

Insights on the role of TLR-4 in neuroinflammation: a hint on COVID-19 relationship

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ABSTRACT

Neuroinflammation plays a crucial role in cognitive decline and neurodegenerative diseases, such as Alzheimer's. Within the central nervous system, microglia express Toll-like receptor 4 (TLR-4) abundantly, which prompts the secretion of proinflammatory cytokines like TNF- α , PGE₂, IL-1 β , and NO that are considered essential components of neuroinflammation. The emergence of neurological complications in patients with COVID-19 has spurred investigations into the potential involvement of TLR-4. Particularly intriguing is its contribution to the cytokine storms triggered by SARS-CoV-2 and SARS-CoV-1 infections. This comprehensive review investigates TLR-4-induced neuroinflammation, focusing on its potential connection to cognitive decline and neurological symptoms triggered by COVID-19. By unraveling the intricate mechanisms by which TLR-4 mediates neuroinflammation, this review aims to shed light on its possible role in the context of COVID-19. Understanding the implications of TLR-4 activation could pave the way for targeted interventions to alleviate the cognitive and neurological impacts of COVID-19. As the world seeks to comprehend the far-reaching effects of the pandemic, grasping the nuances of TLR-4-associated neuroinflammation stands as a crucial step in addressing the challenges posed by cognitive decline and neurological manifestations in patients with COVID-19.

Keywords: *Microglia; Inflammation; Toll-Like Receptor 4; MyD88, NF-kB; SARS-CoV-2.*

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1. Pathophysiology of neuroinflammation

The concept of "neuroinflammation" made its inaugural appearance in the scientific literature in 1995, designating the glial reaction and lymphocyte infiltration observed within the central nervous system (CNS) of individuals afflicted by infectious and autoimmune neurological disorders, including multiple sclerosis [1]. Neuroinflammation has a substantial function in the cognitive impairment and neurodegenerative conditions' development such as Huntington's disorder, Parkinson's

disease (PD), amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and multiple sclerosis (MS) [1-3]. Within the brain, microglia, acting as resident macrophages, have a vital role in the onset and progression of neuroinflammation. Although acute neuroinflammation offers protection, chronic neuroinflammation is detrimental to nervous tissue [1, 2]. Consequently, neuroinflammation yields positive or negative outcomes depending on both the period of inflammatory response and the stimulation nature of the microglial [2]. Under normal circumstances, microglia remove

waste and toxins, yet they can also migrate to lesions and eliminate cellular debris when triggered [2-4]. While essential for defense, excessive or prolonged microglial activation results in neuronal loss and heightened release of proinflammatory cytokines, particularly in the hippocampus [2-4].

1.1. TLR-4 Overview

TLRs represent a category of transmembrane proteins of type I that function as pattern-recognition receptors PRRs. They are extremely maintained and are stimulated by various pathogen-associated molecular patterns (PAMPs). This activation subsequently initiates an innate immune response and inflammatory reactions [5, 6]. TLRs are noticed in various kinds of innate immune cells, involving dendritic cells, natural killer cells, macrophages, and circulating leukocytes as monocytes and neutrophils. They are also placed on the adaptive immune cells B and T lymphocytes, and also on non-immune cells involving endothelial cells, fibroblasts, and epithelial cells [3, 6, 7].

Toll-like Receptor 4 is responsible for detecting and becoming active upon exposure to the bacterial lipopolysaccharide (LPS), a primary molecular element found in the cell wall of Gram -ve bacteria [5, 8]. TLR-4 plays a crucial role in identifying an extensive array of substances, encompassing lipopolysaccharide (LPS), which constitutes the cell wall's fundamental molecular building block, within Gram-negative bacteria [7, 8]. Lipopolysaccharides (LPS) can induce persistent neuroinflammation through the activation of Toll-like receptor 4 (TLR4), subsequently triggering the activation of microglia and the release of proinflammatory cytokines [8]. Additionally, TLR-4 exhibits the capacity to detect viruses, fungi, and mycoplasma. In the context of recognizing LPS and viruses, TLR-4 employs a supplementary protein known as myeloid differentiation protein-

