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Toll-Like Receptors as Interferon-Regulated Genes and Their Role in Disease

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The Toll-like receptors (TLRs) are innate sensors that recognize both microbial and endogenous ligands, initiating the host defense response. TLRs initiate the potent proinflammatory response to infection, are the target for adjuvants, and are essential for the establishment and maturation of adaptive immunity. As such they have been the interest of widespread research and the target of therapeutic intervention on multiple diseases. It has become apparent that expression of a subset of TLRs (TLR1, TLR2, TLR3, TLR5, and TLR7) is induced by Type I interferons (IFN). The role and impact of IFN expression on TLR responses is therefore critical in understanding the role of TLRs in disease, particularly as IFN itself is a downstream gene induced by specific TLRs. In this review we discuss the function and role of IFN-regulated TLRs in disease and how the role of IFN may impact upon TLR induction of the immune response in diseases, particularly in mouse models.

Introduction

The Toll-like receptor (TLR) family consists of germline encoded, membrane-bound pathogen recognition receptors (PRRs) that recognize well-conserved microbial structures known as pathogen-associated molecular patterns in the event of infection. TLR-pathogen-associated molecular pattern engagement initiates downstream innate immune signaling leading to the activation of proinflammatory cytokines and maturation of adaptive immunity. TLR1, TLR2, TLR4, TLR5, TLR6, TLR10, and TLR11 (present in mice but not human) are associated with the plasma membrane and are essential in recognizing bacterial cell wall components, bacterial flagellin, viral particles, and other unidentified pathogenic components, respectively (Hoshino and others 1999; Hayashi and others 2001; Takeuchi and others 2001; Zhang and others 2004; Hasan and others 2005; Jin and others 2007). TLR3, TLR7, TLR8, and TLR9 are endosomally localized and recognize bacterial or viral nucleic acids (Bell and others 2005; Haas and others 2008). Viral recognition by TLR3, TLR7, and TLR9 activates Type I interferons (IFNs), which are important for anti-viral immune responses (Kato and others 2005; Le Goffic and others 2006). TLRs are widely expressed in innate immune cells such as dendritic cells (DCs) and macrophages, as well as a wide variety of non-professional cells, including epithelial cells and fibroblasts (Kato and others 2005; Le Goffic and others 2006; Haas and others 2008).

TLRs are members of a family of proteins containing leucine-rich repeats (LRRs), which form an extracellular domain required for ligand interaction. The LRR modules form a solenoid structure with the hydrophobic residues packed within the interior allowing for a ligand-binding hydrophobic pocket (Jin and others 2007; Kim and others 2007; Liu and others 2008a; Kang and others 2009). The LRR modules fold into parallel β-sheets that bend into a concave surface, resulting in the unique horseshoe shape of TLRs. Intracellular domains of TLRs include the transmembrane domain and Toll/interleukin-1 receptor (TIR) domains. A conserved proline on the BB loop of the TIR domain is crucial for the recruitment of downstream TIR domain-containing adaptor proteins, such as myeloid differentiation primary response protein 88 (MyD88), TIR domain-containing adapter protein/MyD88 adaptor-like (Mal), and TIR domain-containing adapter inducing IFNβ (TRIF) (Xu and others 2000).

After ligand interaction, all TLRs recruit the adaptor protein MyD88, with the exception of TLR3, which in turn activates the interleukin (IL)-1R-associated kinase (IRAK) signaling complex (See Fig. 1) (Kawagoe and others 2008). Mal acts as a bridging adaptor between TLR and MyD88 (Fitzgerald and others 2001). The IRAKs then activate tumor necrosis factor receptor (TNFR)-associated factor (TRAF6), an E3 ubiquitin ligase that leads to polyubiquitination of a complex consisting of transforming growth factor-β-activated kinase 1 (TAK1) and TAK1-binding protein 1. This leads to the subsequent phosphorylation cascade of the IκB kinase α (IKKα), IKKβ, and nuclear factor (NF)-κB essential modulator complex, and IκB (Chen 2005). Degradation of IκB allows NF-κB to translocate into the nucleus and induce expression of a host of proinflammatory cytokines (Chen 2005). Type I IFN activation by TLR7 and TLR9 is also MyD88-dependent,

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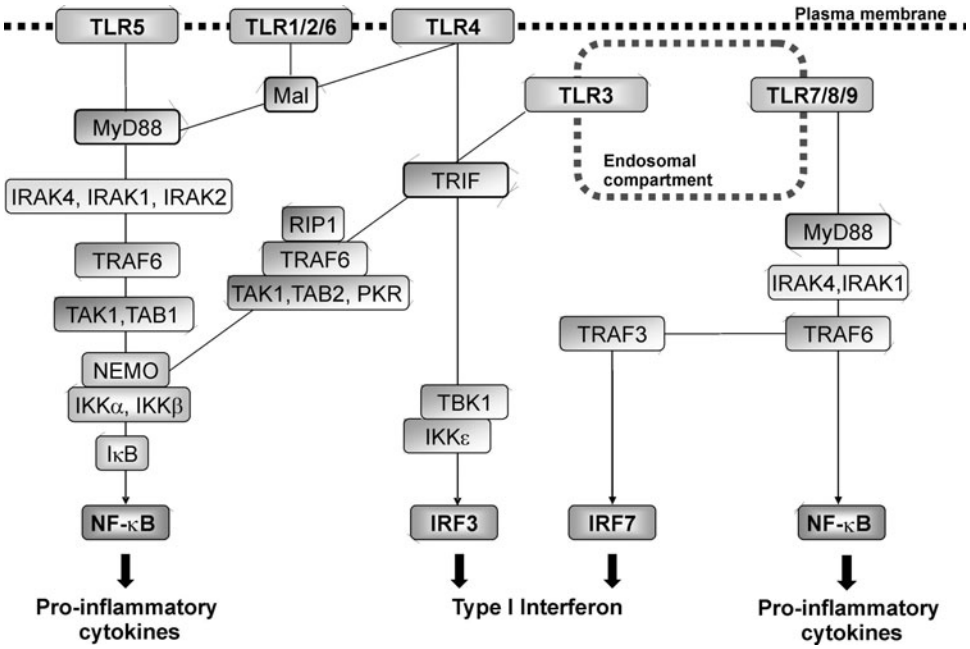

