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Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid

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Palmitic acid (PubChem CID: 985)
Pam_3Csk_4 (CID: 130704)
Lipopolysaccharide core (CID: 53481794)

ABSTRACT

Saturated fatty acids can activate Toll-like receptor 2 (TLR2) and TLR4 but polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA) inhibit the activation. Lipopolysaccharides (LPS) and lipopetides, ligands for TLR4 and TLR2, respectively, are acylated by saturated fatty acids. Removal of these fatty acids results in loss of their ligand activity suggesting that the saturated fatty acyl moieties are required for the receptor activation. X-ray crystallographic studies revealed that these saturated fatty acyl groups of the ligands directly occupy hydrophobic lipid binding domains of the receptors (or co-receptor) and induce the dimerization which is prerequisite for the receptor activation. Saturated fatty acids also induce the dimerization and translocation of TLR4 and TLR2 into lipid rafts in plasma membrane and this process is inhibited by DHA. Whether saturated fatty acids induce the dimerization of the receptors by interacting with these lipid binding domains is not known. Many experimental results suggest that saturated fatty acids promote the formation of lipid rafts and recruitment of TLRs into lipid rafts leading to ligand independent dimerization of the receptors. Such a mode of ligand independent receptor activation defies the conventional concept of ligand induced receptor activation; however, this may enable diverse non-microbial molecules with endogenous and dietary origins to modulate TLR-mediated immune responses. Emerging experimental evidence reveals that TLRs play a key role in bridging diet-induced endocrine and metabolic changes to immune responses.

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1. Introduction

Toll-like receptors (TLRs) are one of the major pattern recognition receptor families that recognize pathogen associated molecular patterns (PAMPs) and mount innate immune responses for host defense against invading pathogens. However, certain TLRs can be activated by non-microbial endogenous molecules leading to induction of sterile inflammation. TLR4 and TLR2 can be activated by saturated fatty acids (SFAs), but inhibited by omega-3 polyunsaturated fatty acids (PUFAs) in particular docosahexaenoic acid (DHA). Receptor dimerization is a prerequisite and sufficient to induce the activation of TLRs. SFAs induce the dimerization and translocation of TLR4 or TLR2 into lipid raft fractions in plasma membrane where the downstream signaling molecules are recruited and activated. This SFA-induced dimerization of TLR4 or TLR2 reflects ligand independent activation of the receptors. Therefore, SFA-induced activation of TLR4 or TLR2 needs to be assessed in the context of biophysical interaction of fatty acids with TLRs in plasma membrane. Such a mode of activation of TLRs deviates from the conventional view on the key and lock relationship for ligands and receptor activation, but renders a possibility that the ligand independent activation of these TLRs can be modulated by diverse non-microbial agonists or inhibitors with dietary origin. This finding advanced our understanding of the mechanisms by which SFAs activate, and DHA inhibits pro-inflammatory signaling pathways. Understanding the mechanism of such a modulation would help us develop dietary or pharmacological strategy to reduce risk of chronic diseases caused in part by dysregulated TLR-mediated inflammatory responses. In this article, putative molecular mechanisms by which saturated fatty acids and DHA reciprocally modulate the activation of TLR4 and TLR2 are reviewed and discussed. Functional consequences of the modulation of TLR-derived pro-inflammatory signaling pathways by fatty acids with regard to metabolic diseases were reviewed

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elsewhere (Arranz et al., 2012; Lee et al., 2010).

2. Pro-inflammatory saturated fatty acids and anti-inflammatory n-3 PUFAs

Replacing dietary SFA with PUFA reduces risk of coronary heart disease (Jakobsen et al., 2009). Higher plasma level of C16:0 and C18:0 is associated with increased incidence of type 2 diabetes (Mozaffarian, 2014). A diet high in saturated fat induces insulin resistance, which is associated with low grade inflammation in adipose tissue and liver in experimental animals (Orr et al., 2012; Tsukumo et al., 2007; Yeop Han et al., 2010). By contrast, DHA has anti-inflammatory and insulin sensitizing effects both in animal and human studies (Albert et al., 2014; Fedor and Kelley, 2009). How SFAs exert pro-inflammatory effects and n-3 PUFA DHA exerts anti-inflammatory effects remained as an important question. SFAs activate TLR2/4-derived pro-inflammatory signaling pathways, and TLR2 or TLR4 deficient mice are protected from high saturated fat diet-induced inflammation and insulin resistance (Glass and Olefsky, 2012; Lee et al., 2010). These findings established a causal link of TLR-mediated inflammation to metabolic diseases. However, the mechanism by which saturated fatty acids activate TLR2/4 is still not clearly understood.

