Review

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Trafficking of endosomal Toll-like receptors

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Over the past decade we have learned much about nucleic acid recognition by the innate immune system and in particular by Toll-like receptors (TLRs). These receptors localize to endosomal compartments where they are poised to recognize microbial nucleic acids. Multiple regulatory mechanisms function to limit responses to self DNA or RNA, and breakdowns in these mechanisms can contribute to autoimmune or inflammatory disorders. In this review we discuss our current understanding of the cell biology of TLRs involved in nucleic acid recognition and how localization and trafficking of these receptors regulates their function.

Innate immune receptors and pattern recognition

The immune system utilizes a variety of receptors to detect potentially harmful microbes such as bacteria, viruses, and fungi. The adaptive immune system consists of millions of lymphocytes, each expressing a distinct antigen receptor generated through gene rearrangement, and provides an extremely broad repertoire of specificities. The innate immune system employs a distinct strategy of microbial recognition with a limited set of receptors expressed broadly on multiple cell types. Thus, compared to the adaptive immune system, the innate immune system sacrifices breadth for the ability to respond rapidly [1].

Over the past 15 years several families of innate immune receptors have been described, including TLRs, RIG-I (retinoic acid inducible gene 1)-like receptors (RLRs), AIM2 (absent in melanoma 2)-like receptors (ALRs), NOD (nucleotide-binding oligomerization domain)-like receptors (NLRs), and C-type lectin receptors (CLRs) [2–6]. Because the specificities of innate receptors are germline-encoded, they have been shaped through evolution to recognize highly conserved features of microbes, termed pathogen-associated molecular patterns (PAMPs) [1]. Examples of PAMPs are lipopolysaccharide (LPS) and lipoteichoic acid, both conserved features of broad bacterial classes [2] (Figure 1). Targeting PAMPs enables innate immune cells to recognize diverse microbes with relatively few receptors. Signals induced by innate receptors not only initiate rapid antimicrobial defenses but also control activation of adaptive immunity [7]. In this way the innate immune system provides key information about the nature of antigen; that is, activation of innate receptors determines when an immune response is appropriate.

Although innate immune recognition of microbes is crucial for effective immunity, it is also important that the immune system avoids responses to self molecules that could lead to autoimmunity. This possibility may appear unlikely, based on the specificity of innate receptors for PAMPs, but there is now considerable evidence that recognition of self ligands by innate immune receptors contributes to many autoimmune or autoinflammatory disorders [8]. This concept is particularly well-established for members of the TLR family, a subset of which recognizes nucleic acids, which can be foreign or self-derived [8] (Figure 1). Numerous regulatory mechanisms exist to prevent the activation of TLRs by self molecules, but one major aspect appears to be tight control of receptor localization and trafficking. In this review we discuss recent findings that examine the cell biological mechanisms that regulate nucleic acid recognition by TLRs and the consequences of when these mechanisms fail.

Nucleic acid recognition by TLRs

TLRs comprise a family of type I transmembrane proteins, each with an N-terminal ectodomain consisting of multiple leucine-rich repeat (LRR) domains involved in ligand binding, as well as a C-terminal cytosolic region containing a Toll/interleukin-1 receptor (TIR) domain that mediates recruitment of signaling components [2]. Based on existing structures of TLR ectodomains, the activated, ligand-bound state appears to be a dimer [9–16]. TLRs utilize common signaling adaptor molecules, MyD88 (myeloid differentiation primary response gene 88) and/or TRIF (TIR domain-containing adaptor inducing interferon-β), to initiate signaling [2].

Viruses and bacteria typically enter cells through endocytic or phagocytic pathways, which can lead to their degradation and release of microbial nucleic acids [17] (Figure 1). Multiple TLRs recognize specific nucleic acid structures: TLR3 recognizes double-stranded RNA (dsRNA) [18], TLR7 and TLR8 recognize single-stranded RNA (ssRNA) [19–22], and TLR9 recognizes DNA [23]. In addition, TLR13, until recently an orphan receptor, recognizes ssRNA [24–26]. For some of these TLRs, specific sequence motifs within RNA or DNA are required for

