The Yin and the Yang of STAT1 Downstream of TLR4 Endocytosis: STAT1 beyond Interferon Signaling

Hozaiifa Metwally, Tadamitsu Kishimoto*
Laboratory of Immune Regulation, Immunology Frontier Research Center, Osaka University, 565-0871 Osaka, Japan

*Correspondence should be addressed to Tadamitsu Kishimoto; kishimoto@ifrec.osaka-u.ac.jp

Received date: June 16, 2020, Accepted date: July 08, 2020

Abstract

Lipopolysaccharide (LPS)–induced toll-like receptor 4 (TLR4) endocytosis has emerged as a key step for the production of interferon (IFN)-β, which activates the transcription of antiviral response genes through Janus kinase (JAK)/pTyr701 signal transducer and activator 1 (STAT1) signaling. TLR4 endocytosis also promotes proinflammatory cytokines production, at least in part through mediating a late-phase of nuclear factor (NF)-κB activation. However, NF-κB activation alone cannot explain the full spectrum of how TLR4 endosomal signaling conduits the production of proinflammatory cytokines. Our study identified STAT1 as a proinflammatory effector downstream of TLR4 endocytosis independent of IFN-β signaling or NF-κB activity. In human macrophages, TLR4 endocytosis activates noncanonical phosphorylation of STAT1 at Thr749 (pThr749), which subsequently promotes the proinflammatory response rather than the IFN response. pThr749 STAT1 prolongs the half-life of interleukin (IL)-6 mRNA through activating the transcription of AT-rich interactive domain-containing protein 5A (ARID5A), which stabilizes IL-6 mRNA. Furthermore, pThr749 STAT1 promotes a late-phase of the transcription of IL-12. We demonstrated that pThr749 confers STAT1 with distinct gene-regulatory prosperities and facilitates STAT1 binding to a noncanonical DNA motif (5’...TTTTGANN...3’) at the promoter regions of ARID5A and IL-12. Our results indicate that different phosphorylation of STAT1 confers distinct DNA binding and gene regulation downstream of TLR4 endocytosis where pTyr701 promotes the IFN response while pThr749 promotes the proinflammatory response. By unveiling an alternative activation of STAT1, our study adds another piece to the puzzle of how TLR4 endocytosis regulates the production of proinflammatory cytokines, which may help in developing biologics or diagnostics for proinflammatory diseases.

Commentary

Developing defense mechanisms by the host is fundamental to ensure its survival against various microbial pathogens. At the heart of the host defense against microbes is its ability to initiate an immune response to detect and eliminate potential microbial threats. However, in many cases the aberrant immune response is the cause of the host’s clinical symptoms of infections rather than the microbe itself [1]. Therefore, understanding the mechanisms governing the initiation and the regulation of the host’s immune response against various microbial encounters is critical for our understanding of the host-microbe interaction. Over three decades ago, Charles Janeway Jr. proposed a model of pathogen detection describing two characteristics of innate immune receptors: first, the ability to distinguish between self and non-self molecules, and second is the ability to promote adaptive immune response to the non-self microbial products [2]. Toll-like receptors (TLRs) were the first among many other innate immune receptors to fulfill Janeway’s prediction. TLRs were discovered as the human homolog of Drosophila Toll protein and were subsequently identified for their ability to recognize conserved pathogens-associated molecular patterns (PAMPs) followed by driving an innate immune response and adaptive immunity [3]. TLR4 was the first member to be characterized, followed by the identification of bacterial lipopolysaccharide (LPS) as the microbial ligand activating the TLR4 [3-6]. TLRs are type I transmembrane proteins, which share conserved functional domains. The N-terminal extracellular domain consisting of leucine-rich repeats in a horseshoe-like structure for ligand recognition, a single transmembrane domain and an intracellular Toll-interleukin (IL)-1 receptor (TIR) domain for signaling transduction [7-9]. TLRs
can be classified according to their cellular localization into cell surface or intracellular TLRs. Cell surface TLRs are located at the plasma membrane and recognize microbial cell surface molecules such as TLR1, 2 and 6 (bacterial lipoprotein), TLR4 (LPS) and TLR5 (flagellin) [10-15]. On the other hand, intracellular TLRs localize in endosomes and detect nucleic acids such as TLR3 (double-stranded RNA), TLR 7 and 8 (single-stranded RNA) and TLR9 (unmethylated CpG containing single-stranded DNA) [16-22]. This compartmentalization of TLRs is fundamental for the specificity of their ligand recognition and the subsequent engagement of specific adaptor molecules that initiate signaling cascades and culminate in an appropriate immune response [23,24].