2 (MD-2) [7, 9]. TLR-4 is predominantly present in microglia within the central nervous system. Upon activation, these microglia cells generate proinflammatory cytokines including TNF- α , PGE2, IL-1 β , and NO. [2, 9]. These cytokines play a pivotal role as vital mediators in the neuroinflammatory process. It is noteworthy that TLR-4 plays a critical role in facilitating extensive neuronal cell death [2, 3]. TLR-4 is importantly present in brain microglia, and the overabundant inflammation triggered by the activation of this pathway has been linked to both depressive conditions and the emergence of neurodegenerative diseases [3, 10]. Much like its fellow TLRs, TLR4 showcases a modular arrangement comprised of a segment characterized via LRR situated in the extracellular region. This component is linked to an intracellular TIR domain, which serves as the conduit for transmitting signals [3, 5]. The TLR-4 receptor system detects even trace quantities of circulating LPS endotoxin at the molecular level. Upon binding, this process induces the dimerization of receptors located on the cellular membrane, thereby launching a cascade of interactions between proteins. These interactions ultimately lead to the pro-inflammatory cytokines and interferons generation. Consequently, this biological mechanism elicits both inflammatory and immunological reactions [3, 5].

1.2. The extracellular TLR-4 receptor system

The process of obtaining lipopolysaccharide (LPS) involves the first extraction from bacterial membranes and the subsequent release of LPS-containing vesicles. This extraction is facilitated by the action of LPS binding protein (LBP) present in serum [11, 12]. The process of LPS transfer from LBP to CD14 occurs, wherein CD14 can exist in either soluble form or be attached to the cell surface through a glycosyl phosphatidyl inositol anchor [11, 12]. CD14 separates LPS aggregates into monomeric

molecules, which are then presented to the TLR4-MD-2 complex [11, 12]. TLR-4 does not directly attach to LPS; instead, it necessitates the presence of the adaptor protein MD-2. MD-2 specifically binds to and identifies the lipophilic portion of LPS (lipid A), resulting in the formation of a distinct complex [5, 12]. Conformational alterations result in the cytoplasmic Toll/interleukin-1 Receptor (TIR) dimerization upon aggregation of the TLR4-MD-2 complex upon binding LPS [11].

1.3. The Intracellular TLR4/MyD88/NF- κ B (MyD88-dependent) pathway

After the successive involvement of CD14 and LBP has facilitated the creation of the active TLR4/MD-2 heterodimer on the cellular surface, the intracellular signaling can proceed through either the TLR4/MyD88/NF- κ B (MyD88-dependent cascade) or the TLR4/TRIF/IRF3 routes (MyD88-independent cascade) [5, 12, 13]. The cytoplasmic segment of TLR-4 and all the TLR-4 adaptor molecules contain Toll/interleukin-1 receptor (TIR) domains, which act a crucial function in their reciprocal interactions [5, 12, 13]. The initiation of the TLR-4/MyD88 cascade occurs through the complex formed by LPS, MD-2, and TLR-4, which is located on the cellular membrane [5, 12, 13]. In the MyD88-dependent signaling pathway shown in **Fig 1**, the TIRAP adaptor engages with the TIR-TIR dimer of the two TLR4 units that form the activated heterodimer through its TIR domain [5, 12, 13]. TIRAP, an intracellular protein, plays a role in membrane anchoring through its phosphatidylinositol 4,5-bisphosphate-binding domain, and as a homodimer, it binds to TLR-4 TIR-domains, creating an interface that facilitates the recruitment of MYD88 in the context of the MyD88-dependent pathway for TLR4 activation [5, 12, 13]. The formation of a complex involving MYD88 molecules and subsequent

binding to the serine/threonine kinases IRAK2 and IRAK4 results in the assembly of a molecular structure known as the meddlesome [5, 12, 13]. As the myddosome is established, it facilitates the autophosphorylation of IRAK4, and within this structure, IRAK1 can also interact with the MYD88-IRAK4 complex, leading to its phosphorylation by IRAK4 [5, 12, 13]. The recruitment of TNF receptor-associated factor 6 (TRAF6) subsequently leads to the assembly of a trimeric complex. The molecule in question forms a complex with phosphorylated IRAK1 and TRAF6 E3 ubiquitin ligase, hence enhancing its polyubiquitination at the Lys63 residue [5, 13]. The TAB2/TAB3 adaptor proteins and the IKK component of the IKK γ -complex are responsible for the identification of polyubiquitin chains of TRAF6 [5]. It enables the activation and recruitment of TAK1, as well as the phosphorylation of the I κ B complex. This ultimately results in the breakdown of the I κ B complex and subsequent release of NF- κ B. The activation of TAK1 results in the trigger of several mitogen-activated protein kinases. Additionally, TAK1, in conjunction with NF- κ B, initiates the proinflammatory cytokines' synthesis and secretion involving IL-1 β , TNF- α , and IL-6 [5, 13].

