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REVIEW

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Role of Toll-like receptors in the pathogenesis of COVID-19

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Abstract

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a pandemic since March 2020. The exact pathogenesis of SARS-CoV-2 and the role of each component of the innate and adaptive immune system is still unknown. However, available data from other coronavirus families, such as SARS-CoV and the Middle East respiratory syndrome and also new findings could be useful for a better understanding of SARS-CoV-2. Toll-like receptors (TLR) play an important role in recognition of viral particles and activation of the innate immune system. Activation of TLR pathways leads to secretion of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α, as well as type 1 interferon. Different TLRs, like TLR2, TLR3, TLR4, TLR6, TLR7, TLR8, and TLR9 are potentially important in COVID-19 infection. It is also worth mentioning that we should bear in mind both the beneficial and harmful effects of TLR in confronting COVID-19 infection. TLRs could be a potential target in controlling the infection in the early stages of disease and production of vaccine against SARS-CoV-2.

KEYWORDS
COVID-19, cytokines, SARS-CoV-2, Toll-like receptors

1 | INTRODUCTION

Toll-like receptors (TLRs) belong to the family of innate immune receptors, which play an important role in the activation of innate immunity, regulation of cytokine expression, indirect activation of the adaptive immune system, and the recognition of pathogen-associated molecular patterns (PAMPs).[1–3] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as novel coronavirus disease 2019 (COVID-19), has appeared in Wuhan, China, spread rapidly and led to a pandemic in 2020.[4–6] The innate immune system protects the body against pathogens. Interestingly, new studies on mesenchymal stem cells (MSCs) have revealed that MSCs can promote an anti-inflammatory condition in affected tissues by increasing anti-inflammatory cytokines and M2 macrophages (anti-inflammatory phenotype) in COVID-19 patients, especially in the severe form of the disease.[7] TLRs' pathways, as a component of innate immunity, could be involved in the pathogenesis of SARS-CoV-2 because several studies have shown that TLRs play an important role in the pathogenesis of SARS-CoV and the Middle East respiratory syndrome (MERS).[1] The aim of this article is to discuss the possible role of different TLRs in COVID-19.

2 | TOLL-LIKE RECEPTORS

TLRs have ten family members in humans. Some of the TLRs are located in the cell membrane, and the others are situated in endosomes, such as TLR3, TLR7, TLR8, and TLR9.[8] TLRs are expressed on different immune cells, such as dendritic cells (DCs), macrophages, natural killer cells, and cells of the adaptive immunity – T cells and B cells.[9] TLR3 recognizes double-strand RNA (ds RNA), TLR4 recognizes lipopolysaccharide (LPS), TLR7/8 recognizes single-strand RNA (ssRNA), and TLR9 recognizes unmethylated CpG DNA.[8] Myeloid differentiation primary response 88 (MyD88) and TIR-domain containing adaptor, inducing interferon-β (IFN-β) (TRIF, also known as TICAM1) are two main pathways for the transduction

of TLR's signals. TRAF and IRAK proteins in signaling pathways cause activation of nuclear factor-kB (NF-kB) and Interferon regulatory factor (IRF), which in turn leads to the production of type 1 IFN and pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), and IL-12. Besides this, TLRs indirectly play role in the adaptive immune system by monitoring the expression of costimulatory molecules (Figure 1).

3 | TLRs AND VIRUSES

Many viruses activate the innate immune system with TLRs, which contributes to the elimination of viruses, although it can also harm the host due to persistent inflammation and tissue destruction. For instance, severity of COVID-19 is associated with production of IL-6 in patients which could be produced by induction of TLR pathways. Activation of TLRs by SARS-CoV-2 leads to activation of inflammasome and production of IL-1β, which induces IL-6. Excessive activation of the inflammasome is associated with poor outcome in COVID-19 patients.[10] In addition, activation of Janus kinase transducers (JAK/STAT), which is induced by TLRs, could lead to macrophage activation syndrome. Therefore, TLRs have a dual role in viral infections.[8,11–15] Besides this, TLRs also contribute to the activation of the adaptive immune system via the upregulation of major histocompatibility complex on dendritic cells.[16]

