Toll-like receptors in ocular surface diseases: overview and new findings

Alessandro LAMBIASE*, Alessandra MICERA†, Marta SACCHETTI*, Flavio MANTELLI* and Stefano BONINI*
*Department of Ophthalmology, University of Rome Campus Bio-Medico, Via Alvaro del Portillo, 21, 00128 Rome, Italy, and †Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Gian Battista Bietti Eye Foundation, Rome, Italy

ABSTRACT

The ocular surface is the first line of defence in the eye against environmental microbes. The ocular innate immune system consists of a combination of anatomical, mechanical and immunological defence mechanisms. TLRs (Toll-like receptors), widely expressed by the ocular surface, are able to recognize microbial pathogens and to trigger the earliest immune response leading to inflammation. Increasing evidence highlights the crucial role of TLRs in regulating innate immune responses during ocular surface infective and non-infective inflammatory conditions. In addition, recent observations have shown that TLRs modulate the adaptive immune response, also playing an important role in ocular autoimmune and allergic diseases. One of the main goals of ocular surface treatment is to control the inflammatory reaction in order to preserve corneal integrity and transparency. Recent experimental evidence has shown that specific modulation of TLR pathways induces an improvement in several ocular inflammatory conditions, such as allergic conjunctivitis, suggesting new therapeutic anti-inflammatory strategies. The purpose of the present review is to summarize the current knowledge of TLRs at the ocular surface and to propose them as potential targets of therapy for ocular inflammatory conditions.

INTRODUCTION

The ocular surface is a complex functional unit composed of the eyelid, lacrimal gland, tear film, cornea and conjunctiva. All of these structures collaborate through mechanical and biological mechanisms (such as activation of cells, release of mediators and cell–cell interaction) to provide a first line of defence [1]. The corneal and conjunctival epithelia are the interface with the environment and represent an active barrier against pathogens, commensal bacteria, toxic stimuli and allergens, playing an important role in the cellular immune response [2–4].

Pathogens coming from the environment trigger an inflammatory reaction, which is related to innate and adaptive immune responses. Innate immunity represents the early host defence against microbial infection and involves several resident and immune cells (i.e. antigen-presenting cells, dendritic cells and natural killer cells), cytokines and chemokines [5,6]. One of the key

Key words: eye, inflammation, innate immunity, ocular surface, pathogen, Toll-like receptor (TLR).

Abbreviations: dsRNA, double-stranded RNA; HCEC, human corneal epithelial cell; HCF, human corneal fibroblast; HSK, herpetic stromal keratitis; HSV, herpes simplex virus; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; ISS, immunostimulatory sequence; LPS, lipopolysaccharide; LBP, LPS-binding protein; LTA, lipoteichoic acid; MIP, macrophage inflammatory protein; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor κB; NSAID, non-steroidal anti-inflammatory drug; PGN, peptidoglycan; ssRNA, single-stranded RNA; TLR, Toll-like receptor; TNF, tumour necrosis factor; TRIF, Toll/IL-1 receptor domain-containing adaptor protein inducing IFNβ; VKC, vernal keratoconjunctivitis.

1 These authors contributed equally to the manuscript and appear in alphabetical order

Correspondence: Professor Stefano Bonini (email s.bonini@unicampus.it).
mechanisms to initiate the innate immune response is pathogen recognition by specific receptors. TLRs (Toll-like receptors) are a family of mammalian innate receptors able to recognize microbial pathogens and to trigger the earliest immune response leading to inflammation. Ten TLRs (TLR1–TLR10) have been shown to be present in humans [7–9]. TLR signalling is mediated by MyD88 (myeloid differentiation factor 88). Additionally, certain TLRs can also induce TRIF [TIR (Toll/IL-1 receptor) domain-containing adaptor protein inducing IFN (interferon) β]-dependent signalling [10]. The activation of intracellular signalling pathways induces the expression of cytokines, chemokines and adhesion molecules. TLR expression and signalling pathways activated are summarized in Figure 1. TLRs are expressed at the ocular surface where they trigger an immediate innate response specific to pathogenic strains (sparing commensal bacteria) and activate adaptive immunity [11,12].

