Future VIROLOGY

EDITORIAL
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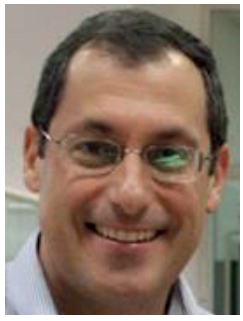
How do viruses interfere with Toll-like receptor 4?


Laure Perrin-Cocon**[,1], Francesco Peri***[,2] & Vincent Lotteau*[,1]

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Toll-like receptors (TLRs) belong to the family of pattern recognition receptors and participate to immune surveillance by detecting pathogen-associated molecular patterns (PAMPs). Upon recognition of PAMPs, TLRs recruit adapter molecules and initiate a wide range of reactions leading to both innate and adaptive immune responses.

TLR4 is expressed at the surface of innate immune cells (monocytes, macrophages and dendritic cells [DCs]) and also on some nonimmune cells like epithelial and endothelial cells. TLR4 forms a heterodimer with MD-2 and this association is necessary for recognition of a wide variety of PAMPs from bacteria, fungi, viruses, plants and cyanobacteria. The majority of these PAMPs are agonists of the TLR4–MD-2 complex.

Detection of bacterial endotoxins (e.g., Gram-negative bacterial lipopolysaccharides [LPS] or lipooligosaccharides) by TLR4 initiate an inflammatory reaction necessary for optimal immune responses to invading Gram-negative bacteria. Lipopolysaccharide binding protein and CD14 are essential to the recognition process leading to the formation of (TLR4.MD–2.LPS)$_2$ heterodimer at the plasma membrane. The surface heterodimer eventually undergoes endocytosis and activates two different intracellular pathways, one involving the formation of the Myddosome and the other, the recruitment of TRAM and TRIF adaptors, leading to the production of inflammatory cytokines. CD14 is essential for the endocytosis of (TLR4.MD–2.LPS)$_2$ and the subsequent activation of TRAM–TRIF pathway leading to the production of type I interferon. Although TLR4 is essential in mounting host responses to Gram-negative bacteria, an excessive or prolonged TLR4 response can promote life-threatening disorders, such as acute sepsis and septic shock.

TLR4 can also be activated by endogenous factors generally defined as damage-associated molecular patterns (DAMPs). DAMPs are extremely diverse molecules

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[1]CIRI – International Center for Infectiology Research, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, Ecole Normale Supérieure de Lyon, Univ Lyon, F-69007, Lyon, France
[2]Department of Biotechnology & Biosciences, University of Milano-Bicocca, Piazza della Scienza, 2; 20126 Milano, Italy
*Author for correspondence: vincent.lotteau@inserm.fr
**Author for correspondence: laure.perrin@inserm.fr
***Author for correspondence: francesco.peri@unimib.it

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derived from damaged, necrotic or infected tissues ranging from proteins to lipoproteins and oxidized phospholipids [1]. DAMPs-mediated TLR4 activation is suspected to play a major role in infectious and noninfectious inflammatory diseases.

The TLR4 pathway is not an 'isolated system' and is under the influence of other pathways involved in the initiation and regulation of the inflammation process. By regulating the intensity of TLR4-mediated responses, these pathways are becoming interesting therapeutic targets for the control of TLR4 activity in pathological situations. A good example is the synergistic activity of TREM-1 that leads to a massive increase in TLR4-mediated proinflammatory cytokine production. This molecular crosstalk is restricted to monocytes–macrophages and neutrophils that co-express TREM-1 and TLR4. Another example is the modulation of TLR4 response to LPS by MIF. In the absence of MIF, TLR4 expression is reduced on macrophages that become hyporesponsive to LPS. By contrast to TREM-1, MIF is co-expressed with TLR4 by various nonimmune cells including epithelial and endothelial cells which are in direct contact with microbial pathogens. Therefore, TLR4 stimulation triggers signaling pathways depending on cell types, expression of co-receptors, concomitant stimulation of regulatory receptors and regulates the expression of many genes involved in antimicrobial response, innate immunity, endocytosis and metabolic reprogramming.

"…Virus-induced DAMPs–TLR4 activation can be deleterious for the host by inducing an uncontrolled inflammatory reaction."

Metabolic reprogramming has emerged as a key checkpoint for the activation of innate immune cells such as murine macrophages and DCs, for which TLR4 activation results in the preferential use of glycolysis rather than mitochondrial catabolic pathways [2–4]. This process recalls the Warburg effect observed in tumor cells. Upon TLR4–MD-2 engagement, cells adapt their metabolism so that glucose remains a source of energy while becoming a source of carbon for new biosynthetic intermediates. We have shown that the stimulation of TLR4 in human DCs triggers a metabolic shift resulting in increased glycolytic activity, which is important for the fine-tuning of DC function [5]. Mechanisms involved in the coupling of TLR4 signaling with glycolysis regulation mainly remain to be identified.

