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TLR8: The Forgotten Relative Revindicated

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MINI REVIEW

TLR8: the forgotten relative revindicated

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The endosomal Toll-like receptors (TLRs) TLR3, TLR7, TLR8 and TLR9 are important in sensing foreign nucleic acids encountered by phagocytes. Because TLR8 was initially thought to be non-functional in mice, less is known about TLR8 than the genetically and functionally related TLR7. Originally associated with the recognition of single-stranded RNA of viral origin, there is now evidence that human TLR8 is also able to sense bacterial RNA released within phagosomal vacuoles, inducing the production of both nuclear factor (NF)-κB-dependent cytokines and type I interferons (IFNs), such as IFN-β. The functions of TLR8 extend beyond the recognition of foreign pathogens and include cross-talk with other endosomal TLRs, a process that may also have a role in the generation of autoimmunity.
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INTRODUCTION

Over the past two decades, our understanding of the innate immune system has increased exponentially,[1–3] and the role of Toll-like receptors (TLRs) in pathogen sensing has been at the forefront of innate immunity research. TLRs are transmembrane pattern recognition receptors capable of recognizing generic pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns. Their activation induces intracellular signaling pathways that result in production of inflammatory cytokines as well as type I interferons (IFNs). TLRs are composed of an ectodomain containing leucine-rich repeats that facilitate PAMP recognition, a transmembrane domain and the intracellular Toll IL-1 receptor (TIR) domain which enables downstream signal transduction.[2]

There are 10 known functional TLRs in humans and 12 in mice;[3] TLRs 1–9 are conserved in both species.[3] Each TLR is associated with the recognition of specific PAMPs, and the response that ensues upon their activation is dependent upon the particular pathogen and the immune cell subtype involved.[4] TLR-mediated recognition of its cognate PAMPs can occur at the plasma membrane or at endosomal and/or endolysosomal membranes.[2] TLR1, 2, 4, 5, 6 and 11 are primarily, although not exclusively, expressed on the plasma membrane of immune cells. These TLRs recognize a variety of unique microbial membrane components like lipids, lipoproteins and proteins. Conversely, TLR 3, 7, 8 and 9 are expressed on intracellular vesicular membranes and are commonly involved in recognition of nucleic acids (Figure 1).[5] Besides the endosomal TLRs, several other nucleic acid sensing systems have been characterized in the cytosol. These include the retinoic acid-inducible gene I, and retinoic acid-inducible gene I-like receptors melanoma differentiation-associated protein 5, and LGP2, which are capable of recognizing RNA of different structures,[6,7] and the pyrin and HIN domain-containing protein family member absent in melanoma (AIM2) which senses dsDNA.[8,9] The signal transduction pathways activated by these pattern recognition receptors are quite well characterized, and will not be discussed in this review.

Until recently, very little attention was paid to TLR8, as this TLR was initially considered to be inactive in mice.[10] Several recent reports have demonstrated that TLR8 is not only involved in the production of type I IFNs in response to viral pathogens, but also triggered upon ligation of bacterial RNA.[11–14]

ENDOSOMAL TLRS

The prototypical intracellular TLRs (TLR3 and TLR7–9) are generally associated with sensing nucleic acids released within endosomal compartments. Their activation leads to the production of a variety of nuclear factor (NF)-κB-mediated cytokines (i.e. tumor necrosis factor) and type I IFNs.[6] TLR3 recognizes double-stranded RNA and double-stranded RNA synthetic analogs (poly I:C).[3] The genes which code for TLR7 and TLR8 are located in the X chromosome. TLR7 and TLR8 are phylogenetically similar[15] and both are capable of recognizing single-stranded RNA and short double-stranded RNA,[16] hence their role in sensing different viral pathogens.[17] TLR7 and TLR8 can also detect oligoribonucleotides and a variety of synthetic chemical agonists such as imidazoquinolines.[15,18,19] TLR9, by contrast, recognizes non-methylated 2′-(cytidine-phosphate-guanosine) DNA motifs which are present in some viruses and bacteria.[3,20]

TLR8 is known to be primarily expressed in monocytes/macrophages and myeloid dendritic cells (DCs),[21,22] while TLR7 is predominately expressed in plasmacytoid DCs and, to some extent, in B cells and monocytes/macrophages.[15,22] There is minimal or absent expression of TLR9 in monocytes, so their endosomal TLR profile constitutes essentially the opposite of that of DCs.[15,22]