For the unique ability of TLRs to recognize pathogens from commensal bacteria, their role in infectious diseases of the ocular surface has been extensively studied and well characterized [13]. However, there is also emerging evidence showing a prominent role of TLRs in the modulation of ocular surface allergic and autoimmune diseases, representing a link between innate and adaptive immunity [8].

The aim of the present review is to outline recent evidence demonstrating that the involvement of TLRs in inflammatory conditions of the ocular surface is not limited to infections and allergic reactions, but extends to autoimmune diseases. The potential development of novel therapies targeting TLRs in such conditions is also discussed.

**OCULAR SURFACE AND INNATE IMMUNITY**

In the eye, as in other parts of the body, the early response against pathogens is given by innate immunity, which provides a non-specific surveillance system and activates specific adaptive immune responses [5,14]. The ocular surface innate immune system consists of a combination of anatomical, mechanical and immunological defence mechanisms. Physical barriers, such as the bony orbit and eyelids, protect against traumatic events; tears,
the cornea and the conjunctiva glycocalyx provide an additional barrier [2,15]. Cell–cell junctions of corneal and conjunctival epithelia contribute to forming this first line of mucosal defence [2].

The conjunctiva is a mucous membrane that covers the inner surfaces of the eyelids and folds back to cover the front surface of the eyeball extending to the edge of the cornea. The conjunctiva is composed of two layers: the conjunctival epithelium and the stroma with connective tissue rich in lymphoid tissue, blood and lymphatic vessels. The corneal epithelium is a multilayer epithelium composed of superficial cells, wing and basal cells. Immediately beneath this layer of epithelial cells is the stroma (fibroblasts are its principal cellular components), followed by an innermost single layer of endothelial cells (Figure 2). The corneal innate immune system consists of multiple cell types. Epithelial cells may secrete cytokines such as IL-1α, TNF (tumour necrosis factor)-α, IL-6 and IL-8. Fibroblasts in the stroma may produce IL-1α, TNF-α, IL-6, IL-8 and α-defensin in response to microbial infection, and they probably contribute to the accumulation and activation of leucocytes in the cornea. In addition, Langerhans cells in the cornea and conjunctiva modulate B- and T-lymphocyte activity [5].

Tears produced by the lacrimal gland cover the cornea and conjunctival epithelia with a tear film, which contributes to their trophic support. Tears are able to flush foreign particles from the ocular surface and to protect against pathogens by the presence of antimicrobial proteins, such as lactoferrin, lysozyme, lipocalin and β-lactam and immunoglobulins IgA and IgG [16,17].

All of these structures, cells and mechanisms collaborate to provide protection in the first minutes to hours after an ocular surface injury with an immediate response and to trigger an appropriate adaptive immune response.

**TLR EXPRESSION AT THE OCULAR SURFACE**

TLRs are expressed by a variety of cells of the ocular surface and play an important role in triggering the earliest immune responses that lead to inflammation. TLRs may be classified based on their localization (intracellular or surface) or by their ligands, which are highly conserved structures expressed by pathogens (not expressed by host cells), enabling the innate immune system to distinguish between self and non-self. Table 1 summarizes the common ligands recognized by TLRs and evidence for the expression of TLRs on corneal and conjunctival epithelia [7,12,18,19]. TLR2 mainly forms heterodimers with either TLR1 or TLR6 to recognize several bacterial products. TLR1/TLR2 heterodimers recognize a variety of bacterial lipopeptides, and TLR6/TLR2 heterodimers recognize mycoplasma lipoproteins and peptidoglycan. TLR2 may also act as a homodimer in the recognition of other ligands, such as Gram-positive cell walls, LTA (lipoteichoic acid), mycobacterial lipoarabinomannan, zymosan and heat-shock protein 60 [12,18,20].