In noninfectious diseases, chronic stimulation of TLR4–MD-2 pathway by DAMPs is suspected to contribute to the development of a wide array of inflammatory syndromes, including type II diabetes [6], atherosclerosis [7], neurodegenerative and autoimmune diseases like rheumatoid arthritis and multiple sclerosis. Several studies have shown that increased TLR4 expression and signaling is an important mechanism in insulin resistance. It has been established that free fatty-acid-induced insulin resistance required the presence of fetuin-A, an endogenous serum ligand of TLR4 produced by the liver [8]. Ischemia/reperfusion injury is another example where activation of TLR4 pathway by DAMPs exacerbates tissue damage.

Viruses have evolved strategies to interfere with TLR4 signaling by several means. The TLR4–MD-2 pathway can be activated by viral proteins like the RSV F protein [9], the circulating Ebola shed-GP [10] and NS1 protein from dengue, West Nile and yellow fever flaviviruses [11]. Although mechanisms of TLR4–MD-2 targeting may vary, it is clear that this pathway plays a crucial role in a number of viral infections by participating to the inflammatory responses orchestrated by immune cells as well as to epithelium and endothelium integrity. Since TLR4-dependent metabolic reprogramming can promote innate antiviral responses while providing enough energy and biosynthetic intermediates for a virus to replicate, pathogens may exploit this TLR4-dependent regulation of glycolysis to respond to increased energetic demands or to modulate the function of antigen-presenting cells.

Although TLR4 stimulation by viral protein could trigger a protective immune response to infection [12], it may also be detrimental to the host by triggering inappropriate or excessive inflammation. TLR4 activation by the envelop protein of mouse mammary virus induced IL-10 secretion that could favor viral persistence [13]. The endogenous multiple sclerosis-associated retrovirus stimulates TLR4 and induces a proinflammatory Th1 response that could contribute to chronic inflammatory and autoimmune pathologies [14]. Our previous work has shown that viral particles from hepatitis C virus chronically infected patients altered the signaling of TLR4 in DCs, resulting in a deficient Th1 response that could favor viral persistence [15,16].

Secreted viral proteins can target the TLR4–MD-2 pathway, triggering signaling or altering its activation/regulation by other ligands. This strategy affects TLR4-expressing cells whether they are infected or not. Interestingly, the soluble Ebola virus glycoprotein that is shed

from infected cells and the secreted NS1 protein of dengue virus both disrupt endothelial cell monolayer integrity in a TLR4-dependent manner [11,17]. Viral proteins can also interfere with intracellular actors of the TLR4 signaling. For example, RSV G protein selectively inhibits the TRIF-dependent signaling pathway of TLR3/4, inhibiting IFN-β production by DCs stimulated by LPS [18].

Viral infection can also lead to the release of DAMPs from infected cells. Some of these danger signals, such as HMGB1 [1], are MD-2 ligands triggering TLR4 signaling. Virus-induced DAMPs–TLR4 activation can be deleterious for the host by inducing an uncontrolled inflammatory reaction. This is the case for instance when influenza virus infection is inducing a cytokine storm with accumulation of oxidized phospholipids and HMGB1 in the infected lungs.

Molecules mimicking lipid A, the part of LPS that directly interacts with MD-2 and CD14 receptors, have been developed as TLR4 antagonists. Eritoran (E5564, developed by Eisai) is a synthetic lipid A mimetic that acts as a TLR4 antagonist by binding with high affinity to MD-2 in a manner similar to lipid A. In a murine model of TLR4-dependent severe influenza infection, the treatment by Eritoran prevented influenza-induced lethality and acute lung injury, reducing the secretion of inflammatory cytokines [19]. Unfortunately, despite high activity in animal models, Eritoran failed to pass clinical Phase III as antisepsis agent in human.

The therapeutic potential of TLR4 antagonists in the treatment or prevention of symptoms directly related to PAMP–TLR4 and DAMP–TLR4 activation motivated the development of novel classes of compounds like FP7. FP7 is a synthetic monosaccharide bearing two lipid chains and two phosphate groups that mimics the monosaccharide lipid X, a natural TLR4 antagonist [20]. FP7 is a potent TLR4 antagonist that can inhibit DAMP-induced inflammation *in vitro* and in a series of animal models of inflammatory diseases. For instance, FP7 inhibits HMGB1-mediated TLR4 cell activation and reduces lung tissue damages and lethality in the TLR4-dependent severe influenza infection model [5]. Because of its monosaccharide structure, FP7 requires less synthesis steps than Eritoran or other disaccharidic lipid A analogs. Even though its pharmacokinetic has to be fully characterized, FP7 shows better water solubility than other lipid A derivatives, good bioavailability and low toxicity. The chemical structure of the FP7 scaffold is now serving as a starting point for the design of novel compounds for selective TLR4-directed therapeutics.

