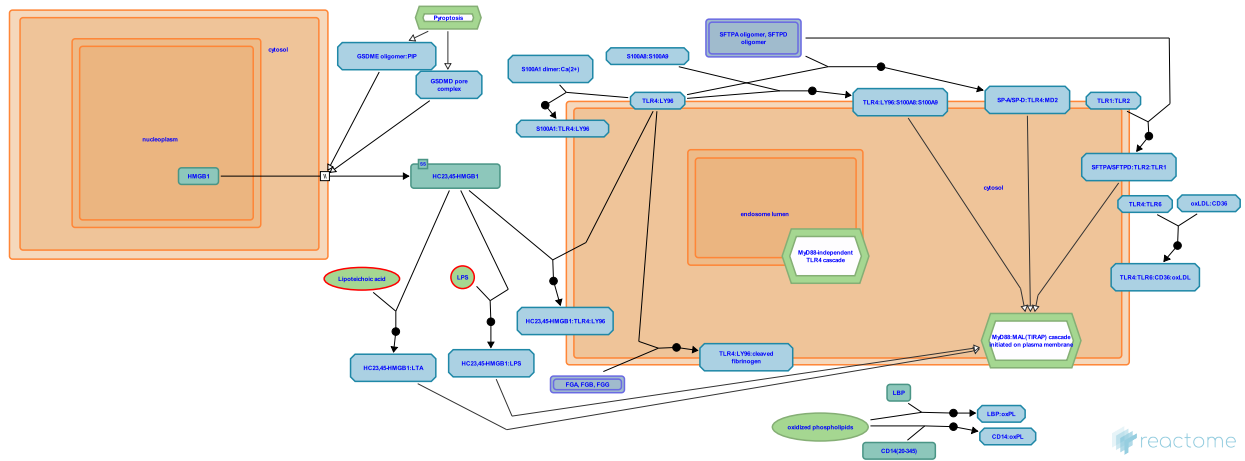


Regulation of TLR by endogenous ligand



D'Eustachio, P., Granucci, F., Kanneganti, TD., Shamovsky, V., Shao, F., Zanoni, I.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the [Reactome Textbook](https://www.reactome.org/textbook/).

31/01/2023

Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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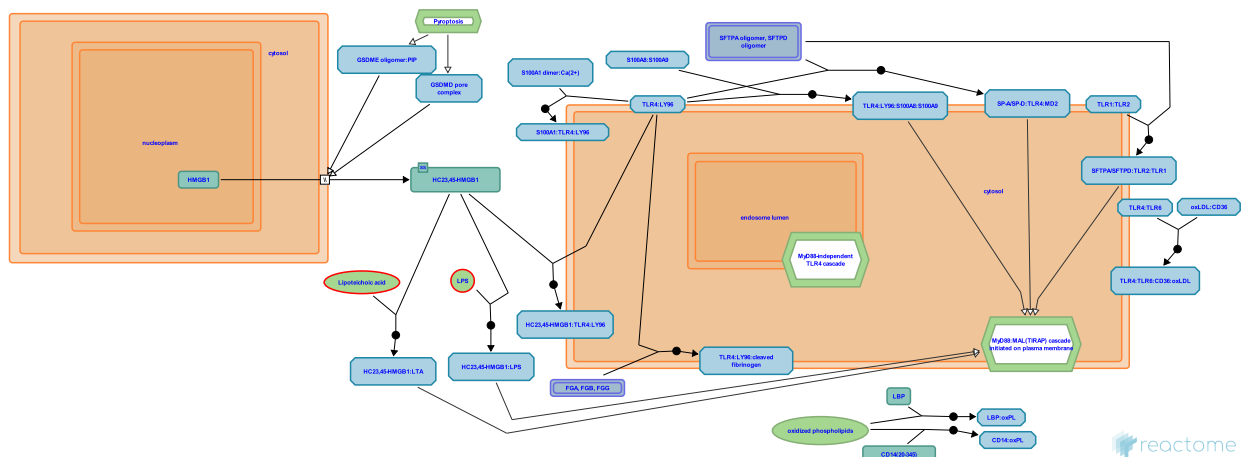
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Reactome database release: 83

This document contains 1 pathway and 12 reactions ([see Table of Contents](#))

Regulation of TLR by endogenous ligand ↗

Stable identifier: R-HSA-5686938



Diverse molecules of host-cell origin may serve as endogenous ligands of Toll-like receptors (TLRs) (Erridge C 2010; Piccinini AM & Midwood KS 2010). These molecules are known as damage-associated molecular patterns (DAMPs). DAMPs are immunologically silent in healthy tissues but become active upon tissue damage during both infectious and sterile insult. DAMPs are released from necrotic cells or secreted from activated cells in response to tissue damage to mediate tissue repair by promoting inflammatory responses. However, DAMPs have also been implicated in the pathogenesis of many inflammatory and autoimmune diseases, including rheumatoid arthritis (RA), cancer, and atherosclerosis. The mechanism underlying the switch from DAMPs that initiate controlled tissue repair, to those that mediate chronic, uncontrolled inflammation is still unclear. Recent evidence suggests that an abnormal increase in protein citrullination is involved in disease pathophysiology (Anzilotti C et al. 2010; Sanchez-Pernaute O et al. 2013; Sokolove J et al. 2011; Sharma P et al. 2012). Citrullination is a post-translational modification event mediated by peptidyl-arginine deaminase enzymes which catalyze the deimination of proteins by converting arginine residues into citrullines in the presence of calcium ions.