TLR4 is expressed by several ocular surface cells, including corneal and conjunctival epithelial cells, fibroblasts and resident immune cells (mostly dendritic cells and macrophages). The ability of TLR4 to form a complex with CD14 and MD2 allows LPS (lipopolysaccharide) to be recognized, making this receptor a ‘sensor’ of Gram-negative bacteria [21–24]. Another major sensor of epithelial cells to detect Gram-negative bacteria is...
**ROLE OF TLRs IN OCULAR SURFACE INFECTION DISEASES**

Under normal conditions, the cornea is highly resistant to microbial invasion. However, when epithelial integrity is breached, pathogens may invade the cornea, resulting in infectious keratitis, which is one of the leading causes of visual impairment and blindness worldwide. Once trauma or chemical injury facilitates bacterial invasion of the stroma, bacterial products activate resident cells and induce an inflammatory response with intense neutrophil infiltration and cornea opacification. Bacterial keratitis is mainly associated with predisposing factors such as contact lens wear, cornea surgery and trauma [31] (Figure 2).

Different TLRs play a key role in the regulation of the response to infectious agents in the cornea: TLR4 and TLR5 are involved in Gram-negative and TLR2 in Gram-positive bacterial infections, TLR2 and TLR4 are also activated in fungal keratitis, and TLR3, TLR7 and TLR8 in viral infections. Both the bacterial and viral genomes are able to activate a TLR9 response.

As stated previously, the role of TLR4 as a sensor of Gram-negative bacteria has been well characterized. TLR4 recognizes LPS (or endotoxin), a highly conserved molecular pattern of Gram-negative bacteria. Four extracellular and cell-surface proteins, LBP (LPS-binding protein), CD14, TLR4, and MD2, are required for endotoxin signalling [21]. LBP and CD14 proteins are present in human tears. Corneal epithelia express LBP on the surface of superficial and basal epithelial cells, whereas TLR4 expression is limited to the wing and basal epithelial cells [22]. The lack of expression of TLR4 on the surface of apical cells of the corneal epithelium may be related to the capacity of TLR4 to trigger an immune response only after the epithelium is breached, thus preventing an inappropriate inflammatory response when the epithelium is intact and by stimulation of

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**Table 1 TLR ligands and expression at ocular surfaces**

*TLR2 forms heterodimers with TLR1 and TLR6 to initiate the signalling cascade.

<table>
<thead>
<tr>
<th>TLR</th>
<th>Ligand</th>
<th>Pathogen</th>
<th>Cornea</th>
<th>Conjunctiva</th>
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<tbody>
<tr>
<td>TLR1</td>
<td>Lipoproteins</td>
<td>Gram-positive and Gram-negative bacteria</td>
<td>mRNA</td>
<td>mRNA and protein</td>
</tr>
<tr>
<td>TLR2 (TLR1)*</td>
<td>Triacylated lipopeptides</td>
<td>Mycoplasma</td>
<td>mRNA and protein</td>
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<tr>
<td>TLR2 (TLR6)*</td>
<td>Diacylated lipopeptides</td>
<td>Gram-positive and Gram-negative bacteria</td>
<td>mRNA and protein</td>
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<tr>
<td>TLR3</td>
<td>dsRNA</td>
<td>dsRNA viruses</td>
<td>mRNA and protein</td>
<td>mRNA and protein</td>
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<tr>
<td>TLR4</td>
<td>LPS</td>
<td>Gram-negative bacteria</td>
<td>mRNA and protein</td>
<td>mRNA and protein</td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>Gram-negative bacteria</td>
<td>mRNA and protein</td>
<td>mRNA and protein</td>
</tr>
<tr>
<td>TLR6 (TLR2)*</td>
<td>Diacylated lipopeptides</td>
<td>Gram-positive and Gram-negative bacteria</td>
<td>mRNA</td>
<td>mRNA</td>
</tr>
<tr>
<td>TLR7</td>
<td>Imidazoquinolone antiviral drug</td>
<td>ssRNA viruses</td>
<td>mRNA and protein</td>
<td>mRNA</td>
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<tr>
<td>TLR8</td>
<td>ssRNA and imidazoquinolone antiviral drug</td>
<td>ssRNA viruses</td>
<td>mRNA</td>
<td>mRNA</td>
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<tr>
<td>TLR9</td>
<td>CpG-rich DNA</td>
<td>Gram-positive and Gram-negative bacteria</td>
<td>mRNA and protein</td>
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<td>TLR10</td>
<td>Unknown</td>
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TLR5, which is considered to be specific for recognizing flagellin, a major bacterial protein [25].

