



Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica,
Alergología y Asma Pediátrica

www.elsevier.es/ai



REVIEW

Mycoplasma pneumoniae and toll-like receptors: A mutual avenue



M. Naghib^a, M. Hatam-Jahromi^a, M. Niktab^a, R. Ahmadi^b, A. Kariminik^{a,*}

^a Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran

^b Department of Clinical Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

Received 10 July 2017; accepted 13 September 2017

Available online 10 January 2018

KEYWORDS

Toll-like receptor;
Mycoplasma pneumoniae;
Complications

Abstract *Mycoplasma pneumoniae* is an intracellular bacterium leading to several complications in humans. *M. pneumoniae* is cleared in some cases and induces complications in others. The main responsible mechanisms regarding the controversy are yet to be cleared. Toll-like receptors (TLRs) are the important cell membrane and intracellular receptors which recognize a wide range of microbial macromolecules. The roles of TLRs in the eradication of several pathogens and also induction of their related complications have been demonstrated. This review article presents recent data about the roles of TLRs in the induction of immune responses which lead to *M. pneumoniae* eradication and related complications.

© 2018 SEICAP. Published by Elsevier España, S.L.U. All rights reserved.

Introduction

It has been reported that *Mycoplasma* are considered as the smallest cell-wall deficient prokaryote. The microbes are wall-less and malleable organisms which are able to grow and proliferate under cell-free conditions.¹ Genetic investigations revealed that *Mycoplasma* contain a small genome (600–1350 kbp) with 23–35% GC ratio. They belong to the bacterial class Mollicutes and nowadays seven species of *Mycoplasma* have been discovered which are pathogenic for humans, including *Mycoplasma pneumoniae*, *M. genitalium*, *M. urealyticum*, *M. fermentation*, *M. hominis*, *M. penetrans*

and *M. pirum*.² *M. pneumoniae* is the most evaluated species and is the most frequent cause of *Mycoplasma*-related human disorders.³ Epidemiological investigations demonstrated that although *M. pneumoniae* is distributed globally, its prevalence is local. Recent investigations demonstrated that the rate of *M. pneumoniae* infection is increasing annually.⁴ *M. pneumoniae* is the main cause of primary atypical and community-acquired pneumonia.⁵ The diseases induce predominantly respiratory symptoms.⁵ The bacteria can also be considered as the cause of autoimmune hemolytic anemia and other blood-related diseases which lead to damage to the cardiovascular system and gastrointestinal tract. Thus, it participates in the pathogenesis of *M. pneumoniae*-related myocarditis, pericarditis, nephritis and also meningitis.⁶ *M. pneumoniae* infection is complex because it is associated with several complications includ-

* Corresponding author.

E-mail address: a.kariminik@iauk.ac.ir (A. Kariminik).

ing cytoadherence, cytotoxicity, membrane fusion damage, invasive damage, toxic damage, nutrition depletion, and inflammation.⁶ However, the essential mechanisms used by *M. pneumoniae* to induce its complications remain to be elucidated.

It has been hypothesized that chronic recognition of *M. pneumoniae* by innate immune cells and consequently chronic activation of the cells may be considered as a main candidate to induce some of the important *M. pneumoniae* complications.⁷ Innate immune cells use several cytoplasmic membrane and intracytoplasmic receptors to recognize prokaryotes including *M. pneumoniae*.⁸ Toll-like receptors (TLRs) are the main well known receptors which play important roles in the recognition of prokaryotic macromolecules, entitled pathogen associated molecular patterns (PAMPs), and also endogenous damage associated molecular patterns (DAMPs).^{9,10} Thus, it has been hypothesized that the receptors may participate in the recognition of *M. pneumoniae* PAMPs to either the induction of appropriate immune responses against the microbe and clearance of the infection or the induction of chronic inflammation, which is a reason for *M. pneumoniae* complications.

Based on the introduction, this review article aims to present the roles of TLRs in the immune responses against *M. pneumoniae* and also their roles in the induction of *M. pneumoniae* complications.