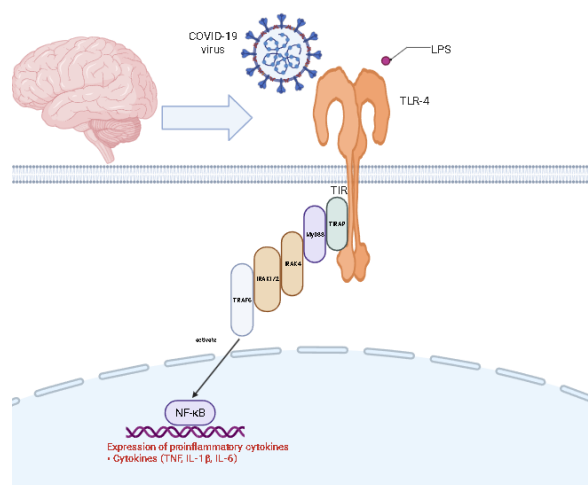


Fig. 1. The role of TLR-4 in the pathophysiology of inflammation and COVID-19 created with BioRender.com

1.4. TLR-4 and neuroinflammation

Various cells within the central nervous system, involving astrocytes, neurons, and microglia, exhibit diverse reactions upon TLR-4 activation. TLR-4 triggering with particular agonists leads to changes in glial cell morphology, heightened proliferation rates, and increased expression of activation markers. Activation of TLR-4 in human microglia and astrocytes prompts the proinflammatory cytokines' secretion like IL-6, IL-1 β , and TNF- α [14]. The cellular actions mediated by TLR-4 have the potential to accelerate neuroinflammation, astrogliosis, and excitotoxicity, while also disrupting the CNS injury-repair mechanisms [14].

The critical involvement of TLR4-triggered NF- κ B signaling is crucial for provoking cerebral inflammation in various CNS conditions. This triggering results in the transcription of multiple proinflammatory genes encoding elements like cytokines, chemokines, COX-2, and MMP-9 [15]. These mediators contribute to the progression of secondary brain injury after traumatic brain injury. The increased expression of cytokines and chemokines might activate microglia, initiating the inflammatory cells' enrollment into the brain, thereby potentially culminating in neuronal loss [15].

A research investigation into the function and process of the TLR-4 /NF- κ B cascade in cognitive damage stimulated by cerebral small vascular disease (CSVD) determined that this cascade has a responsibility in the CSVD-related cognitive impairment's initiation and progression [16]. Its involvement is connected to the regulation of oxidative stress and cellular apoptosis [16].

TLR-4-dependent mechanisms are of paramount importance in the context of neuroinflammation, as they exert regulatory

control on myelination, excitotoxicity, and diverse clearance mechanisms such as autophagy and phagocytosis [14]. The aforementioned mechanisms are commonly observed in a range of central nervous system illnesses, such as AD, PD, traumatic brain injury, ischemic stroke, MS, multiple system atrophy, and Huntington's disease. As a result, investigating drugs that specifically target TLR-4 action could offer potential therapeutic options for these conditions [14, 17].

2. COVID-19 and neuroinflammation

Neurological symptoms, affecting around one-third of COVID-19 cases, encompass fatigue, myalgia, headache, olfactory dysfunction and taste, delirium, and cognitive decline [18]. Even without active infection, the immune-activated S1 protein can impact the brain through various pathways. This could involve disrupting the blood-brain barrier (BBB), changing BBB transporters, altering transporter substrate levels, enhancing immune cell movement into the brain, elevating cytokines that cross the BBB, and prompting brain endothelial cells to secrete neuroimmune constituents precisely into the brain [18].

Various interrelated factors have been suggested as possible contributors to the neurological symptoms noticed in COVID-19 cases, encompassing mechanisms like hypoxia, intense cytokine storm during infection, autoimmune responses following infection, hypercoagulability, endothelial dysfunction, multiple organ failure, and even potential direct invasion of the nervous system [19]. This potential neuro-invasiveness could be linked to the presence of angiotensin-converting enzyme-2 receptors in the brain [19].