FIG. 1. Schematic representation of TLR signaling cascade in brief. TLR downstream signaling proteins lead to the activation of NF-κB-dependent proinflammatory cytokines and Type I IFN response. TLR, Toll-like receptor; IFN, interferon.

involving a complex of TRAF6, TRAF3, IRAK-1, and IRAK-4, which activates interferon regulatory factor 7 (IRF7) (Kawai and others 2004). In contrast, TLR3 downstream signaling is dependent on the adaptor molecule TRIF, which is also recruited by TLR4. TRIF recruits TRAF6, consequently forming a complex containing TAK1, TAK1-binding protein 2, and protein kinase R, which mediates downstream NF-κB activation (Jiang and others 2003, 2004). Protein kinase R contributes to the double-stranded RNA (dsRNA)-induced activation of the p38 mitogen-activated protein kinase pathway by interaction with MKK6 (Silva and others 2004). TRIF also recruits TBK1 and IKKε, which activate IRF3 (Fitzgerald and others 2003). IRF3, as well as IRF7 downstream of TLR7/9, then translocates into the nucleus and induce expression of Type I IFN (Sato and others 1998; Kawai and others 2004). The receptor interacting protein 1 kinase is also found to be essential for NF-κB activation downstream of TRIF, but it is not required for IRF3 activation by TRIF (Meylan and others 2004; Cusson-Hermance and others 2005).

IFN Regulation of TLRs

The Type I IFNs regulate a vast array of protein coding genes, including 2′-5′-OAS, MX1, and ISG15. The advent of high-throughput expression profiling technologies has facilitated the identification of wide-scale transcriptional changes in a tissue-, cell-, or time-specific manner. The recent publication of the Interferome (Samarajiwa and others 2009) involved analysis of over 40 IFN-regulated datasets with the resulting IFN regulated gene (IRG) list accessible online (www.Interferome.org) (see current issue). Intriguingly, the list of IRG included a number of TLRs, including TLR1, 2, 3, 4, 5, and 7. This suggests a capacity for signal potentiation through positive feedback mechanisms associated with the innate immune response. Experimental validation of this phenomenon has been documented, particularly in TLR3-mediated responses (Tanabe and others 2003; Ciencewicki and others 2009). Computational promoter analysis investigating the putative promoter regions in the TLR gene family members produced consistent results. Promoter sequences, defined as 1500 bp upstream and 300 bp downstream relative to the transcription start site, were retrieved from the Ensembl database (Hubbard and others 2009). TRANSFAC Match 2010–based analysis identifies ISRE, IRF, and STAT1 sites commonly associated with IFN response (Kel and others 2003). Significantly, such sites were not enriched in unstimulated TLR family members (Fig. 2). The nature of IFN-regulated TLR induction forms the basis of this review.

TLR2 and Its Co-Receptors, TLR1 and TLR6

TLR2 interacts with a wide variety of bacterial cell wall or mycoplasmal components, as well as fungal components such as zymosan and viruses (Takeuchi and others 2001; Gantner and others 2003; Nilsen and others 2008; Barbalat and others 2009; Kang and others 2009). TLR2 is localized to the plasma membrane, endosomes, and lysosomes in resting monocytes but internalized into phagosomes after ligand binding (Ozinsky and others 2000; Nilsen and others 2008). TLR2 heterodimerizes with its co-receptors TLR1 or TLR6 (Ozinsky and others 2000; Takeuchi and others 2001, 2002). TLR1-TLR2 recognizes predominantly tri-acylated lipopeptide structures as shown by studies using the synthetic lipopeptide, PAM_3CSK (Omueti and others 2005). TLR2-TLR6 identifies di-acylated lipopeptide, specifically the mycoplasmal lipopeptide, diacylated mycoplasmal macrophage-activating lipopeptide-2 kD (MALP-2) (Takeuchi and others 2001). TLR10 also interacts with TLR2, but the possible agonists are still unclear (Hasan and others 2005).

The interactions between TLR1-TLR2 with PAM_3CSK and TLR2-TLR6 with PAM_2CSK are well defined from crystal structure studies (Jin and others 2007; Kang and others 2009). The LRR solenoid structure of TLR1, TLR2, and TLR6 (also TLR4) is slightly different from that of the other LRR protein family members in having distortions at the parallel β-sheets giving rise to N-terminal, central, and C-terminal domains at the extracellular portion of the receptors (Jin and