Other TLRs are considered to be key components of the innate immune response to viral infection, including TLR3 and TLR7. For instance, it has been described that human corneal epithelial cells express functional cell-surface TLR3, acting as a receptor for viral dsRNA (double-stranded RNA) and capable of responding to its ligand by the induction of pro-inflammatory cytokines and IFNβ production [26–28]. Lastly, other TLRs, such as TLR9, are specialized in recognizing DNA containing unmethylated CpG motifs common to both bacterial and viral genomes [29].

TLR activation leads to immune cells recruitment, cytokine release and modulation of corneal healing. In fact, Johnson et al. [30] have demonstrated that stimulation of TLR2 and TLR9 by specific ligands induced corneal neutrophil recruitment and increased corneal thickness and opacity through the common adaptor molecule MyD88.

A fine-tuned TLR regulation is required at the ocular surface, where corneal and conjunctival epithelial cells are in constant contact with commensal microbes and their products. Several mechanisms have been reported to avoid unnecessary immune activation. For example, TLR4 and TLR5 are expressed at the basal and wing cell layers, but not at the apical layers of the corneal epithelium, in order to trigger TLR-mediated innate immune response only when the epithelial barrier has been breached. Ueta et al. [23] have shown that TLR2 and TLR4 are expressed intracellularly in the healthy cornea, where they contribute to the immunosilent environment of the ocular mucosal epithelium. In line with this hypothesis, Zhang et al. [21] have demonstrated that fibroblasts, but not cultured corneal epithelial cells, express MD2, an essential component of LPS–TLR4 signalling, suggesting an additional mechanism of the LPS unresponsiveness of human corneal epithelial cells [21].
the commensal ocular flora. LPS recognition at the ocular surface is, therefore, mainly initiated by tear LBP and CD14, which transfer LPS to MD2 on the stromal fibroblasts if the epithelium is breached. The binding of lipid-A to MD2 causes the rearrangement of TLR4, allowing infection to be rapidly recognized and to trigger the expression of various inflammatory and immunoregulatory genes [21,22,32,33]. In animal models, TLR4-deficient BALB/c mice were susceptible to infection with Pseudomonas aeruginosa and had more severe corneal disease when compared with wild-type BALB/c mice [34,35]. Corneal fibroblasts also express TLR4, CD14 and MD2, and participate in the recognition of the presence of LPS and subsequently express adhesion molecules [ICAM-1 (intercellular adhesion molecule-1)] and chemokines [such as IL-8 and MCP-1 (monocyte chemoattractant protein-1)] that promote leucocyte infiltration [36]. In addition, an essential role of resident corneal macrophages and dendritic cells in the development of corneal inflammation in response to LPS has been demonstrated recently [37].