Toll-like receptors introducing

TLRs are the most important receptors that are expressed either on the cytoplasmic membrane (TLR1, TLR2, TLR4, TLR5 and TLR6) or in the intracytoplasmic vesicles (TLR3, TLR7, TLR8 and TLR9).^{10–12} The innate immune cell receptors have a similar structure containing three main domains including leucine rich repeat (LRR), intracytoplasmic and toll-IL-1 receptor domains.^{10–12} TLRs are different regarding LRR domain; so, they recognize various ranges of ligands that are microbial PAMPs. TLRs recognize their ligands in homodimeric and heterodimeric forms. TLR3, 4, 5, 7, 8 and 9 make homodimeric forms, while TLR1 and 6 make heterodimeric forms with TLR2.^{10–13} TLR2 is the only receptor which recognizes its ligands in both homodimeric and heterodimeric forms.¹⁴ The popular ligands for TLR 2 (in both forms), 3, 4, 5, 7, 8 and 9 are cell wall bacterial macromolecules, double strand RNA (dsRNA), lipopolysaccharide (LPS), bacterial flagellin, single strand RNA (ssRNA), ssRNA and prokaryotic DNA, respectively.¹⁵ TLRs use two popular distinct pathways, myeloid differentiation primary response gene 88 (MYD88) and TIR-domain-containing adapter-inducing interferon- β (TRIF) dependent pathways which are the responsible pathways to activate pro-inflammatory transcription factors including activator protein 1 (AP-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), interferon regulatory factor (IRF) 3, IRF5 and IRF7.¹⁵ MYD88 dependent pathway are used by TLR2 (in both homodimeric and heterodimeric forms), TLR5, 7, 8 and 9, while TRIF dependent pathway is used by TLR3.¹⁶ TLR4 uses either MYD88 or TRIF dependent pathways.¹⁶ Activation of TLRs related transcription factors is associated with up-regulation of several molecules involved in the inflammation including co-stimulatory molecules and pro-inflammatory cytokines.¹⁶

Toll like receptors and *Mycoplasma pneumoniae* infection

Based on the introduction, all TLRs, except TLR5, can target *M. pneumoniae* because the bacterium contains the ligands for the innate immunity receptors. Interestingly, previous investigations have explored the roles played by TLR2 and TLR4 in the pathogenesis of *M. pneumoniae* only.

TLR2 and TLR4 play dual roles in *M. pneumoniae* infection, induction of appropriate immune responses to clear *Mycoplasma pneumoniae* and induction of *M. pneumoniae* complications (hyper-responsiveness).

Roles played by toll like receptors in the induction of appropriate immune response against *Mycoplasma pneumoniae*

Investigations have revealed that TLR2 induces immune responses against *M. pneumoniae* including up-regulation of pro-inflammatory cytokines and short palate lung and nasal epithelium clone 1 (SPLUNC1) which participate in fighting against the bacteria in the human lung.¹⁷ TLR2 dependent up-regulation of SPLUNC1 has also been reported by Chu et al.¹⁸ Another study demonstrated that TLR2 gene transfer to TLR2 knockout (KO) airway epithelium of *M. pneumoniae* infected BALB/c mice via adenoviral vector intranasal inoculation led to significantly reduced *M. pneumoniae* counts.¹⁹ The positive roles played by TLR2 in the clearance of *M. pneumoniae* in the established mouse allergic lungs have also been previously reported.²⁰ Shimizu et al. reported that *M. pneumoniae* derived lipid-associated membrane protein activates TLR-2 homo/heterodimer leading to the activation of NF- κ B to elicit immune responses to *M. pneumoniae*.²¹ The crucial roles of MYD88, as an adaptor protein for TLRs, in the clearance of *M. pneumoniae* has also been documented by Lai et al.²² Fig. 1 illustrates the roles played by TLR2 against *M. pneumoniae*.