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Editions

2015-09-12	Reviewed	D'Eustachio, P.
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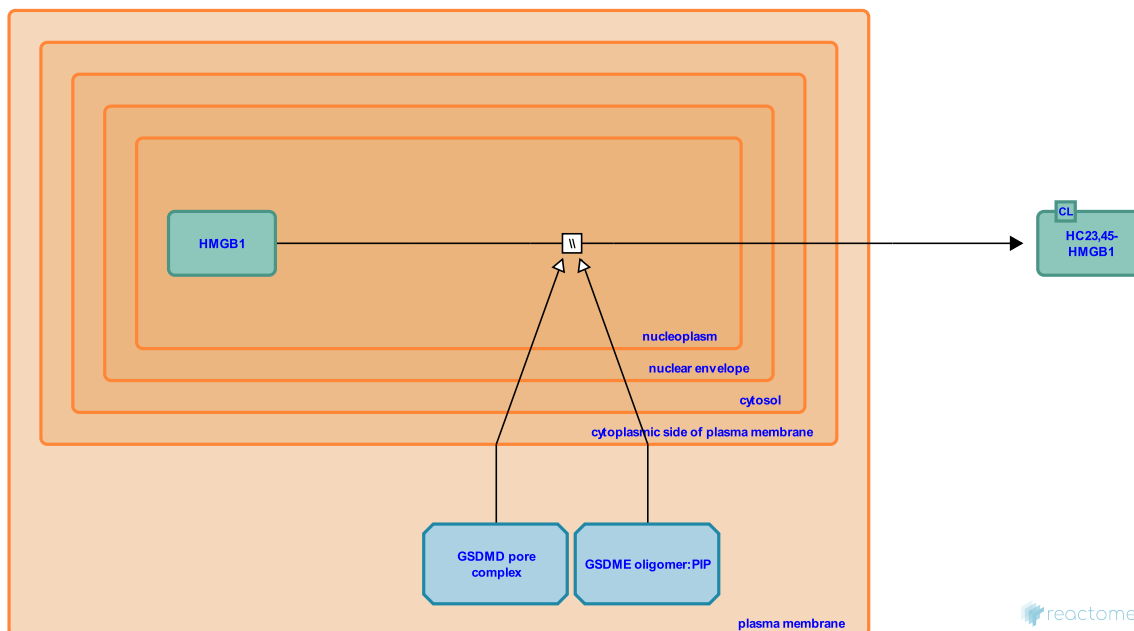
HMGB1 release from cells ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-6805981

Type: omitted

Compartments: nucleoplasm, extracellular region



High mobility group box protein 1 (HMGB1) is a ubiquitous nuclear protein that under normal conditions binds and bends DNA and facilitates gene transcription. In response to infection or injury, HMGB1 is actively secreted by innate immune cells and/or released passively by necrotic or damaged cells to function as an alarmin (Andersson U et al. 2000; Scaffidi P et al. 2002; Bonaldi T et al. 2003; Chen G et al. 2004; Lamkanfi M et al. 2010; Beyer C et al. 2012; Yang H et al. 2013). Earlier studies reported that HMGB1 did not diffuse out of cells undergoing apoptosis as HMGB1 was found to be tightly associated with the chromatin in apoptotic cells, even when the cell membrane was permeabilized artificially with detergents (Scaffidi P et al. 2002). This finding is in agreement with the general observation that apoptosis does not promote inflammation. However, further work showed that cells that undergo apoptosis do release HMGB1 (Bell CW et al. 2006; Yamada Y et al. 2011; Spencer DM et al. 2014). In human apoptotic cells (acute myeloid leukemia H60, HeLa, Jurkat T lymphocyte, pancreatic carcinoma PANC1 cell lines) HMGB1 was found to translocate into membrane-bound vesicles which are generated and released by cells during apoptosis (Spencer DM et al. 2014; Schiller M et al 2013). Outside the cell, HMGB1 can serve as an alarmin to activate innate immune responses including chemotaxis and cytokine release in both normal and aberrant immunity (Andersson U et al. 2000; Zetterström CK et al. 2002; Voll RE et al. 2008; Harris HE et al. 2012; Diener KR et al. 2013; Yang H et al. 2013).

Followed by: [HMGB1 binds LTP](#), [HMGB1 binds LPS](#), [HMGB1 binds TLR4:LY96](#)

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Giessel, A., Pisetsky, DS., Distler, JH., Schett, G., Stearns, NA., Beyer, C. (2012). The extracellular release of DNA and HMGB1 from Jurkat T cells during in vitro necrotic cell death. *Innate Immun*, 18, 727-37. [↗](#)

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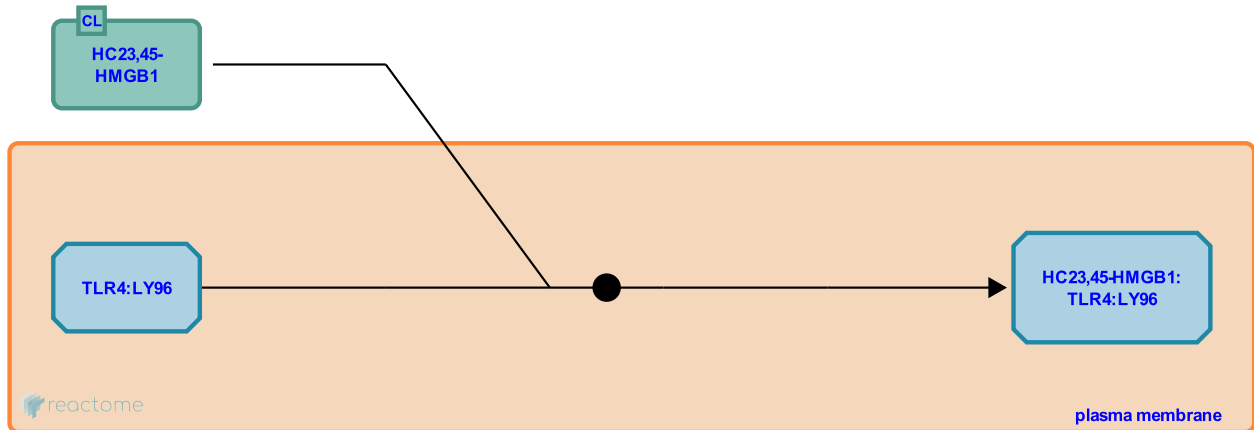
HMGB1 binds TLR4:LY96 ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-5432825

Type: binding

Compartments: plasma membrane, extracellular region



High mobility group box protein 1 (HMGB1) is an endogenous molecule that upon stress can be released into the extracellular milieu (Andersson U et al. 2000; Scaffidi P et al. 2002; Bonaldi T et al. 2003; Chen G et al. 2004; Bell CW et al. 2006; Beyer C et al. 2012; Yang H et al. 2013).