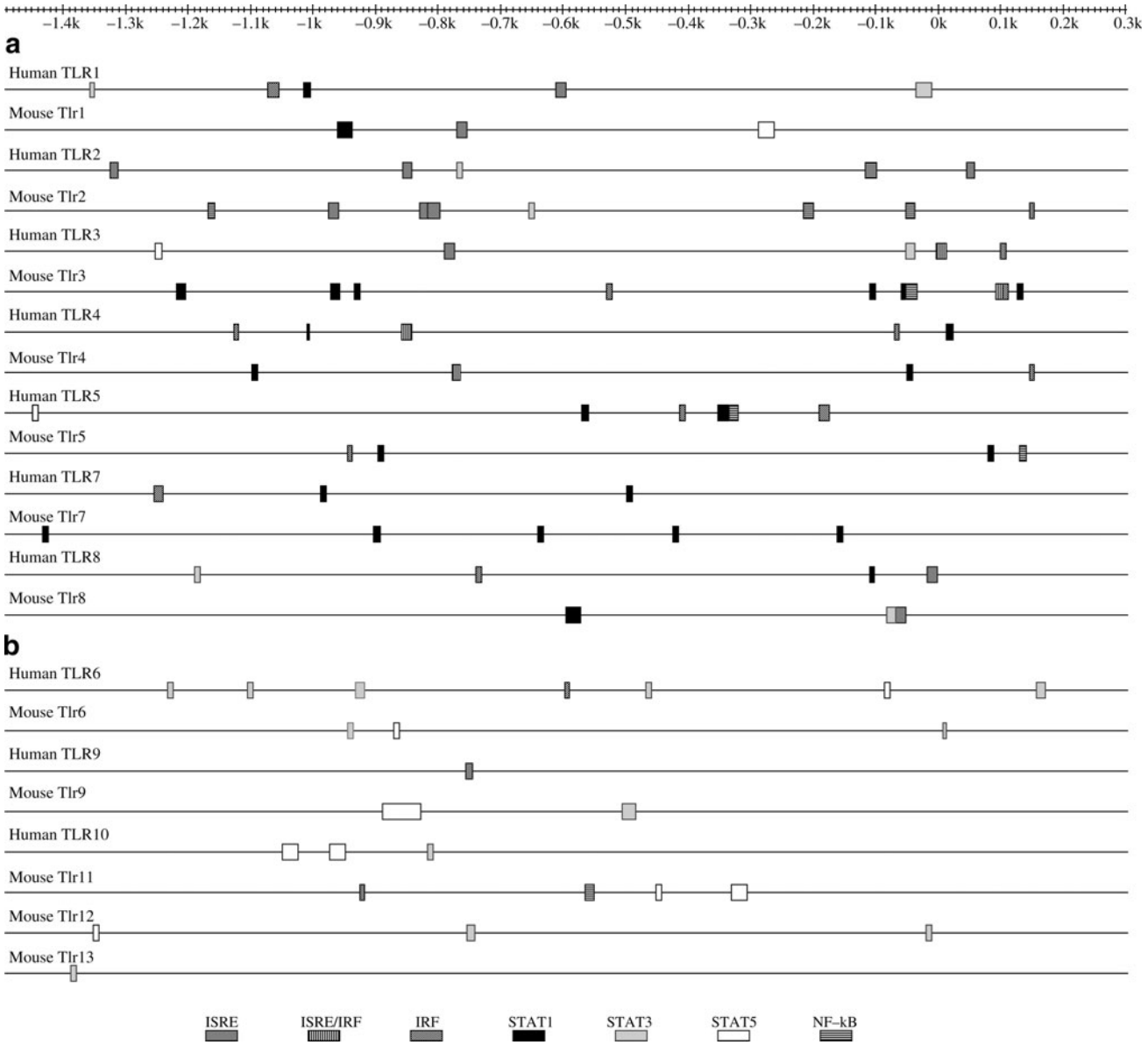

FIG. 2. Match-based putative promoter region analysis of human and mouse TLR genes. Promoter regions are defined as 1,500 bp upstream and 300 bp downstream of the transcription start site with false-positive minimization filtering applied. **(a)** TLR genes identified as IFN regulated through the Interferome database. This gene set demonstrates enrichment of conserved ISRE, ISRE/IRF, and STAT1 sites. **(b)** Analysis of those TLRs that are not identified as Interferon regulated does not demonstrate this enrichment or site conservation.

others 2007; Kim and others 2007; Kang and others 2009). TLR1 and TLR2 form an M-shaped heterodimer with the N-termini facing away from each other and the C-termini converged in the middle (Jin and others 2007). Both ester-bound acyl chains of PAM_3CSK are inserted into the internal hydrophobic pocket in TLR2 via an opening at the convex surface near the central and C-terminal domains. The third amide-linked acyl chain is inserted into the hydrophobic lipid-binding channel in TLR1. The heterodimer structure is stabilized by hydrophobic, hydrogen bonds and ionic interactions between the receptors, which are largely driven by ligand binding (Jin and others 2007). The TLR2-TLR6 heterodimer has an M-shaped structure similar to TLR1-TLR2, but with great differences at the ligand interaction and heterodimerization regions (Kang and others 2009). The hydrophobic pocket in TLR2 still accommodates the ester-linked di-acyl chain of PAM_2CSK. The truncated lipid-binding channel in TLR6 possibly confers specificity of ligand interaction as it excludes the binding of a third amide-linked acyl group, such as from PAM_3CSK. This is consistent with reports showing that TLR1-TLR2 and TLR2-TLR6 discriminate ligands partly based on the acylation state, whereby TLR1-TLR2 recognizes mono-/di-/tri-acylated synthetic lipopeptides, but TLR2-TLR6 has more restrictive requirements on both the acylation state and position of the lipid chains (Omueti and others 2005). The proposed heterodimer structures are further validated by mutational studies showing that ligand binding and signaling are prevented when hydrogen bonds on TLR2 surface are disrupted (Kajava and Vasselon 2010). TLR2 was also shown to interact

directly with lipotechoic acid (LTA) from *Streptococcus pneumoniae* (Kang and others 2009). TLR2 is found to interact with other co-receptors, including CD14, CD36, and Dectin-1, which are involved in the recognition of LTA and zymosan (Gantner and others 2003; Nilsen and others 2008).