2.1. CNS symptoms post COVID-19

Post-COVID-19 or Long COVID-19 emerges as a subsequent phase in the health journey of

numerous COVID-19 survivors, affecting more than 50% of non-hospitalized individuals [20]. Symptoms indicating central nervous system (CNS) involvement, such as fatigue and cognitive difficulties, persist for months without substantial improvement [20]. Notably, a significant proportion (75-90%) of patients with Long COVID-19 had experienced milder COVID-19 cases that did not necessitate hospitalization, eliminating the possibility of attributing the symptoms solely to post-intubation effects or near-death experiences [21]. There exists a prevailing concern regarding the potential for long-term COVID-19 to progress into a chronic neurodegenerative disorder marked by cognitive deterioration, hence eliciting apprehension regarding its enduring consequences [21].

2.2. Neurological complications post COVID-19 and role of TLR-4

Neurological problems are frequently observed as a prominent manifestation of COVID-19 infection in cases after respiratory symptoms [22]. Scholars are currently investigating the potential association between COVID-19 and neurological complications, with TLR-4 appearing as a plausible candidate for mediating this connection. The ACE-2 serves as the principal receptor for the SARS-CoV-2 spike protein. However, it is worth noting that the spike protein also engages in interactions with TLR-4 [22]. This interaction triggers a cytokine storm, causing neuroinflammation and degeneration in patients with COVID-19 [22]. This suggests TLR-4 could be targeted to address COVID-19-related neurological complications [22].

2.3. TLR-4 and COVID-19

In a study conducted by Fontes-Dantas et al. (2023), they presented evidence indicating that administering Spike protein directly into the brains of mice resulted in delayed cognitive impairment, resembling post-COVID-19

syndrome [23]. Their findings also reveal that neuroinflammation and increased microglial activity in the hippocampus contribute to Spike-induced memory problems by triggering the complement-dependent removal of synapses [23]. Blocking TLR-4 signaling either genetically or pharmacologically shields animals from synapse loss and memory decline caused by Spike protein infusion into the brain [23]. Similarly, in a group of 86 individuals who had recovered from mild COVID-19, those possessing the GG genotype for TLR4-2604G>A (rs10759931) exhibited unfavorable cognitive outcomes [23]. Those outcomes highlight TLR-4 as a crucial factor to explore for understanding persistent cognitive malfunction post-COVID-19 infection in both humans and rodents [23].

The TLR-4 signaling cascade has a direct role in cytokine storms caused by SARS-CoV-2 and SARS-CoV-1 infections. Predicted initially through immunoinformatic methods, the subsequent experimental validation of the molecular and cellular interactions between the spike protein of SARS-CoV-2 and TLR-4 was conducted [24]. Furthermore, the Spike protein, when purified, triggers the inflammatory cytokines' expression in human monocyte cells as effectively as LPS does. Interestingly, the inhibitor which targets TLR-4, significantly prevents the inflammatory cytokines' initiation by both LPS and Spike protein in human monocyte cell lines [24]. The evidence presented strongly suggests that the interaction between the Spike protein of SARS-CoV-2 and the TLR-4 signaling cascade follows a similar process to that observed with LPS, leading to an increased synthesis of pro-inflammatory cytokines [24]. Elevated levels of circulating endotoxin could indicate compromised immune reaction versus co-infections in severe COVID-19 cases [25]. The inflammatory reaction of patients with COVID-19 monocytes upon exposure to LPS was assessed [25]. Blood samples from healthy

individuals, mild COVID-19 cases, and severe COVID-19 cases were treated with LPS for 2 h [25]. In instances of severe COVID-19 cases, plasma samples demonstrated elevated LPS levels and serum CD14 concentrations in comparison to both healthy individuals and patients with mild COVID-19 symptoms [25]. Furthermore, systemic changes were noticed in COVID-19 cases' peripheral blood, involving elevated LPS and cytokine levels, alongside TLR-4 increased expression and NF- κ B trigger [25].

