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Review

Differential Expression Patterns of Toll-like Receptors in COVID-19 Patients

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Abstract

Since Toll-like receptors (TLRs) recognize the earliest signs of infection or cell damage, they play fundamental roles in innate immunity. This review summarizes the numerous studies on the expression of TLRs in patients with Coronavirus disease 2019 (COVID-19). We show that infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can stimulate at least six of the ten TLRs in humans and that this can shape the severity of COVID-19. Specifically, TLR2, TLR4, and TLR9 appear to play pathogenic roles while TLR3, TLR7, and TLR8 may be protective. Most have mutations that could partly explain the susceptibility phenotypes of COVID-19. Further understanding the roles of TLRs in COVID-19 immunopathogenesis could reveal prognostic biomarkers and help drive the development of novel and effective therapeutics for COVID-19.

Keywords: TLR; COVID-19; SARS-CoV-2; biomarkers

1. Introduction

Toll-like receptors (TLRs) are key regulators of the innate immune system. They belong to the pathogen-recognition receptor (PRR) family and sense host infection by a variety of pathogens by recognizing structurally-conserved molecules called pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [1]. PAMPs include genomic materials such as viral RNA and microbial membrane components while DAMPs include host-derived nucleic acids from damaged cells and products from stressed cells. In humans, there are ten known TLRs (designated TLR1 to TLR10). TLRs are expressed by immune cells and in some cases by non-immune cells such as epithelial cells. As shown by Fig. 1, they are localized on the cell surface or in intracellular compartments such as the endoplasmic reticulum, endosome, lysosome, or endolysosome. In one case (TLR4), the TLR localizes to both the cell surface and the endosome [2]. When TLRs on or in the cell bind to a PAMP or DAMP, they are activated: their cytosolic domains dimerize and are recognized by various adaptor proteins, including myeloid differentiation primary response-88 (MyD88), MyD88-like adaptor protein (MAL; also known as TIR domain-containing adaptor protein [TIRAP]), TIR-domain-containing adaptor-inducing interferon-β (TRIF), and TRIF-related adaptor molecule (TRAM), tumor necrosis factor-receptor-associated factor (TRAF)-6 (TRAF6), and TRAF3 (Table 1, Ref. [3–15]). These adaptor proteins in turn induce downstream signaling. The adaptor protein(s) used and the signaling outcomes vary depending on the TLR and the TLR-bearing cell, as follows. In innate immune cells, TLRs induce the expression of pro-inflammatory cytokines and/or type-I interferons (IFNs). In the case of all TLRs except TLR3 and endosomally-located TLR4 (discussed further below), MyD88 binds to the dimerized TLR cytoplasmic domain; in the case of TLR2 and cell surface-TLR4, this interaction requires MAL/TIRAP. MyD88 then complexes with members of the interleukin (IL)-1 receptor-associated kinase (IRAK) family, which causes TRAF6 to bind. This activates TRAF6 and induces it to activate transforming growth factor beta-activated kinase 1 (TAK1) with its binding partners TAK1-binding proteins (TAB2 and TAB3). TAK1 then activates mitogen-activated protein kinase (MAPK) family members, including p38 and c-Jun N-terminal Kinase (JNK), culminating in the activation of several transcription factors, namely, cAMP response element-binding protein (CREB) and activator protein-1 (AP-1) [16,17]. TAK1 also stimulates the inhibitor of NF-κB kinase (IKK) complex, which phosphorylates the NF-κB inhibitory protein IκBα, marking it for proteasome degradation and leading to NF-κB activation. This entire cascade ultimately results in pro-inflammatory cytokine secretion. MyD88 also participates in a separate pathway that elicits type-I IFNs: it interacts with TRAF3 and forms a complex containing TRAF3, TRAF6, IKKα, and other kinases that phosphorylates the transcription factor interferon regulatory factor (IRF)7; this causes IRF7 to translocate into the nucleus and induce type-I IFN expression. TLR3 and endosomally-located TLR4 undergo somewhat different cascades when