This paradigm is clear in the case of TLR4, which exploits different adaptors to induce distinct signaling pathways, thus expanding the repertoire of transcribed genes and potentiating the production of a wide array of immune mediators. Among these immune mediators is IL-6, which is a pleotropic cytokine with diverse effects on immune and non-immune cells, and affects the host homeostasis [25-27]. Thus, coordinated regulatory mechanisms exist to regulate TLR4 driven production of immune mediators, especially IL-6. At the plasma membrane, TLR4 recognizes LPS through a multi-receptor complex of LPS-binding protein (LBP), CD14 and MD2, which triggers TLR4 dimerization [28-33]. Dimerized TLR4 at the plasma membrane interact with a sorting adaptor, TIR Domain Containing Adaptor Protein (TIRAP), which recruits the signaling adaptor protein Myeloid differentiation primary response 88 (Myd88) [34-38]. Myd88 conduits TLR4 surface signaling as a part of a large oligomeric supra-molecular organizing center (SMOC) called Myddosome consisting of oligomers of TLR4, TIRAP, Myd88 and IL-1 receptor-associated kinases (IRAKs) [39-43]. The Myddosome through IκB kinases (IKK) and mitogen-activated protein kinases (MAPK) signaling activates nuclear factor-kappa B (NF-κB) and activator protein 1 (AP-1), respectively, culminating in the transcription of a multitude of proinflammatory cytokines such as tumor necrosis factor (TNF), IL-12 and IL-6 [44-47]. Although activating the transcription of proinflammatory cytokines is a key step for initiating the immune response, non-transcriptional regulation is critical for tailoring the immune response and prevent aberrant production of these cytokines. A clear example of the Myddosome-dependent non-transcriptional regulation is the regulation LPS stimulated IL-6 and IL-12, conceivably because of their important role in driving the proinflammatory response and shaping the adaptive immunity [25,48]. At the resting state, Regnase-1 prevents aberrant production of IL-6 and IL-12, but not TNF, by targeting their mRNA for degradation [49]. Upon LPS stimulation the IKK complex phosphorylates the DSGXXS motif of Regnase-1 resulting in its degradation and subsequently promotes IL-6 and IL-12 mRNA stability and production [50].

The association of activated TLR4 with CD14 promotes its endocytosis, which is clathrin- and dynamin-dependent [51,52]. From endosomes, TLR4 dimers interacts with another sorting adaptor called TRIF-related adaptor molecule (TRAM), which seeds the formation of another SMOC, the Triffosome that initiate TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent signaling. TRIF mediates the activation of IKK-related kinase ε (IKKe) and TANK-binding kinase (TBK1), which phosphorylate and activates the transcriptional regulator interferon regulatory factor 3 (IRF3) and the subsequent expression of genes encoding type I interferons (IFNs) [53]. Binding of type I IFNs to their receptor (IFNAR) on the same cell (autocrine) or adjacent cells (paracrine) activates Janus kinase 1 (JAK1) and tyrosine kinase 2 (Tyk2), which in turn promotes Tyr701 phosphorylation of signal transducer and activator 1 (STAT1), a key step for its transcriptional activity [54]. This phosphorylation event results in the binding of STAT1 with STAT2 and IRF9 to form the heterotrimer called IFN-stimulated gene factor 3 (ISGF3) complex, which binds to IFN-stimulated response element (ISRE) sites and initiates the transcription of multiple IFN-stimulated genes important for antiviral response [55]. Moreover, TRIF-mediated type I IFNs production promotes Caspase-11-dependent NLRP3 inflammasome activation followed by cell death and the release of IL-1β and IL-18 [56]. Although type I IFNs are fundamental for initiating the antiviral immune response, their contribution to host defense against bacterial pathogens is elusive with increasing evidence showing that they are dispensable for the production of proinflammatory cytokines [57-60]. Although TRIF signaling promotes proinflammatory cytokine production through sustaining a late-phase activation of NF-κB [61,62], several observations have challenged this idea and showed that neither TRIF deficiency nor interfering with TLR4 endocytosis affects the kinetics of NF-κB activation [63-65]. Thus, it remains unclear how TLR4 signaling from endosomes promotes the production of proinflammatory cytokines.