4 | TLRs IN SARS-CoV AND MERS-CoV

Unfortunately, the exact pathway of the COVID-19 pathogenesis is still unknown. Therefore, most of the information regarding the COVID-19 pathogenesis arises from the available data about SARS-CoV and MERS-CoV.[9] Results of studies suggest that type 1 IFN plays an important role in SARS-CoV and MERS-CoV. They interfere with signaling pathways of the host cell and decrease the expression of IFN receptors, which in turn lead to a systemic inflammatory response.[9] As the production of type 1 IFN is mediated by TLRs, they could play a vital role in the pathogenesis of CoVs. Moreover, a cytokine storm exists in MERS-CoV, SARS-CoV, and SARS-CoV-2 that plays a crucial role in deterioration of these infections.[17] Several studies have shown that TLR3 via the TIRF pathway leads to a protective response in SARS-CoV and MERS-CoV infections. Although TLR3 in mouse models leads to activation of IRF3 and NF-kB pathways and production of type 1 IFN and pro-inflammatory cytokines, in knock-out mice for TLR3, no reduction in the secretion of these cytokines is seen in coronavirus infection. Therefore, other pathways are also involved in the production of pro-inflammatory cytokines and type 1 IFN.[1] Induction of the TLR3 pathway, but not TLR2/4/7, in murine coronavirus infection in mouse models, stimulates the production of IFN-β in macrophages and hinders infection.[18] Besides this, MERS-CoV 4a protein can bind to dsRNA and suppress the production of type 1 IFN.[19] TLR4 also activates the same pathway. Although TLR4 plays a vital role in bacterial infections, it can be activated by oxidized phospholipids, which also appears in viral lung