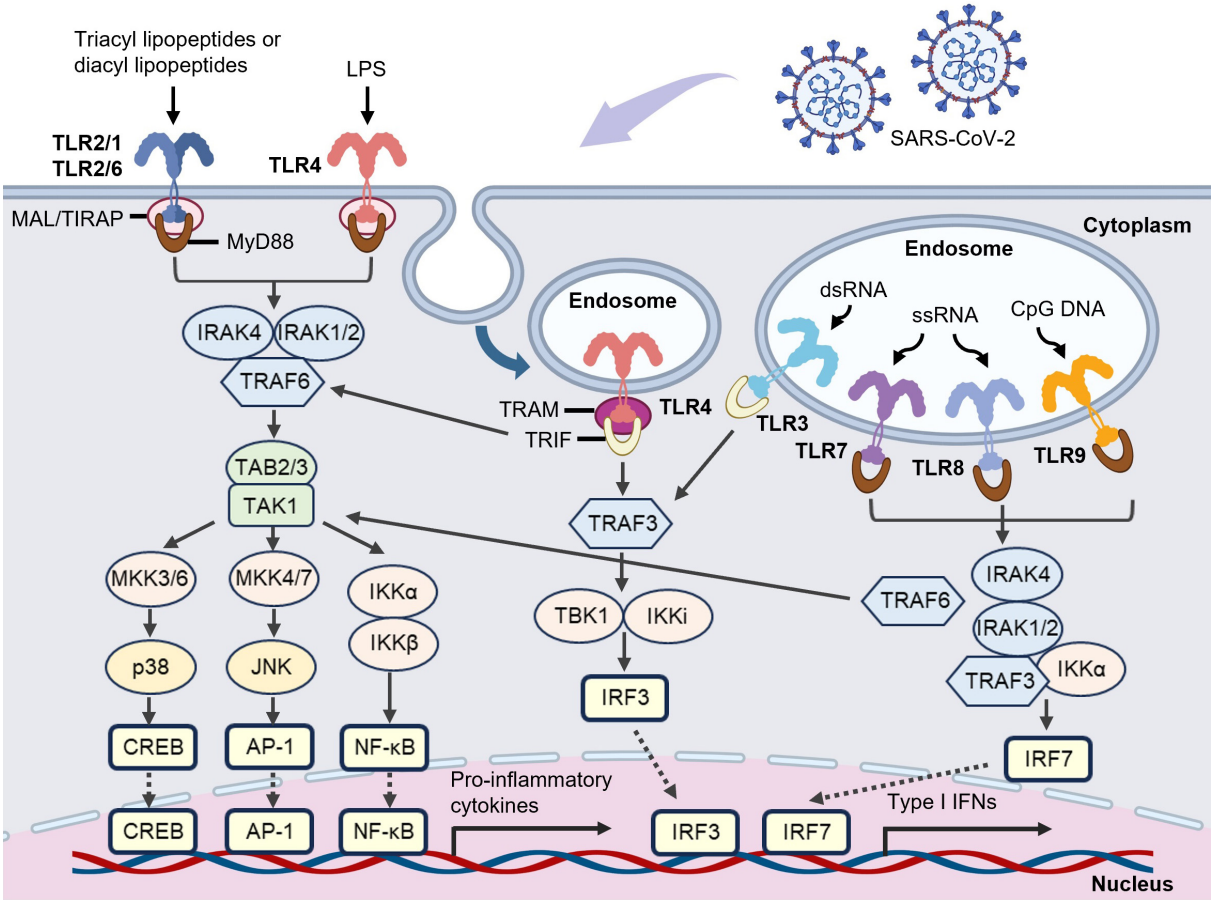

Fig. 1. TLR signaling pathways that are activated by SARS-CoV-2 infection. Six human TLRs demonstrate altered expression in COVID-19 patients. They function as homodimers (TLR3, TLR4, TLR7, TLR8, and TLR9) or heterodimers (TLR2/1 and TLR2/6) and are located on the cell surface (TLR2 and TLR4) or within intracellular compartments (TLR3, TLR4, TLR7, TLR8, and TLR9). The six TLRs are activated by PAMPs or DAMPs produced by SARS-CoV-2 infection. Their cytosolic domains then dimerize, which induces the binding of adaptor proteins, including MyD88 (brown horseshoe shape), MAL/TIRAP (light-pink oval shape), TRIF (yellow horseshoe shape), and TRAM (purple oval shape). These proteins in turn initiate downstream signaling pathways which include interactions between IRAK family and TRAFs. TRAF6 activates TAK1 with its adaptor proteins, TAB2 and TAB3. Activation of MAPK family members, such as p38 and JNK, are followed. TAK1 also stimulates the IKK complex and leads to NF-κB activation. TRAF3 causes IRFs to translocate into the nucleus by forming a complex containing TRAF6, IKKα, and other kinases, or by recruiting TBK1 and IKKi. These downstream signaling pathways result in the secretion of pro-inflammatory cytokines and/or type-I IFNs. dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; IFN, interferon; LPS, Lipopolysaccharide; MyD88, Myeloid differentiation factor 88; MAL, MyD88-adaptor-like protein; TIRAP, TIR-domain containing adaptor protein; TRAM, TRIF-related adapter molecule; TRIF, TIR-domain-containing adapter-inducing interferon-β; IRAK, Interleukin (IL)-1 receptor-associated kinase; TRAF, TNF receptor-associated factor; TAK1, Transforming growth factor beta-activated kinase 1; TAB, TAK1-binding protein; MAPK, mitogen-activated protein kinase; TBK1, Tank-binding kinase 1; IKK, Inhibitor of NF-κB kinase; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; COVID-19, Coronavirus disease 2019; TLR, Toll-like receptor; PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; JNK, c-Jun N-terminal Kinase; IRF, interferon regulatory factor; AP, activator protein; CREB, cAMP response element-binding protein; MKK, mitogen-activated protein kinase kinase. Created with https://www.biorender.com.

they are dimerized. Endosomally-located TLR4 is directly recognized by first TRAM and then TRIF, while TLR3 is recognized by TRIF alone. TRIF in both cases then engages with both TRAF3 and TRAF6, which recruit Tank-binding kinase-1 (TBK1) and IKKi. This induces IRF3 to translocate into the nucleus, where it drives type-I IFN expression [16]. TLRs not only induce cytokine production in innate immune cells, they also activate adaptive immune cells by stimulating dendritic cell (DC) maturation. Moreover, adaptive immune cells can express TLRs and can therefore be activated directly [1,18]. This TLR-signaling system is finely regulated but can be dysregulated and thereby induce disease.

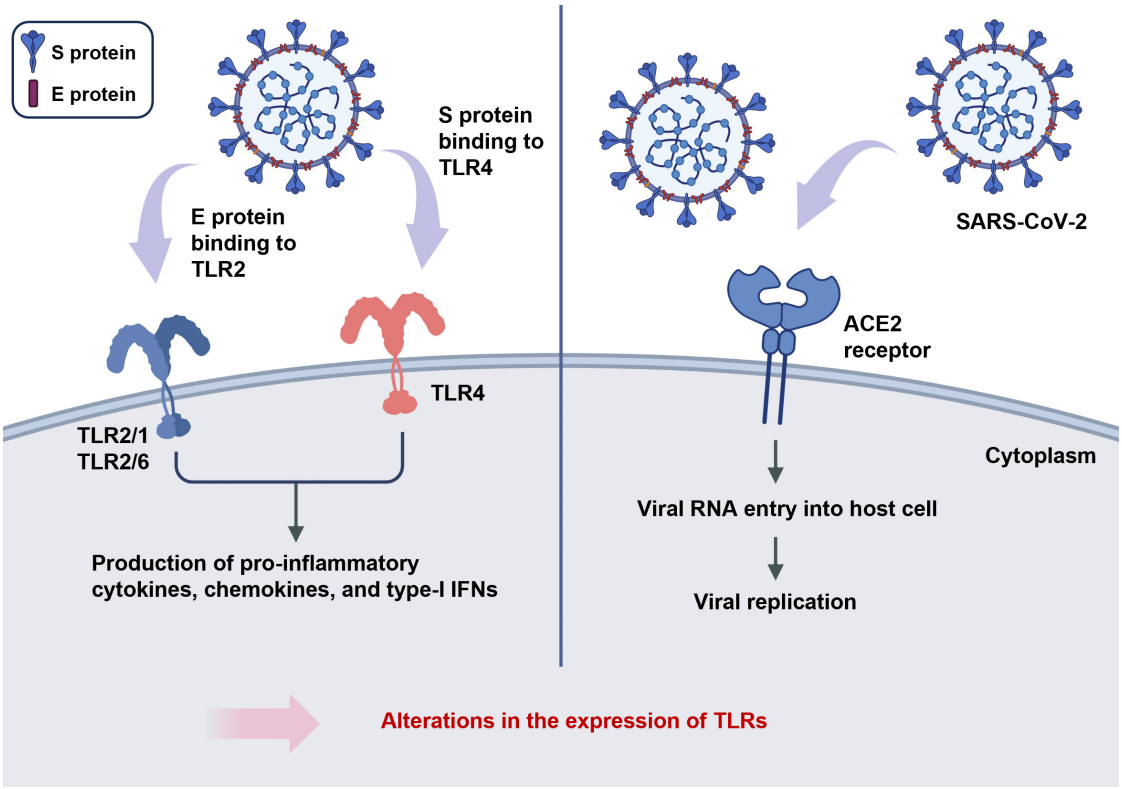

Fig. 2. Schematic depiction of the interactions of SARS-CoV-2 with TLRs and the ACE2 receptor. SARS-CoV-2 enters the host cell by binding to the angiotensin-converting enzyme-2 (ACE2) receptor. The envelope protein of SARS-CoV-2 interacts with TLR2, while the Spike protein binds to TLR4. The dsRNA and ssRNA produced by the virus are recognized by TLR3 and TLR7/8, respectively. LPS produced by translocation of microbial products from leaky intestines may also stimulate TLR4, while mitochondrial (mt) DNA released by infected cells activates TLR9. The TLR activation events initiate downstream signaling pathways that lead to the secretion of pro-inflammatory cytokines, chemokines, and type-I IFNs. Deranged expression and mutations of each of these TLRs can lead to impaired cytokines secretion and may be associated with severe COVID-19. Created with https://www.biorender.com.

