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Review article

Saturated fatty acids trigger TLR4-mediated inflammatory response

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ABSTRACT

Toll-like receptors (TLR) mediate infection-induced inflammation and sterile inflammation by endogenous molecules. Among the TLR family, TLR4 is the best understood. However, while its downstream signaling pathways have been well defined, not all ligands of TLR4 are currently known. Current evidence suggests that saturated fatty acids (SFA) act as non-microbial TLR4 agonists, and trigger its inflammatory response. Thus, our present review provides a new perspective on the potential mechanism by which SFAs could modulate TLR4-induced inflammatory responses: (1) SFAs can be recognized by CD14-TLR4-MD2 complex and trigger inflammatory pathways, similar to lipopolysaccharide (LPS). (2) SFAs lead to modification of gut microbiota with an overproduction of LPS after a high-fat intake, enhancing this natural TLR4 ligand. (3) In addition, this metabolic endotoxemia leads to an oxidative stress thereby producing atherogenic lipids – oxLDL and oxidized phospholipids – which trigger CD36-TLR4-TLR6 inflammatory response. (4) Also, the high SFA consumption increases the lipemia and the mmLDL and oxLDL formation through oxidative modifications of LDL. The mmLDL, unlike oxLDL, is involved in activation of the CD14-TLR4-MD2 inflammatory pathway. Those molecules can induce TLR4 inflammatory response by MyD88-dependent and/or MyD88-independent pathways that, in turn, promotes the expression of proinflammatory transcript factors such as factor nuclear kappa B (NF-κB), which plays a crucial role in the induction of inflammatory mediators (cytokines, chemokines, or costimulatory molecules) implicated in the development and progression of many chronic diseases.

1. Introduction

The microbial recognition process is mediated by pattern recognition receptors (PRRs) which are linked to the innate immune system, and are mostly expressed in macrophages and dendritic cells, but they are also present in nonimmune cells [1,2]. The major PRRs include toll-like receptors (TLRs) and C-type lectin receptors. The TLRs are transmembrane proteins expressed on cell surfaces which recognize mainly microbial membrane components. They are the best characterized PRRs and are linked to

Abbreviations used: AKT, PKB – protein kinase B; ASC, apopto-sis-associated speck-like protein containing a CARD; CD14, cluster of differentiation 14; CD36, cluster of differentiation 36; COX2, ciclo-oxigenase-2; DAMP, danger-associated molecular pattern; Gro 1, Cxcl1 – chemokine (C-X-C Motif) ligand 1; IFN, type I interferon; IKK, IκB kinase; IL, interleukin; IRAK, IL-1R-associated kinase; IRF3, interferon regulating factor 3; IκB, inhibitor of NF-κB; LBP, LPS-binding protein; LDL, low-density lipoprotein; LPS, lipopolysaccharides; MAPK, mitogen-activated protein kinase; MCP1, monocyte chemoattractant protein 1; MD2, myeloid differential protein-2; MIP, macrophage inflammatory protein; mmLDL, minimally modified low-density lipoprotein; MyD88, myeloid differentiating primary response gene 88; NF-κB, factor nuclear kappa B; NLRP3, NOD-like receptor family, pyrin domain containing 3; oxLDL, oxidize low density lipoprotein; oxPL, oxidize phospholipids; PAMP, pathogen-associated molecular pattern; PI3K, phosphatidylinositol 3-kinase; PRR, pattern recognition receptors; PUFA, polyunsaturated fatty acid; RANTES, regulated on activation, normal t cell expressed and secreted; RIP1, receptor-interacting protein 1; ROS, reactive oxygen species; SFA, saturated fatty acid; TAB1, TGF-beta activated kinase 1/MAP3K7 binding protein 1; TAK1, transforming growth factor-β-activate kinase; TBK1, TRAF family member-associated NF- kB activator (TANK) binding kinase-1; TIR, toll-interleukin receptor; TIRAP, TIR domain containg adaptor protein; TLR, toll-like receptor; TNF, tumor necrosis factor; TRAF, TNF-receptor associated factor; TRAM, TRIF related adaptor molecule; TRIF, TIR domain–containing adaptor-inducing IFN-β; VCAM1, vascular cell adhesion molecule-1.