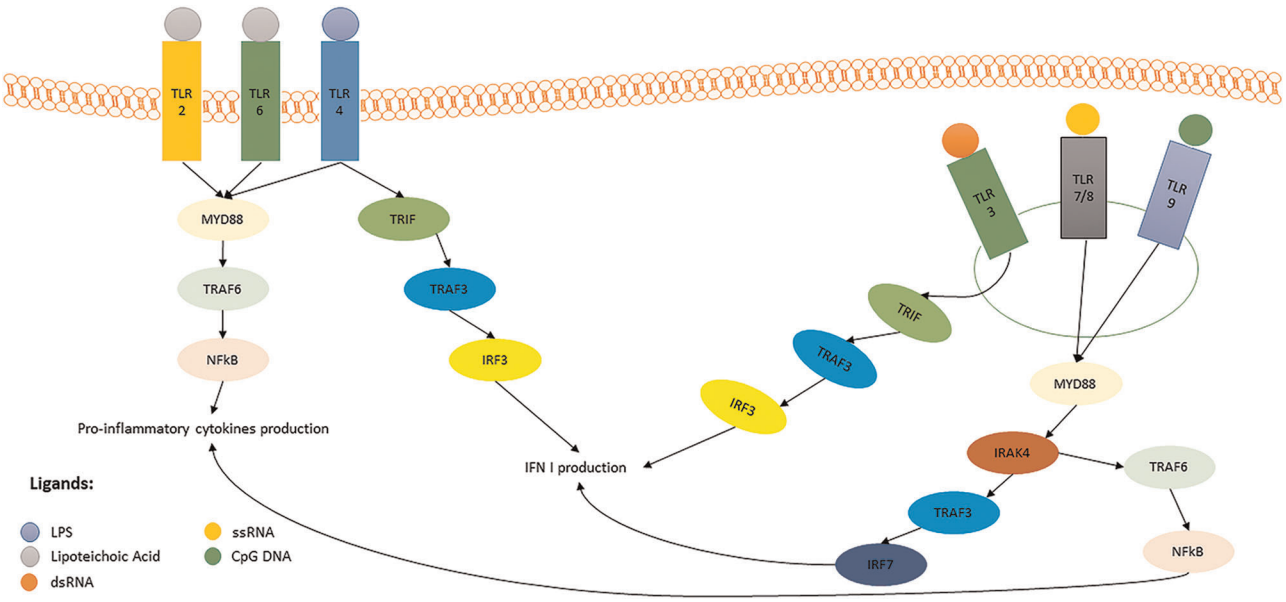

FIGURE 1 Toll-like receptor signaling pathways. TLR2/6 and 4 localize in the cell membrane, and TLR3, TLR7/8, and 9 localize in the endosome surface. Activation of two major adaptive downstream proteins, MYS88 and TRIF, leads to production of pro-inflammatory cytokines and IFN I. Activation of TRIF induces activation of TRAF3, which activates IRF3 and leads to production of IFN I. MYD88 induces activation of TRAF6 in TLR2/6 as well as TLR4 pathway but leads to activation of IRAK4 and indirectly TRAF6 and TRAF3 in TLR7/9 and TLR9 pathway. Activation of NF-kB signaling through TRAF6 increases production of pro-inflammatory cytokines and activation of IRF7 leads to production of IFN I. dsRNA, double strand RNA; IFN I, interferon type 1; LPS, lipopolysaccharides; MYD88, myeloid differentiation primary response 88; NF-kB, nuclear factor k-light-chain-enhancer; ssRNA, single strand RNA; TRAF, tumor necrosis factor receptor-associated factor; TRIF, TIR-domain-containing adapter-inducing interferon-β

infections.[1] In addition, neutrophil extracellular traps (NETs) are activated by the TLR4 signaling pathway. Studies have shown that NET formation in COVID-19 patients could lead to sustained inflammation which could deteriorate the condition of patients. Moreover, the higher production of TLR4 in men is due to a higher testosterone level; this fact can explain the higher level of pro-inflammatory cytokines in men compared to women.[20] Interestingly, neutrophil myeloperoxidase, which produces oxidized phospholipids, has a higher level in COVID-19 patients and also TLR4 pathway activation in the pulmonary phase of infection causes oxidative injury. Therefore, TLR4 could also be involved in the pathogenesis of COVID-19.[21,22] An in silico study has also revealed a close relationship between bat SARS-CoV and SARS-CoV-2 (based on the spike protein and ACE2) and found that SARS-CoV-2 spike protein binds with TLR1, 4, and 6, with a higher affinity for TLR4. TLR4 antagonist could be administered for treating COVID-19 patients.[23] According to another study, TLR8 is seen in the lung, and TLR7 and TLR8 could lead to a cytokine storm in SARS-CoV-1 and cause different side effects.[24]

5 | TLRS IN SARS-COV-2

SARS-CoV-2 has ssRNA^{+} like other CoVs. Spike S glycoproteins on the SARS-CoV-2 envelope bind to angiotensin-converting enzyme 2 (ACE2) and the virus enters inside the cell by receptor-mediated endocytosis.[9] The results of several studies have shown that most of the patients suffer from lymphopenia and have elevated serum levels of pro-inflammatory cytokines, such as TNF-α, IL-6, and interleukin-2 receptor (IL-2R). It is also worth mentioning that higher serum levels of TNF-α, IL-1, IL-6, and IL-8, a higher number of neutrophils, as well as the decrease of lymphocytes are associated with the severity of the disease.[9] Besides this, IL6$^{-/-}$ mice are characterized by less lung damage in viral lung infections.[1] Based on the studies on other CoVs and COVID-19, TLRs could play a crucial role in COVID-19 disease. Conti et al.[25] suggested that activation of the TLRs in COVID-19 infection could lead to the production of pro-inflammatory cytokines, such as IL-1β. Moreover, immunopathological consequences causing death in COVID-19 patients are due to the interaction of TLRs with virus particles.[26] A study by Totura et al. revealed that both TRIF-driven and MyD88-driven pathways, induced by TLRs, provide the most effective antiviral defense against SARS-CoV and lethal SARS-CoV disease is seen in removal of TLR signaling pathways. For instance, TLR3$^{-/-}$, TLR4$^{-/-}$ mice are more susceptible to SARS-CoV without an increase in mortality, but TLR3/TLR4 adaptor TRIF deficient mice are highly susceptible to SARS-CoV with high risk of mortality. Also, irregular signaling pathways and pro-inflammatory cytokine production following infection of TRIF$^{-/-}$ mice, were similar to patients with severe outcomes in SARS-CoV or MERS-CoV infection.[27] However, other studies on SARS-CoV-2 have revealed the pathologic role of TLR4 in excessive inflammation in COVID-19 patients as it leads to NETosis and activation of inflammasome, as mentioned before.[20,22,28] TLR agonists could also be used as prophylactic drugs for SARS-CoV-2. Proud et al.[29] revealed that prophylactic administration of TLR2/6 agonist reduces SARS-CoV-2 transmission and provides protection against COVID-19. Stimulation of TLR2 leads to activation of the innate immune response, suppression of excessive inflammation and tissue damage, as well as promotion of the integrity of local epithelial barrier function.