TLR2 and Diseases

TLR2 has been implicated in the innate immune response to infection of various pathogens, including bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Helicobacter pylori*; viruses such as Vaccinia virus and Herpes simplex virus; as well as fungal pathogen such as *Paracoccidioides brasiliensis* (Smith and others 2003; Kurt-Jones and others 2004; Muir and others 2004; Loures and others 2009). As TLR2 is capable of engaging a large variety of pathogens, it has become increasingly apparent that TLR2 and its co-receptors have important roles in the progression of infectious or inflammatory diseases. In human bronchial epithelial cell lines, TLR2 is required for NF-κB activity in response to *S. aureus* and *P. aeruginosa* infection, which are dominant pathogens exacerbating lung airway inflammation in cystic fibrosis (CF) (Muir and others 2004). Critically, single-nucleotide polymorphisms (SNP) in *TLR1* and *TLR2* have been linked to human diseases, such as acute rheumatic fever and leprosy (Berdeli and others 2005; Johnson and others 2007). The *TLR1* I602S SNP in Europeans prevents the localization of TLR1 to the cell surface and subsequent signaling with TLR2, which in turn confers protection to leprosy, a *Mycobacterium leprae*-associated disease (Johnson and others 2007). The *TLR2* R753Q SNP is associated with group A streptococcal ARF in Turkish patients (Berdeli and others 2005). In a recent report, TLR2 was further shown to induce Type I IFN (IFNβ and IFNα4) expression in response to Vaccinia virus but not bacterial ligands (Barbalat and others 2009). The production of Type I IFN was attributed to a very limited specific population of monocytes ($Ly6C^{hi}$ $CD11b^{+}CD11c^{-}B220^{-}$ cells) identified from bone marrow (BM) and spleen from C57BL/6 mice and is dependent on MyD88, IRF3, and IRF7.

An increasing body of evidence has further shown that TLR2 signaling contributes to diseases involving both pathogen-induced and sterile inflammation, namely, atherosclerosis and asthma (Chisholm and others 2004; Mullick and others 2005; Kawakami and others 2008; Kormann and others 2008; Naiki and others 2008). Atherosclerosis is characterized by chronic inflammation at the blood vessel wall, where immune cells such as T-cells and macrophages constitute an important part of the atherosclerotic lesion (reviewed in Hansson 2005). In BM-reconstitution experiments, $Tlr2^{-/-}$ BM-derived cells protected $Ldlr^{-/-}$ mice from atherogenesis after stimulation with PAM_3CSK, suggesting that hematopoietically derived cells such as macrophages may exacerbate atherogenesis in response to infectious agonists (Mullick and others 2005). In contrast, TLR2 expressed in nonhematopoietic cells such as endothelial cells are responsible for atherogenic effect in response to endogenous ligands (Mullick and others 2005). In support of this study, TLR2 deficiency in $ApoE^{-/-}$ mice, which spontaneously develop hypercholesterolemia on a normal diet, exhibited reduced atherosclerotic lesions and level of the monocyte chemoattractant protein 1, an inflammatory factor essential in macrophage recruitment and atherogenesis (Liu and others 2008b). TLR2 is further shown to interact with numerous endogenous ligands, believed to be released by necrotic cells at atherosclerotic lesions, including the pro-inflammatory cytokine high-mobility group box 1 and apolipoprotein-CIII (Scaffidi and others 2002; Park and others 2004; Kawakami and others 2008). Infectious ligands, such as those from *Chlamydia pneumoniae*, were found to accelerate atherosclerosis in $ApoE^{-/-}$ mice dependent on TLR2, TLR4, and MyD88 (Naiki and others 2008).

Asthma is an allergic airway disorder characterized by chronic inflammation involving eosinophil and/or neutrophils infiltration to the lung airways and the deregulation of adaptive immunity in favor of activation of CD4 T helper 2 (Th2) cells and Th2-associated cytokines, promoting the production of IgE by B cells (reviewed in Barrett and Austen 2009). TLR2, TLR4, and CD14 mRNA levels were found to be increased in the sputum of human subjects of neutrophillic asthma (characterized by the infiltration of both neutrophils and eosinophils) (Simpson and others 2007). Human genotyping studies also showed that SNPs in the TLR2 co-receptors, TLR1, TLR6, and TLR10 are associated with reduced risk of childhood atopic asthma (Kormann and others 2008). Peripheral blood mononuclear cells (PMBCs) isolated from the carriers of the TLR1 and TLR6 SNPs showed lower Th2-associated IL-4 production in response to ligands of TLR1-TLR2 and TLR2-TLR6 heterodimers but not ligands of TLR2 only (Kormann and others 2008). However, mouse model studies on the role of TLR2 agonists on asthma-associated inflammation have produced contradictory results. Studies using BALB/c mice showed that co-immunization with ovalbumin (OVA) and TLR2 ligand peptidoglycan induces Th2 associated cytokine expression and increased airway responsiveness (Chisholm and others 2004). Another study using C57BL/6 and 129/SvEv mice shown similar Th2 immune response from co-immunization of OVA and PAM_3CSK (Redecke and others 2004). Conflicting results were observed in studies demonstrating that PAM_3CSK treatment promotes Th1 response (ie, IL-12 and IFNγ) and decreased asthmatic inflammation in BALB/c mice (Patel and others 2005). The basis for this discrepancy is still unknown, but it may be due to the difference in the experimental design such as the administration of OVA and TLR2 ligands during the sensitization and challenge phase or the genetic background of the mice. Nevertheless, studies reporting possible TLR2 ligands found in common allergens such as cockroach frass and house dust mites still emphasized the potential contribution of TLR2 agonists to the exacerbation of asthma (Page and others 2008; Phipps and others 2009).

Mouse Models Used in Asthma Studies

C57BL/6 and BALB/c mice are 2 common mouse strains used in asthma studies, but they produce a different asthma response when sensitized and challenged with OVA (Gueders and others 2009). BALB/c mice were hyperresponsive to methacholine airway challenge and appeared to promote Th2 responses by producing CCL11 and Th2-associated cytokines (IL-4 and IL-14) in lung tissue (Gueders and others 2009). On the other hand, C57BL/6 mice were hyporesponsive to the airway challenge but produced cytokines promoting eosinophillic inflammation in the bronchoalveolar lavage fluid (Gueders and others 2009). Given that there may

be a bias in BALB/c mice in IFN expression and activation compared to C57Bl/6 mice (Wells and others 2003), differences in TLR1 and TLR2 expression due to disparate IFN regulation could possibly be the underlying reason for the distinct cytokine expression and distribution patterns in these mouse types during asthma challenge.