Toll-like receptors participate in the induction of *Mycoplasma pneumoniae* complications

Fan et al. reported that TLR2, but not TLR4, expression had positive correlations with severity of wheeze and IgE serum levels in the *M. pneumoniae* infected children.²³ Another investigation by Shao et al. revealed that the expression of both TLR2 and TLR4 on the dendritic cells (DCs) had significant positive correlations with the severities of asthma in the *M. pneumoniae* infected patients.²⁴ An investigation by Steib et al. demonstrated that TLRs activation on the nonparenchymal liver cells by *M. pneumoniae* is a main reason for induction of portal hypertension, especially in patients suffering from mal-liver functions such as liver cirrhosis.²⁵ The positive association between expression levels of TLR4 and acute myocardial infarction in the *M. pneumoniae* infected patients have been reported by Lima-Neto et al.²⁶ As mentioned previously, cytoadherence is a *M. pneumoniae* related complication which is associated with respiratory epithelium damage and immune response hypersensitivities.²⁷ Accordingly, Shimizu et al. demonstrated that *M. pneumoniae* related cytoadherence

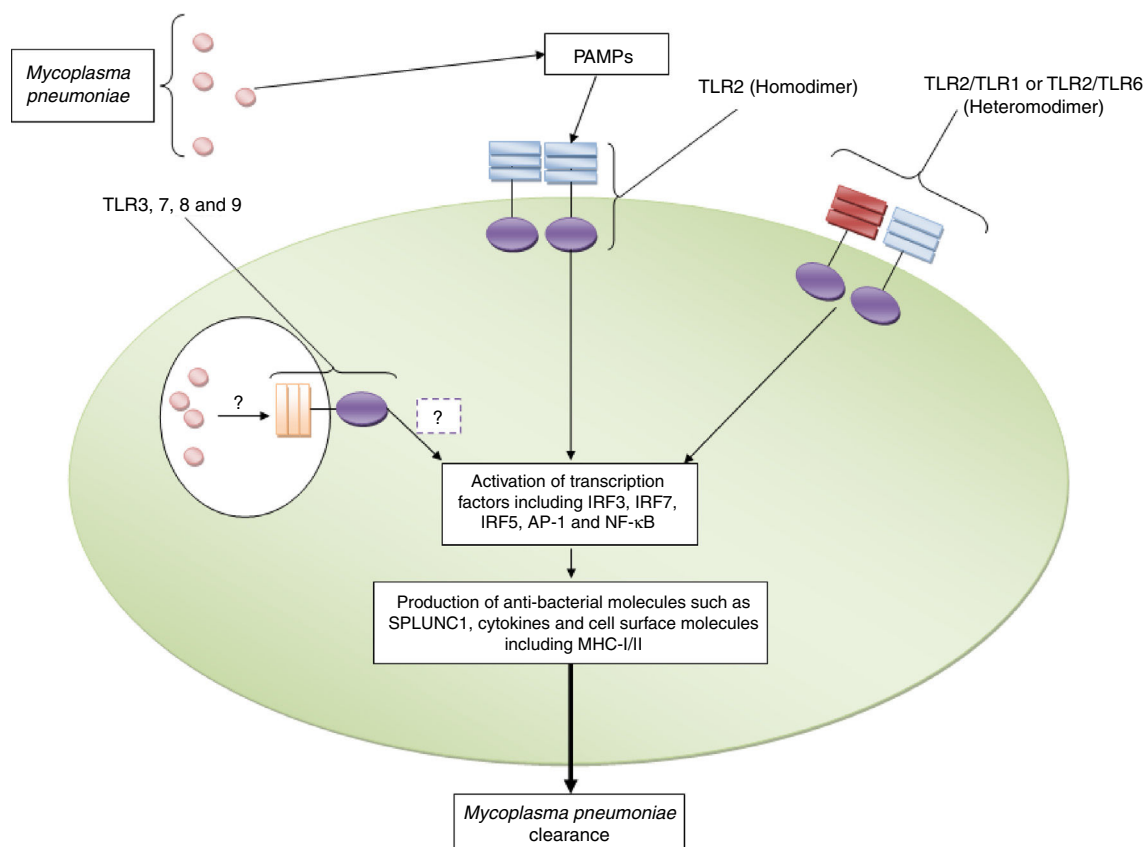


Figure 1 TLR2 plays key roles in the recognition of *Mycoplasma pneumoniae*. The figure illustrates that TLR2 in both homodimeric and heterodimeric forms (with TLR1 and TLR6) recognizes *Mycoplasma pneumoniae* and induces appropriate immune responses to clear the bacterium. The roles played by TLR3, TLR7, TLR8 and TLR9 need to be explored.