Using surface plasmon resonance (SPR) analysis recombinant HMGB1 was shown to bind TLR4:LY96(MD2) in a concentration-dependent manner (Yang H et al. 2010; Yang H et al. 2015). The binding required cysteine at the position 106 whereas the C106A HMGB1 mutant failed to bind TLR4:LY96 (Yang H et al. 2010). In addition, C106A and C106S HMGB1 failed to stimulate TNF release in mouse peritoneal macrophages (Yang H et al. 2010). The activity of HMGB1 was found to depend on the redox state of three cysteines at positions 23, 45 and 106 (C23, C45 and C106) (Urbonaviciute V et al. 2009; Venereau E et al. 2012, 2013; Yang H et al. 2012, 2013). Tandem mass spectrometric analysis revealed that the inflammatory activities of HMGB1 required both the formation of an intramolecular disulfide bond between C23 and C45 and the reduced state of C106 (thiol state, C106-SH) (Yang H et al. 2012; Venereau E et al. 2012). Both terminal oxidation of these cysteines to sulfonates (CySO₃⁻) with reactive oxygen species (ROS) and their complete reduction to thiols (CySH) abrogated the cytokine-stimulating activity of HMGB1 in cultured human primary macrophages and mouse macrophage-like RAW 264.7 cells (Yang H et al. 2012; Venereau E et al. 2012). Biosensor-based SPR analysis confirmed that only the disulfide bond (C23-S-S-C45)-containing HMGB1 binds to LY96 (MD2) with high affinity (apparent K_d = 12 nM) regardless of whether LY96 or HMGB1 was immobilized on the sensor chip (Yang H et al. 2015). Moreover, TLR4 and LY96 (MD2) were recruited into CD14-containing lipid rafts of mouse RAW264.7 macrophages after stimulation with HMGB1, suggesting that an optimal HMGB1-dependent TLR4 activation in vitro required the co-receptor CD14 (Kim S et al. 2013). In addition to stimulating cells by direct interaction with innate immune receptors, HMGB1 was found to form immunostimulatory complexes with cytokines and other endogenous and exogenous ligands such as bacterial lipopolysaccharide (LPS) (Youn JH et al. 2008; Wahamaa H et al. 2011; Hreggvidsdottir HS et al. 2009) HMGB1 in complex with LPS, IL1alpha or IL1beta boosted proinflammatory cytokine- and matrix metalloproteinase (MMP3) production in synovial fibroblasts obtained from rheumatoid arthritis (RA) and osteoarthritis (OA) patients (Wahamaa H et al. 2011; He ZW et al. 2013). HMGB1 was reported to associate and amplify the activity of LPS (TLR4 ligand), CpG-ODN (TLR9 ligand) or Pam3CSK4 (TLR1:TLR2 ligand) in a synergistic manner when added to the cultures of human peripheral blood mononuclear cell (PBMC) (Hreggvidsdottir HS et al. 2009).

Preceded by: [HMGB1 release from cells](#)

Literature references

Ding, A., Fenton, MJ., Wang, H., Yu, M., Tracey, KJ., Latz, E. et al. (2006). HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock*, 26, 174-9. [↗](#)

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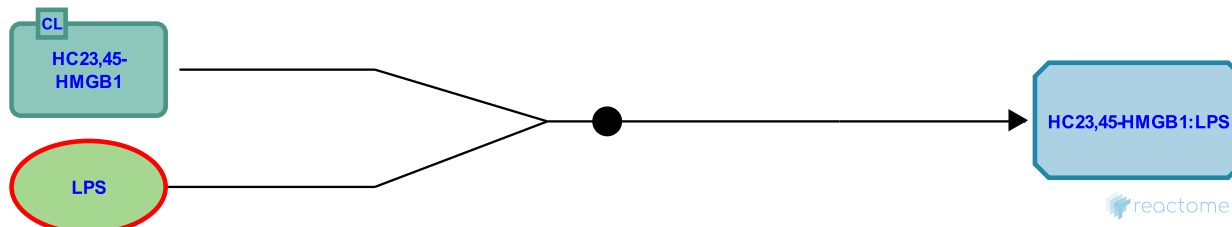
HMGB1 binds LPS ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-6804100

Type: binding

Compartments: extracellular region



High mobility group box 1 (HMGB1) is an ubiquitous nuclear protein that is actively secreted by innate immune cells and/or released passively by necrotic or damaged cells in response to infection or injury (Andersson U et al. 2000; Scaffidi P et al. 2002; Bonaldi T et al. 2003; Chen G et al. 2004; Beyer C et al. 2012; Yang H et al. 2013). Outside the cell, HMGB1 can serve as an alarmin to activate innate immune responses including chemotaxis and cytokine release in both normal and aberrant immunity (Andersson U et al. 2000; Zetterström CK et al. 2002; Voll RE et al. 2008; Harris HE et al. 2012; Diener KR et al. 2013; Yang H et al. 2013).

HMGB1 can form immunostimulatory complexes with cytokines and other endogenous and exogenous ligands such as bacterial lipopolysaccharide (LPS) to potentiate proinflammatory response (Youn JH et al. 2008, 2011; Wähämaa H et al. 2011; Hreggvidsdottir HS et al. 2009). The activity of HMGB1 depended on the redox state of three cysteines at positions 23, 45 and 106 (C23, C45 and C106) (Urbonaviciute V et al. 2009; Venereau E et al. 2012, 2013; Yang H et al. 2012, 2013). Tandem mass spectrometric analysis revealed that the inflammatory activities of HMGB1 required both the formation of an intramolecular disulfide bond between C23 and C45 and the reduced state of C106 (thiol state, C106-SH) (Yang H et al. 2012; Venereau E et al. 2012). Both terminal oxidation of these cysteines to sulfonates (CySO₃⁻) with reactive oxygen species (ROS) and their complete reduction to thiols (CySH) abrogated the cytokine-stimulating activity of HMGB1 in cultured human primary macrophages and mouse macrophage-like RAW 264.7 cells (Yang H et al. 2012; Venereau E et al. 2012).

HMGB1 binding to LPS facilitated transfer of LPS to CD14 and enhanced TNF α production in human peripheral blood mononuclear cells (PBMCs) (Youn JH et al. 2008). HMGB1 in complex with LPS boosted proinflammatory cytokine- and matrix metalloproteinase (MMP3) production in synovial fibroblasts obtained from rheumatoid arthritis (RA) and osteoarthritis (OA) patients (Wähämaa H et al. 2011; He ZW et al. 2013).

In addition to its ability to act in a synergy with LPS and other ligands, HMGB1 was shown to stimulate cells by direct interaction with innate immune receptors such as TLR4:LY96 (Yang H et al. 2010; Yang H et al. 2015).