TLR3

TLR3 contributes to innate immune signaling in response to viral infection. TLR3 ligands include viral dsRNA from viruses, released by damaged or dying cells and the synthetic dsRNA polyinosine–polycytidylic acid (poly(I:C)) (Alexopoulou and others 2001; Karikó and others 2004; Le Goffic and others 2006). dsRNA activation and induction of NF-κB, expression of pro-inflammatory cytokines such as IL-6, IL-12, and tumor necrosis factor alpha (TNFα), as well as Type I IFN are ablated in *Tlr3*$^{-/-}$ mice (Alexopoulou and others 2001). Like many other TLRs, TLR3 is expressed in a variety of cells, but TLR3 is expressed in conventional myeloid DCs (cDCs) but absent from plasmacytoid DCs (pDCs), where TLR7 and TLR9 are highly expressed and are able to secrete large amounts of IFNα in response to viral infection (Kadowaki and others 2001; Diebold and others 2004). This suggests that the separate DC subtypes have different roles in fighting viral infections. Expression of TLR3 can be upregulated by Type I IFN, viral infection, or poly(I:C) in various cell types, indicating that a positive feedback mechanism may drive TLR3 activation and signaling during viral infection (Miettinen and others 2001; Heinz and others 2003; Le Goffic and others 2006).

The intracellular localization of TLR3 is cell type dependent; it is found to be purely endosomal in human immature DCs and CD11c^{+} myeloid DCs, but can be located on the cell surface in fibroblast cell lines (Matsumoto and others 2002, 2003). In humans, a cytoplasmic linker region consisting of 2 critical residues (R740 and V741) between the transmembrane and TIR domains is essential for the targeting of TLR3 to the intracellular membranes (Nishiya and others 2005). In resting cDCs, TLR3 was expressed in the endoplasmic reticulum but translocated to the endosomal compartments in response to dsRNA where endosomal maturation is required for TLR3-ligand interaction (de Bouteiller and others 2005; Johnsen and others 2006). It was further observed that TLR3 is expressed proximal to phagosomes, suggesting that the fusion of phagosomes to endosomal compartments containing TLR3 may assist in presenting dsRNA from apoptotic cells to TLR3 (Nishiya and others 2005).

The ectodomain domain structure of TLR3 is the typical horse-shoe-shaped solenoid with a heavily glycosylated concave surface but a convex surface free from glycosylation (Bell and others 2005). Upon ligand interaction, TLR3s form dimers and multiple TLR3 dimers were observed to bind to long dsRNAs (de Bouteiller and others 2005; Leonard and others 2008). The TLR3 dimer has an M-shaped crystal structure similar to the TLR1-TLR2 or TLR2-TLR6 heterodimers, with the C-termini converging in the middle (Bell and others 2005; Liu and others 2008a). Two nucleotide interaction sites have been identified on mouse TLR3 via crystal structure studies, including one closer to the C-terminus of the extracellular domain (LRR19 to 21) and the second at the N-terminus (LRR-N-terminus to LRR3) on the glycan-free surface, and the interaction is stabilized by hydrogen bonding and electrostatic interactions (Liu and others 2008a). The TLR3 dimer has an overall dimension similar to the CD14 dimer, and it was found that TLR3 signaling is enhanced by interaction with CD14 by aiding poly(I:C) uptake (Choe and others 2005; Lee and others 2006a). The TIR domain of TLR3 is different from other TLRs as the BB loop in TLR3 TIR lacked a conserved proline, which is replaced with an alanine (Oshiumi and others 2003). This alanine is required for TLR3 interaction with its adaptor protein, TRIF.

The role of TLR3 signaling in viral infection is controversial since it has been shown to be both detrimental or protective for the host (Wang and others 2004; Le Goffic and others 2006; Daffis and others 2008). *Tlr3*$^{-/-}$ mice had a survival advantage from influenza A virus (IAV) infection over wild-type mice despite a higher viral load (Le Goffic and others 2006). It could be reasoned that TLR3 deficiency led to decreased levels of inflammatory cytokines, such as RANTES, IL-6, and IL-12p40/p70, in the bronchoalveolar space and reduced CD8^{+} T cells and macrophages. This indicates that TLR3 signaling may be important for viral clearance but could also lead to detrimental inflammatory response in the host. In addition, TLR3 signaling is primarily induces an NF-κB-dependent pro-inflammatory response, whereas the RNA helicase rectinoic-inducible gene I (RIG-I) signaling regulates both pro-inflammatory and Type I IFN antiviral responses, indicating that IAV-induced antiviral responses may require cooperative efforts from separate PRRs (Le Goffic and others 2007). TLR3 was also found to mediate inflammatory response to Coxsackievirus B4 (CVB4), a virus that has been implicated in viral myocarditis and autoimmune diabetes (Richer and others 2009). *Tlr3*$^{-/-}$ mice had a lower survival rate compared to wild-type mice after CVB4 infection and had increased viral replication and reduced inflammatory cytokines. In a more recent study, the RNA helicase melanoma differentiation-associated gene 5 (*MDA5*) was shown to be essential in mediating CVB3-induced Type I IFN response, suggesting that TLR3 and RIG-I/MDA5 may recognize different subsets of CVB strains and more studies will be required to define the specific role of each PRRs (Wang and others 2010).

Tlr3$^{-/-}$ mice displayed increased survival rate compared to wild-type mice after challenged with lethal dose of West Nile virus (WNV), with reduced inflammation and viral load in the brain tissues (Wang and others 2004). More importantly, this study also reported that the blood–brain barrier was compromised in wild-type mice but not in *Tlr3*$^{-/-}$ mice after WNV infection, suggesting that TLR3 may be important in mediating the movement of the virus across the blood–brain barrier into the brain in WNV-induced encephalitis. However, another report showed that *Tlr3*$^{-/-}$ mice were more susceptible to WNV infection and that blood–brain barrier permeability during WNV infection was not affected by TLR-deficiency (Daffis and others 2008). The discrepancies between both studies are still unexplained, but may be due to differences in experimental design (ie, the virus stock preparation or administration route). Moreover, TLR3 deficiency had no effect on WNV-induced Type I IFN levels, suggesting that other viral PRRs may be responsible for the IFN production (Daffis and others 2008).