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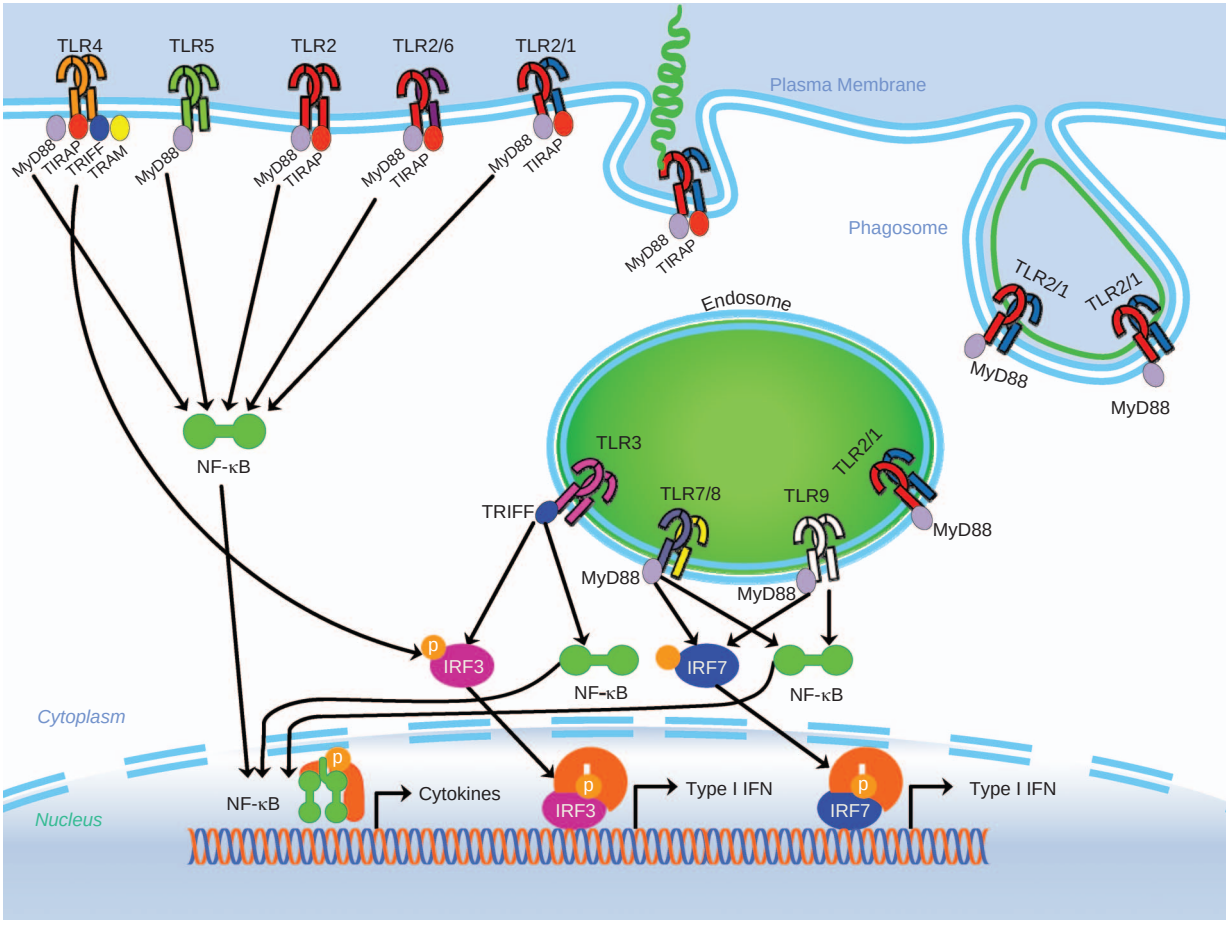