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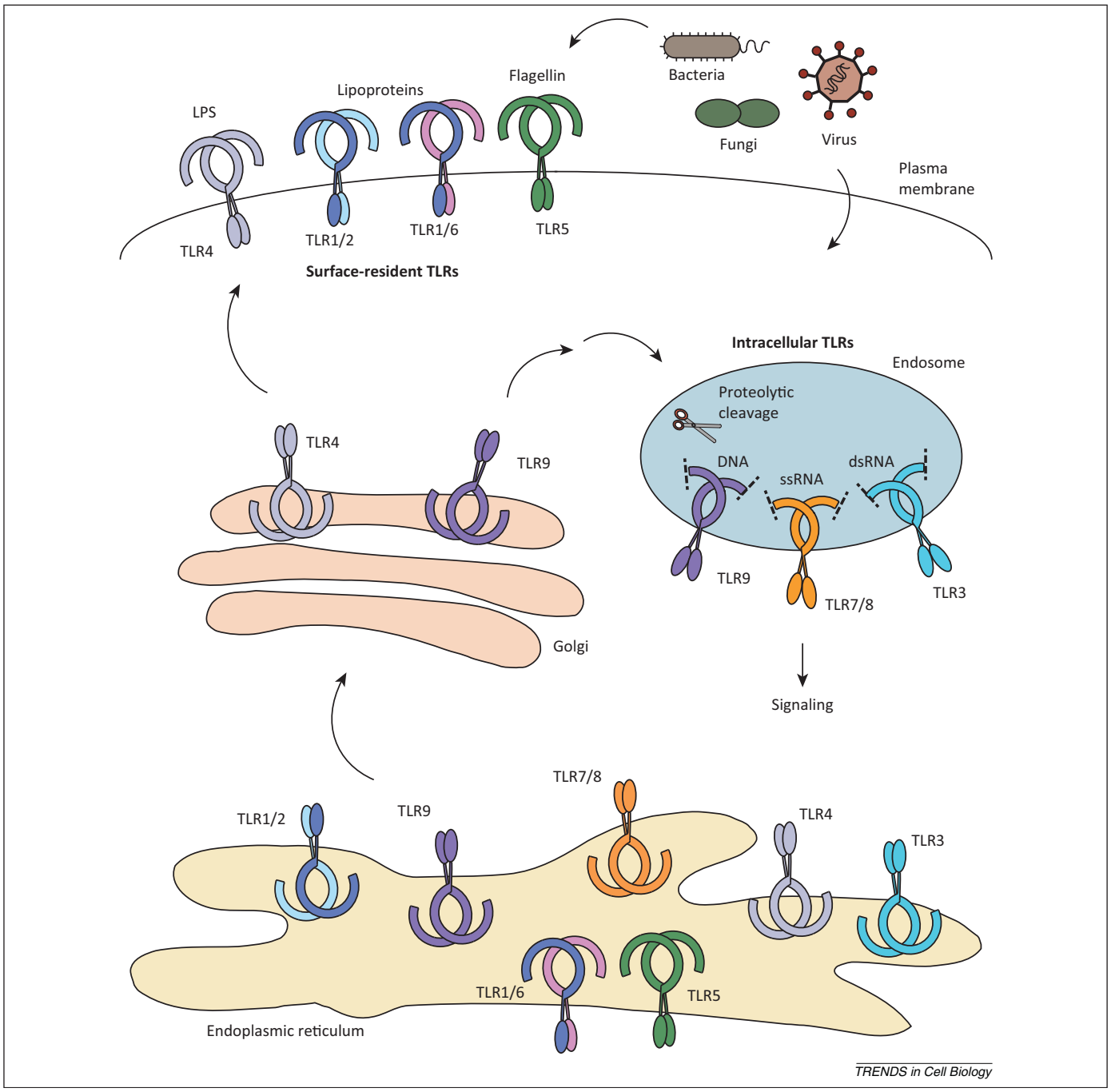

Figure 1. Overview of Toll-like receptor (TLR) trafficking. TLRs are synthesized in the endoplasmic reticulum (ER), traffic to the Golgi, and ultimately localize to the cell surface or remain intracellular in endosomes or lysosomes. All TLRs have a horseshoe-like ectodomain structure and interact with their ligands as dimers. Surface resident TLRs 1, 2, 4, 5, and 6 recognize microbial ligands such as lipopolysaccharide (LPS), bacterial lipoproteins, and flagellin. Most endosomal TLRs recognize microbe-derived nucleic acids. The ectodomains of these nucleic acid sensing TLRs undergo proteolytic processing in endosomes to generate functional receptors capable of signaling upon ligand recognition.

optimal ligand binding, and there is evidence that the optimal motifs differ between species [27–33]. Activation of nucleic acid sensing TLRs triggers downstream signaling and activation of transcription factors such as nuclear factor κB (NF-κB) and interferon regulatory factors (IRFs) to promote proinflammatory and antiviral responses, respectively [2].

The use of nucleic acids to detect the presence of microbes may seem paradoxical given the potential for inappropriate recognition of host nucleic acids. In fact, nucleic acids can be present in the extracellular milieu, either through non-apoptotic forms of cell death, secondary necrosis of uncleared apoptotic debris, or via release of DNA by neutrophils during NETosis (from NET, neutrophil extracellular trap) [34–36]. Inappropriate recognition of these host-derived nucleic acids can lead to autoimmune or autoinflammatory disorders such as systemic lupus erythematosus (SLE) [37,38]. Although these potential risks are great, the emergence of nucleic acid recognition as a general strategy of innate immune recognition suggests an evolutionary benefit that outweighs the risk of responding to self nucleic acids. Therefore, mechanisms must exist that reduce the likelihood of responses to self nucleic acids. For example, nucleases present in the