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TLR2 – promiscuous or specific? A critical re-evaluation of a receptor expressing apparent broad specificity

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Abstract

Of all pattern recognition receptors (PRR) in innate immunity, Toll-like receptor 2 (TLR2) recognizes the structurally broadest range of different bacterial compounds known as pathogen-associated molecular patterns (PAMPs). TLR2 agonists identified so far are lipopolysaccharides (LPSs) from different bacterial strains, lipoproteins, (synthetic) lipopeptides, lipoarabinomannans, lipomannans, glycosylphosphatidylinositol, lipoteichoic acids (LTA), various proteins including lipoproteins and glycoproteins, zymosan, and peptidoglycan (PG). Because these molecules are structurally diverse, it seems unlikely that TLR2 has the capability to react with all agonists to the same degree. The aim of this review is to identify and describe well-defined structure–function relationships for TLR2. Because of its biomedical importance and because its genetics and biochemistry are presently most completely known among all Gram-positive bacteria, we have chosen *Staphylococcus aureus* as a focus. Our data together with those reported by other groups reveal that only lipoproteins/lipopeptides are sensed at physiologically concentrations by TLR2 at picomolar levels. This finding implies that the activity of all other putative bacterial compounds so far reported as TLR2 agonists was most likely due to contaminating highly active natural lipoproteins and/or lipopeptides.

Keywords: Lipoprotein; Lipopeptide; Lipoteichoic acid; Pathogen-associated molecular patterns (PAMPs); Peptidoglycan; TLR2

Abbreviations: ai 15:0, (ω−2)-methyl-tetradecanoic acid (*anteiso*-pentanoic acid); *ai*17:0, (ω−2)-methyl-hexatetradecanoic acid (*anteiso*-heptanoic acid); 27:0-dioic acid, heptacosane-1, 27-dioic acid; 28:0(27-OH), 27-hydroxy-octacosanoic acid; Ara-LAM, uncapped LAM; CMC, critical micellar concentration; ESI, electrospray ionization; FSL-1, synthetic N-terminal part of lipoprotein LP44 of *Mycoplasma salivarum*, *S*-[2,3-bis(palmitoyloxy)-(2*S*)-propyl]-CGDPKHSPKSF; FT-ICR, Fourier transform ion cyclotron resonance; GLC, gas–liquid chromatography; GLC–MS, combined gas–liquid chromatography/mass spectrometry; GPI, glycosylphosphatidylinositol; LAM, lipoarabinomannan; LM, lipomannan; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MALP-2 or *R*-MALP-2, *S*-[2,3-bis(acyloxy)-(2*R*)-propyl-cysteinyl-GNNDESNISFKEK]; *S*-MALP-2, *S*-[2,3-bis(acyloxy)-(2*S*)-propyl-cysteinyl-GNNDESNISFKEK]; Man-LAM, mannose capped LAM; MDP, muramyldipeptide; MS, mass spectrometry; Nod, nucleotide-binding oligomerization domain; PAMP, pathogen-associated molecular pattern; Pam_2CSK_4, *S*-[2,3-bis(palmitoyloxy)-(2*S*)-propyl]-CSKKKK; Pam_3CSK_4, *N*-Palmitoyl-*S*-[2,3-bis(palmitoyloxy)-(2*S*)-propyl]-CSKKKK; PE, phosphatidylethoanolamine; PG, peptidoglycan; PILAM, phospho-*myo*-inositol capped LAM; PC, phosphatidylcholine; PRR, pattern recognition receptor; PS, phosphatidylserine; TLR, Toll-like receptor; WTA, wall teichoic acid.