Preceded by: [HMGB1 release from cells](#)

Literature references

Aveberger, AC., Andersson, U., Schierbeck, H., Wähämaa, H., Harris, HE., Hreggvidsdottir, HS. et al. (2011). High mobility group box protein 1 in complex with lipopolysaccharide or IL-1 promotes an increased inflammatory phenotype in synovial fibroblasts. *Arthritis Res. Ther.*, 13, R136. ↗

Ding, A., Fenton, MJ., Wang, H., Yu, M., Tracey, KJ., Latz, E. et al. (2006). HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock*, 26, 174-9. [↗](#)

Choi, JE., Youn, JH., Shin, JS., Kim, ES., Oh, YJ. (2008). High mobility group box 1 protein binding to lipopolysaccharide facilitates transfer of lipopolysaccharide to CD14 and enhances lipopolysaccharide-mediated TNF-alpha production in human monocytes. *J. Immunol.*, 180, 5067-74. [↗](#)

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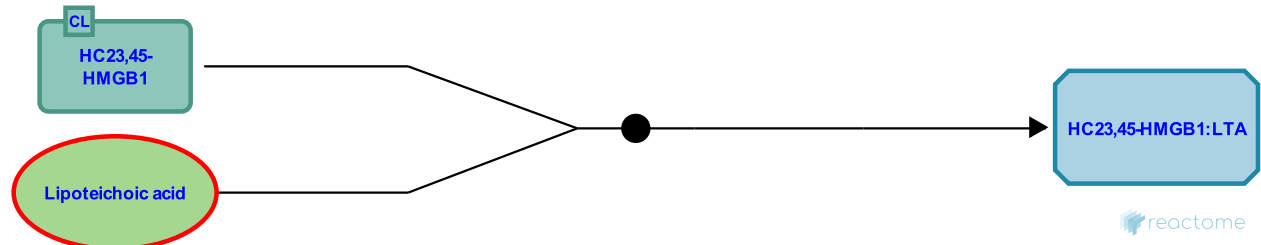
HMGB1 binds LTP ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-6804099

Type: binding

Compartments: extracellular region



Lipoteichoic acid (LTA) is a component of the cell wall of Gram-positive bacteria. LTA induces a toll-like receptor 2 (TLR2)-mediated inflammatory response upon initial binding to coreceptors CD36 and CD14 (Nilsen NJ et al. 2008).

High mobility group box protein 1 (HMGB1) is a ubiquitous nuclear protein that under normal conditions binds and bends DNA and facilitates gene transcription. In response to infection or injury, HMGB1 is actively secreted by innate immune cells and/or released passively by necrotic or damaged cells (Andersson U et al. 2000; Scaffidi P et al. 2002; Bonaldi T et al. 2003; Chen G et al. 2004; Beyer C et al. 2012; Yang H et al. 2013). Outside the cell, HMGB1 can serve as an alarmin to activate innate immune responses including chemotaxis and cytokine release in both normal and aberrant immunity (Andersson U et al. 2000; Zetterström CK et al. 2002; Voll RE et al. 2008; Harris HE et al. 2012; Diener KR et al. 2013; Yang H et al. 2013). HMGB1 has been implicated in TLR2-mediated inflammation (Yu M et al. 2006; Park JS et al. 2006). Addition of HMGB1 induced cellular activation and TLR2- and TLR4-mediated NFkappaB-dependent transcription in TLR2- or TLR4-transfected human embryonic kidney-293 (HEK293) cells (Park JS et al. 2006). Mouse Tlr2 was found to associate with immunoprecipitated Hmgb1 from mouse macrophage-like RAW264.7 cell lysates (Park JS et al. 2006). Anti-TLR2 antibodies dose-dependently attenuated HMGB1-induced IL-8 release in TLR2-expressing HEK293 cells and markedly reduced HMGB1 cell surface binding on murine macrophage-like RAW 264.7 cells (Yu M et al. 2006). Moreover, results of ELISA, surface plasmon resonance and native PAGE electrophoretic mobility shift analyses indicated that HMGB1 binds LTA in a concentration-dependent manner and that this binding is inhibited by LBP (Kwak MS et al. 2015). Native PAGE, fluorescence-based transfer and confocal imaging analyses indicated that HMGB1 catalytically disaggregated LTA transferring LTA to CD14. NFkappaB p65 nuclear transmigration, degradation of Ikbalpha and reporter assay results demonstrated that NFkappaB activity in HEK293-hTLR2/6 cells was significantly upregulated by a mixture of LTA and soluble CD14 in the presence of HMGB1 (Kwak MS et al. 2015). Furthermore, the production of TNFalpha and IL6 in murine J774A.1 and RAW264.7 cells increased significantly following treatment with a mixture of LTA and HMGB1 compared with treatment with LTA or HMGB1 alone (Kwak MS et al. 2015). Thus, HMGB1 was proposed to play an important role in LTA-mediated inflammation by binding to LTA and transferring LTA to CD14, which is subsequently transferred to TLR2:TLR6 to induce an inflammatory response.

Preceded by: [HMGB1 release from cells](#)

Literature references

Ding, A., Fenton, MJ., Wang, H., Yu, M., Tracey, KJ., Latz, E. et al. (2006). HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. *Shock*, 26, 174-9. ↗

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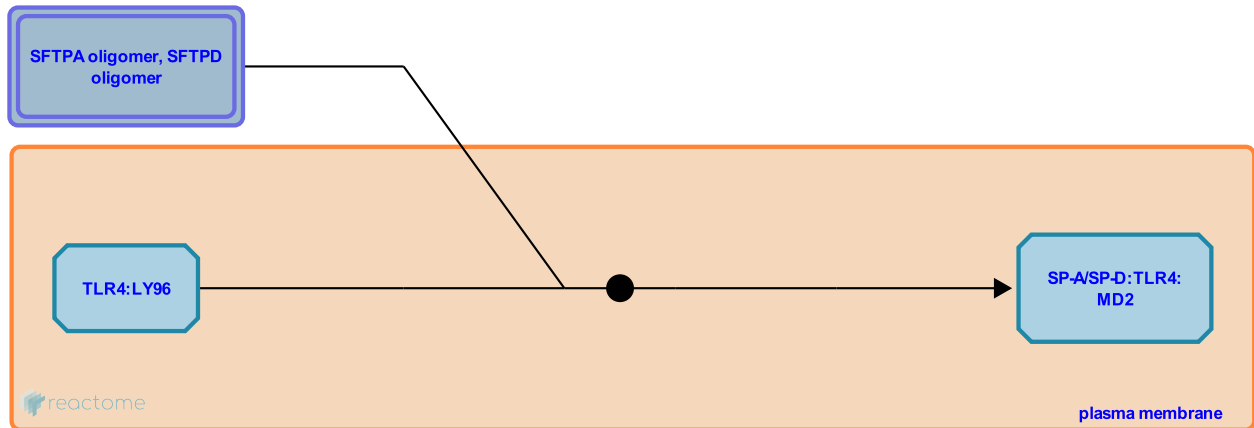
SFTPA/SFTPD binds TLR4:LY96 ↗

