutic interventions. Some of the recent studies have justified this line of thought and have come up with striking results. In one of the approaches what has been exploited is the well-known phenomenon of 'endotoxin tolerance', where repeated low-dose administration of TLR4 agonist LPS renders the host desensitized to subsequent LPS stimulation⁸². This is due to some kind of redirection of the immune response during priming events and is characterized by impaired NF- κ B and AP-1 activities and suppressed cytokine responses⁸³. A similar approach has been undertaken for TLR5 which acts as a sensor for epithelial cells to recognize Gram-negative bacteria and subsequently mediates the mucosal surface innate immunity⁸⁴. Treatment with TLR5 ligand flagellin results in cellular tolerance through some kind of reprogramming in cultured human corneal epithelial cells (HCECs). Specifically, prolonged flagellin treatment impairs NF- κ B activation and thus reduces proinflammatory cytokine production, but augments expression of antimicrobial molecules⁸⁵. Most importantly subconjunctival injection of flagellin prior to infection attenuated the development of *Pseudomonas aeruginosa* (PA) keratitis⁸⁶. Moreover, similar protection can be conferred against *Pseudomonas* keratitis by topical application of flagellin thus making this approach ideal for clinical use in future. The underlying mechanism for this protection is attributed to a great extent on expression of the antimicrobial peptide CRAMP which has previously been reported to be a determinant factor for corneal susceptibility to PA keratitis⁸⁵. These studies point towards some sort of reprogramming of the immune response which is mediated through TLR5 upon flagellin treatment. Based upon aforementioned observations, it was suggested that incorporation of low dosage of flagellin in a contact lens solution or eye drops could serve as a prophylactic measure for

contact lens wearer or as a post-ocular surgery remedy to reduce the incidence of bacterial keratitis⁸⁵.

Recently, similar approach has been successfully employed in the model of *S. aureus* endophthalmitis⁸⁷. Bacterial endophthalmitis arises due to introduction of bacteria in the vitreous cavity either during ocular surgeries or due to some penetrating ocular injury, and is associated with serious visual impairment⁸⁸. Kumar *et al*⁸⁷ demonstrated that intravitreal injections of TLR2 ligand Pam3Cys prior to bacterial inoculation prevented the development of *S. aureus* endophthalmitis. They have also shown that Pam3Cys activated microglial cells in the retina; pretreatment of microglia with Pam3Cys attenuated the inflammatory response to *S. aureus* challenge, but substantially enhanced their phagocytic activity⁸⁹ and the expression of cathelicidin-related anti-microbial peptide (CRAMP) which has a direct bactericidal action⁸⁷. Similar observations were noted for the primary retinal microglia. The phenomenon of tolerance has also been observed for other TLRs like TLR5, -7, -9 but not for TLR3. This observation has been rationalized on the basis of the adaptor molecules through which different TLRs signal. For instance, most of the TLRs which signal through MyD88 could not only be tolerized, but also be cross-tolerized as these all share the common adaptor. On the other hand, TLR3 signals in a MyD88 independent manner and does not show the property of tolerance, which connotes that this approach cannot be endorsed in conditions which involve TLR3 such as in viral-mediated ophthalmologies and, therefore, additional alternative approaches should be explored. Thus preconditioning with TLR ligands (with the exception of TLR3) can be a viable and handy approach in a number of TLR-mediated ocular pathologies. However, tolerance is not merely a hyporesponsive state and stating that would be an over-simplification. For example, while tolerance to TLR4 downregulates the production of proinflammatory cytokines TNF- α , IL-1 and IL-6, NOS2 levels are upregulated and TLR4 tolerized macrophages show enhanced phagocytic activity⁹⁰. Similarly as mentioned previously, while glial cells primed for TLR5 show attenuated cytokine production, these show heightened phagocytic activity and enhanced expression of anti-microbial peptide. Furthermore, there may be subtle differences in the actual outcome of tolerance to different TLRs in different pathological models which may need further manipulations. TLR antagonism can be another strategy to attenuate TLR signalling which has been tried in several disease models of infection using small

molecule inhibitors (e.g. eritoran for TLR4 or ODN-based inhibitors of TLR7), neutralizing antibodies and siRNAs mediated gene silencing⁹¹. Although the administration of the drug seems to be a major issue, this approach still looks promising and may also be a workable strategy. Alternatively, components downstream of TLRs may as well be targeted, but only in limited cases owing to the wider range of their effects.

Ocular diseases represent a very small fraction of the systemic disorders where TLRs directly play a role. Therefore, developing a strategy which targets any specific TLR in one of the ocular disease models may potentially open avenues even for seemingly unrelated maladies. Similarly, clues from other disease models may also be borrowed and applied to ocular pathologies. For example, after establishing the role of flagellin as a prophylactic intervention in *Pseudomonas* keratitis⁸⁶, Yu *et al*⁹² have demonstrated profound stimulatory effect of flagellin on lung mucosal innate immunity, a response that might be exploited therapeutically to prevent the development of Gram-negative bacterial infection of the respiratory tract. Priming with LPS has been shown to cause tolerance to brain ischaemia in the mouse model of stroke and porcine model of deep hypothermic circulatory arrest^{93,94}. Identically, pretreatment with TLR9 ligand CpG has been shown to provide neuronal protection in both *in vitro* and *in vivo* models of stroke⁹⁵.

Conclusion and future prospective

A major advance in our understanding of infection and immunity occurred with the discovery of TLRs. TLRs enable the host immune system to recognize and respond to microbes by their "signature" molecular component(s), triggering the earliest immune responses that lead to inflammation. TLRs are likely to have wide implications in ocular immunology, not only in inflammatory eye diseases but also in other areas such as corneal transplantation and intraocular tumours. The initial molecular mechanisms that lead to the loss of the normally sight protective state of ocular immune privilege and the development of various forms of ocular inflammation are currently poorly understood. Microbial agents or their PAMPs, via their interaction with TLRs and other pattern recognition receptors (PRRs), may be critically important in the pathogenesis of inflammatory eye diseases. A better understanding of these mechanisms is of fundamental importance to expand our knowledge of ocular infection and immunity but also would be of major clinical significance,

as it may identify potential new therapeutic targets that may be more selective, effective, and safer than the currently available therapies for treating sight threatening inflammatory eye diseases. However, from a pharmacological point of view, this area of research is still in infancy. More knowledge of the TLR signaling pathways as well as increasing evidence for the role of TLR ligands in the molecular pathogenesis of diseases will be needed for the development of new drugs targeting TLRs, especially for ocular infectious diseases. Understanding the complex mechanisms underlying Toll-like receptor localization and function will provide additional data that might help devise novel therapeutic approaches involving Toll-like receptors and their agonists, in an attempt to modulate the immune system.

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