In their 2020 computational analysis, Choudhury and colleagues identified that TLR-4 exhibited the most robust interaction with the SARS-CoV-2 spike glycoprotein compared to further TLRs. This finding suggests that among the various TLRs present on cell surfaces, TLR4 is likely the primary candidate for identifying molecular displays of SARS-CoV-2 and initiating inflammatory reactions [26]. The study gains credibility from the well-established strong binding between the human ACE2 entrance receptor and the Spike protein [26]. This interaction implies potential implications for the introduction of SARS-CoV-2 into human cells and the activation of the cytokine storm, with potential impacts on multiple organs [26]. The cytokine storm is a significant complication in severe COVID-19 cases, and TLR-4's role in producing key cytokines like IL-6 and TNF- α makes it especially pertinent in understanding severe disease manifestations [26].

Shon and colleagues (2020) conducted a study involving 48 participants, including 28 COVID-19 cases comprising 20 with mild/moderate symptoms 8 with severe symptoms, and 20 healthy controls [26]. The patients diagnosed with COVID-19 demonstrated heightened concentrations of inflammatory signaling molecules in their PBMCs, with the activation of TLR-4 acting a significant function

in this immune response [26]. There was a considerable upregulation observed in the expression of TLR-4 and its downstream signaling markers, including MYD88, CD14, IRAK1, IRAP, TRAF6, and TICAM [26]. Notably, several genes linked with the NF- κ B signaling cascade, involving NFKBIA, NFKB1, RELA, and NFKB2, showed substantial elevation, indicative of hyperactivation in response to COVID-19 infection [26]. The outcomes of this study indicate that the increased inflammatory reactions observed in individuals with COVID-19 are a result of the triggering of the NF- κ B signaling cascade via the TLR-4 receptor [26]. Therefore, the data suggests that the molecular cause of COVID-19 effects is associated with the inflammatory signaling triggered by TLR-4 activation, rather than being purely ascribed to viral activity [26].

The ongoing investigation aimed to validate the impacts of the S1 subunit of the SARS-CoV-2 Spike protein on pro-inflammatory actions in macrophages derived from both murine and human sources. In reaction to S1 exposure, mouse peritoneal exudate macrophages generated pro-inflammatory mediators [27]. S1 exposure also initiated the trigger of NF- κ B and JNK signaling cascades [27]. The induction of pro-inflammatory cytokines by S1 was mitigated via specific inhibitors targeting the NF- κ B and JNK cascades [27]. The generation of pro-inflammatory cytokines and stimulation of intracellular signaling cascades caused through both S1 and LPS were diminished when a TLR-4 antagonist was administered to mouse peritoneal exudate macrophages and human THP-1 cell-derived macrophages [27]. The results of this study demonstrate that the S1 subunit of the SARS-CoV-2 Spike protein induces the activation of TLR-4 signaling, hence facilitating the initiation of pro-inflammatory reactions in macrophages of both murine and human origin

[27].

In a research conducted by Frank et al., adult male Sprague-Dawley rats received intra-cisterna magna (ICM) injections of either vehicle or S1 [7]. Behavioral observation in their cages; 8 hours after ICM; indicated that S1 led to decreased behaviors like total action, wall-rearing, and self-grooming [7]. Furthermore, S1 demonstrated an increase in social avoidance during the juvenile social exploration test, which persisted for a period of 24 hours following the administration of ICM [7]. The expression of neuroimmune genes (Iba1, MhcII α , Cd11b, Cd200r1, Tlr2, Gfap, Tlr4, Il1b, Nlrp3, Hmgb1) and the levels of associated proteins (CXCL1, IL-1 β , TNF, IL-2, IFN γ , IL-10) were observed to be impacted by the administration of S1 in various brain regions (specifically, the hippocampus, hypothalamus, and frontal cortex) at various points in time (specifically, 24 h and 7 days following S1 treatment) [7]. The microglia exhibited elevated gene expression (Il6, Tnf, Il1b, Nlrp3) and protein levels (TNF, IL-6, CXCL1, IL-10, IL-1 β) during direct exposure to S1 [7]. In addition, the activation of TLR-2 and TLR-4 receptor signaling was observed in HEK293 transgenic cells upon exposure to S1 [7]. The cumulative evidence suggests that the structural proteins derived from SARS-CoV-2 possess the ability to function autonomously as PAMPs. This capability enables them to initiate neuroinflammatory responses by interacting with pattern recognition receptors [7].