is associated with inflammatory responses up-regulation of TLR4.²⁸ However, the investigation was unable to find the roles of TLR2 in the induction of cytoadherence, as TLR2 KO mice reveal a normal cytoadherence complication like wild type (WT) mice.²⁸ Another investigation revealed that *M. pneumoniae* cytopathic effects in the respiratory tracts may be associated with the interaction of *M. pneumoniae* lysate (MPL) with TLR2.²⁹ Accordingly, Lee et al. used *M. pneumoniae* lysate to activate human airway epithelial cells to produce pro-inflammatory molecules such as cytokines.²⁹ Their results demonstrated that *M. pneumoniae* lysate leads to up-regulation of pro-inflammatory molecules by human airway epithelial cells and using anti-TLR2 antibody and siRNA TLR2 leads to decrease expression of IL-8 as a pro-inflammatory cytokines.²⁹ Nevertheless, Chmura et al. were unable to find the relation between expression of TLR2 and induction of IL-8 by *M. pneumoniae*.³⁰ The important roles played by TLR2 in the induction of reactive oxygen species (ROS) and IL-8 by pulmonary epithelial cells have been documented by Choi et al.³¹ Another study showed that TLR2 also induces *M. pneumoniae* related complications via up-regulation of prostaglandins PGD² and PGE² and also harmful pro-inflammatory cytokines such as TNF- α in a human macrophage cell line, RAW264.7 cells.³² Additionally, it has been documented that excess expression of mucins in the lung is the main complication of asthma.³³ Kraft et al.

demonstrated that *M. pneumoniae* induces expression of MUC5AC (the major airway mucin) via interaction with TLR2 in a pathologic manner.³³ Chu et al. also confirmed the critical roles played by TLR2 in the up-regulation of mucin in the lung of *M. pneumoniae* infected patients.³⁴ Nevertheless, the negative roles of TLR2 on the expression of mucin in the *M. pneumoniae* infected asthmatic patients have also reported previously.³⁵ Interestingly, a study by Shimizu et al. revealed that *M. pneumoniae* derived triacylated lipoproteins activate NF- κ B to induce excessive immune responses via interaction with TLR2 homodimer and also TLR1 and 2 heterodimer, but not TLR2 and 6 heterodimer.³⁶ Another investigation by Chu et al. showed that inhaled fluticasone propionate (FP), a member of corticosteroids, leads to decreased inflammation and bronchial hyper-responsiveness via down-regulation of TLR2 in the lung.³⁷ Furthermore, the significant roles played by TLR2 in the induction of cardiac and hepatic damages in the *M. pneumoniae* infected patients have been documented by Fan et al.³⁸

Conclusion

As mentioned previously, TLR2 and TLR4 are the only members that have been investigated regarding their roles in the induction of immune responses against *M. pneumoniae* and