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bacterial and viral infection response [2,3].

Among TLRs, TLR4 is unique due to its ability to activate both MyD88-dependent and MyD88-independent pathways expressing predominantly inflammatory molecules and type I interferons (IFNs), respectively. Also, TLR4 is the only known member of the TLR family that engages all four toll-interleukin receptor (TIR) domain-containing adaptor proteins to signaling the inflammatory response [3,4].

Different sources of pathogen-associated molecular patterns (PAMPs) derived from bacterial, viral and fungus pathogens activate the TLR signaling [5]. The LPS of Gram-negative bacterial cell walls are the major PAMP, and a natural TLR4 ligand [5]. Also, TLR4 are activated by endogenous danger-associated molecular patterns (DAMPs) released as a consequence of injury and inflammation, such as oxidized low density lipoprotein (oxLDL) and oxidized phospholipids (oxPL) [5,6]. This sterile inflammatory, likewise microbial induced inflammation, can recruit neutrophils and macrophages leading to production of pro-inflammatory cytokines and chemokines, mainly tumor necrosis factor (TNF) and interleukin (IL)-1 [7].

Other possible nonmicrobial agonists for TLR4 include saturated fatty acids (SFA), but little has been explored on this subject. In fact, evidence suggests that SFA and LPS share the same inflammatory signaling pathway as TLR4, thus promoting expression of pro-inflammatory transcript factors, such as factor nuclear kappa B (NF-κB) and cyclooxygenase 2 (COX2) [8].

In order to improve our understanding on the inflammatory process mediated by SFA, we present a new perspective on the potential mechanism by which SFAs could act as TLR4-activating promoters and trigger pro-inflammatory responses involved in the development and progression of many chronic diseases and metabolic disorders, including cancer, cardiovascular diseases, metabolic syndrome and obesity-induced insulin resistance.

2. SFA trigger TLR4 inflammatory pathways

SFA particularly lauric acid (C12:0), similarly to LPS, modulate the activation of TLR4 [8–10]. In fact, the LPS is recognized by an accessory protein cluster of differentiation 14 (CD14) which is a glycoprotein, either glycosylphosphatidylinositol present in two forms: membrane bounded – at the outer leaflet of the plasma membrane – or soluble in blood [1,11]. The CD14 is best characterized for its capability to interact with LPS-binding protein (LBP) and transfer LPS to the TLR4 accessory molecule myeloid differential protein-2 (MD2). The TLR4-MD2 forms a dimer in the plasma membrane lipid [1,6]. Upon LPS recognition, the CD14-TLR4-MD2 complex engages TIRAP-MyD88 adaptors and leads do MyD88-dependent response, subsequently the CD14-TLR4-MD2 complex is endocytosed and recruits TRAM-TRIF adaptors which elicits MyD88-independent response [1,3], as we will describe below. Lee et al. (2003) suggest that both CD14 and MD2 are required for TLR4 activation by a lauric acid like LPS, signaling the inflammatory pathway of CD14-TLR4-MD2 complex [8].

Gut microbiota is a huge reservoir of LPS, which under normal conditions causes no harm in the intestinal lumen. However, a high-fat diet has been shown to induce gut microbiota alterations, raising the proportion of Gram-negative bacteria with an over-expansion of LPS, and increasing the intestinal permeability. Thus, this process promotes a bacterial translocation of Gram-negative bacteria, and endotoxin-produced bacteria from intestinal mucosa to the blood stream. This metabolic endotoxemia caused by LPS can activate the TLR4 mediated by LBP, CD14 and MD2, which leads to a MyD88-dependent and MyD88-independent response [12].