6 | TLR7/8 IN COVID-19

TLR7/8 are tandem duplicated genes on the X-chromosome, which are located in the endosome membrane and recognize ssRNA and synthetic oligoribonucleotides, such as imidazoquinoline, imiquimod, and R-848. Therefore, they could be involved in the recognition of the SARS-CoV-2 genome.[24] Binding of the superficial S glycoprotein on the envelope of the virus to ACE2 could be sensed by TLR7. TLR7 is expressed on monocyte-macrophages and DC and its activation leads to the production of IL-1, IL-6, monocyte chemoattractant protein-1, MIP-1A, TNF-α, and type 1 IFN.[30] Also, a gender-dependent response could be seen in ssRNA viruses such as SARS-CoV-2 as the TLR7/8 gene is on the X chromosome. Higher expression of TLR7 could lead to a better prognosis in ssRNA viral infections, inducing a higher immune response. Interestingly, the level of IL-6 after viral infection is lower in women compared with men.[24,31] Moreover, van der Made et al. found loss of function variants of X-chromosomal TLR7 in 4 male patients with severe COVID-19 infection that leads to impairment of type 1 and 2 IFN response. Whole-genome sequencing of SARS-CoV, MERS-CoV, and SARS-CoV-2 revealed that TLR7 could be more involved in the pathogenesis of SARS-CoV-2 in comparison to SARS-CoV and MERS-CoV as it has more ssRNA motifs that could bind to TLR7.[32] Agonists of TLRs could lead to a strong immune response in COVID-19.[16] For instance, an innate immunity stimulator, Imiquimod, could be an option to control COVID-19. Imiquimod binds to TLR7/8 and induces the production of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-2, IL-6, IL-8, IL-12, as well as IFN-α. However, it should be noticed that Imiquimod in the late stage of the infection could lead to cytokine storm and side effects of consistent inflammation. Therefore, Imiquimod is appropriate in the early stages of COVID-19 infection.[9] It should be mentioned that another candidate for triggering NET formation in COVID-19 patients is TLR7 and activation of TLR7/8 can induce a strong pro-inflammatory response in patients, leading to acute lung injury. Therefore, it may have a dual role in progression of the disease.[17,33] Another clinical trial in China (ChiCTR2000029776) evaluates the therapeutic effects of the induction of TLR pathways in COVID-19 patients. Moreover, the TLR2/6/9 agonist is also administered to activate innate immune cells and lung epithelial cells to produce various factors against infection. Interestingly, several clinical trials evaluate the effect of anti-inflammatory factors to reduce death caused by persistent inflammation of the lung in COVID-19 patients. For instance, CD24Fc conjugate is used to block TLR activation.[16] Also, using TLR4 antagonists such as glycyrrhetinic acid has anti-inflammatory effects in the lung of mice with acute respiratory distress syndrome, and protects the tissue from

destruction due to inflammation. It may also stimulate an anti-inflammatory activity downstream of the less active ACE2. Therefore, it could be a potential approach to control COVID-19.[34] Ramaiah suggested that mTOR inhibition and p53 activation could have therapeutic effects in COVID-19 patients. mTOR inhibitors play their role in the association of MyD88, IRF7, and the TLR9 pathways.[35]

7 | CONCLUSION

Hence, it could conceivably be hypothesized that TLRs have both harmful and beneficial effects in COVID-19 infection. Using the available data regarding SARS-CoV and MERS could be useful for a better understanding of the exact role of each component of innate and adaptive immunity in COVID-19 infection. Although only TLR7/8 recognizes ssRNA, the genetic material of COVID-19, other TLRs, such as TLR3, TLR4, and TLR6, could also be involved in COVID-19 infection. Both antagonists and agonists of TLRs, based on the type of the TLR, should be examined to determine the therapeutic and harmful effects in COVID-19 infection. The stage of the disease is also important in the type of the targeting (agonist/antagonist) TLRs. Not only TLRs but also related pathways should be studied as related pathways have shown an association with mortality and susceptibility to the virus in other coronavirus families. Suppression of excessive activation of the inflammasome and NET formation could also be considered as a therapeutic goal. Several trials are being conducted regarding TLRs' pathways in COVID-19[26] that could lead to finding a new drug or vaccine for the infection. Bioinformatics studies could also help better understanding of interactions of TLRs with proteins and RNA of COVID-19.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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