2.4. Recovery from COVID-induced cognitive impairment

To date, there has been no regulatory approval for pharmaceutical interventions designed to address cognitive dysfunction resulting from COVID-19. Ongoing clinical trials are actively investigating potential therapeutic options [28]. Meanwhile, it is prudent to

contemplate the incorporation of vitamin D monitoring and supplementation as a strategic consideration within the therapeutic framework. This approach is viewed through the lens of neuroprotection and neurorepair and applies to both current patients with COVID-19 and individuals in post-recovery phases [29]. Empirical evidence suggests that luteolin, a naturally occurring flavonoid, may mitigate cognitive impairment by attenuating the activation of mast cells and microglia [30]. Furthermore, within the scientific literature, EGb 761[®], a specialized extract derived from Ginkgo biloba, consistently demonstrates the capacity to safeguard endothelial cells, exhibit potent anti-inflammatory attributes, and enhance neuroplasticity [31]. Given its mechanistic foundations, established efficacy in ameliorating cognitive deficits, and commendable tolerability profile, the proposition of employing EGb 761[®] as a therapeutic agent to alleviate post-COVID-19 cognitive symptoms emerges as a promising avenue for further exploration [31].

Cognitive rehabilitation represents a valuable approach for assisting patients suffering from cognitive dysfunction post-COVID-19 [28, 32]. This comprehensive strategy encompasses various components, including reminiscence therapy involving discussions of past and current events, engagement with topics of personal interest, exposure to music, and engagement in practical activities such as baking or indoor gardening [28]. Additionally, it encompasses computer-based cognitive training, participation in puzzles, word games, numerical challenges, and reading exercises. This multifaceted approach aims to enhance cognitive function and overall mental well-being in individuals facing cognitive difficulties [32].

Conclusion

The objective of this review is to elucidate the relationship between SARS-CoV-2 and the

neuroinflammation initiated through Toll-like receptor 4 (TLR-4), resulting in cognitive impairment that could persist even after the resolution of respiratory symptoms following COVID-19. These discoveries imply that the suppression of TLR-4 signaling during the acute phase of SARS-CoV-2 infection may offer a potential strategy to alleviate certain neurological and neuropsychiatric consequences that may arise after COVID-19. Given the significant role played by TLR-4 in neuroinflammation, the TLR-4 pathway emerges as a crucial therapeutic target, particularly due to its implication in various diseases, some of which carry a high mortality rate, such as sepsis and COVID-19. Consequently, there exists substantial therapeutic promise in the formulation and advancement of drugs specifically tailored to target this pathway. However, further research and clinical trials must be conducted to identify potential agents capable of effectively inhibiting this pathway.

Abbreviations

CNS, Central Nervous System; AD, Alzheimer's Disease; TNF- α , Tumor Necrosis Factor Alpha; IL-1 β , Interleukin 1 Beta; PGE2, Prostaglandin E2; NO, Nitric Oxide; COVID-19, Coronavirus Disease 2019; ACE-2, Angiotensin Converting Enzyme 2; CSVD, Cerebral Small Vascular Disease; IL-6, Interleukin 6; IL-2, Interleukin 2; IFN, interferon; IFN γ , Interferon Gamma; CXCL1, Chemokine (C-X-C Motif) Ligand 1; TRIF, toll/interleukin-1 receptor domain-containing adaptor protein inducing IFN- β ; HEK293, Human Embryonic Kidney 293 Cells; CD14, Cluster of differentiation 14; IL, interleukin; IRAK, IL-1 receptor associated kinases; LBP, lipid binding protein; TNF, tumour necrosis factor; BBB, Blood-Brain Barrier; MD-2, myeloid differentiation factor 2; MyD88, myeloid differentiation primary-response protein 88; LPS, lipopolysaccharide; NF- κ B, nuclear factor kappa-light-chain-

enhancer of activated B cells; IRF3, interferon regulatory factor 3; TRAF6, TNF receptor associated factor 6; TLR, toll-like receptor; TIRAP, toll/interleukin-1 receptor homology domain-containing adaptor protein; TRAM, TRIF-related adaptor molecule.

Declarations

Consent to publish

All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

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Author contribution

Salma A. Elshafey: review idea and outline, Writing - original draft, manuscript revision. Dalia A. ElKhouly: editing, Supervision. Esther T. Menze: editing, Supervision. Mariane G. Tadros: editing, Supervision, all authors approved the final manuscript.

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