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Research review paper

Therapeutic targeting of innate immunity with Toll-like receptor 4 (TLR4) antagonists

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ABSTRACT

Early recognition of invading bacteria by the innate immune system has a crucial function in antibacterial defense by triggering inflammatory responses that prevent the spread of infection and suppress bacterial growth. Toll-like receptor 4 (TLR4), the innate immunity receptor of bacterial endotoxins, plays a pivotal role in the induction of inflammatory responses. TLR4 activation by bacterial lipopolysaccharide (LPS) is achieved by the coordinate and sequential action of three other proteins, LBP, CD14 and MD-2 receptors, that bind lipopolysaccharide (LPS) and present it to TLR4 by forming the activated $(TLR4\text{-}MD\text{-}2\text{-}LPS)_2$ complex. Small molecules active in modulating the TLR4 activation process have great pharmacological interest as vaccine adjuvants, immunotherapeutics or antisepsis and anti-inflammatory agents. In this review we present natural and synthetic molecules active in inhibiting TLR4-mediated LPS signalling in humans and their therapeutic potential. New pharmacological applications of TLR4 antagonists will be also presented related to the recently discovered role of TLR4 in the insurgence and progression of neuropathic pain and sterile inflammations.

Contents

1. Introduction: Role of TLR4 and therapeutic applications of TLR4 antagonists . . . 252
2. The structural biology of LPS recognition and TLR4 signalling . . . 253
3. Inhibitors of TLR4 signalling . . . 253
   3.1. LPS sequestrants . . . 253
   3.2. Inhibitors of LPS-LBP and LPS-CD14 interaction . . . 254
   3.3. MD-2-TLR4 antagonists . . . 255
      3.3.1. E5531 and E5564 . . . 255
      3.3.2. MD-2-TLR4 antagonists with a chemical structure unrelated to lipid A . . . 255
   3.4. Compounds that bind directly to TLR4 . . . 256
   3.5. Other TLR4-active compounds . . . 257
      3.5.1. Heme and its derivatives . . . 257
      3.5.2. Thalidomide . . . 258
      3.5.3. Opioids . . . 258
4. Future perspectives . . . 258
Acknowledgement . . . 259
References . . . 259

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1. Introduction: Role of TLR4 and therapeutic applications of TLR4 antagonists

A fundamental concept in immunology is the distinction between innate and adaptive immune response. The two are distinguished by whether the antigen receptors are encoded in the germ line (innate immunity) or generated somatically by gene rearrangement or hypermutation (adaptive immunity). Innate immunity recognition relies on a diverse set of germ line encoded receptors, termed pattern recognition receptors (PRR), which recognize broad classes of molecular structures common to groups of microorganisms. One of the largest and best studied families of PRR is the Toll family of receptors (Toll-like receptors, TLRs) that detect microbial components with high sensitivity and selectivity (Miyake, 2007). Ten functional TLRs in humans (TLR1-10) can be divided into two distinct groups with regard to subcellular distribution and ligand specificity. Cell surface TLRs including TLR1, 2, 4 and 6 recognize microbial membrane lipids and lipopeptides, TLR5 recognizes bacterial flagellin protein, whereas TLR3, 7, 8, 9 reside in intracellular organelles (endosomes/lysosomes) and recognize nucleic acids (Akira et al., 2006; Akira and Takeda, 2004).

Among TLRs, TLR4 selectively recognizes bacterial lipopolysaccharide (LPS) or endotoxin, (Beutler, 2002; Beutler et al., 2001; Poltorak et al., 1998), which results the rapid triggering of pro-inflammatory processes. The induction of inflammatory responses by LPS is achieved by the coordinate and sequential action of four principal endotoxin-binding proteins: the LPS-binding protein (LBP), the cluster differentiation antigen 14 (CD14), the myeloid differentiation protein (MD-2) and the Toll-like receptor 4 (TLR4). This process starts with the binding of LBP to LPS aggregates, in form of micelles or membrane blebs, and ends up with the formation of the activated $(TLR4\text{-}MD\text{-}2\text{-}LPS)_2$ complex that has a pivotal role in initiating the inflammatory cascade (Fig. 1). LPS recognition by TLR4 mediates a rapid cytokine production and the recruitment of inflammatory cells to the site of infection (Hayashi et al., 2003). TLR4 activation also regulates adaptive responses (Iwasaki and Medzhitov, 2004).