Location: Regulation of TLR by endogenous ligand

Stable identifier: R-HSA-5432852

Type: binding

Compartments: plasma membrane, extracellular region



The hydrophilic pulmonary surfactant proteins SP-A (SFTPA) and SP-D (SFTPD) belong to the C-type lectin family. Members of the C-type lectin family contain an N-terminal collagen-like domain and a C-terminal carbohydrate recognition domain (CRD) (Kishore U et al. 2006). The CRD allows binding to various components, including carbohydrates, phospholipids or charge patterns found on microbes, allergens and dying cells, while the collagen region can interact with receptor molecules present on immune cells in order to initiate clearance mechanisms (Kishore U et al. 2006). SP-A and SP-D are known to bind to a range of microbial pathogens that invade the lungs (Eggleton P & Reid KB 1999; Crouch E & Wright JR 2001; McCormack FX1 & Whitsett JA 2002; Nayak A et al. 2012; Jakel A et al. 2013). SP-A and SP-D form large oligomeric structures to orchestrate the pulmonary innate immune defense by mechanisms that may involve binding and agglutinating pathogens (Kuan SF et al 1992; Griese M & Starosta V 2005; Yamada C et al. 2006; Kishore U et al. 2006; Zhang L et al. 2001). The direct interaction of SP-A with macrophages was shown to promote phagocytosis of *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Van Iwaarden JF et al. 1994; Hickman-Davis JM et al. 2002; Ding J et al. 2004; Mikerov AN et al. 2008; Gil M et al. 2009).

SP-A and SP-D were found to bind to the recombinant soluble form of extracellular TLR4 domain (sTLR4) and MD2 in a Ca^{2+} -dependent manner, with involvement of the CRD region (Yamada et al. 2006; Yamazoe M et al. 2008). SP-A was also shown to interact with CD14 (Sano H. et al. 1999). Studies involving gene knock-out mice, murine models of lung hypersensitivity and infection together with functional characterization of cell surface receptors revealed both pro- and anti-inflammatory functions of SP-A and SP-D in the control of lung inflammation in mammals (Guillot L et al. 2002; Madan T et al. 2001, 2005, 2010; Wang JY & Reid KB 2007; Yamada et al. 2006; Yamazoe M et al. 2008; Wang G et al. 2010). Anti-inflammatory effects of SP-A caused inhibition of NF- κ B activation and accumulation of inhibitory protein I kappa B-alpha (I κ B-alpha) in LPS-challenged alveolar macrophages (AM) (Wu Y et al. 2004). SP-A also inhibited tumor necrosis factor-alpha (TNFalpha) expression induced by smooth LPS but not by rough LPS in the human macrophage-like cell line U937 cells (Sano H. et al. 1999). In addition, SP-A attenuated cell surface binding of smooth LPS and subsequent NF- κ B activation in TLR4/MD2 expressing human embryonic kidney (HEK293) cells (Yamada et al. 2006). Like SP-A, SP-D bound to complex of sTLR4:MD2 was found to down regulate a secretion of TNFalpha and activation of NF- κ B in LPS-stimulated AM and TLR4/MD-2-transfected HEK293 cells (Yamazoe M et al. 2008). SP-A and SP-D are thought to prevent LPS-

elicited inflammatory responses by altering LPS binding to its receptors, TLR4:MD2 or CD14 (Sano H. et al. 1999; Yamada et al. 2006; Yamazoe M et al. 2008).

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Kishore, U., Madan, T., Shrive, AK., Greenhough, TJ., Waters, P., Chakraborty, T. et al. (2006). Surfactant proteins SP-A and SP-D: structure, function and receptors. *Mol Immunol*, 43, 1293-315. [↗](#)

Mitsuzawa, H., Yamazoe, M., Voelker, DR., Kuroki, Y., Takahashi, H., Ariki, S. et al. (2008). Pulmonary surfactant protein D inhibits lipopolysaccharide (LPS)-induced inflammatory cell responses by altering LPS binding to its receptors. *J. Biol. Chem.*, 283, 35878-88. [↗](#)

Mitsuzawa, H., Himi, T., Sano, H., Yamada, C., Kuroki, Y., Nishitani, C. et al. (2006). Surfactant protein A directly interacts with TLR4 and MD-2 and regulates inflammatory cellular response. Importance of supratrimeric oligomerization. *J. Biol. Chem.*, 281, 21771-80. [↗](#)

Mitsuzawa, H., Ohya, M., Yamada, C., Sano, H., Kuroki, Y., Saito, T. et al. (2006). Human pulmonary surfactant protein D binds the extracellular domains of Toll-like receptors 2 and 4 through the carbohydrate recognition domain by a mechanism different from its binding to phosphatidylinositol and lipopolysaccharide. *Biochemistry*, 45, 8657-64. [↗](#)

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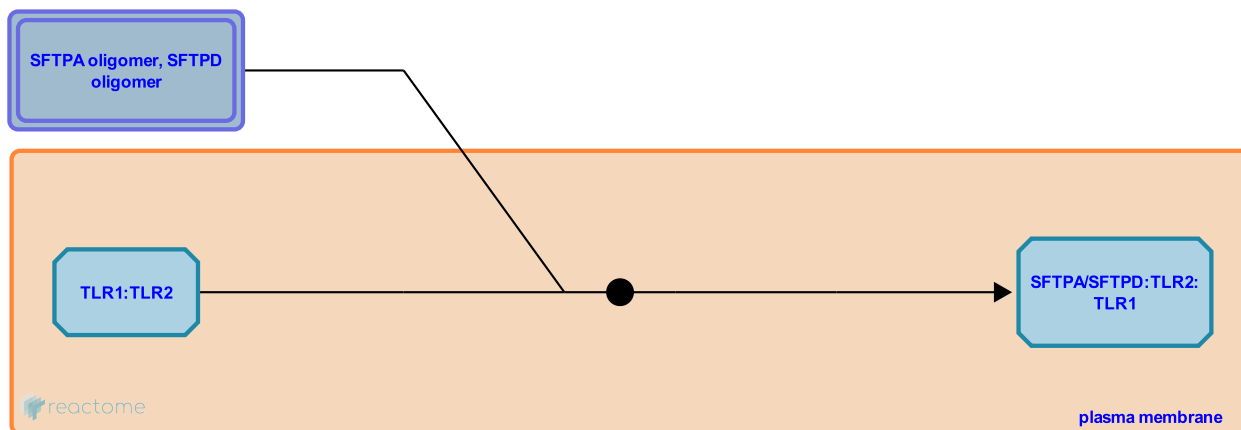
SFTPA/SFTPD binds TLR2:TLR1 ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-5432814