Overall, TLR3-induced inflammatory responses to viruses may be beneficial or detrimental to the host, and other viral PRRs are very likely to have either a cooperative role or a more dominant role in inducing an antiviral Type I IFN

Table 1. TLR Mouse Models of Disease

Receptor	*Disease*	*Mouse model and ligand*	*Mouse genetic background*	*Outcome*	*References*
TLR2	Atherosclerosis	$Ldlr^{-/-}$ mice PAM_3CSK	C57BL/6	Increased atherosclerotic lesion and levels of serum amyloid A (SAA)	Mullick and others (2005)
TLR2	Atherosclerosis	$ApoE^{-/-}$ mice *Chlamydia pneumoniae*	C57BL/6	Increased MCP1, IL-12p40 TNFα, and IL-6 Increased DC recruitment, rescued by TLR2, and TLR4 deficiency	Naiki and others (2008)
TLR2	Atherosclerosis	$TLR2^{-/-}ApoE^{-/-}$ mice	C57BL/6	Reduced atherosclerotic lesion and MCP1	Liu and others (2008b)
TLR2	Asthma	*Staphylococcus aureus* peptidoglycan (co-immunized with OVA)	BALB/c	Increased Th2 associated cytokines (IL-4, IL-5, IL-13) and airway responsiveness	Chisholm and others (2004)
TLR2	Asthma	PAM_3CSK (co-immunized with OVA)	C57BL/6 129/SvEv	Increased Th2-associated cytokines (IL-13, IL-1β, GM-CSF) Reduced Th1-associated cytokines (IL-12, IFNβ, IL-18, IL-27)	Redecke and others (2004)
TLR2	Asthma	PAM_3CSK	BALB/c	Reduced inflammatory cells infiltration	Patel and others (2005)
TLR3	Influenza	$TLR3^{-/-}$ IAV	C57BL/6	Reduced lethality despite higher viral load Decreased inflammatory response (ie, RANTES, IL-6, IL-12p40/p70) Reduced $CD8^+$ T cells and macrophages	Le Goffic and others (2006)
TLR3	WNV encephalitis	$TLR3^{-/-}$ WNV	C57BL/6	Increased survival Reduced inflammation in the brain but increased inflammatory cytokines (IL-6, TNFα) in peripheral blood	Wang and others (2004)
TLR3	WNV-induced encephalitis	$TLR3^{-/-</BP/>}$ WNV	C57BL/6	Decreased survival but has no effect on Type I IFN induction myeloid cells	Daffis and others (2008)
TLR5		$TLR5^{-/-}$ Flagellin *Salmonella typhimuriium*	C57BL/6	Reduced pro-inflammatory cytokine (IL-6) expression Increased resistant to oral *S. typhimuriium* infection	Uematsu and others (2006)

TLR5	Colitis	TLR5$^{-/-}$	C57BL/6	Developed spontaneous colitis Increased pro-inflammatory cytokines and genes associated with TLR4-LPS signaling	Vijay-Kumar and others (2007)
TLR5	Metabolic syndrome	TLR5$^{-/-}$	C57BL/6	Mild intestinal inflammation Metabolic syndrome features such as insulin resistance, hyperlipidemia, increased adipocity	Vijay-Kumar and others (2010)
TLR5	Lung infection	TLR5$^{-/-}$ *Pseudomonas aeruginosa*	C57BL/6	Impaired bacterial clearance, neutrophil infiltration in the bronchoalveolar air space, and expression of MCP1 and TNFα	Morris and others (2009)
TLR7	Influenza	TLR7$^{-/-}$ IAV	C57BL/6	Impaired IFNα production	Diebold and others (2004)
TLR7	Influenza	TLR7$^{-/-}$ IAV	C57BL/6	Impaired development of adaptive immune response (reduced virus-specific IgG1 and IgG2a and IFNγ)	Koyama and others (2007)
TLR7	Neonatal sepsis	TLR7$^{-/-}$ MyD88$^{-/-}$ Group B streptococci	C57BL/6	Neonatal mice highly susceptible Reduced IFNβ production in MyD$^{-/-}$ mice	Mancuso and others (2009)

DC, dendritic cell; IFN, interferon; IL, interleukin; MCP1, monocyte chemoattractant protein 1; TLR, Toll-like receptor; TNFα, tumor necrosis factor alpha; WNV, West Nile virus.

response specific to particular viruses. The presence of TLR3 only in myeloid DCs but not pDCs suggests the possibility of TLR3 being required for modulating the adaptive immune response rather than mediating Type I IFN in antiviral signaling (Kadowaki and others 2001). Myeloid DCs are antigen-presenting cells essential for processing and cross-presenting exogenous antigens to cytotoxic $CD8^+$ T cells (reviewed in William and others 2004). Murine $CD8\alpha^+$ DCs are activated by exogenous dsRNA or apoptotic bodies from virally infected cells acquired from phagocytosis, resulting in cytotoxic T cell cross-priming, which is dependent on TLR3 (Schulz and others 2005). BM reconstitution experiments showed that immunization of C57BL/6 mice with virally infected cells or dsRNA with OVA antigen induces OVA-specific $CD8^+$ T cells expansion, which required TLR3-expressing DCs (Schulz and others 2005). Thus, TLR3 may have multiple roles in eliciting immune responses to viral infection by bridging innate and adaptive immunity.