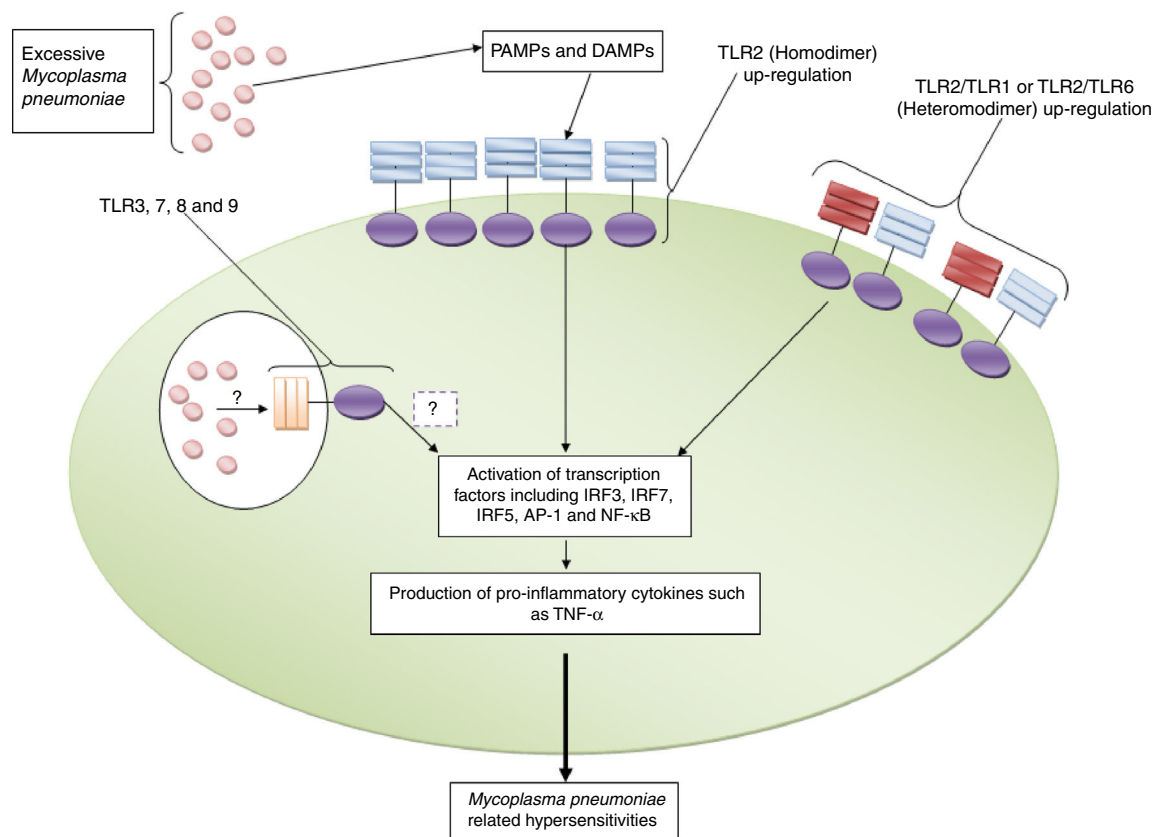


Figure 2 TLR2 and its roles in the induction of *Mycoplasma pneumoniae* related complications. The figure illustrates that excessive expression of TLR2 in both homodimeric and heterodimeric forms (with TLR1 and TLR6) and also high counts of *Mycoplasma pneumoniae* induce pro-inflammatory complications directly and by up-regulation of DAMPs (such as HSP-1) indirectly. The roles played by TLR3, TLR7, TLR8 and TLR9 in the induction of *Mycoplasma pneumoniae*-related complications need to be explored.

also their roles in the pathogenesis of the bacterium. Based on the fact that *M. pneumoniae* is a cell wall free microbe and its lipoproteins, which are anchored in the membrane, are exposed to immune cells, hence, it has been hypothesized that TLR2 is the most important TLR which participates in recognition of *M. pneumoniae* (Fig. 1). Accordingly, the investigations have revealed that TLR2 not only plays key roles in *M. pneumoniae* recognition; it plays significantly in the induction of *M. pneumoniae* related complications, especially hypersensitivities. It appears that expression levels of TLR2 and also counts of *M. pneumoniae* which are exposed to immune cells are the most important reason to determine the outcome of immune responses. Accordingly, excessive expression of TLR2 and its interaction with high numbers of *M. pneumoniae* results in hyper activation of immune cells and consequently induction of *M. pneumoniae* related complications (Fig. 2). Additionally, a study by Gally et al. showed that *M. pneumoniae* induces expression of TLR2 in a heat shock protein-1 (HSP-1) dependent manner.³⁹ Therefore, it may be hypothesized that induction of endogenous DAMPs, such as HSPs, may be a mechanism used to induce hypersensitivities. Moreover, there is another hypothesis regarding the affinity of TLR2 to *M. pneumoniae* lipoproteins. Accordingly, it may be hypothesized that there is a magnitude affinity of TLR2 to *M. pneumoniae* diacylated lipopeptides which leads to induction of hypersensitivities.⁴⁰