In contrast to IFN-pTyr701 STAT1, STAT1 deficiency results in diminished production of IL-6 and enhanced survival in response to bacterial infections [66,67], indicating that the role of STAT1 extends beyond that of its Tyr701 phosphorylation in the context of shaping the proinflammatory response. Our group has identified AT-rich interactive domain-containing protein 5α (Arid5a) as a post-transcriptional stabilizer of IL-6 mRNA through counteracting the Regnase-1 effect [68]. Notably, Arid5a/Regnase-1 regulation extends beyond IL-6 to
other immune modulatory molecules such as OX40 [69]. Deficiency of Arid5a phenocopied that of TRIF and STAT1 in enhancing mice survival in murine endotoxic shock model [70], denoting a potential signaling pathway connecting these molecules. In this regard, we found that TLR4 endocytosis promotes the formation of a noncanonical TBK1/IKKβ kinase complex, which in turn mediates a noncanonical STAT1 phosphorylation at Thr749. Intriguingly, pThr749 STAT1 augments the TRIF-dependent macrophage proinflammatory cytokine production through distinct mechanisms independently of its pTyr701 or the NF-κB activity. Of note, phosphorylation of Thr749 did not affect STAT1 nuclear translocation. Instead, it facilitated STAT1 binding to a noncanonical DNA motif (5’-TTGANNCC-3’) at the promoter regions of ARID5A and IL-12 resulting in augmented production of IL-12 and IL-6 through augmenting their transcription and mRNA stabilization, respectively [71]. Collectively, our study highlights the importance of the spatiotemporal regulation of TLR4 signaling and its impact on mediating differential phosphorylation of STAT1 resulting in altering its DNA binding specificity and transcriptional outcome. Thus, our study provides a potential mechanistic explanation of how TLR4 signaling from endosomes promotes proinflammatory cytokines production independent of NF-κB activation. It requires future research for better understanding of the TLR4 proinflammatory endosomal signaling and to answer whether the balance between pTyr701 and pThr749 STAT1 dictates the fate of the macrophage immune response towards antiviral or proinflammatory, respectively; what is the in vivo biological effects of the pThr749 STAT1 on the host’s immune response; and, what are the molecular mechanisms regulating this phosphorylation.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Funding

This work was supported by the Kishimoto Foundation. H.M. is supported by KAKENHI Research Activity Start-up grant (No. 19K23864).

Acknowledgements

We thank Mari Okawa for her assistance.

References

1. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008 Jul;454(7203):428-35.
2. Janeway CA. Approaching the asymptote? Evolution and revolution in immunology. In Cold Spring Harbor symposia on quantitative biology 1989 Jan 1 (Vol. 54, pp. 1-13). Cold Spring Harbor Laboratory Press.
3. Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997 Jul;388(6640):394-7.
4. Qureshi ST, Lariviè re L, Leveque G, Clermont S, Moore KJ, Gros P, Malo D. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). The Journal of Experimental Medicine. 1999 Feb 15;189(4):615-25.
5. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science. 1998 Dec 11;282(5396):2085-8.
6. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, et al. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. The Journal of Immunology. 1999 Apr 1;162(7):3749-52.
7. Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO. The structural basis of lipopolysaccharide recognition by the TLR4–MD-2 complex. Nature. 2009 Apr;458(7242):1191-5.
8. Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, et al. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell. 2007 Sep 21;130(6):1071-82.
9. Kobe B, Kajava AV. The leucine-rich repeat as a protein recognition motif. Current opinion in structural biology. 2001 Dec 1;11(6):725-32.
10. Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, et al. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. The Journal of Immunology. 2002 Jul 1;169(1):10-4.
11. Takeuchi O, Kawai T, Mühlradt PF, Morr M, RudolfJD, Zychlinsky A, et al. Discrimination of bacterial lipoproteins by Toll-like receptor 6. International immunology. 2001 Jul 1;13(7):933-40.
12. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity. 1999 Oct 1;11(4):443-51.
13. Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, et al. Recognition of lipopeptide patterns by Toll-like
receptor 2-Toll-like receptor 6 heterodimer. Immunity. 2009 Dec 18;31(6):873-84.

14. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Eugene CY, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 2001 Apr;410(6832):1099-103.

15. Gewirtz AT, Navas TA, Lyons S, Godowski PJ, Madara JL. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. The Journal of Immunology. 2001 Aug 15;167(4):1882-5.

16. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A Toll-like receptor recognizes bacterial DNA. Nature. 2000 Dec;408(6813):740-5.

17. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature. 2001 Oct;413(6857):732-8.

18. Diebold SS, Kaisto T, Hemmi H, Akira S, e Sousa CR. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004 Mar 5;303(5663):1529-31.

19. Greulich W, Wagner M, Gaidt MM, Stafford C, Cheng Y, Linder A, et al. TLR8 is a sensor of RNase T2 degradation products. Cell. 2019 Nov 27;179(6):1264-75.

20. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 2004 Mar 5;303(5663):1526-9.

21. Hemmi H, Kaisto T, Takeuchi O, Sato S, Sanjo H, Hoshino K, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88–dependent signaling pathway. Nature Immunology. 2002 Feb;3(2):196-200.

22. Krüger A, Oldenburg M, Chebrulovu C, Beisser D, Kolter J, Sigmund AM, et al. Human TLR 8 senses UR/URR motifs in bacterial and mitochondrial RNA. EMBO reports. 2015 Dec;16(12):1656-63.

23. Kagan JC. Defining the subcellular sites of innate immune signal transduction. Trends in Immunology. 2012 Sep 1;33(9):442-8.

24. Brubaker SW, Bonham KS, Zanoni I, Kagan JC. Innate immune pattern recognition: a cell biological perspective. Annual Review of Immunology. 2015 Mar 21;33:257-90.

25. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harbor perspectives in biology. 2014 Oct 1;6(10):a016295.

26. Kishimoto T. Interleukin-6: from basic science to medicine—40 years in immunology. Annual Review of Immunology. 2005 Apr 23;23:1-21.

27. Kang S, Narazaki M, Metwally H, Kishimoto T. Historical overview of the interleukin-6 family cytokine. Journal of Experimental Medicine. 2020 May 4;217(5).

28. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science. 1990 Sep 21;249(4975):1431-3.

29. Meng J, Gong M, Björkbacka H, Golenbock DT. Genome-wide expression profiling and mutagenesis studies reveal that lipopolysaccharide responsiveness appears to be absolutely dependent on TLR4 and MD-2 expression and is dependent upon intermolecular ionic interactions. The Journal of Immunology. 2011 Oct 1;187(7):3683-93.

30. Nagai Y, Akashi S, Nagafuku M, Ogata M, Iwakura Y, Akira S, et al. Essential role of MD-2 in LPS responsiveness and TLR4 distribution. Nature Immunology. 2002 Jul;3(7):667-72.

31. Gioannini TL, Weiss JP. Regulation of interactions of Gram-negative bacterial endotoxins with mammalian cells. Immunologic Research. 2007 Nov 1;39(1-3):249-60.

32. da Silva Correia J, Soldau K, Christen U, Tobias PS, Ulevitch RJ. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex transfer from CD14 to TLR4 and MD-2. Journal of Biological Chemistry. 2001 Jun 15;276(24):21129-35.

33. Eckert JK, Kim YJ, Kim JI, Gürtler K, Oh DY, Sur S, et al. The crystal structure of lipopolysaccharide binding protein reveals the location of a frequent mutation that impairs innate immunity. Immunity. 2013 Oct 17;39(4):647-60.

34. Bonham KS, Orzalli MH, Hayashi K, Wolf AI, Glanemann C, Weninger W, et al. A promiscuous lipid-binding protein diversifies the subcellular sites of toll-like receptor signal transduction. Cell. 2014 Feb 13;156(4):705-16.