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It emerged in late 2019 and led to a global public-health crisis. Like other coronaviruses, SARS-CoV-2 is a positive-sense single-stranded RNA (ssRNA) virus. The Spike glycoproteins on its surface mediate its entry into the host cell by binding to angiotensin-converting enzyme-2 (ACE2) receptor, which is expressed on the cell membrane by epithelial and other cells in the lungs, intestines, heart, kidney, and other tissues [19–21] (Table 2, Ref. [19,21–28]; and Fig. 2).

Since TLRs serve as the first line of defense against viral pathogens, it was quickly suspected that they may also be activated by SARS-CoV-2. Indeed, there is emerging evidence that many human TLRs can directly sense SARS-CoV-2 molecules. For example, human TLR4 recognizes the Spike protein of SARS-CoV-2 [29] while human TLR2 recognizes its envelope protein [22] (Table 2 and Fig. 2). Moreover, many studies suggest that while TLRs are important for protecting the majority of patients who are infected with SARS-CoV-2, some are responsible for the cytokine storm in the minority of patients who develop severe COVID-19 [30]. Many of these studies involve transcriptome analyses of COVID-19 patients: together they show that the expression levels of six TLRs in the blood or tissues are altered by SARS-CoV-2 infection [31–33]. These TLRs are TLR2, TLR3, TLR4, TLR7, TLR8, and TLR9. However, the expression of the different TLRs in human patients remains poorly understood to date. Since improving our understanding of TLRs in COVID-19 pathogenesis will likely aid the development of therapies and biomarkers for COVID-19, we here comprehensively review the transcriptome studies on TLR expression in COVID-19 patients.

2. Toll Like Receptor 2

TLR2 forms a heterodimer with TLR2 or TLR6 and is expressed on the surface of many cell types, including macrophages, DCs, neutrophils, B cells, T cells, and non-immune cells such as endothelial and epithelial cells [3,4]. It recognizes bacterial lipopeptides and lipoproteins and fungal and parasite components. It can also detect viral components such as viral envelope proteins [34]. Its adaptor proteins include MyD88 and MAL/TIRAP (Table 1 and Fig. 1).

Table 1. Expression of TLRs by human cells.

TLR	Ligand	Adaptor proteins	Location	Cell-type expression in humans
TLR2/1	Triacyl lipopeptides	MAL/TIRAP, MyD88	Cell surface	Monocytes [4], macrophages, myeloid DCs [5], plasmacytoid DCs, T cells [3], B cells, NK cells [4], endothelial cells [6], epithelial cells [7,8]
TLR2/6	Diacyl lipopeptides	MAL/TIRAP, MyD88	Cell surface	Monocytes [9], myeloid DCs [5], T cells [3], epithelial cells [8]
TLR3	dsRNA	TRIF	Endosome	Monocytes, macrophages, myeloid DCs [5], plasmacytoid DCs, T cells [3], B cells, epithelial cells [8], fibroblasts, nerve cells [10]
TLR4	LPS	MAL/TIRAP, MyD88, TRAM, TRIF	Cell surface, endosome	Monocytes [11], macrophages, myeloid DCs [5], plasmacytoid DCs, T cells [3], B cells, epithelial cells [7], keratinocytes [12]
TLR7	ssRNA	MyD88	Endosome	Monocytes, macrophages, myeloid DCs, plasmacytoid DCs, T cells [3], B cells, endothelial cells [6]
TLR8	ssRNA	MyD88	Endosome	Monocytes, macrophages, myeloid DCs [5], plasmacytoid DCs, T cells [3], B cells [13]
TLR9	CpG DNA	MyD88	Endosome	Monocytes, macrophages, NK cells [14], plasmacytoid DCs [15], T cells [3], B cells [15]

TLR, Toll-like receptor; MAL, MyD88-adaptor-like protein; TIRAP, TIR-domain containing adaptor protein; MyD88, Myeloid differentiation factor 88; DCs, dendritic cells; NK, natural killer; dsRNA, double-stranded RNA; TRIF, TIR-domain-containing adapter-inducing interferon-β; LPS, lipopolysaccharide; TRAM, TRIF-related adapter molecule; ssRNA, single-stranded RNA.

Table 2. The components of SARS-CoV-2, their role(s) in COVID-19 pathogenesis, and their known links to TLRs.

Viral structural protein and other components	Role in COVID-19 pathogenesis	Known link to TLR
Glycoprotein membrane (M) protein	Virus assembly, membrane budding [27] Maintaining the shape of the viral envelope [26]	
Spike (S) protein	Binding of the virus to host cell receptor ACE2 [21]	Recognized by TLR4 [24]
Envelope (E) protein	Virus assembly, maturation, budding, and proliferation [26] Maintaining the structural integrity [26] Ion conduction as a viral ion channel [27]	Recognized by TLR2 [22]
Nucleocapsid (N) protein	Packaging of ssRNA genome [27] Viral replication, cellular response to infection in the host cellular machinery [26]	
Viral dsRNA	Produced early during the infection cycle as a result of genome replication and mRNA transcription [28]	Recognized by TLR3 [23]
Viral ssRNA	SARS-CoV-2 genomic RNA [19]	Recognized by TLR7/8 [25]

dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; ACE2, angiotensin-converting enzyme-2; mRNA, messenger RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; COVID-19, Coronavirus disease 2019.

Zheng *et al*. [22] showed that TLR2 can detect SARS-CoV-2 surface proteins prior to virus entry into host cells. Specifically, their transcriptome analysis of whole blood samples from patients with differing COVID-19 severity showed that TLR2 and MyD88 expression associates with increasing disease severity. They then found that when human peripheral blood mononuclear cells (PBMCs) are infected with SARS-CoV-2, their TLR2 molecules are activated by the envelope protein but not the Spike protein, and that this induces MyD88-dependent inflammatory-cytokine production by the PBMCs [22].

The upregulation of TLR2 by SARS-CoV-2, particularly in severe COVID-19 cases, was also observed by several other studies. Thus, Taniguchi-Ponciano *et al.* [35] reported that PBMCs from critically ill COVID-19 patients express much higher levels of TLR2 mRNA (messenger RNA) than PBMCs from healthy individuals. Similarly, Sultan *et al*. [36] found that while blood samples from patients with moderate and severe COVID-19 expressed similar levels of TLR2 mRNA, these expression levels were significantly higher compared to samples from healthy controls. Moreover, single cell RNA-sequencing (scRNA-

seq) analyses showed that the macrophages, monocytes, and neutrophils in the peripheral blood or bronchoalveolar lavage fluid (BALF) of patients with severe COVID-19 expressed TLR2 at higher levels than the equivalent cells from healthy volunteers or patients with moderate COVID-19 [31]. Another scRNA-seq analysis of BALF also found that TLR2 was upregulated in COVID-19 patients relative to healthy individuals, and that this expression increased with disease severity, especially in the CD14-CD16+ myeloid-cell populations [32]. Similarly, Theobald *et al.* [37] showed that PBMC-derived CD14+ macrophages from convalescent COVID-19 patients expressed more TLR2 than the same cells from healthy individuals. Significantly, the TLR2 expression in the patient macrophages rose further when they were stimulated with the Spike protein *in vitro*. In addition, when these macrophages were treated with Spike protein and then nigericin, which is needed for the formation of IL-1β, they produced large amounts of IL-1β that was specifically blocked by an antibody against TLR2 [37]. Thus, the engagement of TLR2 on macrophages with Spike protein may cause the cells to produce abundant IL-1β.