Type: binding

Compartments: plasma membrane, extracellular region



The lung surfactant proteins SP-A (also known as SFTPA) and SP-D (SFTPD) have been implicated in the regulation of pulmonary host defense and inflammation. SP-A and SP-D were found to bind to the recombinant soluble form of extracellular TLR2 domain (TLR2) via its C-terminal carbohydrate recognition domain (CRD) in a Ca²⁺-dependent manner (Murakami S et al. 2002; Ohya M et al. 2006). SP-A downregulated TLR2-mediated signaling and tumor necrosis factor alpha (TNFalpha) secretion in TLR2-transfected human embryonic kidney 293 (HEK293) cells upon stimulation with TLR2 ligands such as fungal cell surface component zymosan or bacterial peptidoglycan (PGN) (Murakami S et al. 2002; Sato M et al. 2003). Similarly, SP-A significantly reduced PGN-elicited TNFalpha secretion by human leukemic monocyte lymphoma U937 cell line and rat alveolar macrophages (Murakami S et al. 2002). In primary human monocyte-derived macrophage SP-A regulated TLR2 and TLR4 activity by diminishing proinflammatory cytokine production as the result of a decreased phosphorylation of a key regulator of NFkB, IkbAlpha. Nuclear translocation of NFkB-p65 (RELA) was also inhibited (Henning LN et al. 2008). SP-A downregulated kinases upstream of IkbAlpha by decreasing the phosphorylation of Akt and MAPKs in response to either LPS (TLR4 ligand) or Pam3Cys (TLR2 ligand) (Henning LN et al. 2008). In addition, SP-A upregulated surface protein expression of TLR2 on macrophages, while it did not affect TLR4 surface expression. The increased TLR2 expression is thought to enhance pathogen recognition by TLR2, while SP-A mediated inhibition of TLR signaling may protect from an overreactive inflammatory response (Henning LN et al. 2008).

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Mitsuzawa, H., Sano, H., Voelker, DR., Kuroki, Y., Takahashi, H., Akino, T. et al. (2002). Surfactant protein A inhibits peptidoglycan-induced tumor necrosis factor-alpha secretion in U937 cells and alveolar macrophages by direct interaction with toll-like receptor 2. *J. Biol. Chem.*, 277, 6830-7. [↗](#)

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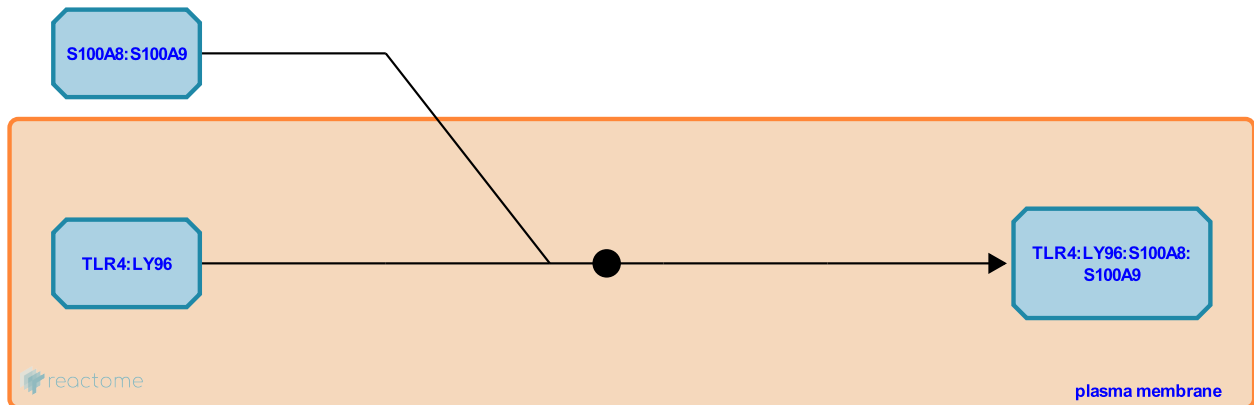
S100A8:S100A9 binds TLR4:LY96 ↗

Location: Regulation of TLR by endogenous ligand

Stable identifier: R-HSA-5432849

Type: binding

Compartments: plasma membrane, extracellular region



S100A8 (also known as MRP8) and S100A9 (MRP14) are Ca²⁺-binding proteins that are associated with acute and chronic inflammation and cancer (Ehrchen JM et al. 2009; De Jong HK et al. 2015). S100A8 & S100A9 have been identified as important damage-associated molecular patterns (DAMPs) recognized by TLR4 (Foell D et al. 2007; Vogl t et al. 2007; 2012; Kang JH et al. 2015). Surface plasmon resonance studies showed that S100A8 can directly interact with TLR4:MD2 complex with K_d of 1.1-2.5 x 10⁻⁸ M ((Vogl T et al. 2007). Human embryonic kidney cells stably transfected with TLR4,CD14 and MD2 demonstrated a strong induction of proinflammatory cytokines like TNF α and IL8 after stimulation with LPS as well as with S100A8 (Vogl T et al. 2007). Induction of NF κ B responses by S100A9 in human monocytic THP-1 cell line and mouse bone marrow-derived dendritic cells was TLR4-dependent (Riva M et al. 2012). Moreover, induction of MUC5AC mRNA and protein in normal human bronchial epithelial cells as well as NCI-H292 lung carcinoma cells occurred in a dose-dependent manner through TLR4 signaling pathway (Kang JH et al. 2015). In addition, S100A8:S100A9 was reported to regulate cell survival of human neutrophils through a signaling mechanism involving an activation of MEK:ERK1 via TLR4 (Atallah M et al. 2012). In experimental mouse models the proinflammatory and TLR4-dependent activities of S100A8:S100A9 were further confirmed (Vogl t et al. 2007; Loser K et al. 2010; Kuipers MT et al. 2013; Deguchi A et al. 2015).

S100A8 & S100A9 are constitutively expressed in neutrophils, myeloid-derived dendritic cells, platelets, osteoclasts and hypertrophic chondrocytes (Hessian PA et al. 1993; Kumar A et al. 2003; Healy AM et al. 2006; Schelbergen RF et al 2012). In contrast, these molecules are induced under inflammatory stimuli in monocytes/macrophages, microvascular endothelial cells, keratinocytes and fibroblasts (Hessian PA et al. 1993; Eckert RL et al. 2004; Viemann D et al. 2005; McCormick MM et al. 2005; Hsu K et al. 2005). S100A8 & S100A9 tend to form homodimers and heterodimers (Kumar RK et al. 2001; Riva M et al. 2013; Korndorfer IP et al. 2007). The heterodimeric S100A8:S100A9 complex is termed calprotectin and is considered as the predominantly occurring form. In response to stress S100A8:S100A9 is primarily released from activated or necrotic neutrophils to extracellular milieu where it functions as an innate immune mediator of infection, autoimmunity, and cancer (Ehrchen JM et al. 2009; Rammes A et al. 1997; Frosch M et al. 2000; Loser K et al. 2010).