Gram-negative bacteria are also recognized by TLR5 through flagellin. Flagellated bacteria are able to activate TLR5, leading to the production of pro-inflammatory mediators such as IL-6 and IL-8. Similar to TLR2 and TLR4, TLR5 is expressed on the cell surface of basal and wing corneal and conjunctival epithelial cells, thus allowing an inflammatory response to be initiated only when internal epithelial layers are exposed to pathogens [25,38,39]. TLR2 plays a role in the recognition of Gram-positive bacteria at the human ocular surface, where it can be activated by several bacterial products, including lipoproteins, PGN (peptidoglycan) and LTAs, presumably in combination with TLR1 or TLR6 [20]. Conjunctival epithelial cells express TLR2 and may play an active role in the chronic ocular inflammatory response to Staphylococcus aureus [40]. Kumar et al. [41] reported that HCECs (human corneal epithelial cells) recognize the synthetic lipopeptide Pam3Cys (tripalmitoylcysteine) and S. aureus exoproducts, and have suggested that TLR2 is a sentinel for the detection of Gram-positive bacteria through the recognition of lipoproteins. In fact, they have demonstrated that exposure of HCECs to S. aureus activates TLR2 and induces production of pro-inflammatory cytokines (IL-6, IL-8 and ICAM-1) and antimicrobial molecules [18,41,42]. Sun et al. [43] have shown that the exposure of C57BL/6 mouse corneal epithelium to S. aureus induces neutrophil recruitment to the corneal stroma, and increases corneal thickness and haze in a TLR2-dependent manner. On the other hand, Ueta et al. [23] have shown that TLR2 is expressed intracellularly and that HCECs do not respond to S. aureus PGN, suggesting that the unresponsiveness of HCECs to this TLR2 ligand is a contributing factor to an immunosilent environment at the ocular surface.

Moreover, TLR4 and TLR2 have been implicated in the inflammatory response to the parasitic nematode Onchocerca volvulus, the causative agent of so-called river blindness. Corneal damage is thought to occur as a result of the host inflammatory responses to the endosymbiotic bacteria Wolbachia [44–46]. Studies reported that TLR4 and TLR2 are involved in the development of fungal keratitis. In vitro studies have shown that exposure of HCECs to Aspergillus fumigatus and Fusarium resulted in an up-regulation of TLR2 and TLR4, and the release of IL-1β, IL-6, IL-8 and IL-10 [47–50]. In an animal model of Fusarium keratitis, TLR4−/− mice had more severe corneal disease than C57BL/6 mice [51].

Recently, the involvement of TLR4 in the recognition of the ubiquitous protozoa Acanthamoeba has been described [52]. Although humans are largely resistant to Acanthamoeba, infection may occur following a minor corneal abrasion, resulting in a painful vision-threatening disease. TLR4 exerts a major role on Acanthamoeba keratitis through the TLR4–MyD88–NF-κB (nuclear factor eB) and TLR4–ERK1/2 (extracellular-signal-regulated kinase 1/2) pathways to induce the secretion of inflammatory cytokines [52].

Lastly, TLR3, expressed by human corneal epithelium and fibroblasts, plays a major role against viral infection, responding to dsRNA from viruses by production of IFN-β. TLR3 expression in HCECs is up-regulated after exposure to poly(I:C), a synthetic dsRNA analogue, with the production of IL-6, IL-8 and IFN-β, MIP (macrophage inflammatory protein)-1α, MIP-1β, RANTES (regulated upon activation, normal T-cell expressed and secreted), IFN-β and TLR3 [26,27,53,54], suggesting that corneal epithelial cells are important sentinels of the corneal innate immune system against viral infection. Ueta et al. [55] recently reported that TLR3 at the conjunctival epithelium could not only induce antiviral innate immune responses, but may also regulate allergic reactions. TLR7 and TLR8 have also been implicated in viral recognition. They recognize ssRNA (single-stranded RNA), leading to IFN-α production by virus-infected macrophages and dendritic cells [56]. HSV (herpes simplex virus)-1 infection of HCECs resulted in the induction of TLR7 expression and secretion of IL-6, IL-8 and TNF-α, suggesting that TLR7 may have a role in the corneal immune response in herpetic keratitis [57]. In addition, TLR4, TLR7, TLR8 and TLR9 were found to be up-regulated in human cornea with active HSK (herpetic stromal keratitis) compared with healthy cornea [58].