TLR5

TLR5 recognizes flagellin from Gram-positive and Gram-negative bacteria, such as from *Salmonella typhimurium*, *Listeria monocytogenes*, *Bacillus subtilis*, *Legionaire pneumophila*, and *P. aeruginosa* (Hayashi and others 2001; Gewirtz and others 2001; Hawn and others 2003; Morris and others 2009). TLR5-induced signaling in response to flagellated bacteria is MyD88 dependent, as $myd88^{-/-}$ mice were unresponsive to purified flagellin (Gewirtz and others 2001; Hayashi and others 2001). TLR5 has further been shown to have a crucial role in intestinal immunity in several studies (Gewirtz and others 2001; Vijay-Kumar and others 2007; Uematsu and others 2008). Although TLR5 is expressed on the basolateral surface of intestinal epithelial cells and induced signaling in response to flagellin, TLR5 was observed to be highly expressed in the $CD11c^+CD11b^+$ DC subset of lamina propria (LPDC) in intestines of mice, which may be the dominant producer of pro-inflammatory cytokines and chemokines from TLR5 (Gewirtz and others 2001; Uematsu and others 2006, 2008). LPDCs from wild-type mice were found to express pro-inflammatory cytokines in response to flagellin and *S. typhimurium*, which was prevented in LPDCs from $Tlr5^{-/-}$ mice (Uematsu and others 2006). LPDCs expressing TLR5 were able to modulate adaptive immune signaling by promoting B-cells differentiation into IgA^+ plasma cells and development of antigen-specific Th1/Th17 response, which may modulate the pathogenesis of intestinal bowel diseases (Uematsu and others 2008). However, $Tlr5^{-/-}$ mice demonstrated higher resistance to oral *S. typhimurium* infection, and the translocation of *S. typhimurium* from the intestinal tract to the draining mesenteric lymph nodes was impaired in $Tlr5^{-/-}$ mice, suggesting that TLR5 may required for the establishment of *S. typhimurium* systemic infection (Uematsu and others 2006).

$Tlr5^{-/-}$ mice were also shown to develop spontaneous colitis or histopathologic features of colitis, and had higher commensal bacterial load, aberrant pro-inflammatory cytokines expression, and increased body weight in a proportion of the mice (Vijay-Kumar and others 2007). However, a dominant negative *TLR5* SNP is associated with reduced risk for Crohn's disease in human, which may be reasoned by the differences in TLR5 expression profile or commensal microflora in human and mice, as well as dysregulated signaling via other PRRs (Gewirtz and others 2006). In a more recent report, $Tlr5^{-/-}$ mice with standardized gut microflora were shown to have reduced incidence of colitis, and uniformly exhibited mild intestinal inflammation as well obesity (Vijay-Kumar and others 2010). These $Tlr5^{-/-}$ mice displayed impaired glycemic control driven by insulin resistance, hyperlipidemia, increased adiposity, and pancreatic inflammatory infiltrates after fed with high-fat diet, which are characteristic of metabolic syndrome in human (Vijay-Kumar and others 2010). Moreover, depleting gut microbiota in $Tlr5^{-/-}$ mice reduced metabolic syndrome features, and the transfer of $Tlr5^{-/-}$ mice gut microbiota to wild-type germ-free mice conferred $TLR5^{-/-}$ phenotype to the recipient mice, including obesity, insulin resistance, and increased pro-inflammatory cytokines. This study emphasized that TLR5 deficiency may result in alteration of the gut microflora and mild inflammation leading to the manifestation of metabolic syndrome features, suggesting that changes in host–microbiota interaction can contribute to metabolic syndrome.

TLR5 has also been implicated in lung diseases, such as in *P. aeruginosa* and *L. pneumophila* infection (Morris and others 2009). $Tlr5^{-/-}$ mice displayed impaired bacterial clearance, neutrophil infiltration in the bronchoalveolar air space, and expression of monocyte chemoattractant protein 1 and TNFα after challenged with low dose of *P. aeruginosa*, but the effects were less prominent in high doses of *P. aeruginosa*, indicating that other TLRs may compensate for the loss of TLR5 (Morris and others 2009). Consistently, *Tlr5* was found to be highly expressed in CF lung epithelial cell lines and TLR5-flagellin signaling contributes to the aggravated inflammation in response to *P. aeruginosa* in the CF cell lines, which was not observed when challenged with flagellin-deficient *P. aeruginosa* (Blohmke and others 2008).

TLR7

TLR7 was initially shown to recognize synthetic antiviral low-molecular-weight compounds such as imiquimod and R-848, but now it is known to be activated by RNA species from mainly ssRNA viruses, including human deficiency virus 1 (HIV-1), IAV, and Lymphocytoid Choriomeningitis virus in mice (Hemmi and others 2002; Heil and others 2004; Koyama and others 2007; Jung and others 2008). Together with TLR9, TLR7 is highly expressed in pDCs, and is critical in signaling Type I IFN production by pDCs in response to viral ssRNA induction (Kadowaki and others 2001; Diebold and others 2004). TLR8 is closely related to TLR7, but it signals primarily in human monocytic DCs and not expressed in mice (Heil and others 2004; Gorden and others 2005). In a study using a human macrophage-like cell line, TLR7 and TLR8 display sequence-specific ssRNA stimulation; however, it appears that TLR7 may be more important in recognizing the various ssRNAs used compared to TLR8 (Gantier and others 2008). Further studies are required to define the role of human TLR7 and TLR8 in recognizing ssRNA and the effect of its downstream cytokine induction in response to pathogen infection.

TLR7 expression was shown to be induced and upregulated in human microphages by Sendai virus and IAV (Miettinen and others 2001). Similar to TLR3, TLR7 has intracellular localization primarily in endosomal compartments (Diebold and others 2004; Nishiya and others 2005; Mancuso and others 2009). In contrast to TLR3, the trans-

membrane domain in TLR7 is sufficient for targeting to endosomal membranes (Nishiya and others 2005). Internalization of ligands into the intracellular compartments such as phagolysosomes or endosomes is required for TLR7 recognition (Diebold and others 2004; Mancuso and others 2009). In BM-derived DCs, TLR7 was found to be expressed in the endoplasmic reticulum during resting state, but translocated to the lysosomal or endosomal compartment after imiquimod stimulation (Kim and others 2008).