35. Yamamoto M, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisto T, et al. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. Nature. 2002 Nov;420(6913):324-9.

36. Horng T, Barton GM, Flavell RA, Medzhitov R. The adaptor molecule TIRAP provides signalling specificity
for Toll-like receptors. Nature. 2002 Nov;420(6913):329-33.

37. Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jeffieres CA, Mansell AS, Brady G, et al. Mal (MyD88-adaptor-like) is required for Toll-like receptor-4 signal transduction. Nature. 2001 Sep;413(6851):78-83.

38. Horng T, Barton GM, Medzhitov R. TIRAP: an adapter molecule in the Toll signaling pathway. Nature Immunology. 2001 Sep;2(9):835-41.

39. Motshwene PG, Moncrieffe MC, Grossmann JG, Kao C, Ayaluru M, Sandecker AM, et al. An oligomeric signaling platform formed by the Toll-like receptor signal transducers MyD88 and IRAK-4. Journal of Biological Chemistry. 2009 Sep 11;284(37):25404-11.

40. Lin SC, Lo YC, Wu H. Helical assembly in the MyD88–IRAK4–IRAK2 complex in TLR/IL-1R signalling. Nature. 2010 Jun;465(7300):885-90.

41. Motshwene PG, Moncrieffe MC, Grossmann JG, Kao C, Ayaluru M, Sandecker AM, et al. An oligomeric signaling platform formed by the Toll-like receptor signal transducers MyD88 and IRAK-4. Journal of Biological Chemistry. 2009 Sep 11;284(37):25404-11.

42. Lin SC, Lo YC, Wu H. Helical assembly in the MyD88–IRAK4–IRAK2 complex in TLR/IL-1R signalling. Nature. 2010 Jun;465(7300):885-90.

43. Suzuki N, Suzuki S, Duncan GS, Millar DG, Wada T, Mirtsos C, et al. Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. Nature. 2002 Apr;416(6882):750-4.

44. Wang C, Deng L, Hong M, Akkaraju GR, Inoue JI, Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature. 2000 Jul;412(6844):346-51.

45. Emmerich CH, Ordureau A, Strickson S, Arthur JS, Pedrioli PG, Komander D, et al. Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. Proceedings of the National Academy of Sciences. 2013 Sep 17;110(38):15247-52.

46. Deng L, Wang C, Spencer E, Yang L, Braun A, You J, et al. Activation of the IκB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell. 2000 Oct 13;103(2):351-61.

47. Muzio M, Ni J, Feng P, Dixit VM. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. Science. 1997 Nov 28;278(5343):1612-5.

48. Vignali DA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. Nature Immunology. 2012 Aug;13(8):722.

49. Matsushita K, Takeuchi O, Standley DM, Kumagai Y, Kawagoe T, Miyake T, et al. Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay. Nature. 2009 Apr;458(7242):1185-90.

50. Iwasaki H, Takeuchi O, Teraguchi S, Matsushita K, Uehata T, Kuniyoshi K, et al. The IκB kinase complex regulates the stability of cytokine-encoding mRNA induced by TLR–IL-1R by controlling degradation of regnase-1. Nature immunology. 2011 Dec;12(12):1167-75.

51. Zanoni I, Ostuni R, Marek LR, Barresi S, Barbalat R, Barton GM, et al. CD14 controls endocytosis of Toll-like receptor 4. Cell. 2011 Nov 11;147(4):868-80.

52. Husebye H, Halaas O, Stenmark H, Tunheim G, Sandanger Ø, Bogen B, et al. Endocytic pathways regulate Toll-like receptor 4 signaling and link innate and adaptive immunity. The EMBO Journal. 2006 Feb 22;25(4):683-92.

53. Fitzgerald KA, Kagan JC. Toll-like receptors and the control of immunity. Cell. 2020 Mar 11.

54. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. Nature reviews Immunology. 2014 Jan;14(1):36-49.

55. Wiesauer I, Gaumannmüller C, Steinparzer I, Strobl B, Kovarik P. Promoter occupancy of STAT1 in interferon responses is regulated by processive transcription. Molecular and cellular biology. 2015 Feb 15;35(4):716-27.