TLR9 has also been implicated in HSV keratitis. Sarangi et al. [59] have demonstrated that HSV keratitis lesions are reduced in TLR2−/− and TLR9−/− mice. Consistent with this finding, Wuest et al. [60] have shown that TLR9 is necessary for the early augmentation of chemokines following HSV-1 infection in mouse cornea.
TLR9 recognizes DNA containing unmethylated CpG motifs common to both bacterial and viral genomes, leading to an inflammatory response characterized by a Th1 cytokine profile. In C57BL/6 mice, the result of such a Th1 response to *P. aeruginosa* is corneal perforation [35]. In that study, silencing the TLR9 signalling reduced inflammation, but probably contributed to decreased bacterial killing in the cornea.

**TLRs IN OCULAR SURFACE IMMUNE DISEASES**

In an intact immune system, stimulation of TLRs is regulated to trigger an appropriate immune response. Activation of a different TLR pathway induces the specific expression of cytokines, and co-stimulatory and MHC molecules. These molecules influence the adaptive immune response by polarizing Th differentiation towards the Th1 or Th2 response. In most cases, TLR signalling is crucial for the development of Th1 immune responses (characterized by IL-12 and IFN-γ production), consequently allowing an adequate antimicrobial immune response [61]. However, the influence of TLR signalling on Th responses is still controversial, and a role for TLRs in activation of Th2 responses (characterized by IL-4, IL-5 and IL-13 production) has also been reported, with convincing evidence that TLRs are linked to systemic allergic diseases [62,63]. Specifically, there is evidence that TLR2 and TLR4 activation may lead to a Th2 response, whereas TLR9 results in Th1 immunity [64,65]. Interestingly, it has been proposed that some ocular surface immune disorders might be caused by abnormalities of mucosal innate immunity, leading to a Th1/Th2 imbalance that results in autoimmune diseases or allergy [66].

The possible role of TLRs in the development of allergic diseases has been the subject of intense investigation. Epidemiological studies have shown that the prevalence of allergies has increased over recent decades in industrialized countries [67]. This finding resulted in the proposal of the ‘hygiene hypothesis’, which postulates that this increase in atopy may be due to increased environmental cleanness, resulting in a decline in exposure to environmental microbial products in early life, which drives the maturing immune system towards a Th1 phenotype and thus away from a Th2 phenotype [67]. TLRs that are involved in recognition of many microbial products may play a crucial role in the maturation of the normal adult immune system. In fact, several TLR-gene mutations and polymorphisms are associated with the development of allergic diseases such as asthma and conjunctivitis [68]. In a mouse model of allergic conjunctivitis, treatment with the TLR2 agonist Pam3CSK4 [tripalmitoylcysteinylseryl-(lysyl)₅] resulted in a significant decrease in eosinophil infiltration into the conjunctiva, which was associated with inhibition of both the Th1 and Th2 responses [69]. In contrast, Chung et al. [70] have reported that, in a mouse experimental allergic conjunctivitis model, inoculation of *S. aureus* markedly accelerated the signs of allergic conjunctivitis. Recently, TLR3 has also been reported to enhance the late-phase reaction of experimental allergic conjunctivitis [71].

In humans, we [72] have demonstrated a different pattern of TLRs expression in children with VKC (vernal keratoconjunctivitis), a form of allergic conjunctivitis, compared with healthy subjects. Specifically, we observed that TLR4 was up-regulated, TLR9 was down-regulated and TLR2 was slightly reduced in the conjunctiva of VKC patients, suggesting that TLRs play a role in the pathogenesis of VKC [72] (Figure 3). In line with this hypothesis, patients with VKC had an improvement in the signs and symptoms of ocular allergy associated with the down-regulation of ICAM-1 and TLR4 after 4 weeks of treatment with *Lactobacillus acidophilus* eye drops [73]. Similarly, in a mouse model of allergic conjunctivitis, administration of a TLR9 agonist inhibited the immediate immune response and the subsequent infiltration of inflammatory cells [74,75]. Other evidence from Cook et al. [40] has shown that TLR2 expression is increased in the conjunctiva of patients with AKC.
(atopic keratoconjunctivitis), another form of allergic conjunctivitis [40].

