Experimental Eye Research 90 (2010) 679–687

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Review

Toll-like receptors in ocular surface disease

Rachel L. Redfern*, Alison M. McDermott

University of Houston, College of Optometry, 505 J. Davis Armistead Building, 4901 Calhoun Rd., Houston, TX 77204-2020, USA

ARTICLE INFO

Article history:
Received 26 November 2009
Accepted in revised form 16 March 2010
Available online 24 March 2010

Keywords:
toll-like receptors
cornea
conjunctiva
inflammation
infection

ABSTRACT

The ability of the ocular surface to mount an immune response is in part attributed to a family of proteins called toll-like receptors (TLRs). The latter are evolutionary conserved receptors that recognize and respond to various microbes and endogenous ligands. In addition to their recognition function, TLR activation triggers a complex signal transduction cascade that induces the production of inflammatory cytokines and co-stimulatory molecules, thus initiating innate and adaptive immunity. Toll-like receptor expression at the ocular surface is modulated during infection (e.g. Herpes simplex, bacterial keratitis and fungal keratitis) as well as during various inflammatory conditions (allergic conjunctivitis and dry-eye syndrome). Here recent findings regarding TLR expression and their involvement in various ocular surface diseases are discussed.

Published by Elsevier Ltd.

1. Introduction

Toll-like receptors (TLRs) are a family of highly conserved glycoprotein pattern recognition receptors that recognize conserved motifs on pathogen associated molecular patterns (PAMPs) on bacteria, viruses, fungi and protozoa. TLRs are expressed on a wide variety of cell types including epithelia, endothelia, antigen presenting cells and lymphocytes. They are type I transmembrane glycoproteins which have an extracellular leucine-rich domain and a cytoplasmic domain that is homologous to the signaling domain of the interleukin (IL)-1 receptor hence is referred to as the Toll/IL-1 receptor (TIR) domain. The latter mediates activation of intracellular signaling pathways, leading to functional changes including cytokine, chemokine and adhesion molecule expression.

To date, 10 functional human TLRs have been identified; their microbial ligands and signaling pathways are depicted in Fig. 1. TLR1, 2, 4, 5, 6, and 10 are typically located at the cell surface. TLR2 forms heterodimers with TLR1 and with TLR6 and recognizes a variety of microbial lipoproteins. TLR2/6 and TLR2/1 heterodimers recognize bacterial diacyl and triacyl lipopeptides respectively (von Aulock et al., 2003; Takeda et al., 2002). TLR4 forms a complex with MD-2 and CD14 and recognizes lipopolysaccharide (LPS) from Gram-negative bacteria (Beutler, 2000), and TLR5 recognizes flagellin, a component of bacterial flagella (Hayashi et al., 2001). TLR10 is able to dimerize with TLR1 and TLR2, but the microbial ligand for this receptor has yet to be identified (Hasan et al., 2005). TLR 3, 7, 8, and 9 are typically located intracellularly, on endosomal membranes and recognize nucleic acids. TLR3 recognizes double stranded RNA, a by-product of viral replication (Alexopoulou et al., 2001) whereas TLR7 and 8 recognize viral single stranded RNA (Diebold et al., 2004; Heil et al., 2004). TLR9 responds to unmethylated cytosine-phosphate-guanosine dinucleotide (CpG) motifs found in both bacterial and viral DNA (Hemmi et al., 2000; Tabeta et al., 2004).

Although TLRs were first recognized for their capacity to bind PAMPs recently a number of endogenous ligands have come to light. Many of these are molecules indicative of tissue trauma, such as intracellular components of ruptured cells, nucleic acids, heat shock proteins and extracellular matrix breakdown products such as hyaluronan fragments, fibrinogen and high-mobility group box 1 proteins (Kluwe et al., 2009). Thus, TLRs maybe part of a surveillance system to monitor tissue injury and progress of re-modeling as well as infection. On the downside, TLR activation by endogenous ligands is also associated with disease; activation of TLR9 by endogenous DNA is implicated in the development of autoimmune disorders such as systemic lupus erythematosus in both humans and murine models of the disease (Lamphier et al., 2006).