TLR4 trigger can be remarkably sensitive and robust, stimulating prompt and powerful host defence responses to different species of invading bacteria. However, an excessively potent host response generates life-threatening syndromes such as acute sepsis and septic shock. Gram-negative sepsis is the first cause of deaths in intensive care units, and it is associated to a mortality of about 45% (Martin et al., 2003). The most important clinical and pharmacological use of TLR4 antagonists would therefore be to contrast Gram-negative bacterial sepsis and septic shock (Cribbs and Martin, 2007; Martin et al., 2003). Molecules with endotoxin antagonistic activity that can inhibit TLR4 activation are potential lead compounds for antisepsis drug development.

Recent findings reveal a previously unappreciated role for TLRs and in particular TLR4 in some types of sterile inflammation, namely, inflammation not caused by viruses and microbes that is often the product of tissue injury (Kanzler et al., 2007; Okun et al., 2009). Indeed, TLR4 responds to endogenous ligands such as heat shock proteins, extracellular matrix degradation products, HMGB-1, β-defensin, surfactant protein A, minimally modified LDL (Miyake, 2007). Recent work demonstrates that in the absence of LPS or an exogenous pathogen TLR4 is a key microglial receptor for the initiation of nerve injury-induced behavioural hypersensitivity (De Leo et al., 2006). TLR4 might therefore be a key contributor in microglial activation to connect the innate immunity with the initiation of neuropathic pain (Tanga et al., 2005). The contribution of CD14 in TLR4-dependent neuropathic pain has also

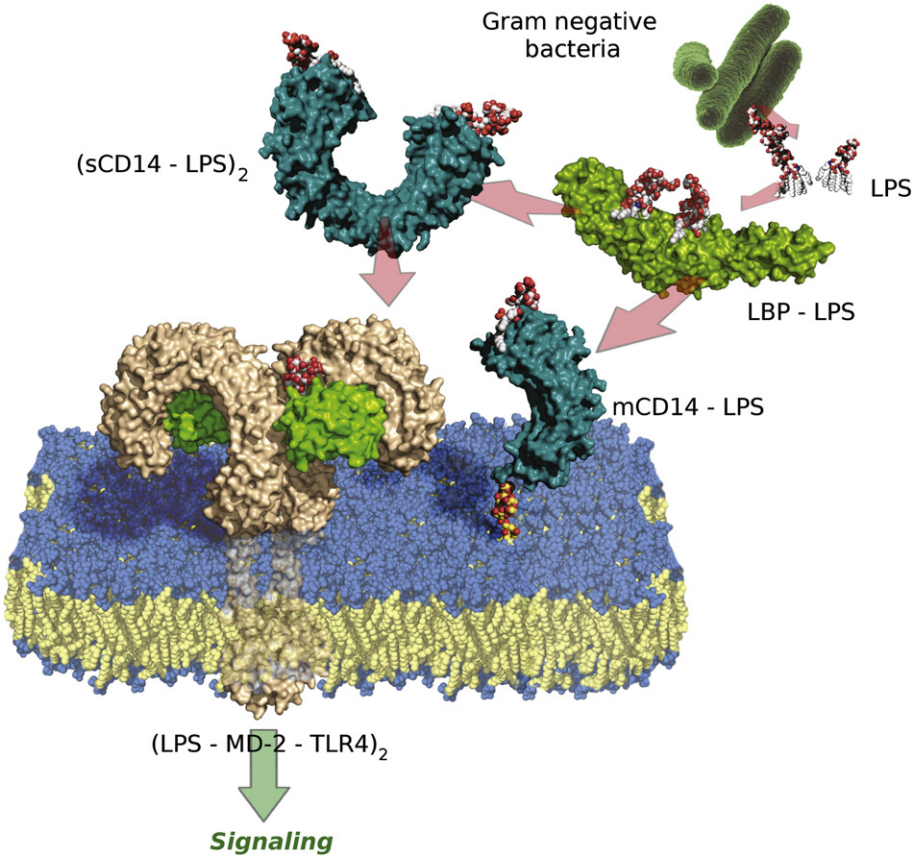

Fig. 1. The LPS transport chain and signal amplification: from LPS aggregates to the $(TLR4\text{-}MD\text{-}2\text{-}LPS)_2$ activated complex. Picture from PDB ID: 3FXI (for TLR4·MD-2 complex), 1O77 (for the TIR domain of TLR4), 2OBD and 1BPI (for LBP, 1WWL (for CD14) and 1QFG (for LPS).