Preliminary data from our group [76] have indicated that differences in TLR expression in the conjunctiva may be present between Th1- and Th2-driven diseases. In fact, we have shown an opposite conjunctival pattern of expression of TLR2, TLR4 and TLR9 mRNA in VKC (a Th2-driven disease) and SS (Sjögren syndrome) (a Th1-driven disease) [76].

TLR activation has also been reported as a pathogenic event in systemic autoimmune diseases such as SLE (systemic lupus erythematosus), diabetes, MS (multiple sclerosis) and IBD (inflammatory bowel disease). Ueta et al. [77] have shown polymorphisms in the TLR3 gene in patients with SJS (Stevens–Johnson syndrome) in a Japanese population and hypothesized that viral infection and/or drugs may trigger a disorder in the host innate immune response, leading to an abnormal inflammatory reaction of the mucosa, ocular surface and skin [8,13,19,77].

**OCULAR SURFACE TLRs AS POTENTIAL TARGETS OF THERAPY**

The results discussed in the present review highlight the role of TLRs in ocular surface infective and immune diseases. TLR agonists have already proved useful in clinical trials in allergic, infectious and autoimmune diseases [78]. Therefore TLRs are becoming novel potential therapeutic targets for the modulation of ocular surface inflammatory reactions.

Currently, available ocular anti-inflammatory therapies include NSAIDs (non-steroidal anti-inflammatory drugs), steroids and cyclosporine. It has also been suggested that these drugs are involved in TLR pathways. For instance, NSAIDs are thought to be involved in TLR4 signalling [79,80] and cyclosporine in both TLR2 and TLR4 signalling [81,82]. Moreover, steroids, the most widely used topical agents and the standard treatment for many inflammatory diseases of the ocular surface [83], have been shown to influence TLR expression and signalling. However, their role in modulating innate immune responses of the cornea or its response to infections has yet to be fully elucidated.

Jin et al. [84] have shown that hydrocortisone increased the mRNA expression of TLR2 and TLR4 in HCECs, whereas it down-regulated TLR2 and TLR4 mRNA and protein expression in cultured HCFs (human corneal fibroblasts). The authors speculated that the suppression of the innate immunity in HCFs through TLRs by hydrocortisone may explain the observation that topical steroid treatment can promote opportunistic corneal infections [84]. Moreover, it has been reported that hydrocortisone may decrease the expression of VEGF (vascular endothelial growth factor), an important factor in the development of corneal neovascularization in response to inflammation, through inhibiting TLR2 and TLR4 activity in cultured HCFs [85,86]. In addition, in a recent study, Hara et al. [53] demonstrated that dexamethasone may increase the susceptibility of HCECs to viral infections by altering the TLR3 signalling pathways [53].

To date, steroids are the most used and potent ocular anti-inflammatory agents, but their use is limited by relevant side effects such as increased intra-ocular pressure, cataract development and the promotion of opportunistic corneal infection. Therefore studies are focusing on identifying novel potential targets for anti-inflammatory therapy in ocular surface conditions. Recently, several approaches have been taken to target TLR responses to reduce ocular surface inflammation. Targeting of the TLR2, TLR4, TLR5 and TLR9 pathways has been shown to be a promising approach for modulating infection-related inflammation in experimental studies. Kumar et al. [39] have demonstrated that pre-treatment with the TLR5 ligand flagellin may induce protective mechanisms against bacterial infection in the cornea by modulating the host inflammatory response and enhancing innate defence. Huang et al. [35] have shown that silencing TLR9 signalling reduces inflammation and decreases bacterial killing in *P. aeruginosa* keratitis. Sun et al. [87] have demonstrated that short-chain ceramide (C₆) in nanoparticle formulations inhibited corneal inflammation induced by LPS or by killed *S. aureus* by blocking JNK (c-Jun N-terminal kinase) phosphorylation. In that study, topical application of C₆ nanoparticles blocked TLR2- and TLR4-induced corneal inflammation [87]. Recently, the same authors have shown [51] that the TLR4 antagonist eritoran tetrasodium inhibits LPS- and *P. aeruginosa*-induced corneal inflammation, but not TLR2-induced inflammation, and is therefore a more selective antagonist for corneal infiltrates caused by Gram-negative bacteria.