Figure 1 TLR signaling overview: the TLR family can be subdivided into two categories. TLR1, TLR2, TLR4, TLR5 and TLR6 are located primarily on the cell surface. Conversely, TLR3, TLR7, TLR8 and TLR9 are located within intracellular vesicles. Furthermore, TLRs can be found as homo or heterodimers (such as TLR1/2 and TLR2/6). Upon recognition of a PAMP, the TLR changes its conformation and recruits adapter proteins such as TRIF, TRAM, MyD88 and TIRAP. The cell surface TLRs, including the heterodimers TLR1/2 and TLR2/6, recruit MyD88 and TIRAP adaptor proteins to signal the NF-κb pathway to produce NF-κb-mediated cytokines. Additionally, TLR4 uses the TRIF and TRAM proteins to produce type I IFNs through the IRF3 pathway. Of the endosomal TLRs, TLR8 and TLR9 utilize adaptor protein MyD88 to signal through NF-κb and IRF7 pathways. Finally, endosomal TLR3 enlists the TRIF adaptor to create both type I IFNs as well as NF-κb cytokines through IRF3 and NF-κb pathways. The model depicts internalization of an extracellular pathogen, *Borrelia burgdorferi* (Bb), which is internalized into the phagosome where TLR2 and TLR8 signalling occurs. Phagocytosis of live Bb, induces transcription of IFN-β through IRF-7, a phenomenon entirely dependent on the availability of TLR8, while induction of NF-κb-dependent cytokines is due to a cooperative action of TLR2 and TLR8.[10] IFN, interferon; IRF, IFN regulatory factor; PAMP, pathogen-associated molecular pattern; NF, nuclear factor; TLR, Toll-like receptor.

Shuttling of the TLR from the endoplasmic reticulum compartment to the endosome is now considered to be critical for their activation.[23] Recent evidence suggests that endosomal TLRs are delivered from the endoplasmic reticulum to the endosome by the endoplasmic reticulum-resident membrane protein UNC93B1.[24,25] In the case of TLR8, UNC93B1 appears to be required not only for intracellular trafficking, but also for cell signaling.[25] After activation by its cognate ligands, TLR8 is modified to yield species of different molecular mass, including a monoubiquitinated form which might serve as a cell signal for trafficking in the endocytic pathway.[23] Attachment of monoubiquitin to membrane proteins serves a signal for internalization of the TLR into the endosome, the site of active signaling.

TLR8 IS ACTIVATED BY BACTERIAL RNA

While the role of TLR7 in sensing bacterial RNA is now well accepted,[26,27] a similar role for TLR8 has only began to be uncovered. Using TLR7 and TLR8 stably transfected HEK cell lines, it was previously shown that *Escherichia coli* total RNA induced activation of TLR7 and TLR8.[28] Two relatively recent studies suggested that bacterial nucleic acids could induce TLR8 activation. In the first study, TLR8 was upregulated following phagocytosis of *Mycobacterium bovis* by THP-1 cells.[11] In the same study, the investigators showed that there was an association of *TLR8* gene variants with susceptibility to pulmonary tuberculosis. In the second study, phagocytosis of *Helicobacter pylori* by THP-1 cells induced TLR8 activation.[12] We recently showed that phagocytosis of live *Borrelia burgdorferi*, the spirochetal bacterial agent of Lyme disease, induced transcription of IFN-β[29] and that this phenomenon was entirely dependent on the availability of TLR8,[13] while induction of NF-κb-dependent cytokines was due to a cooperative action of TLR2 and TLR8. More recently, we provided *ex vivo* evidence that TLR8 activation is triggered by recognition of borrelial RNA delivered to endosomal vacuoles.[14] In these same studies, we demonstrated that in highly purified human monocytes, TLR8 is self-amplifying and solely responsible for induction of IFN-β through IRF-7 (Figure 1),[13] a similar signaling pathway associated with recognition of RNA viruses.[30]

Bacterial ribosomal RNA appears to be the major stimulatory fraction to elicit production of IFN-α response by human peripheral blood mononuclear cells,[27] although transfer RNA from some bacteria may also induce production of IFN-α through TLR7 activation.[31]

MURINE TLR8 ACTIVATION

Though mouse and human TLR8 are highly related, they have shown differential receptor specificity both to natural/physiological and to synthetic TLR ligands.[10,32] Historically, murine TLR8 was thought to be non-functional as it was initially observed that TLR7$^{-/-}$ mice did not respond to the TLR7/8 agonist R848,[10] or TLR8 RNA ligands.[33] However, when murine peripheral blood mononuclear cells were treated simultaneously with TLR8 selective imidazoquinoline 3M-002 and polyT oligonucleotides, enhanced TLR8 activation and suppression of TLR7 was observed.[34] A recent report provided evidence that TLR8 can be activated in murine plasmacytoid DCs by vaccinia virus DNA, an A/T rich DNA virus.[35] This finding is controversial not only because DNA has not been previously linked to TLR8 activation, but also because murine plasmacytoid DCs are generally known to express TLR7 and TLR9, and not TLR8.[36]