With the exception of aforementioned self-nucleic acid signaling via TLR9, endogenous TLR ligands trigger TLR2 or TLR4. Owing to similarities among the cytokine effects of these endogenous ligands and TLR2/4 microbial agonists it has been suggested that contamination with bacterial LPS or lipoprotein is actually

Abbreviations: TLR, toll-like receptor; IL, interleukin; PA, *Pseudomonas aeruginosa*; SA, *Staphylococcus aureus*; LPS, lipopolysaccharide; NFκB, nuclear factor κB; MyD88, myeloid differentiation protein 88; IFN, interferon; HCEC, human corneal epithelial cells; HSV, herpes simplex virus; VKC, vernal keratoconjunctivitis; SS, Sjögren's syndrome.

* Corresponding author. Tel.: +1 713 743 1943; fax: +1 713 743 2053.
E-mail address: redfernrachel@gmail.com (R.L. Redfern).

0014-4835/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.exer.2010.03.012

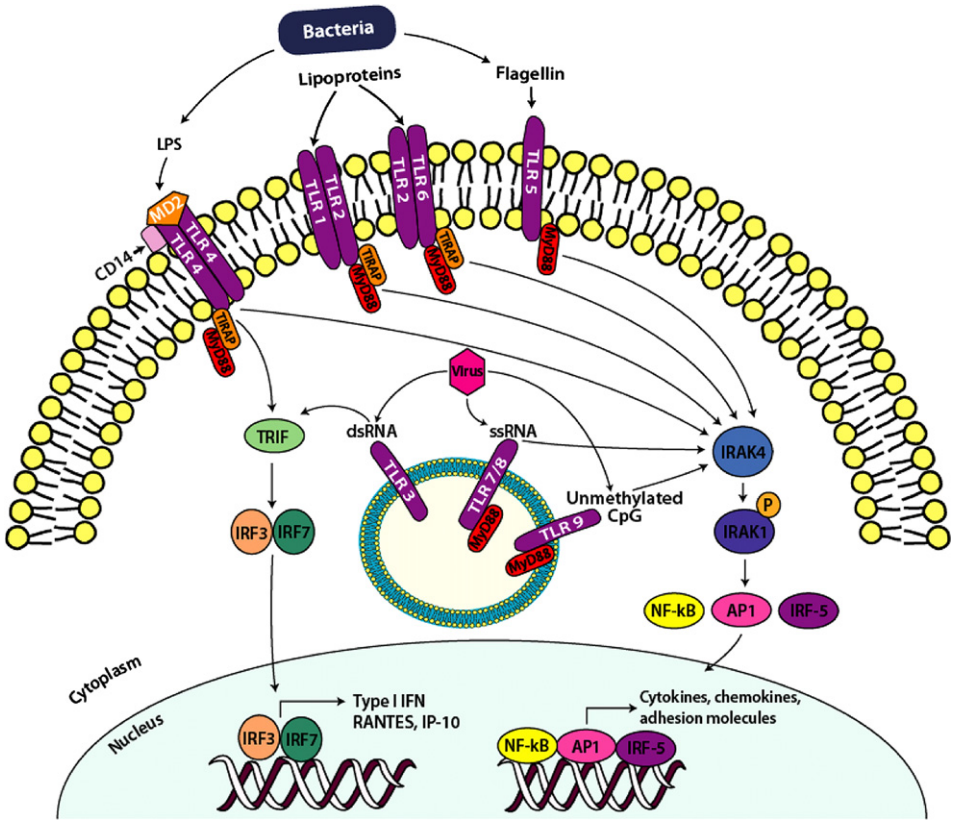

Fig. 1. Simplified overview of TLR signaling. Cell surface TLR2, 4 and 5 recognize bacterial PAMPs lipoproteins, LPS and flagellin respectively, whereas intracellular TLR3, 7/8, and 9 recognize microbial dsRNA, ssRNA and unmethylated CpG motifs respectively from either replicating or infecting viruses or bacteria in the endosome of the cell. The activation of TLRs initiates a MyD88-dependent (all TLRs except TLR3) or TRIF-dependent (TLR3 and TLR4) pathway. The MyD88-dependent pathway utilizes adaptor molecule TIRAP (except TLR7, 8 and 9) leading to IRAK-4 and IRAK-1 recruitment, activated IRAK-4 phosphorylates IRAK-1 which ultimately leads to the activation of transcription factors AP-1, NFκB and IRF-5. TLR3 and TLR4 signal via a MyD88-independent pathway that is mediated via the adaptor protein, TRIF, which leads to the activation of transcription factors IRF-3 and IRF-7 that induce the expression of type I IFN genes.

responsible for at least some of the effects attributed to endogenous ligands (Tsan and Gao, 2007). Thus, studies claiming identification of an endogenous TLR ligand need to be scrutinized to ensure adequate controls were in place to account for possible bacterial product contamination.

All TLRs, except TLR3, signal via the adaptor molecule myeloid differentiation protein 88 (MyD88) which associates with the TLR cytoplasmic domain via a homophilic interaction between the TIR domains (Fig. 1). IL-1R-associated kinase (IRAK)-4 and IRAK-1 are recruited, activated IRAK-4 phosphorylates IRAK-1 which ultimately leads to the activation of transcription factors activating protein (AP)-1, nuclear factor κB (NFκB) and interferon regulatory factor (IRF)-5. This stimulates the