The genotyping study of Bagheri-Hosseinabadi *et al.* [38] also showed recently that a point mutation in the *TLR2* gene (rs5743708 G>A) associated with a greater risk of COVID-19 infection and severe COVID-19, which suggests that a missense mutation of TLR2 promotes severe COVID-19.

Recent studies also suggest that TLR2 may participate in the thromboinflammation that characterizes severe COVID-19. The mechanism involves the activation of platelets by SARS-CoV-2 and their production of extracellular vesicles. These vesicles in turn induce neutrophils to produce neutrophil extracellular traps (NETs), which then promote the formation of thrombo-emboli. Specifically, Zuo *et al.* [39] and Sung *et al.* [40] showed together that extracellular vesicles in the serum from COVID-19 patients induced normal neutrophils to produce NETS. Sung *et al.* [40] also showed that incubating normal neutrophils with normal platelets and SARS-CoV-2 *in vitro* induced NET formation, and that this was abrogated by blocking TLR2. Thus, TLR2 may promote COVID-19-induced thromboinflammation.

Sultan *et al.* [36] also observed that the TLR2 mRNA levels in the blood of patients with moderate and severe COVID-19 correlated with their serum levels of biomarkers of impaired renal (creatine) and cardiac (troponin) function. This suggests that TLR2 may also promote the myocardial damage and renal dysfunction induced by COVID-19.

Thus, there is substantial evidence that suggests that TLR2 is broadly upregulated in COVID-19 patients and participates in various COVID-19-related pathologies. The fact that TLR2 expression correlates positively with COVID-19 severity suggests that TLR2 could be a potential therapeutic target in COVID-19. This is supported by Zheng *et al.* [22]: they found that treating human SARS-CoV-2-infected PBMCs with the TLR2 antagonist Oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (oxPAPC) reduced their secretion of inflammatory cytokines and chemokines, namely, Tumor necrosis factor-α (TNF-α), IFN-γ, IL-1α, IL-6, and C-X-C motif chemokine ligand 10 (CXCL10). Moreover, they observed that TLR2-deficient mice did not develop lung inflammation after intratracheal instillation of SARS-CoV-2 envelope protein, and that oxPAPC treatment protected human ACE2 receptor-expressing mice from SARS-CoV-2-induced mortality [22]. In addition, Sung *et al.* [40] showed that a murine model of SARS-CoV-2 infection was strongly protected from SARS-CoV-2-induced thromboinflammation when TLR2 had been knocked out.

3. Toll Like Receptor 3

TLR3 plays a vital role as an innate immune sensor. It is located in the endosome and is expressed by a wide range of non-immune cells such as epithelial cells, nerve cells, and fibroblasts as well as by immune cells [23,41]. It was first identified as a sensor of double-stranded RNA (dsRNA), which is an intermediate replication product of most viruses. Consequently, TLR3 is considered to be a major effector of host responses against viral infection. TLR3 forms a homodimer and TRIF is the key adaptor protein (Table 1 and Fig. 1).

TLR3 recognizes the dsRNA of SARS-CoV-2 after replication (Table 2 and Fig. 2). This recognition event triggers the binding of TRIF to the TLR3 homodimer, which in turn induces intracellular signaling and the secretion of cytokines [42].

Many studies show that COVID-19 associates with TLR3-expression changes in various tissues, and that it may play a protective role. For example, Menezes *et al.* [43] found that while patients with severe COVID-19 had higher TLR3 mRNA levels in their peripheral blood on admission day than the uninfected control group, the patients who died and/or needed mechanical ventilation had lower TLR3 mRNA levels than the patients who survived and did not need mechanical ventilation. A very recent study by Farkas *et al.* [44] also suggested that TLR3 may prevent the vascular remodeling in the lung that is induced by SARS-CoV-2. Specifically, their immunohistochemical and mRNA analyses of human lung samples showed that COVID-19 associates both with thickening of the pulmonary artery wall and concomitant loss of pulmonary artery endothelial-cell expression of TLR3. Their *in vitro* analyses then showed that SARS-CoV-2 infection repressed the TLR3 expression of human pulmonary artery endothelial cells [44]. Croci *et al.* [45] further demonstrated the potential protective role of TLR3: they found that severe COVID-19 associates with a single-nucleotide polymorphism (rs3775291 [Leu412Phe]) in TLR3 that decreases autophagy, which is a key anti-viral mechanism. Similarly, Zhang *et al.* [46] found that at least

3.5% of patients with life-threatening COVID-19 pneumonia had mutations in genes that are involved in the TLR3-dependent induction and amplification of type-I IFNs.

In addition, impairment of TLR3 expression and TLR3-dependent type-I IFN secretion may reflect TLR3 tolerance due to hyperstimulation induced by the abundance of TLR3 agonists during infection: Naqvi *et al.* [47] showed with HEK-TLR3 reporter cell lines that the serum and endotracheal aspirate from intensive-care unit (ICU) COVID-19 patients strongly activate TLR3. Moreover, an *in vitro* analysis of PBMCs from SARS-CoV-2-infected individuals showed that when the plasmacytoid DCs in the samples were stimulated with synthetic TLR3 agonists, they produced less IFN-α than the equivalent cells from healthy individuals [48].

These observations suggest together that TLR3 and its induction of type-I IFNs play a protective role in COVID-19 patients that can become overwhelmed by excessive levels of TLR3 agonists during infection. Thus, therapeutically upregulating TLR3 could potentially be effective for ameliorating COVID-19 pathogenicity. Indeed, Farkas *et al.* [44] showed that treating mice with a TLR3 agonist ameliorated the pulmonary damage induced by mouse-adapted SARS-CoV-2.

4. Toll Like Receptor 4

TLR4 plays crucial roles in initiating and regulating inflammatory responses against infectious organisms. In humans, it is predominantly expressed by myeloid-lineage cells. It is a plasma membrane receptor that can be taken up during endocytosis. It initiates intracellular signaling in both locations when it encounters PAMPs such as lipopolysaccharide (LPS), which is a component of the cell wall of Gram-negative bacteria [49]. However, it also senses viral proteins and some DAMPs. It forms homodimers when triggered, and its protein adaptors include MyD88, MAL/TIRAP, TRAM, and TRIF (Table 1 and Fig. 1).