S100A8 and S100A9 protein levels were elevated in patients with a wide range of inflammatory diseases, including rheumatoid arthritis, juvenile idiopathic arthritis, inflammatory bowel disease, acute lung in-

flammation, sepsis and vasculitis (Ehrchen JM et al. 2009; van Zoelen MA et al. 2009; Vogl T et al. 2012; Holzinger D et al. 2012; Rahman MT et al. 2014; Anink J et al. 2015). Increased S100A8 and S100A9 serum levels have been also identified as independent risk predictors for various cardiovascular diseases such as acute coronary syndrome and myocardial infarction (Yonekawa K et al. 2011; Cotoi OS et al. 2014; Larsen SB et al. 2015).

Literature references

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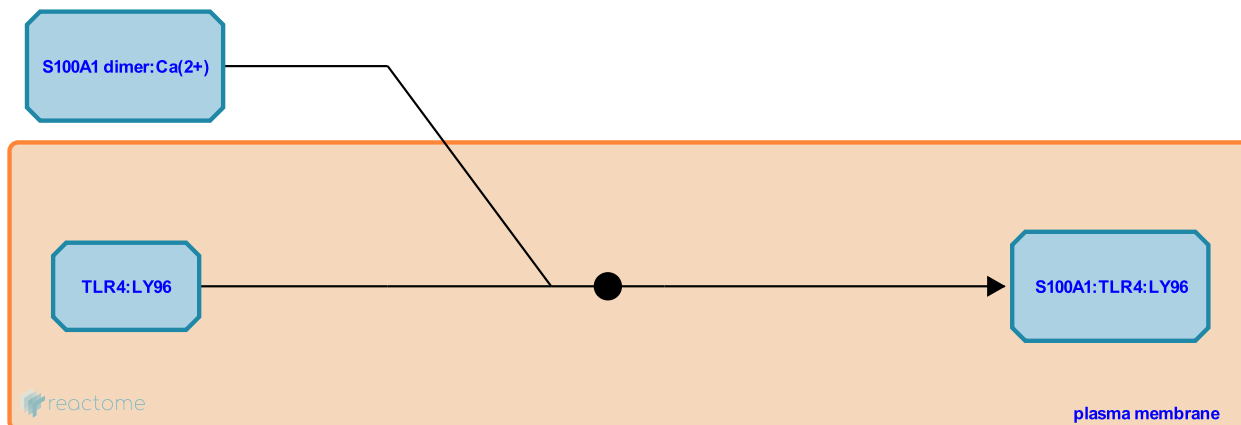
S100A1 binds TLR4:LY96 [↗](#)

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-6805943

Type: binding

Compartments: plasma membrane, extracellular region



The human event of S100A1 is inferred from the mouse data.

S100A1 is a Ca(2+)-sensing protein of the EF-hand family. S100A1 is expressed predominantly in cardiomyocytes, where it regulates Ca(2+)-dependent signaling events (Wright NT et al. 2005; Cannon BR et al. 2011; Brinks H et al. 2011; Yu J et al. 2015; Rohde D et al. 2014; Ritterhoff J & Most P 2012). In response to ischemic/hypoxic damage of cardiomyocytes, S100A1 is released or transferred to the extracellular region through open channels on membrane (Rohde D et al. 2014). The extracellular S100A1 activates signal and promotes cell survival pathways, including inflammation response via Toll-like receptor 4 (TLR4) (Brinks H et al. 2011; Yu J et al. 2015; Rohde D et al. 2014). In rodent H9C2 cells S100A1 was found to regulate the inflammatory response and oxidative stress via TLR4/ROS/NFkappaB pathway (Yu J et al. 2015).

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- Li, Y., Wu, L., Xiao, L., Yu, J., Lu, Y., Xing, Y. et al. (2015). Role of S100A1 in hypoxia-induced inflammatory response in cardiomyocytes via TLR4/ROS/NF- κ B pathway. *J. Pharm. Pharmacol.*, 67, 1240-50. [↗](#)
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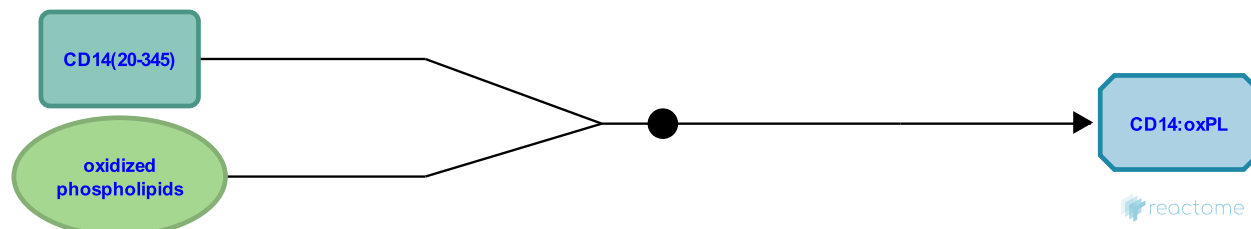
oxPL binds CD14 ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-8869694

Type: binding

Compartments: extracellular region



The generation of reactive oxygen species is a central feature of inflammation that results in the oxidation of host phospholipids. Endogenously formed oxidized phospholipids, such as 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (OxPAPC), have been shown to inhibit TLR4- & TLR2-mediated signaling induced by bacterial lipopeptide or lipopolysaccharide (LPS) in various human cells (Bochkov VN et al., 2002; von Schlieffen E et al., 2009). Oxidized phospholipids were found to bind LPS binding protein (LBP) and soluble CD14 suggesting that the binding prevented recognition of LPS by these proteins thus preventing recognition of LPS and activation of TLR4 (Erridge C et al., 2008; von Schlieffen E et al., 2009). In addition, oxPAPC protected mice treated with a lethal dose of LPS (Bochkov VN et al., 2002). Thus, oxidized phospholipids may function as a negative feedback to blunt innate immune responses.