In line with this protective role of TLR4 antagonists on LPS-mediated ocular surface inflammation, a topical TLR4 antagonist (ibudilast; Banyu/Kyorin Pharmaceutical, Tokyo, Japan) has been launched for the treatment of ocular allergy. Studies performed on SAC (seasonal allergic conjunctivitis) using ibudilast eye drops have shown a significant reduction in allergic symptoms and better outcomes compared with mast-cell stabilizers (sodium cromoglycate) [88,89]. On the basis of these results, it would be interesting to test ibudilast eye drops on VKC, another form of allergic conjunctivitis characterized by an increase in TLR4 in the conjunctiva and associated with a decrease in TLR9. Consequently, in VKC, another potential therapeutic approach could be the selective stimulation of TLR9. In fact, an ISS (immunostimulatory sequence) containing CpG motifs recognized by TLR9 has now been shown to bias the immune response towards the development of...
an antigen-specific Th1 response [90,91]. This response may potentially drive the immune response away from the Th2-type inflammation of allergic reactions. It has been shown that the co-administration of CpG motifs and allergens to mice sensitized to develop Th2-type inflammation causes a rapid alteration in the immune bias to a Th1 response [90,91]. Different studies have shown that intraperitoneal or conjunctival administration of ISS-ODN (ISS oligodeoxynucleotides) prevented the clinical effects of allergic conjunctivitis in a mouse model and effectively suppressed the late-phase reaction in the conjunctiva [74,75]. After decades of ocular anti-allergic treatments based on anti-histamine, mast-cell stabilizers and corticosteroids, the possibility of selectively targeting TLRs to modulate allergic inflammation represents a major advance and a fascinating therapeutic strategy.

CONCLUSIONS

On the ocular surface, the early response against noxious stimuli is provided by innate immunity, which represents a non-specific surveillance system and promotes adaptive immune responses. The evidence discussed in the present review highlights the emerging roles of TLRs in regulating innate immune responses during ocular surface infectious and non-infectious inflammatory conditions. In addition, increasing evidence shows that TLRs play an important role in the pathogenic mechanisms of autoimmune and allergic ocular diseases, modulating the adaptive immune responses.

One of the main goals of ocular surface therapies is to control the inflammatory reaction in order to preserve morphofunctional integrity and corneal transparency. The use of currently available anti-inflammatory drugs is limited by relevant side effects such as opportunistic infection, possibly related to the drugs' effects on TLR pathways.

Recent evidence that TLRs may become therapeutic targets in inflammatory ocular diseases, associated with the possibility to use extracellular TLR agonists and of pharmacologically modulating TLR intracellular signalling pathways, may allow novel therapeutic strategies for avoiding the detrimental effects of prolonged inflammation at the ocular surface. Nevertheless, many aspects of TLR signalling, ligands and cellular expression should be clarified better in order to develop tailored immune treatments that can improve the management and outcome of ocular inflammatory conditions. The evaluation of TLR-modulating molecules in human diseases has already started with several ongoing clinical trials [78]. Given the rapidly growing literature on TLRs in the cornea and conjunctiva, we are optimistic that targeting these molecules will soon become an additional tool for clinicians in the management of ocular surface inflammation.

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