Several studies have shown that the Spike protein of SARS-CoV-2 directly interacts with and stimulates TLR4, thereby inducing pro-inflammatory responses [24, 50]. TLR4 appears to play a pathogenic role in COVID-19: Carnevale *et al.* [51] reported recently that the interaction between the Spike protein and TLR4 on platelets from patients with COVID-19 activates the platelets and promotes platelet-dependent thrombus growth. Multiple studies also showed that TLR4 expression associates with more severe COVID-19. Thus, Menezes *et al.* [43] and Sohn *et al.* [52] found that TLR4 is upregulated in the PBMCs from severe/critically ill COVID-19 patients compared to milder cases or healthy controls. Sohn *et al.* [52] also observed with transcriptome analyses that molecules that act downstream of TLR4 activation (i.e., MAL/TIRAP, MyD88, TRAM, TRIF and NF-κB signaling pathway genes) are upregulated in COVID-19-patient PBMCs compared to healthy-control PBMCs. Similarly, a large cohort study showed that COVID-19 patients with severe symptoms exhibited 2-fold greater TLR4 expression in nasopharyngeal samples than patients with low or mild symptoms. Notably, this study also showed that older adults expressed less TLR4 than younger adults, and that age correlated negatively with severity. Thus, younger patients not only expressed more TLR4, they were also more prone to severe disease in this cohort [53]. Mohamed *et al.* [54] also found that the TLR4 levels in the serum of COVID-19 patients correlated positively with COVID-19 severity, as indicated by severe chest-computed-tomography scores, hypoxia, and lymphopenia. Finally, Teixeira *et al.* [55] showed that COVID-19 non-survivors have higher levels of LPS in their peripheral blood than survivors, possibly due to COVID-19-related intestinal permeabilization and translocation of enteric microorganisms or their products into the blood stream. Moreover, these high LPS levels associated with higher blood levels of pro-inflammatory cytokines such as IL-6 and TNF-α and chemokines such as CCL2 and CCL5 [55]. The latter findings support the pathological role of TLR4 in COVID-19 since LPS is the most canonical ligand of TLR4.

Interestingly, Salem *et al.* [56] found recently that the TLR4 promoter in blood samples from COVID-19 patients is heavily methylated compared to samples from healthy subjects without COVID-19. They suggested that this was due to downregulation of the methyltransferase DNA methyltransferase 3 beta (DNMT3B), whose expression correlated positively with TLR4 promoter methylation [56]. These findings are curious because DNA methylation generally represses gene expression. Nonetheless, since DNMT inhibitors can control coronavirus infection and have been proposed as COVID-19 treatments, Salem *et al.* [56] advised that further research is needed before therapeutic use of such inhibitors can be initiated [57].

These findings show that TLR4 interacts with the Spike protein of SARS-CoV-2 and plays a key role in driving the pathogenic hyperinflammation and poor outcomes of COVID-19. Thus, blocking TLR4 may be an effective treatment for COVID-19. Indeed, there are currently a number of ongoing or recently completed clinical trials on agents that antagonize TLR4, some of which have shown to block the inflammation in COVID-19 [58].

5. Toll Like Receptor 7/8

TLR7 and TLR8 are closely related members of the TLR family in terms of phylogeny and structure, although they do show some functional differences. They are located in the endosome and are abundantly expressed in immune cells, including monocytes, macrophages, and plasmacytoid DCs [3,59,60]. They form homodimers, and are stimulated by ssRNA. MyD88 is the key adaptor protein (Table 1 and Fig. 1).

TLR7/8 binds to SARS-CoV-2: Moreno-Eutimio *et al*. [25] found that the SARS-CoV-2 genome contains multiple ssRNA fragments that could be recognized by TLR7/8. Moreover, several studies show that severe COVID-19 associates with lower expression of TLR7 and TLR8. Thus, Wu and Yang [61] showed with scRNA-seq analysis that compared to mild COVID-19, severe COVID-19 associates with lower mRNA expression of both TLR7 and TLR8 in BALF macrophages and epithelial cells. Similarly, Sorrentino *et al*. [62] observed by RT-PCR that TLR7 and TLR8 are downregulated in the BALF samples from patients with fatal COVID-19 compared to survivors. Menezes *et al*. [43] had similar results for TLR8: compared to healthy controls, patients with severe COVID-19 demonstrated decreased TLR8 mRNA in their peripheral blood on the day of hospital admission. However, they observed that TLR7 mRNA expression was increased in the COVID-19 patients, not decreased [43]. Moreover, Naqvi *et al*. [47] reported that HEK-TLR7 reporter cells were consistently activated by serum and endotracheal aspirate from COVID-19 patients in the ICU. Nonetheless, there is additional evidence that suggests that severe COVID-19 associates with downregulation of TLR7. In particular, there is a growing body of research showing that loss-of-function mutations in TLR7 may drive severe COVID-19 in males because TLR7 is located on the X chromosome [63]. Thus, Van der Made *et al*. [64] observed that four young men from two unrelated families who developed severe COVID-19 had unique loss-of-function mutations in TLR7 that associated with low PBMC expression of type-I IFN on *in vitro* treatment with the TLR7 agonist imiquimod. Fallerini *et al*. [65] also noted that 2.1% of severely affected males and 0% of asymptomatic patients had deleterious missense mutations in TLR7, including the Arg920Lys mutation. This was also observed by a cohort analysis of unrelated male patients with critical COVID-19 under the age of 60: ~1.8% had TLR7 mutations that abrogated their B-cell and myeloid-cell responses to imiquimod. This phenotype was rescued by transfection of wild-type TLR7 [66]. Mantovani *et al*. [67] also recently detected the Arg920Lys TLR7 variant along with a new variant (Asp41Glu) in two severely affected male patients by transcriptome analysis of imiquimod-stimulated PBMCs. Sex-biased downregulation of TLR7 may also promote severe COVID-19 in males: Gómez-Carballa *et al*. [33] showed with RNA-seq and n-Counter datasets that TLR7 is downregulated in the blood, nasal, and saliva of male patients in the ICU compared to female ICU patients and male/female non-ICU patients. This associated with hypermethylation at three differentially methylated positions [33]. It is also possible that similar mechanisms may reduce TLR8 expression since TLR8 is also located on the X chromosome [63]: a pilot study showed recently that macrophage-activation syndrome associated with missense mutations in not only TLR7 but also TLR8, particularly in males [68].

The evidence to date suggests that COVID-19-associated reduction in TLR7/8 expression may lead to deficient IFN-λ [43] or IFN-I [61] production that hampers the ability of the patients to clear the SARS-CoV-2 infection. Thus, TLR7/8 dysfunction may play important roles in COVID-19 severity and their loss-of-function mutations or sex-biased expression could serve as important risk factor for male patients.

6. Toll Like Receptor 9

TLR9 is an intracellular innate immune receptor that is localized in endosomal vesicles. It is expressed by immune cells such as plasmacytoid DCs, macrophages, and natural killer cells [69] and plays a pivotal role in inflammatory responses against viral infection. In particular, it regulates the secretion of IFNs. It specifically recognizes unmethylated CpG-dinucleotides, which are common in bacterial and viral DNA but relatively uncommon in vertebrate genomic DNA [70]. However, these motifs are also present in the mitochondrial (mt) DNA of humans because mtDNA undergoes very little CpG methylation due to its small size and short non-coding control region [71]. Thus, mtDNA can serve as a DAMP and trigger TLR9. TLR9 forms a homodimer and its adaptor protein is MyD88 (Table 1 and Fig. 1).