56. Rathinam VA, Vanaja SK, Waggoner L, Sokolovska A, Becker C, Stuart LM, et al. TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by gram-negative bacteria. Cell. 2012 Aug 3;150(3):666-19.

57. McNab F, Mayer-Barber K, Sher A, Wack A, O’garra A. Type I interferons in infectious disease. Nature Reviews Immunology. 2015 Feb;15(2):87-103.

58. Robinson N, McComb S, Mulligan R, Dudani R, Krishnan L, Sad S. Type I interferon induces necroptosis in macrophages during infection with Salmonella enterica serovar Typhimurium. Nature Immunology. 2012 Oct;13(10):954.

59. Perkins DJ, Rajaiah R, Tennant SM, Ramachandran G, Higginson EE, Dyson TN, et al. Salmonella typhimurium co-opts the host type I IFN system to restrict macrophage innate immune transcriptional responses selectively. The Journal of Immunology. 2015 Sep 1;195(5):2461-71.
60. Karaghiosoff M, Steinborn R, Kvarick P, Kriegshäuser G, Baccarini M, Donabauer B, et al. Central role for type I interferons and Tyk2 in lipopolysaccharide-induced endotoxin shock. Nature Immunology. 2003 May;4(5):471-7.

61. Najjar M, Saleh D, Zelic M, Nogusa S, Shah S, Tai A, et al. RIPK1 and RIPK3 kinases promote cell-death-independent inflammation by Toll-like receptor 4. Immunity. 2016 Jul 19;45(1):46-59.

62. Meylan E, Burns K, Hofmann K, Blancheteau V, Martinon F, Kelliher M, et al. RIP1 is an essential mediator of Toll-like receptor 3-induced NF-κB activation. Nature Immunology. 2004 May;5(5):503-7.

63. Tatematsu M, Yoshida R, Morioka Y, Ishii N, Funami K, Watanabe A, et al. Raftlin controls lipopolysaccharide-induced TLR4 internalization and TICAM-1 signaling in a cell type-specific manner. The Journal of Immunology. 2016 May 1;196(9):3865-76.

64. Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science. 2003 Aug 1;301(5633):640-3.

65. Schappe MS, Szteyn K, Stremska ME, Mendu SK, Downs TK, Seegren PV, et al. Chanzyme TRPM7 mediates the Ca2+ influx essential for lipopolysaccharide-induced Toll-like receptor 4 endocytosis and macrophage activation. Immunity. 2018 Jan 16;48(1):59-74.

66. Herzig D, Fang G, Toliver-Kinsky TE, Guo Y, Bohannon J, Sherwood ER. STAT1-deficient Mice are Resistant to CLP-induced Septic Shock. Shock (Augusta, Ga.). 2012 Oct;38(4):395.

67. Ohmori Y, Hamilton TA. Requirement for STAT1 in LPS-induced gene expression in macrophages. Journal of Leukocyte Biology. 2001 Apr;69(4):598-604.

68. Masuda K, Ripley B, Nishimura R, Mino T, Takeuchi O, Shioi G, et al. Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo. Proceedings of the National Academy of Sciences. 2013 Jun 4;110(23):9409-14.

69. Hanieh H, Masuda K, Metwally H, Chalise JP, Mohamed M, Nyati KK, et al. Arid5a controls IL-6 mRNA stability in murine CD4+ T cells by recognizing a stem-loop structure in its 3’ UTR. European Journal of Immunology. 2018 Apr;48(4):593-604.

70. Zaman MM, Masuda K, Nyati KK, Dubey PK, Ripley B, Wang K, et al. Arid5a exacerbates IFN-γ-mediated septic shock by stabilizing T-bet mRNA. Proceedings of the National Academy of Sciences. 2016 Oct 11;113(41):11543-8.

71. Metwally H, Tanaka T, Li S, Parajuli G, Kang S, Hanieh H, et al. Noncanonical STAT1 phosphorylation expands its transcriptional activity into promoting LPS-induced IL-6 and IL-12p40 production. Science Signaling. 2020 Mar 24;13(624).