TLR7 signaling has been further shown to be critical in stimulating Type I IFN in response to viral infections. During IAV infection, TLR7 is required to induce IFNα production in mouse BM-derived pDCs (Diebold and others 2004). Moreover, The TLR7-MyD88-dependent signaling pathway in mice infected intra-nasally with IAV was shown to be important for the development of adaptive immune response, including B cell production of virus-specific IgG1 and IgG2a and $CD4^+$ T cell-induced IFNγ (Koyama and others 2007). Recently, TLR7 signaling was suggested to have a protective role against HIV-1 in human subjects resistant to HIV-1 infection (HIV-1–exposed seronegative individuals), whereby TLR7 signaling in PMBCs of the subjects resulted in elevated levels of cytokines and chemokines compared with healthy control subjects after stimulation with TLR7 ligands (Biasin and others 2010). TLR7 and TLR8 in humans are activated by HIV-1 ssRNA, and TLR7/TLR8 activation induced by R-848 prevented HIV-1 replication in human lymphoid tissue but allowed for virion production in latently infected promonocytic cells lines (Heil and others 2004; Schlaepfer and others 2006). However, the exact immunologic factor induced by TLR7/TLR8 essential for modulating viral replication is still unidentified as it is independent of the IFN and IFN-associated genes studied (Schlaepfer and others 2006). Another specific TLR7 agonist (SM360320) has also been shown to suppress Hepatitis C virus replication in human hepatoma cell lines carrying the viral replicon and liver tissues, which was also independent of extracellular Type I IFN, as inhibiting Type I IFN receptors with specific neutralizing antibodies did not relieve the suppression (Lee and others 2006b). These studies indicate that TLR7 signaling may be crucial for regulating viral replication by having direct antiviral effects in the infected cells in addition to Type I IFN response.

Despite the majority of literature suggesting a primary function for TLR7 signaling in mediating antiviral immune response, recent studies have shown that TLR7 is also capable of recognizing bacterial RNA ligands and induces Type I IFN response in bacterial infection (Mancuso and others 2009; Petzke and others 2009). TLR7 expressed in mouse BM-derived cDCs but not pDCs was shown to induce IFNβ production in response group B streptococci (GBS), a pathogen important in neonatal sepsis in human and mice (Mancuso and others 2009). $Tlr7^{-/-}$ neonatal mice were highly susceptible to low-dose GBS infection compared with wild-type pups, indicating that TLR7 signaling may contribute to the Type I IFN response, which has been shown to be important for defense against GBS infection in mice (Mancuso and others 2007, 2009). TLR7 and TLR9 have also been recently described to induce IFN response to *Borrelia burgdorferi*, a spirochete associated with Lyme disease (Petzke and others 2009). Stimulating human PMBCs with *B. burgdorferi* resulted in increased expression of Type I and II IFNs, as well as a range of IFN-induced genes. Inhibiting TLR7 and/or TLR9 in human PMBCs reduced IFNα production after *B. burgdorferi* stimulation, suggesting that TLR7/TLR9 signaling may contribute to the IFN and IFN-associated gene expression.

Similar to other TLRs, TLR7 is able to identify endogenous ligands, specifically self-nucleic acids, which may contribute to the development of systemic autoimmune disease such as systemic lupus erythematosus and psoriasis (Vollmer and others 2005; Lee and others 2008; Ganguly and others 2009). U1 small ribonucleoprotein particles, which are highly present in apoptotic bodies, were shown to form immune complex with autoantibodies from systemic lupus erythematosus patient sera and delivered into pDC and monocyte subsets in human PMBCs (Vollmer and others 2005). The RNA sequences within these small ribonucleoprotein particles were able to activate TLR7/TLR8/TLR9, leading to induction of IFNα and TNFα. The anti-microbial peptide LL37, which is highly expressed in inflamed psoriatic skin, was shown to form complex with self-RNA from apoptotic and necrotic cells, transporting them into human pDCs and mDCs, activating TLR7/TLR8 signaling (Ganguly and others 2009). The above studies emphasized the importance of TLR7 and TLR8 intracellular localization in preventing extracellular self-RNA recognition, since autoimmune TLR7/TLR8 signaling is triggered when extracellular self-RNA is aberrantly delivered into the endosomal compartments.

Conclusions

TLRs are involved in a wide variety of diseases and display a broad repertoire of ligands, both microbial and endogenous. Indeed, therapeutic targeting of TLRs is being vigorously investigated by numerous biotechnology companies and researchers as a means of intervening in a multitude of human diseases as described in this review. Interestingly, quite a few TLRs are Type I IFN regulated, yet TLR4, perhaps one of the most widely studied TLRs, is not, which may explain why TLR4 is widely expressed in a variety of tissues and cell types, yet other TLRs, such as those described herein, display more selective or specific distribution and expression.

Interestingly, given our increasing dependence on mouse models for dissecting TLR-mediated human diseases, the genetic background of the mouse may be critical in the disease phenotype or interpretation of the data (see Table 1). An obvious difference between mouse and human disease models is the lack of TLR8 expression in mice, which may bias or complicate the interpretation of nucleic acid response. Further, variations or fluctuations of Type I IFN in in-bred mice colonies such as Balb/c and Bl/6 may not truly reflect the wide variation of IFN expression existent in the human population, which may affect or alter subsets of TLR expression and hence responses. Indeed, as we have noted in this review, while TLR1 and TLR2 are IRGs, TLR6 does not appear to be, which may affect expression and ultimately the responses of TLR2 heterodimers to specific ligands dependent on priming or inflammation-induced IFN expression.

As such, it would appear that the modulation and regulation of the innate immune response via the TLRs is another function that can be attributed to the pleiotropic effects IFNs induce and control, something to keep in mind when using models of disease where expression or immunomodulation of IFNs is altered or deleted.

Author Disclosure Statement

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