As mentioned, excessive expression of TLR2 on the immune cells may be a reason for induction of *M. pneumoniae* related complications. Several mechanisms may induce excessive expression of TLR2 including genetic variations. There is not enough investigation regarding the relation between genetic variations of TLR2 and *M. pneumoniae* complications. Fang et al. reported that the polymorphisms within TLR2 and TLR4 genes of pigs were associated with expression of the molecules in the *M. pneumoniae* infected animal.⁴¹ While, investigation on humans by Lima-Neto et al. showed that TLR4 c.896A> G, TLR4 c.1196> T and TLR2 c.2258G> A were not associated with increased risks of acute myocardial infarction in *M. pneumoniae* infected patients.²⁶ Thus, it seems that more genetic and epigenetic investigation needs to be performed to improve our knowledge regarding the reasons of excessive expression of TLR2 in the patients suffering from *M. pneumoniae*.

Furthermore, due to the fact that intracellular localization is an important mechanism used by *M. pneumoniae* to escape from immune responses,⁴² hence, the intra-vesicular TLRs, including TLR3, TLR7, TLR8 and TLR9, can participate in the recognition of *M. pneumoniae* PAMPs and consequently play important roles in either its eradication or complications (Figs. 1 and 2). Thus, future investigations can be directed to exploring the roles of other TLRs in the immune responses against *M. pneumoniae*.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgment

This review article has been supported by Islamic Azad University, Kerman Branch.