expression of multiple genes such as cytokines, chemokines and adhesion molecules. TLR3 signals via a MyD88-independent pathway that is mediated via the adaptor protein TIR domain-containing adaptor protein-inducing interferon (IFN)-β (TRIF), thus leading to the activation of transcription factors IRF-3 and IRF-7 that induce expression of IFN-α/β and IFN inducible genes such as RANTES and interferon-inducible protein (IP)-10. Fig. 1 shows a general overview of TLR signaling, for comprehensive details of the pathways the reader is referred to a review article by Albiger et al. (2007).

2. Expression of TLRs at the ocular surface

A summary of findings regarding cornea and conjunctival TLR expression from several different published sources cited below is presented in Fig. 2. The first report of the localization of TLRs to the ocular surface came in 2001 when Song et al. showed that freshly isolated and telomerase immortalized human corneal epithelial cells (HCEC) express TLR4. Subsequently the expression of mRNA for TLRs 1–10 has been detected in the corneal epithelium from subjects undergoing various ocular surgeries and from cadaver corneas, although not all subjects expressed all TLRs and the relative expression between subjects was variable, with TLR7 and 8 tending to be lower (Jin et al., 2007; Redfern et al., 2006; Ueta et al., 2005; Wu et al., 2007). Similar results have been observed with primary cultured HCEC and cell lines (Kumar et al., 2004; Redfern et al., 2006; Wu et al., 2007). Expression at the protein level has been confirmed for TLR2, 3, 4, 5 and 7 (Hozono et al., 2006; Kumar et al., 2006a; Li et al., 2005; Song et al., 2001; Ueta et al., 2004, 2005; Wu et al., 2007; Zhang et al., 2003).

Studies of the distribution and functionality of some TLRs at the ocular surface has produced contrasting results in a number of instances. Ueta et al. (2004, 2005) observed intracellular TLR2 expression in HCEC that was unresponsive to peptidoglycan. However, Kumar et al. (2004, 2006b) found cell surface TLR2 expression, stimulation of which activated NFκB and upregulated cytokine and anti-microbial peptide (human β-defensin-2, hBD-2) expression. Similarly, Kumar et al. (2006a) observed functional (as determined by NFκB activation and IL-6 and IL-8 secretion) intracellular expression of TLR3 by HCEC. However Ueta et al. (2005) reported that TLR3, while functional, was expressed at the cell surface. TLR3 is commonly found intracellularly on endosomal membranes, although surface expression has been documented for other cell types including fibroblasts (Matsumoto et al., 2003) and