TLR9 can recognize SARS-CoV-2 infection: when human umbilical vein endothelial cells were infected with SARS-CoV-2, they released mtDNA, which in turn activated their TLR9 molecules and induced the cells to secrete cytokines [72]. This recognition event may associate with more severe COVID-19: Bagheri-Hosseinabadi *et al*. [38] showed that hospitalized COVID-19 patients with clinical symptoms expressed significantly more TLR9 mRNA in nasopharyngeal-swab epithelial cells than asymptomatic patients and symptomatic patients who were not hospitalized. Similarly, scRNA-seq analysis showed that compared to patients with mild COVID-19, patients with severe COVID-19 exhibited greater TLR9 expression in BALF cells and PBMCs [73]. In addition, the genotyping study of Alhabibi *et al*. [74] found that a common TLR9 polymorphism (rs5743836 [1237C>T]) associates with a greater risk of COVID-19 infection and severe COVID-19. Thus, TLR9 may play a pathogenic role in COVID-19.

7. Conclusions

Since TLRs recognize PAMPs and DAMPs, which are the first harbingers of infection, and initiate immune responses, they are a central component of the innate immune system. Of the ten human TLRs, six (TLR2, TLR3, TLR4, TLR7/8, and TLR9) can recognize SARS-CoV-2 infection. The activation of three appears to have pathogenic consequences: TLR2, TLR4, and TLR9 are upregulated in severe COVID-19 patients compared to control subjects. Moreover, TLR2 activation associates with thromboinflammation and renal and cardiac dysfunction, and TLR4 stimu-

lation associates with platelet-dependent thrombosis. By contrast, the remaining three TLRs, namely, TLR3, TLR7, and TLR8, associate with protection from COVID-19: they generally appear to be downregulated in severe COVID-19, and at least with TLR3, this appears to be due to hyperstimulation with the ligand. The variable outcomes of COVID-19 in individuals can also be partly attributed to mutations in TLRs: point mutations in TLR2, TLR3, and TLR9 associate with increased COVID-19 severity. Mutations or sex-biased expression of TLR7/8, which are X-linked, may also partly explain severe cases of COVID-19 in young males. These mutations in host's TLRs may impair the following phosphorylation cascade and cytokines secretion, resulting in inadequate defense against the infection. Further investigations of TLR expression patterns and how these patterns correlate with the clinical manifestations of COVID-19 are needed to improve our understanding of the regulatory role of TLRs in COVID-19 pathophysiology. These studies will also help to determine whether TLR agonists or antagonists could serve as effective therapies for COVID-19 patients and/or whether TLRs could be prognostic markers. This is important background for preclinical and clinical research on TLR agonists or antagonists, which is currently in its infancy [30].

Author Contributions

SYL, RK, and NL conceptualized the paper. NL searched for and analyzed the data from the reviewed literature. NL wrote the original draft and drew the figures. RK and SYL edited the original draft. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Duan T, Du Y, Xing C, Wang HY, Wang RF. Toll-Like Receptor Signaling and Its Role in Cell-Mediated Immunity. Frontiers in Immunology. 2022; 13: 812774.

[2] Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Frontiers in Immunology. 2014; 5: 461.

[3] Zarember KA, Godowski PJ. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. Journal of Immunology. 2002; 168: 554–561.

[4] Flo TH, Halaas O, Torp S, Ryan L, Lien E, Dybdahl B, *et al*. Differential expression of Toll-like receptor 2 in human cells. Journal of Leukocyte Biology. 2001; 69: 474–481.

[5] Schreibelt G, Tel J, Sliepen KHEWJ, Benitez-Ribas D, Figdor CG, Adema GJ, *et al*. Toll-like receptor expression and function in human dendritic cell subsets: implications for dendritic cell-based anti-cancer immunotherapy. Cancer Immunology, Immunotherapy. 2010; 59: 1573–1582.

[6] Triantafilou M, Gamper FGJ, Lepper PM, Mouratis MA, Schumann C, Harokopakis E, *et al*. Lipopolysaccharides from atherosclerosis-associated bacteria antagonize TLR4, induce formation of TLR2/1/CD36 complexes in lipid rafts and trigger TLR2-induced inflammatory responses in human vascular endothelial cells. Cellular Microbiology. 2007; 9: 2030–2039.

[7] Dong Z, Yang Z, Wang C. Expression of TLR2 and TLR4 messenger RNA in the epithelial cells of the nasal airway. American Journal of Rhinology. 2005; 19: 236–239.

[8] Melkamu T, Squillace D, Kita H, O'Grady SM. Regulation of TLR2 expression and function in human airway epithelial cells. The Journal of Membrane Biology. 2009; 229: 101–113.

[9] Salim F, Gunawan H, Suwarsa O, Sutedja E. Increased Expression of Toll-Like Receptor (TLR) 2 and TLR6 on Peripheral Blood Monocytes by Induction of Staphylococcal Enterotoxin B During Exacerbation of Atopic Dermatitis Patients. Clinical, Cosmetic and Investigational Dermatology. 2023; 16: 301–307.

[10] Gao D, Ciancanelli MJ, Zhang P, Harschnitz O, Bondet V, Hasek M, *et al*. TLR3 controls constitutive IFN-β antiviral immunity in human fibroblasts and cortical neurons. The Journal of Clinical Investigation. 2021; 131: e134529.

[11] Visintin A, Mazzoni A, Spitzer JH, Wyllie DH, Dower SK, Segal DM. Regulation of Toll-like receptors in human monocytes and dendritic cells. Journal of Immunology. 2001; 166: 249–255.

[12] Song PI, Park YM, Abraham T, Harten B, Zivony A, Neparidze N, *et al*. Human keratinocytes express functional CD14 and toll-like receptor 4. The Journal of Investigative Dermatology. 2002; 119: 424–432.

[13] Moen SH, Ehrnström B, Kojen JF, Yurchenko M, Beckwith KS, Afset JE, *et al*. Human Toll-like Receptor 8 (TLR8) Is an Important Sensor of Pyogenic Bacteria, and Is Attenuated by Cell Surface TLR Signaling. Frontiers in Immunology. 2019; 10: 1209.

[14] Roda JM, Parihar R, Carson WE 3rd. CpG-containing oligodeoxynucleotides act through TLR9 to enhance the NK cell cytokine response to antibody-coated tumor cells. Journal of Immunology. 2005; 175: 1619–1627.

[15] Jiang W, Lederman MM, Harding CV, Rodriguez B, Mohner RJ, Sieg SF. TLR9 stimulation drives naïve B cells to proliferate and to attain enhanced antigen presenting function. European Journal of Immunology. 2007; 37: 2205–2213.

[16] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006; 124: 783–801.

[17] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nature Immunology. 2010; 11: 373–384.