TLR CROSS-TALK

Several investigators have shown that responses to viral and bacterial pathogens are not merely dependent on activation of individual TLRs, but instead by complex TLR–TLR interactions.[37,38] Synergistic effects have been observed between endosomal TLRs, like augmentation of NF-κb and IFN regulatory factor (IRF) activation, when monocyte-derived macrophages and DCs are stimulated concomitantly with TLR8, as well as with TLR3 and TLR4 ligands.[37,39] TLR8 may also act synergistically with intracellular pattern recognition receptors, such as nucleotide oligomerization domain receptor to induce inflammatory cytokine production.[40]

In line with this idea, we have provided evidence that TLR2 expression is upregulated following TLR8 activation in human monocytes[14] (Figure 2), a phenomenon occurring possibly through an IFN-β autocrine/paracrine effect.[20] A similar phenomenon has been observed in THP-1 cells, where preexposure to the TLR8 ligand 3M-002, increased the response to subsequent stimulation with TLR2 ligands.[41]

The TLR7/8 ligand R848 also increases expression of CD14 in monocytes as they differentiate into DCs.[42] This is an important finding because CD14 facilitates TLR2-dependent signaling by bacterial lipoproteins.[43–45] Upregulation of TLR2 and TLR8 also has been associated with enhanced clearance of human papillomavirus infection.[46] All this evidence suggests beneficial implications in the use of TLR7/8 agonists as vaccine adjuvants.

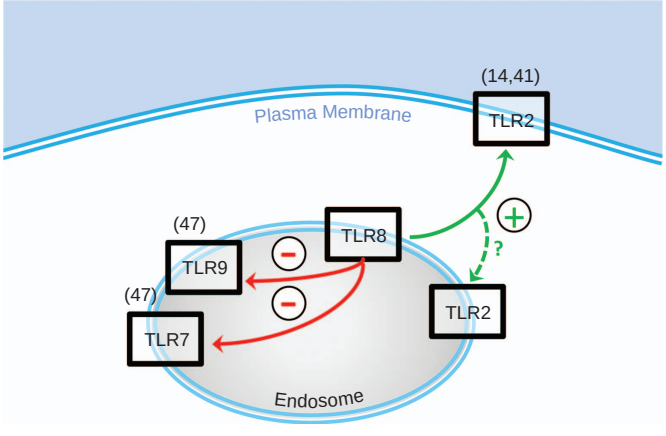

Figure 2 Regulatory effects of TLR8: human TLR8 downregulates TLR7 and TLR9 signalling. Similarly murine TLR8 inhibits TLR7.[47] Conversely TLR8 can upregulate TLR2 expression.[14]

Human TLR8 inhibits TLR7 and TLR9 activation, and murine TLR8 also inhibits TLR7 activation[47] (Figure 2). TLR8 deficiency leads to overexpression of TLR7 in murine DCs with increased NF-κb activation and development of autoimmunity.[48] In humans, TLR7 and TLR9 are upregulated in patients with Sjogren's syndrome, while TLR8 is not.[49]

TLR8 SPLICE VARIANTS

Given that TLR8 appears to play a role in the sensing of phagocytosed bacteria, *TLR8* SNPs could alter or modulate TLR8 function. The *TLR8* gene locus encodes for two splice variants (TLR8v1 and TLR8v2) with alternative translation start sites.[50,51] Their proteins differ in 19 additional amino acids in the *N*-terminus of TLR8v1. A third missense variant, termed G or A1G allele of *TLR8* (rs3764880), affects the coding region of TLR8v2, rendering a truncated form.[12] While all three variants appear to exhibit similar function,[12] TLR8v2 is the most conserved form of TLR8 amongst primates and seems to be the prevalent form of TLR8 expressed in human monocytes.[12] The effect of rs3764880 appears to be mostly related to changes of Kozak context, which reduce the amount of TLR8 which is generated.[12]