References

- Jacobs E, Ehrhardt I, Dumke R. New insights in the outbreak pattern of *Mycoplasma pneumoniae*. *Int J Med Microbiol*. 2015;305:705–8. Epub 2015/09/01.
- Edward D, Freundt E. Proposal for classifying organisms related to *Mycoplasma laidlawii* in a family Sapromycetaceae, genus Sapromyces, within the Mycoplasmatales. *Microbiology*. 1969;57:391–5.
- Hardy RD, Jafri HS, Olsen K, Hatfield J, Iglehart J, Rogers BB, et al. *Mycoplasma pneumoniae* induces chronic respiratory infection, airway hyperreactivity, and pulmonary inflammation: a murine model of infection-associated chronic reactive airway disease. *Infect Immun*. 2002;70:649–54. Epub 2002/01/18.
- Defilippi A, Silvestri M, Tacchella A, Giacchino R, Melioli G, Di Marco E, et al. Epidemiology and clinical features of *Mycoplasma pneumoniae* infection in children. *Respir Med*. 2008;102:1762–8. Epub 2008/08/16.
- Saraya T, Kurai D, Nakagaki K, Sasaki Y, Niwa S, Tsukagoshi H, et al. Novel aspects on the pathogenesis of *Mycoplasma pneumoniae* pneumonia and therapeutic implications. *Front Microbiol*. 2014;5:410. Epub 2014/08/27.
- Waites KB, Balish MF, Atkinson TP. New insights into the pathogenesis and detection of *Mycoplasma pneumoniae* infections. *Future Microbiol*. 2008;3:635–48.
- Shimizu T. Inflammation-inducing factors of *Mycoplasma pneumoniae*. *Front Microbiol*. 2016;7:414. Epub 2016/04/12.
- Chiba H, Pattanajitvilai S, Mitsuzawa H, Kuroki Y, Evans A, Voelker DR. Pulmonary surfactant proteins A and D recognize lipid ligands on *Mycoplasma pneumoniae* and markedly augment the innate immune response to the organism. *Chest*. 2003;123 3 Suppl.:426S. Epub 2003/03/12.
- Karimi-Googheri M, Arababadi MK. TLR3 plays significant roles against hepatitis B virus. *Mol Biol Rep*. 2014;41:3279–86.
- Zare-Bidaki M, Hakimi H, Abdollahi SH, Zainodini N, Arababadi MK, Kennedy D. TLR4 in toxoplasmosis; friends or foe? *Microb Pathog*. 2014;69–70:28–30.
- Shahrakhyahed A, Sanchooli J, Sanadgol N, Arababadi MK, Kennedy D. TLR9: an important molecule in the fight against hepatitis B virus. *Postgrad Med J*. 2014;90:396–401. Epub 2014/06/20.
- Sepehri Z, Kiani Z, Alavian SM, Arababadi MK, Kennedy D. The link between TLR7 signaling and hepatitis B virus infection. *Life Sci*. 2016;158:63–9.
- Bagheri V, Askari A, Arababadi MK, Kennedy D. Can toll-like receptor (TLR) 2 be considered as a new target for immunotherapy against hepatitis B infection? *Human Immunol*. 2014;75:549–54.
- Sepehri Z, Kiani Z, Nasiri AA, Kohan F. Toll-like receptor 2 and type 2 diabetes. *Cell Mol Biol Lett*. 2016;21:2.
- Marinelli C, Di Liddo R, Facci L, Bertalot T, Conconi MT, Zusso M, et al. Ligand engagement of Toll-like receptors regulates their expression in cortical microglia and astrocytes. *J Neuroinflamm*. 2015;12:244.
- Premkumar V, Dey M, Dorn R, Raskin I. MyD88-dependent and independent pathways of toll-like receptors are engaged in biological activity of triptolide in ligand-stimulated macrophages. *BMC Chem Biol*. 2010;10:3. Epub 2010/04/14.
- Thaikootathil J, Chu HW. MAPK/AP-1 activation mediates TLR2 agonist-induced SPLUNC1 expression in human lung epithelial cells. *Mol Immunol*. 2011;49:415–22. Epub 2011/09/09.
- Chu HW, Gally F, Thaikootathil J, Janssen-Heininger YM, Wu Q, Zhang G, et al. SPLUNC1 regulation in airway epithelial cells: role of toll-like receptor 2 signaling. *Respir Res*. 2010;11:155. Epub 2010/11/09.
- Wu Q, Jiang D, Minor MN, Martin RJ, Chu HW. In vivo function of airway epithelial TLR2 in host defense against bacterial infection. *Am J Physiol Lung Cell Mol Physiol*. 2011;300:L579–86. Epub 2011/01/18.
- Wu Q, Martin RJ, Lafasto S, Efav BJ, Rino JG, Harbeck RJ, et al. Toll-like receptor 2 down-regulation in established mouse allergic lungs contributes to decreased mycoplasma clearance. *Am J Respir Crit Care Med*. 2008;177:720–9. Epub 2008/01/19.
- Shimizu T, Kida Y, Kuwano K. A dipalmitoylated lipoprotein from *Mycoplasma pneumoniae* activates NF- κ B through TLR1, TLR2, and TLR6. *J Immunol*. 2005;175:4641–6.
- Lai JF, Zindl CL, Duffy LB, Atkinson TP, Jung YW, Van Rooijen N, et al. Critical role of macrophages and their activation via MyD88-NF κ B signaling in lung innate immunity to *Mycoplasma pneumoniae*. *PLoS ONE*. 2010;5:e14417.
- Fan Q, Gu T, Li P, Yan P, Chen D, Han B. Roles of T-cell immunoglobulin and mucin domain genes and toll-like receptors in Wheezy children with *Mycoplasma pneumoniae* pneumonia. *Heart Lung Circ*. 2016;25:1226–31. Epub 2016/10/27.
- Shao L, Cong Z, Li X, Zou H, Cao L, Guo Y. Changes in levels of IL-9, IL-17. IFN-gamma, dendritic cell numbers and TLR expression in peripheral blood in asthmatic children with *Mycoplasma pneumoniae* infection. *Int J Clin Exp Pathol*. 2015;8:5263–72. Epub 2015/07/21.
- Steib CJ, Schewe J, Gerbes AL. Infection as a trigger for portal hypertension. *Digest Dis*. 2015;33:570–6.
- Lima-Neto L, Hirata R, Luchessi A, Silbiger V, Cavichioli D, Dos Santos E, et al. Chlamydia pneumoniae and increased TLR4 gene expression in leukocytes are associated with acute myocardial infarction. *J Biol Regulat Homeost Agent*. 2013;28:449–60.
- Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. *Clin Microbiol Rev*. 2004;17:697–728.
- Shimizu T, Kimura Y, Kida Y, Kuwano K, Tachibana M, Hashino M, et al. Cytoadherence of *Mycoplasma pneumoniae* induces inflammatory responses through autophagy and toll-like receptor 4. *Infect Immun*. 2014;82:3076–86.
- Lee KE, Kim KW, Hong JY, Kim KE, Sohn MH. Modulation of IL-8 boosted by *Mycoplasma pneumoniae* lysate in human airway epithelial cells. *J Clin Immunol*. 2013;33:1117–25. Epub 2013/06/20.
- Chmura K, Bai X, Nakamura M, Kandasamy P, McGibney M, Kuronuma K, et al. Induction of IL-8 by *Mycoplasma pneumoniae* membrane in BEAS-2B cells. *Am J Physiol Lung Cell Mol Physiol*. 2008;295:L220–30. Epub 2008/05/20.
- Choi SY, Lim JW, Shimizu T, Kuwano K, Kim JM, Kim H. Reactive oxygen species mediate Jak2/Stat3 activation and IL-8 expression in pulmonary epithelial cells stimulated with lipid-associated membrane proteins from *Mycoplasma pneumoniae*. *Inflamm Res*. 2012;61:493–501. Epub 2012/01/25.
- Kandasamy P, Zarini S, Chan ED, Leslie CC, Murphy RC, Voelker DR. Pulmonary surfactant phosphatidylglycerol inhibits *Mycoplasma pneumoniae*-stimulated eicosanoid production from human and mouse macrophages. *J Biol Chem*. 2011;286:7841–53. Epub 2011/01/06.
- Kraft M, Adler KB, Ingram JL, Crews AL, Atkinson TP, Cairns CB, et al. *Mycoplasma pneumoniae* induces airway epithelial cell expression of MUC5AC in asthma. *Eur Resp J*. 2008;31:43–6. Epub 2008/01/02.