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Introduction

Release of pro-inflammatory cytokines by immune cells is one of the most important reactions in sepsis: Secretion is provoked by bacterial antigens, which are recognized by the innate immune system. Pathogen recognition is mediated by a set of germline-encoded receptors that are referred to as "pattern recognition receptors" (PRRs). These receptors are present in all multicellular organisms and they recognize structures termed "pathogen-associated molecular patterns" (PAMPs) (Janeway, 1989). PAMPs represent a limited number of conserved molecular structures produced by micro-organisms in which they play a vital role for their survival and replication. Another prerequisite for bacterial PAMPs is that they be exclusively derived from the microbe and not present in the multicellular host (Janeway and Medzhitov, 1999). Although now well accepted in innate immunity research, the term PAMP has received criticism, since molecules of microbial origin and not patterns interact with their receptors in a specific way (Beutler, 2003). Furthermore, recognition processes are modulated by co-receptors, thus increasing the efficiency and reliability of PAMPs. During the past decade effort has emphasized defining the specific interactions as to how PAMPs are recognized by their PRRs. Extracellular so-called Toll-like receptors (TLRs) (Beutler, 2004) in addition to intracellularly binding nucleotide-binding and oligomerization domain (Nod) receptors (NLRs) have been identified (Girardin et al., 2002). In this review, we concentrate on TLR2, the most intensively studied receptor in innate immunity. Despite the overwhelming number of reports on TLR2 agonists, the structural and biochemical features of the molecules representing "patterns" for TLR2 have been poorly described. It is our hope that a clearer structure–function relationship will result in a more systematic development of new anti-inflammatory drugs.

Incompletely defined agonists (PAMPs) and the lack of structural knowledge of their receptors (PRRs) have resulted in confusion and misinterpretation of PRR specificity. Most of the PAMPs relevant for innate immunity must exhibit significant binding and activation of their receptor at picomolar (pM) concentration. With TLR2, only PAMPs sensed at a very low physiologically relevant concentration are considered. In this review, we critically summarize the identification of various TLR2-agonists guided by the idea that PRRs interact with their corresponding agonists by an unequivocal structure–function relationship. But TLR2 represents the receptors with "complicate recognition" and, therefore, seems to be not specific (Wetzler, 2003). As a source to investigate the agonists of TLR2 we have chosen *Staphylococcus aureus* to investigate TLR2 PAMPs because it is a good representative of Gram-positive bacteria, and because of its increasing biomedical importance (Sriskandan and Cohen, 1999).

At a first glance, putative candidates for PAMPs include a range of diverse microbial molecules structurally distinct from host biomolecules. For example, biologically active lipids representing microbial PAMPs must be distinct from ubiquitous lipids in the eukaryotic cellular membrane. Glycolipids not present in host cells and which are structurally unrelated to glycolipids of the host are suitable candidates for PAMPs. In Gram-negative bacteria the most thoroughly described PAMP is lipopolysaccharide (LPS, endotoxin); its PRR was determined to be TLR4 along with its co-receptors MD2, CD14, and LBP. In contrast to TLR4, a clear structure–function relationship for TLR2 appears to be more difficult to define, since there exists an extremely high number of structurally non-related TLR2 agonists (Henderson et al., 1996).

Most TLR2 PAMPs are glycolipids, lipopeptides, or GPI-anchored structures. These molecules all contain a hydrophobic component, thus having the tendency to form aggregates in water. This feature complicates receptor-specific binding by innate immune system receptors (Seong and Matzinger, 2004). Molecular aggregates are rather complex and variable for the receptors; hence the innate immune system utilizes specific co-receptors, which assist in PAMP identification. The fact that all amphiphilic PAMPs identified to date need co-receptors for full expression of their biological activity clearly supports this conclusion. For example, LPS alone is unable to interact with TLR4, but requires co-receptors such as MD-2 (Gioannini et al., 2004), CD14 (Kitchens, 1999), and LBP (Schumann et al., 1990) to effect a successful interaction. In another example the highly active lipopeptide originally identified in *Mycoplasma fermentan* (MALP-2) is an amphiphile PAMP and requires co-receptors. It reacts in a specific way with TLR2 (as heterodimeric TLR2/TLR6) (Takeuchi et al., 2001) and it utilizes CD36 as a co-receptor (Hoebe et al., 2005).

Other TLR2 agonists presently known are lipoteichoic acid (LTA) from Gram-positive bacteria, lipoarabinomannan (LAM) from mycobacteria, and glycosylphosphatidylinositol (GPI) anchored lipids from *Trypanosoma cruzi*. Common to all of these agonists is their amphiphilic structure. Most surprisingly, however, TLR2 agonists lacking structural relationship to all lipophiles listed above have been reported. Among these are staphylococcal peptidoglycan (PG) and the polysaccharide zymosan isolated from yeast. Finally, TLR2 was described to interact with whole bacteria (*Chlamydia*, *Francisella*) (Table 1). From the structural point of view, many of the PAMPs published for TLR2 have almost nothing in common (Figs. 1 and 2) and the only explanation for their putative activity was the declaration that TLR2 is a "promiscuous" receptor