Interestingly, phagocytosis of *H. pylori* by THP-1 monocytic cells results in significant upregulation of the two main TLR8 variant mRNAs.[12] The attenuated form of *M. bovis*, also known as bacille Calmette-Guérin, is also able to induce TLR8 protein expression in the same cell line, when located in the phagosome.[11] *Mycobacterium tuberculosis* has been reported to induce both TLR8 variant mRNAs in the blood of patients with active TB.[11] The A allele of the TLR8 polymorphism rs3764880 was associated with increased susceptibility to pulmonary tuberculosis, while its allele G was associated with protection against active tuberculosis.[11] A decrease in TLR8v2 translation seen with the G allele of rs3764880[12] may imply a decrease in TLR8 sensing and activation. Protection against HIV-1 and TB disease progression, which has been observed in patients with this *TLR8* allele[11,52] could be due to a resultant lesser inflammatory response. HIV-1 replication in human DCs may require TLR8-dependent NF-κb activation,[17] and in the case of *M. tuberculosis*, compromised phagosomal sensing would translate into a limited immune response. Lastly, TLR8v1, appears to be involved in the positive regulation of TLR8 function in CD16^{+}CD14^{+}-differentiated monocytes,[12] a subset of differentiated human monocytes with enhanced proinflammatory properties.[53]

TLR-8 AGONISTS

TLR8 agonists are attractive targets for use as vaccine adjuvants, due to their efficiency in activating inflammatory immune responses.[54] The use of R848 (Resiquimod) is effective at activating local immune responses,[55] and CL075, a specific TLR8 agonist, stimulates the generation of inflammatory monocyte-derived DCs.[56] There is promising therapeutic potential of TLR8 agonists in cancer, aiming to induce an immune response to tumors and improve clinical responses to clinically approved monoclonal antibody therapies, especially in individuals who show reduced antibody-dependent cell-mediated cytotoxicity activity. A number of endosomal TLR agonists are being tested for their potential to enhance antitumor immunity.[57] Recently a new TLR8 agonist (VTX-2337) that activates monocytes, DCs and natural killer cells was shown to increase IFN-γ production, cytolytic activity, and to enhance rituximab-mediated antibody-dependent cell-mediated

cytotoxicity, even in individuals with genotypes associated with a reduced affinity for therapeutic monoclonal antibodies.[58]

Another TLR8 agonist (VTX-1463) is currently in development for the treatment of allergic rhinitis.[59] The basis for its use relies on the induction of T helper 1 (Th1)-associated inflammatory mediators, such as IL-12, IFN-γ and monocyte chemotactic protein-1 after TLR8 activation. By promoting TLR8 signaling, the cytokine response may activate antigen presenting cells, thereby inducing a Th1 type response.[57] The induction of this Th1 type response aims to shift the balance of the Th1/Th2 ratio in favor of a reduction in the allergic reaction. Taking advantage of this inducible Th1 type response through TLR8 activation, the use of an HIV vaccine where the HIV-gag protein has been coupled with a TLR-7/8 agonists improves the $CD8^+$ T cell response in rhesus macaques.[60] Stimulation of TLR7/8 by R-848 has been reported to suppress HIV-replication in cultured monocytes.[61]

CONCLUSIONS

Bacterial nucleic acid sensing by intracellular innate immune receptors is an important component of the host immune response to microbial infections.[26] Initially associated with the recognition of single-stranded RNA of viral origin, herein we have summarized the evidence that human TLR8 is also able to sense bacterial RNA.[11–14] Bacterial RNA constitutes a viability-associated PAMP (vita PAMP),[62] in contrast to its naked ligand counterpart. Internalization and degradation of bacteria in phagosomes, allows close interaction between bacterial RNA with endosomal TLR8. TLR8 in turn has the ability to upregulate other TLRs and further modulate the immune response to bacterial pathogens.

TLR-mediated recognition of bacterial ligands in the phagosome promotes selection of bacterial antigens for optimal presentation on MHC class II, and leads to the induction of costimulatory molecules and cytokines necessary for activation and differentiation of T lymphocytes. This not only constitutes the basis for the use of TLR8 ligands as vaccine adjuvants,[54] but explains the regulation of adaptive immunity by the innate immune system,[63] and the detrimental effect of endosomal-TLR-mediated autoimmunity.[64] The potential role of TLR8 in the generation of a critical immune response against bacterial infection and cancer has just begun to be uncovered.

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