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References

1 Shirey KA, Lai W, Patel MC *et al.* Novel strategies for targeting innate immune responses to influenza. *Mucosal Immunol.* 9(5), 1173–1182 (2016).

2 Krawczyk CM, Holowka T, Sun J *et al.* Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 115(23), 4742–4749 (2010).

3 Everts B, Amiel E, Huang SC *et al.* TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKvarepsilon supports the anabolic demands of dendritic cell activation. *Nat. Immunol.* 15(4), 323–332 (2014).

4 O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J. Exp. Med.* 213(1), 15–23 (2016).

5 Perrin-Cocon L, Aublin-Gex A, Sestito SE *et al.* TLR4 antagonist FP7 inhibits LPS-induced cytokine production and glycolytic reprogramming in dendritic cells, and protects mice from lethal influenza infection. *Sci. Rep.* 7, 40791 (2017).

6 Reyna SM, Ghosh S, Tantiwong P *et al.* Elevated toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. *Diabetes* 57(10), 2595–2602 (2008).

7 Erridge C. The roles of Toll-like receptors in atherosclerosis. *J. Innate Immun.* 1(4), 340–349 (2009).

8 Pal D, Dasgupta S, Kundu R *et al.* Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat. Med.* 18(8), 1279–1285 (2012).

9 Rallabhandi P, Phillips RL, Boukhvalova MS *et al.* Respiratory syncytial virus fusion protein-induced toll-like receptor 4 (TLR4) signaling is inhibited by the TLR4 antagonists Rhodobacter sphaeroides lipopolysaccharide and eritoran (E5564) and requires direct interaction with MD-2. *MBio* 3(4), e00218–e00212 (2012).

10 Okumura A, Pitha PM, Yoshimura A, Harty RN. Interaction between Ebola virus glycoprotein and host toll-like receptor 4 leads to induction of proinflammatory cytokines and SOCS1. *J. Virol.* 84(1), 27–33 (2010).

11 Modhiran N, Watterson D, Muller DA *et al.* Dengue virus NS1 protein activates cells via Toll-like receptor 4 and disrupts endothelial cell monolayer integrity. *Sci. Transl. Med.* 7(304), 304ra142 (2015).

12 Haynes LM, Moore DD, Kurt-Jones EA, Finberg RW, Anderson LJ, Tripp RA. Involvement of toll-like receptor 4 in innate immunity to respiratory syncytial virus. *J. Virol.* 75(22), 10730–10737 (2001).

13 Jude BA, Pobezinskaya Y, Bishop J *et al.* Subversion of the innate immune system by a retrovirus. *Nat. Immunol.* 4(6), 573–578 (2003).

14 Rolland A, Jouvin-Marche E, Viret C, Faure M, Perron H, Marche PN. The envelope protein of a human endogenous retrovirus-W family activates innate immunity through CD14/TLR4 and promotes Th1-like responses. *J. Immunol.* 176(12), 7636–7644 (2006).

15 Agaugue S, Perrin-Cocon L, Andre P, Lotteau V. Hepatitis C lipo-Viro-particle from chronically infected patients interferes with TLR4 signaling in dendritic cell. *PLoS ONE* 2(3), e330 (2007).

16 Perrin-Cocon L, Agaugue S, Diaz O *et al.* Th1 disabled function in response to TLR4 stimulation of monocyte-derived DC from patients chronically-infected by hepatitis C virus. *PLoS One* 3(5), e2260 (2008).

17 Escudero-Perez B, Volchkova VA, Dolnik O, Lawrence P, Volchkov VE. Shed GP of Ebola virus triggers immune activation and increased vascular permeability. *PLoS Pathogens* 10(11), e1004509 (2014).

18 Shingai M, Azuma M, Ebihara T *et al.* Soluble G protein of respiratory syncytial virus inhibits Toll-like receptor 3/4-mediated IFN-beta induction. *Int. Immunol.* 20(9), 1169–1180 (2008).

19 Shirey KA, Lai W, Scott AJ *et al.* The TLR4 antagonist Eritoran protects mice from lethal influenza infection. *Nature* 497(7450), 498–502 (2013).

20 Cighetti R, Ciaramelli C, Sestito SE *et al.* Modulation of CD14 and TLR4.MD-2 activities by a synthetic lipid A mimetic. *Chembiochem* 15(2), 250–258 (2014).