34. Chu HW, Jeyaseelan S, Rino JG, Voelker DR, Wexler RB, Campbell K, et al. TLR2 signaling is critical for *Mycoplasma pneumoniae*-induced airway mucin expression. *J Immunol*. 2005;174:5713–9.
35. Wu Q, Martin RJ, Rino JG, Jeyaseelan S, Breed R, Chu HW. A deficient TLR2 signaling promotes airway mucin production in *Mycoplasma pneumoniae*-infected allergic mice. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L1064–72. Epub 2006/12/30.
36. Shimizu T, Kida Y, Kuwano K. Triacylated lipoproteins derived from *Mycoplasma pneumoniae* activate nuclear factor- κ B through toll-like receptors 1 and 2. *Immunology*. 2007;121:473–83.
37. Chu HW, Campbell JA, Rino JG, Harbeck RJ, Martin RJ. Inhaled fluticasone propionate reduces concentration of *Mycoplasma pneumoniae*, inflammation, and bronchial hyperresponsiveness in lungs of mice. *J Infect Dis*. 2004;189:1119–27. Epub 2004/03/05.
38. Fan Q, Meng J, Li P, Liu Z, Sun Y, Yan P. Pathogenesis and association of *Mycoplasma pneumoniae* infection with cardiac and hepatic damage. *Microbiol Immunol*. 2015;59:375–80. Epub 2015/05/27.
39. Gally F, Minor MN, Smith SK, Case SR, Chu HW. Heat shock factor 1 protects against lung *Mycoplasma pneumoniae* infection in mice. *J Innate Immun*. 2011;4:59–68.
40. Into T, Dohkan J, Inomata M, Nakashima M, Shibata K, Matsushita K. Synthesis and characterization of a dipalmitoylated lipopeptide derived from paralogous lipoproteins of *Mycoplasma pneumoniae*. *Infect Immun*. 2007;75:2253–9. Epub 2007/02/28.
41. Fang X, Liu X, Meng C, Fu Y, Wang X, Li B, et al. Breed-linked polymorphisms of porcine toll-like receptor 2 (TLR2) and TLR4 and the primary investigation on their relationship with prevention against *Mycoplasma pneumoniae* and bacterial LPS challenge. *Immunogenetics*. 2013;65:829–34.
42. Chaudhry R, Ghosh A, Chandolia A. Pathogenesis of *Mycoplasma pneumoniae*: an update. *Indian J Med Microbiol*. 2016;34:7–16. Epub 2016/01/19.