3. Lipid binding sites in TLR-MD2 and TLR2/1 or TLR2/6 dimers

Phylogenetic, X-ray crystallographic and amino acid sequence analyses of leucine rich repeat (LRR) domains of TLRs provided an important clue about structural features of the ligands and their interactions with TLRs. Phylogenetic analyses of TLRs revealed TLR1, – 2, – 6, – 10 as the same clade and TLR4 or TLR3, – 5, – 7, – 8, – 9 as two separate clades (Hughes and Piontkivska, 2008). Based on X-ray crystallographic analyses of LRR ectodomains of TLRs and analyses of amino acid sequences, Kang and Lee (Kang and Lee, 2011) divided TLRs into two subgroups: the first group containing LRRs with three-domain fold (TLR 1, – 2, – 4, – 6, – 10), and the second group containing LRRs with single-domain fold (TLR3, – 5, – 7, – 8, – 9) (Fig. 1). The LRRs of the first group of TLRs can be divided into distinct three subdomains: N-terminal, central and C-terminal due to the lack of the asparagine networks that are present in the LRR domains of the second group of TLRs. The lack of the asparagine networks creates structural distortions at the boundaries of the subdomains of the first subgroup. Interestingly, the ligand binding pockets of TLR1, – 2, and – 6 are all localized between C-terminal and central domains (Fig. 1C). The ligands for TLR1, – 2, and – 6 contain fatty acyl chains that bind the lipid binding pockets. By contrast, one of the MD2 binding sites in TLR4 is located between the N-terminal and central subdomains. The sequence analysis of the ectodomains of the second group of TLR3, – 5, – 7, – 8, and – 9 revealed that they all have continuous asparagine network exhibiting smooth single-domain fold without having the lipid binding pockets. These single-domain fold TLRs interact with hydrophilic ligands such as nucleic acids through surface-exposed residues. The results demonstrating that SFAs activate TLRs containing lipid binding pockets with three-domain fold group only suggest a direct interaction of SFAs with the lipid binding pockets. More than 100 μM SFAs are required to activate TLR4 or TLR2 in cell culture systems, while pico- or nano-molar ranges of LPS or Pam_3CSK_4 are enough to stimulate TLR4 or TLR2, respectively. Therefore, it is questionable whether SFAs, without polar head groups present in LPS or lipopetides, act as ligands for TLR4-MD2 and TLR2 dimers. Thus, SFAs should be called as agonists rather than the ligands for the TLRs.

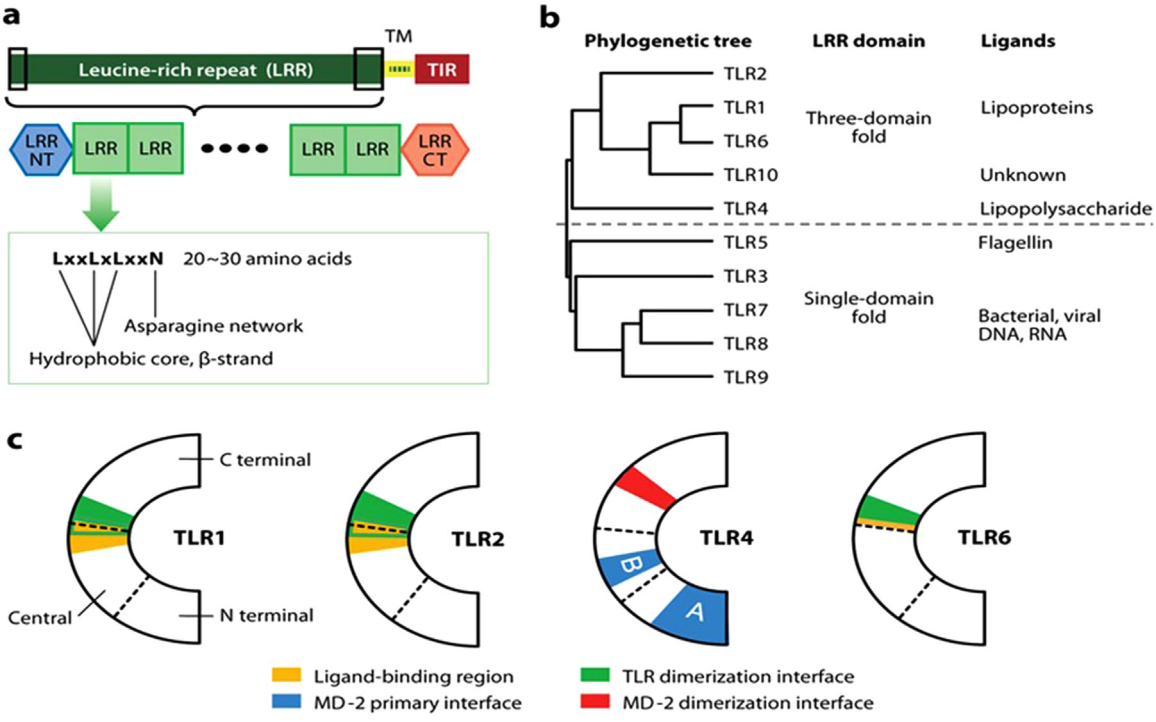

Fig. 1. Arrangement of Toll-like receptor (TLR) domains. (a) TLRs consist of an extracellular leucine-rich repeat (LRR) domain, a transmembrane (TM) domain, and an intracellular Toll/IL-1R homology (TIR) domain. The extracellular LRR domain contains 20~27 LRR modules. LRRNT and LRRCT modules cover the N and C termini of the LRR modules, respectively. (b) Classification of TLRs: phylogenetic analysis, the structures of the LRR domains, and the chemical properties of the ligands suggest that TLRs can be divided into two major subclasses. (c) Structural boundaries are important for function. Boundaries dividing the N-terminal, central, and C-terminal subdomains are marked by broken lines. Functionally important areas are colored. The A and B patches of the primary TLR4-MD-2 interface are marked. (Kang and Lee, 2011).