[18] O'Neill LAJ, Golenbock D, Bowie AG. The history of Toll-like receptors - redefining innate immunity. Nature Reviews. Immunology. 2013; 13: 453–460.

[19] Yang H, Rao Z. Structural biology of SARS-CoV-2 and implications for therapeutic development. Nature Reviews. Microbiology. 2021; 19: 685–700.

[20] Fawzy MS, Ashour H, Shafie AAA, Dahman NBH, Fares AM, Antar S, *et al*. The role of angiotensin-converting enzyme 2 (*ACE2*) genetic variations in COVID-19 infection: a literature review. The Egyptian Journal of Medical Human Genetics. 2022; 23: 97.
[21] Beyerstedt S, Casaro EB, Rangel ÉB. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. European Journal of Clinical Microbiology & Infectious Diseases. 2021; 40: 905–919.
[22] Zheng M, Karki R, Williams EP, Yang D, Fitzpatrick E, Vogel P, *et al*. TLR2 senses the SARS-CoV-2 envelope protein to produce inflammatory cytokines. Nature Immunology. 2021; 22: 829–838.
[23] Lundberg AM, Drexler SK, Monaco C, Williams LM, Sacre SM, Feldmann M, *et al*. Key differences in TLR3/poly I:C signaling and cytokine induction by human primary cells: a phenomenon absent from murine cell systems. Blood. 2007; 110: 3245–3252.
[24] Zhao Y, Kuang M, Li J, Zhu L, Jia Z, Guo X, *et al*. SARS-CoV-2 spike protein interacts with and activates TLR41. Cell Research. 2021; 31: 818–820.
[25] Moreno-Eutimio MA, López-Macías C, Pastelin-Palacios R. Bioinformatic analysis and identification of single-stranded RNA sequences recognized by TLR7/8 in the SARS-CoV-2, SARS-CoV, and MERS-CoV genomes. Microbes and Infection. 2020; 22: 226–229.
[26] Manan A, Pirzada RH, Haseeb M, Choi S. Toll-like Receptor Mediation in SARS-CoV-2: A Therapeutic Approach. International Journal of Molecular Sciences. 2022; 23: 10716.
[27] Zhang Z, Nomura N, Muramoto Y, Ekimoto T, Uemura T, Liu K, *et al*. Structure of SARS-CoV-2 membrane protein essential for virus assembly. Nature Communications. 2022; 13: 4399.
[28] Li Y, Renner DM, Comar CE, Whelan JN, Reyes HM, Cardenas-Diaz FL, *et al*. SARS-CoV-2 induces double-stranded RNA-mediated innate immune responses in respiratory epithelial-derived cells and cardiomyocytes. Proceedings of the National Academy of Sciences of the United States of America. 2021; 118: e2022643118.
[29] Choudhury A, Mukherjee S. In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. Journal of Medical Virology. 2020; 92: 2105–2113.
[30] Mabrey FL, Morrell ED, Wurfel MM. TLRs in COVID-19: How they drive immunopathology and the rationale for modulation. Innate Immunity. 2021; 27: 503–513.
[31] Xu Q, Yang Y, Zhang X, Cai JJ. Association of pyroptosis and severeness of COVID-19 as revealed by integrated single-cell transcriptome data analysis. Immunoinformatics. 2022; 6: 100013.
[32] Desterke C, Turhan AG, Bennaceur-Griscelli A, Griscelli F. PPARγ Cistrome Repression during Activation of Lung Monocyte-Macrophages in Severe COVID-19. iScience. 2020; 23: 101611.
[33] Gómez-Carballa A, Pardo-Seco J, Pischedda S, Rivero-Calle I, Butler-Laporte G, Richards JB, *et al*. Sex-biased expression of the TLR7 gene in severe COVID-19 patients: Insights from transcriptomics and epigenomics. Environmental Research. 2022; 215: 114288.
[34] Compton T, Kurt-Jones EA, Boehme KW, Belko J, Latz E, Golenbock DT, *et al*. Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. Journal of Virology. 2003; 77: 4588–4596.
[35] Taniguchi-Ponciano K, Vadillo E, Mayani H, Gonzalez-Bonilla CR, Torres J, Majluf A, *et al*. Increased expression of hypoxia-induced factor 1α mRNA and its related genes in myeloid blood cells from critically ill COVID-19 patients. Annals of Medicine. 2021; 53: 197–207.
[36] Sultan RH, Elesawy BH, Ali TM, Abdallah M, Assal HH, Ahmed AE, *et al*. Correlations between Kidney and Heart Function Bioindicators and the Expressions of Toll-Like, ACE2, and NRP-1 Receptors in COVID-19. Vaccines. 2022; 10: 1106.
[37] Theobald SJ, Simonis A, Georgomanolis T, Kreer C, Zehner M, Eisfeld HS, *et al*. Long-lived macrophage reprogramming drives spike protein-mediated inflammasome activation in COVID-19. EMBO Molecular Medicine. 2021; 13: e14150.
[38] Bagheri-Hosseinabadi Z, Rezazadeh Zarandi E, Mirabzadeh M, Amiri A, Abbasifard M. mRNA expression of toll-like receptors 3, 7, 8, and 9 in the nasopharyngeal epithelial cells of coronavirus disease 2019 patients. BMC Infectious Diseases. 2022; 22: 448.
[39] Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, *et al*. Neutrophil extracellular traps in COVID-19. JCI Insight. 2020; 5: e138999.
[40] Sung PS, Yang SP, Peng YC, Sun CP, Tao MH, Hsieh SL. CLEC5A and TLR2 are critical in SARS-CoV-2-induced NET formation and lung inflammation. Journal of Biomedical Science. 2022; 29: 52.
[41] Chiba Y, Mizoguchi I, Mitobe K, Higuchi K, Nagai H, Nishigori C, *et al*. IL-27 enhances the expression of TRAIL and TLR3 in human melanomas and inhibits their tumor growth in cooperation with a TLR3 agonist poly(I:C) partly in a TRAIL-dependent manner. PLoS ONE. 2013; 8: e76159.
[42] Bortolotti D, Gentili V, Rizzo S, Schiuma G, Beltrami S, Strazzabosco G, *et al*. TLR3 and TLR7 RNA Sensor Activation during SARS-CoV-2 Infection. Microorganisms. 2021; 9: 1820.
[43] Menezes MCS, Veiga ADM, Martins de Lima T, Kunimi Kubo Ariga S, Vieira Barbeiro H, de Lucena Moreira C, *et al*. Lower peripheral blood Toll-like receptor 3 expression is associated with an unfavorable outcome in severe COVID-19 patients. Scientific Reports. 2021; 11: 15223.
[44] Farkas D, Bogamuwa S, Piper B, Newcomb G, Gunturu P, Bednash JS, *et al*. A role for Toll-like receptor 3 in lung vascular remodeling associated with SARS-CoV-2 infection. bioRxiv. 2023; 2023.01.25.524586. (preprint)
[45] Croci S, Venneri MA, Mantovani S, Fallerini C, Benetti E, Picchiotti N, *et al*. The polymorphism L412F in *TLR3* inhibits autophagy and is a marker of severe COVID-19 in males. Autophagy. 2022; 18: 1662–1672.
[46] Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, *et al*. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020; 370: eabd4570.
[47] Naqvi I, Giroux N, Olson L, Morrison SA, Llanga T, Akinade TO, *et al*. DAMPs/PAMPs induce monocytic TLR activation and tolerance in COVID-19 patients; nucleic acid binding scavengers can counteract such TLR agonists. Biomaterials. 2022; 283: 121393.
[48] Arunachalam PS, Wimmers F, Mok CKP, Perera RAPM, Scott M, Hagan T, *et al*. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. Science. 2020; 369: 1210–1220.
[49] Vaure C, Liu Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. Frontiers in Immunology. 2014; 5: 316.
[50] Bhattacharya M, Sharma AR, Mallick B, Sharma G, Lee SS, Chakraborty C. Immunoinformatics approach to understand molecular interaction between multi-epitopic regions of SARS-CoV-2 spike-protein with TLR4/MD-2 complex. Infection, Genetics and Evolution. 2020; 85: 104587.
[51] Carnevale R, Cammisotto V, Bartimoccia S, Nocella C, Castellani V, Bufano M, *et al*. Toll-Like Receptor 4-Dependent Platelet-Related Thrombosis in SARS-CoV-2 Infection. Circulation Research. 2023; 132: 290–305.
[52] Sohn KM, Lee SG, Kim HJ, Cheon S, Jeong H, Lee J, *et al*.