Besides the LPS, which is a classical PAMP, DAMPs derived from gut microbiota can also activate the TLR4 inflammatory pathway. Those DMAPs, such as oxLDL and oxPL, can be formed from an overproduction of LPS by inflammatory response and oxidative stress [6].

The oxLDL is known as a specific ligand of cluster of differentiation 36 (CD36) [13]. The CD36 is a glycosylated protein member of the class B scavenger receptor family, which plays an important role in glucose and fatty acid metabolism [13]. This scavenger receptor is found on the surface of diverse cell types and is also involved in TLR-dependent inflammatory response induced by various ligands such as lipid-associated products of microbial or endogenous origin [1,13].

oxLDL is sequestered by CD36 and induces intracellular CD36-TLR4-TLR6 heteromerization [14]. The CD36-TLR4-TLR6 signaling propagates by both MyD88 and TRIF adaptors, inducing pro-inflammatory mediators through MyD88-dependent and MyD88-independent pathways in absence of MD2 and CD14 that are essential cofactors for recognition of LPS by the TLR4 complex [14]. Furthermore, the CD36-TLR4-TLR6 complex acting via NF-κB and reactive oxygen species (ROS) primes the NLRP3 (NOD-like receptor family, pyrin domain containing 3) inflammasome in response to oxLDL. Also, oxLDL recognition and endocytosis by CD36 results in the formation of intracellular cholesterol crystals that activates NLRP3 via lysosomal destabilization and stimulates IL-1β and IL-18 formation [2,15]. Moreover, oxPL has been proposed as a -TLR4 agonist, which activates MyD88-independent pathways and induces IL-6 production [16], but to date, little has been explored about this subject.

Furthermore, SFA cause a more pronounced lipemia than mono and polyunsaturated fatty acids (PUFA) [17]. Also, after consumption of a high-fat meal, the lipid peroxidation by ROS results in a minimally modified low-density lipoprotein (mmLDL) formation and the generation of extensive amounts of oxLDL [18]. The mmLDL represents an early product of progressive LDL oxidation, formed before the oxLDL [19]. Both are known as pro-inflammatory and pro-atherogenic lipoproteins [11,20]. In addition, occurs a local production of oxPL [16].

Although the polyunsaturated fatty acids (PUFA) are more prone to oxidation and oxLDL synthesis due to their high degree of unsaturation [21], experimental studies have shown that n-3 PUFA inhibit the TLR4-induced signaling pathways and target gene expression [8,10]. This fact seems to be related to the anti-inflammatory effects of PUFA mediated by the G protein-coupled receptor 120 (GPR120). The stimulation of GPR120 with n-3 PUFA inhibit TLR4 signaling probably by its association with β-arrestin2. This complex is internalized, and β-arrestin2 binds to TAB1 (TGF-beta-activated kinase 1/MAP3K7 binding protein 1), resulting in inhibition of TAK1 (transforming growth factor-β-activate kinase) phosphorylation and activation and, consequently, the inactivation of the TLR4 signaling [22]. In addition, n3 PUFA disrupts the translocation of TLR4 into lipid raft, preventing its activation [9].

Like oxLDL, the mmLDL is an endogenous TLR4 ligand which is not recognized by scavenger receptors but binds to a CD14 receptor, and like LPS, stimulates the classic TLR4 response through a CD14-TLR4-MD2 complex and induces the activating protein-1 (AP1) and phosphatidylinositol 3-kinase (PI3K) activation [1,19]. Notable, unlike oxLDL, the mmLDL is not internalized [18]. Moreover, the increased exposure of mmLDL can enhance sterile inflammation by increased uptake of oxLDL, probably mediated by a higher expression of CD36 [11].

3. TLR4 activation

Specifically, TLR4 engages all four toll-interleukin receptor (TIR) adaptors proteins to signing the chain reaction needed to activate intracellular signaling inflammatory response, unlike other TLRs