Literature references

- Erridge, C., Spickett, CM., Webb, DJ., Kennedy, S. (2008). Oxidized phospholipid inhibition of toll-like receptor (TLR) signaling is restricted to TLR2 and TLR4: roles for CD14, LPS-binding protein, and MD2 as targets for specificity of inhibition. *J. Biol. Chem.*, 283, 24748-59. ↗
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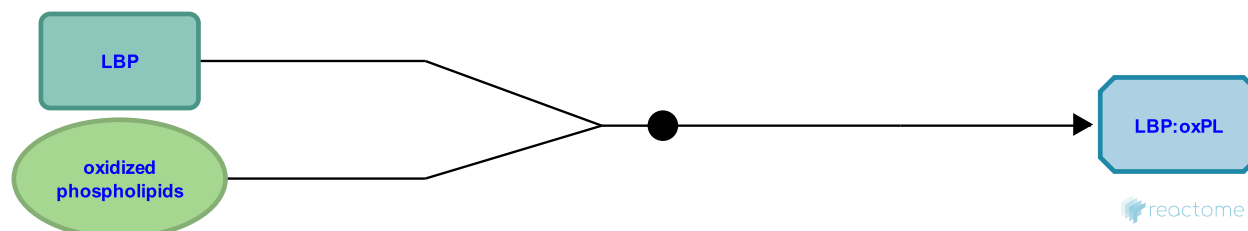
oxPL binds LBP ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-8869683

Type: binding

Compartments: extracellular region



Antibacterial defence involves activation of neutrophils that generate reactive oxygen species (ROS) capable of killing bacteria. The ROS production results in the oxidation of host phospholipids. Endogenously formed oxidized phospholipids, such as 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (OxPAPC), have been shown to inhibit TLR4- & TLR2-mediated signaling induced by bacterial lipopeptide or lipopolysaccharide (LPS) in various human cells (Bochkov VN et al., 2002; von Schlieffen E et al., 2009). Oxidized phospholipids were found to bind LPS binding protein (LBP) and soluble CD14 suggesting that the binding prevented recognition of LPS by these proteins thus preventing recognition of LPS and activation of TLR4 (Erridge C et al., 2008; von Schlieffen E et al., 2009). In addition, oxPAPC protected mice treated with a lethal dose of LPS (Bochkov VN et al., 2002). Thus, oxidized phospholipids may function as a negative feedback to blunt innate immune responses.

Literature references

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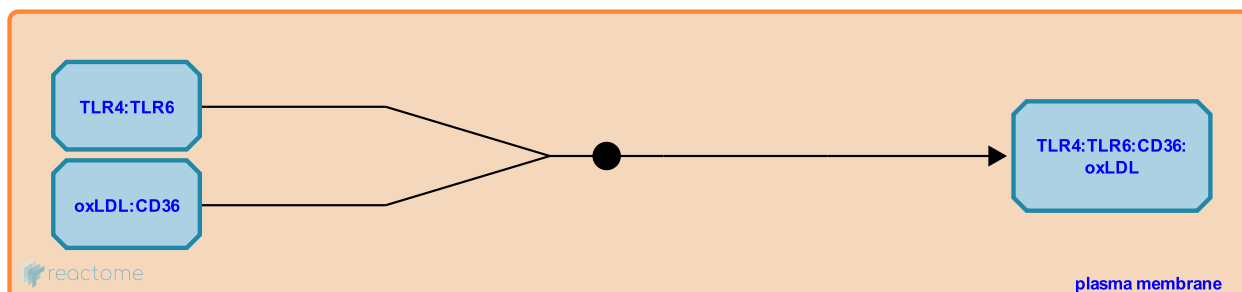
oxLDL:CD36 binds TLR4:TLR6 ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-8869667

Type: binding

Compartments: plasma membrane



Oxidized low-density lipoprotein (oxLDL) and amyloid-beta sequestered by the scavenger receptor CD36 trigger sterile inflammatory signaling through a CD36:TLR4:TLR6 heteromerization (Stewart CR et al., 2010). The heterotrimeric CD36:TLR4:TLR6 signaling complex, acting via NFkappaB and reactive oxygen species, primes the NLRP3 inflammasome in response to oxLDL (Sheedy FJ et al., 2013).

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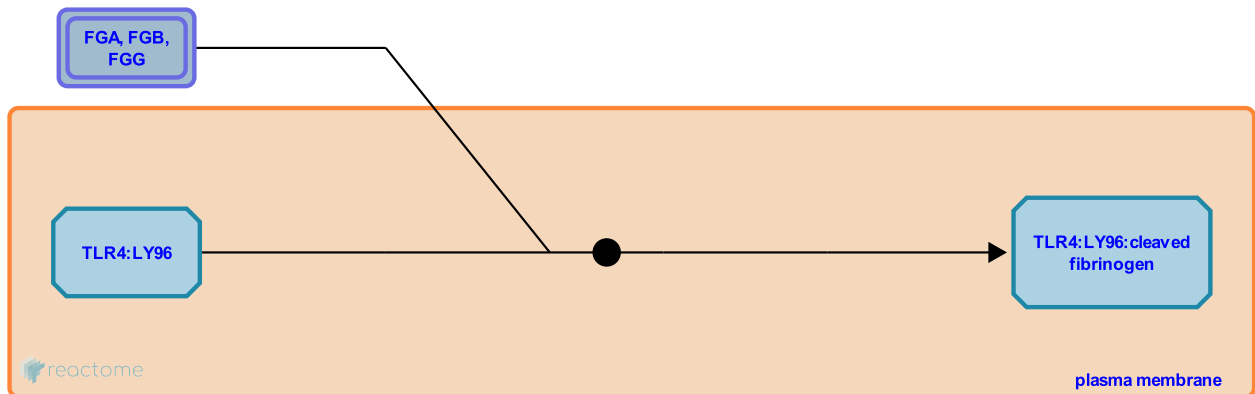
Cleaved fibrinogen products bind TLR4:LY96 ↗

Location: [Regulation of TLR by endogenous ligand](#)

Stable identifier: R-HSA-8870678

Type: binding

Compartments: plasma membrane



Fibrinogen, in addition to its role in coagulation, is also an acute phase protein of inflammation which can induce a cytokine production acting as an endogenous ligand for toll-like receptor 4 (TLR4) expressed on cells including macrophages and airway epithelial cells (Millien VO et al., 2013; Kuhns DB et al., 2007; Smiley ST et al., 2001). In human macrophages fibrinogen stimulated interleukin IL6 expression and extracellular signal-related kinase (ERK) phosphorylation ((Hodgkinson CP et al., 2008). In human embryonic kidney 293 (HEK293)-CD14-MD2 cells expressing TLR4, fibrinogen induced robust phosphorylation of ERK1, p38alpha and JNK and activated transcription factors NFkappaB, Elk1 and AP1 (Hodgkinson CP et al., 2008). Moreover, proteinases, such as thrombin, can cleave fibrinogen. In mice, exposure to endogenous or exogenous proteinases lead to hyperactivation of an antifungal pathway and lead to allergic airway inflammation through activation of TLR4-dependent signaling pathway by fibrinogen cleaved products (Millien VO et al., 2013)

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Hancock, WW., Smiley, ST., King, JA. (2001). Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J Immunol*, 167, 2887-94. ↗

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