COVID-19 Patients Upregulate Toll-like Receptor 4-mediated Inflammatory Signaling That Mimics Bacterial Sepsis. Journal of Korean Medical Science. 2020; 35: e343.
[53] Martinez Marignac VL, Oertlin GS, Favant JL, Fleishmann E, Salinas M, Marchetti G, *et al*. Immune response to SARS CoV2 infection by TLR3, TLR4 and TLR7 gene expression. medRxiv. 2023; 2023.05.12.23288889. (preprint)
[54] Mohamed HA, Abdelkafy AE, Khairy RMM, Abdelraheim SR, Kamel BA, Marey H. MicroRNAs and cytokines as potential predictive biomarkers for COVID-19 disease progression. Scientific Reports. 2023; 13: 3531.
[55] Teixeira PC, Dorneles GP, Santana Filho PC, da Silva IM, Schipper LL, Postiga IAL, *et al*. Increased LPS levels coexist with systemic inflammation and result in monocyte activation in severe COVID-19 patients. International Immunopharmacology. 2021; 100: 108125.
[56] Salem S, Mosaad R, Lotfy R, Elbadry M. Altered expression of DNA methyltransferases and methylation status of the TLR4 and TNF-α promoters in COVID-19. Archives of Virology. 2023; 168: 95.
[57] Rabaan AA, Al Mutair A, Alhumaid S, Al Alawi Z, Al Mohaini M, Alsalman AJ, *et al*. Modulation of host epigenome by coronavirus infections and developing treatment modalities for COVID-19 beyond genetics. European Review for Medical and Pharmacological Sciences. 2021; 25: 5947–5964.
[58] Mukherjee S. Toll-like receptor 4 in COVID-19: friend or foe? Future Virology. 2022; 10.2217/fvl-2021-0249.
[59] Schön MP, Schön M. TLR7 and TLR8 as targets in cancer therapy. Oncogene. 2008; 27: 190–199.
[60] Zhang M, Yan Z, Wang J, Yao X. Toll-like receptors 7 and 8 expression correlates with the expression of immune biomarkers and positively predicts the clinical outcome of patients with melanoma. OncoTargets and Therapy. 2017; 10: 4339–4346.
[61] Wu D, Yang XO. Dysregulation of Pulmonary Responses in Severe COVID-19. Viruses. 2021; 13: 957.
[62] Sorrentino L, Fracella M, Frasca F, D'Auria A, Santinelli L, Maddaloni L, *et al*. Alterations in the Expression of IFN Lambda, IFN Gamma and Toll-like Receptors in Severe COVID-19 Patients. Microorganisms. 2023; 11: 689.
[63] Lind NA, Rael VE, Pestal K, Liu B, Barton GM. Regulation of the nucleic acid-sensing Toll-like receptors. Nature Reviews. Immunology. 2022; 22: 224–235.
[64] van der Made CI, Simons A, Schuurs-Hoeijmakers J, van den Heuvel G, Mantere T, Kersten S, *et al*. Presence of Genetic Variants Among Young Men With Severe COVID-19. JAMA. 2020; 324: 663–673.
[65] Fallerini C, Daga S, Mantovani S, Benetti E, Picchiotti N, Francisci D, *et al*. Association of Toll-like receptor 7 variants with life-threatening COVID-19 disease in males: findings from a nested case-control study. eLife. 2021; 10: e67569.
[66] Asano T, Boisson B, Onodi F, Matuozzo D, Moncada-Velez M, Maglorius Renkilaraj MRL, *et al*. X-linked recessive TLR7 deficiency in ~1% of men under 60 years old with life-threatening COVID-19. Science Immunology. 2021; 6: eabl4348.
[67] Mantovani S, Daga S, Fallerini C, Baldassarri M, Benetti E, Picchiotti N, *et al*. Rare variants in Toll-like receptor 7 results in functional impairment and downregulation of cytokine-mediated signaling in COVID-19 patients. Genes and Immunity. 2022; 23: 51–56.
[68] Bali DFA, Gulen TA, Ozmen E, Yuce ZT, Yildiz O, Turunc T, *et al*. The Effect of Sex-Specific Genetic Factors on the Host Immune Response to COVID-19: A Pilot Study. Erciyes Medical Journal. 2022; 44: 594–602.
[69] Fehri E, Ennaifer E, Bel Haj Rhouma R, Ardhaoui M, Boubaker S. TLR9 and Glioma: Friends or Foes? Cells. 2022; 12: 152.
[70] Krieg AM. CpG motifs in bacterial DNA and their immune effects. Annual Review of Immunology. 2002; 20: 709–760.
[71] Sharma N, Pasala MS, Prakash A. Mitochondrial DNA: Epigenetics and environment. Environmental and Molecular Mutagenesis. 2019; 60: 668–682.
[72] Costa TJ, Potje SR, Fraga-Silva TFC, da Silva-Neto JA, Barros PR, Rodrigues D, *et al*. Mitochondrial DNA and TLR9 activation contribute to SARS-CoV-2-induced endothelial cell damage. Vascular Pharmacology. 2022; 142: 106946.
[73] Shaath H, Vishnubalaji R, Elkord E, Alajez NM. Single-Cell Transcriptome Analysis Highlights a Role for Neutrophils and Inflammatory Macrophages in the Pathogenesis of Severe COVID-19. Cells. 2020; 9: 2374.
[74] Alhabibi AM, Hassan AS, Abd Elbaky NM, Eid HA, Khalifa MAAA, Wahab MA, *et al*. Impact of Toll-Like Receptor 2 and 9 Gene Polymorphisms on COVID-19: Susceptibility, Severity, and Thrombosis. Journal of Inflammation Research. 2023; 16: 665–675.