American Journal of BioMedicine
AJBM 2021;9 (1): 1-35
Research Article DOI: 10.18081/2333-5106/015-09/581-596

TLR4-mediated Neuroinflammation in SLE through phosphorylation of MyD88/IRAK signaling pathway

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Abstract

A role for innate immunity in inflammation of CNS is being increasingly evidenced. This study focused on determining the role of toll-like receptor 4 (TLR4) and phosphorylation of MyD88/IRAK signaling pathway in systemic lupus erythematosus (SLE) pathogenesis. We used mouse models of SLE (BXSB/Yaa mice) and their controls (C57BL/6 mice). TLR4 significantly phosphorylation of MyD88/IRAK activity and enhanced SLE pathogenesis in BXSB/Yaa mice, whereas the small interference RNA-mediated knockdown of TLR4 activity attenuated SLE activity.

Keywords: TLR4; SLE; MyD88; IRAK

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Received August 30, 2020; Accepted November 19, 2020; Published January 30, 2021.

Introduction to Toll-like Receptor 4 (TLR4)

The recognition of pathogen-associated molecular patterns by pattern recognition receptors on immune cells is the first step for the establishment of immune responses against bacterial infections. Toll-like receptor 4 (TLR4) is a conserved receptor involved in innate immunity. To induce the immune response, TLR4 is capable of recognizing lipopolysaccharide (LPS) from Gram-negative bacteria. In fact, TLR4 is the mammalian LPS-sensing receptor which, upon activation, signals through the NF-κB pathway, leading to the production of pro-inflammatory mediators of innate immunity. Furthermore, other TLR4 ligands also play a role in the downstream signaling pathway. In this review, we address TLR4 structure and its actions, not only in LPS recognition but also in providing antimicrobial immune responses that are required for host survival.

Toll-like Receptor 4 (TLR4) The host needs to recognize and discriminate between evolutionarily conserved pathogen-associated molecular patterns present in many infectious microbes and ligands derived from self-substances, so that it can mount protective immune defenses and avoid unnecessary immune activation. Through membrane-bound and intracellular pattern recognition receptors (PRRs), the mammalian host senses infection, thus allowing for fine-tuning of the immune responses. The Toll-like receptors (TLRs) are PRR family members that contribute to form the first line of innate host immune defense against microbial infections. Indeed, TLR binding with these pathogen-associated molecular patterns and

damage-associated molecular patterns creates signals that are translated for the production of antimicrobial mediators which begin combating almost immediately. Such engagement of TLRs is generally non-cognate and does not undergo recombination, although it can be evolutionarily adapted to readily recognize new world pathogen antigens. TLRs play central roles in pathogen recognition and seem to exist in most vertebrate species, providing direct antagonism against pathogens.

Structure and Signaling Pathways of TLR4

Toll-like receptor 4 (TLR4) belongs to the Toll-like receptor (TLR) family and plays a key role in recognizing pathogen-associated molecular patterns (PAMPs) derived from various Gram-negative and Gram-positive bacteria, and in inducing innate and adaptive immunity. TLR4 activation initiates two signaling pathways: the TRIF-dependent pathway, which is MyD88-independent, leading to the production of type I IFN, and the MyD88-dependent pathway, leading to the induction of pro-inflammatory responses. The cytokines produced by TLR4 activation have multiple functions, including the activation of various components of the immune system, and the production of numerous chemokines important in leukocyte recruitment. The crosstalk between TLR4 signaling pathways is very complex and involves multiple molecular crosstalk networks. Different classes of molecules can stabilize TLR4 in different immune response states, and some targeting TLR4 pathway can enhance host resistance against pathogenic infections. As TLR4 is a key molecule related to multiple disease susceptibilities, a comprehensive understanding of these signaling pathways will be helpful for drug development and disease treatment.

The TLR family is key in recognizing pathogen-associated molecular patterns (PAMPs) and initiating the immune response by both innate and adaptive immunity. TLR4 is as an essential pattern recognition receptor (PRR) for recognizing lipopolysaccharide (LPS), which is derived from the outer membrane of Gram-negative bacteria. In recent years, the structure and mechanism of TLR4 has been well studied both in Gram-negative and Gram-positive infections. TLR4 could stimulate an extensive inflammatory response and a specific adaptive immune response. The TLR4 signaling pathway is also an indispensable part of innate immunity, which plays an important role in clearing pathogens from the host and initiating classic inflammatory immune responses. Due to its powerful pathogen recognition and immune activation capabilities, TLR4 has become a target for the treatment of various infectious and chronic inflammatory diseases, and it can be considered as a new target for tumor immunotherapy and vaccination.

Extracellular Domain

One of the first common characteristics found in all members of the TLR family is the presence of a string of thirty to forty leucine-rich repeats (LRR) in the extracellular domain. This region is responsible for the recognition of a wide variety of microbial components, such as LPS,

lipopeptides, flagellin, lipoarabinomannan, zymosan, and others. It is well established that most LRR domains are intimately involved with specific protein-protein interactions, which makes them suitable for mediating the protein-protein interactions known in important events of the immune response, such as the antigen-antibody binding and cytokine-receptor interactions. The crystal structure of the extracellular domain of TLR3 showed structural similarity between the TLR3-TIR domains and that of two proteins involved in pathogen recognition, β-glucan receptors. Although these data provide support for the notion that LRR domains are exclusively involved in mediating protein-protein interactions, these findings have limited application to the LRRs of TLRs because they are organized in a different way from all the known LRRs that have been structurally characterized.

Transmembrane Domain

TLR4 is a signal-transducing cell surface molecule that triggers host defense mechanisms in response to infection by gram-negative bacteria, by recognizing the lipid A moiety of bacterial lipopolysaccharide. The process of lipopolysaccharide binding to TLR4 creates two hydrophobic surfaces on the receptor, capable of membrane association. Since physical confirmation of these statements about the structure of the ligand-receptor complex and the transmembrane tails of TLR4 would be therapeutically relevant, we used synthetic transmembrane construct analogues of the transmembrane domains of TLR4 to understand their chemical and biophysical behavior.

Two constructs mimicking the transmembrane segment of TLR4 were synthesized. The longer sequence has 28 residues and the shorter sequence has 23 residues. The two constructs showed significant conformational differences, demonstrated by circular dichroism studies, HPLC size exclusion chromatography, and chemical crosslinking. The results were consistent with the secondary structure that we resolved by 100 ns molecular dynamics studies and by the 3D de novo structure prediction. Further, the two segments of the TLR4 transmembrane domain had significant differences in their ability to associate with the TLR4 ligand, lipopolysaccharide, in a biologically relevant mimetic micellar system. These results highlight the importance of the interactions of TLR4 constructs with Gram-negative bacterial outer membrane components and offer novel TLR4 construct design ideas that could be therapeutically relevant.

Intracellular Domain

Another conserved structure among mammalian TLRs is the intracellular domain. Although there are notable differences between the TIR domains of TLR2 and TLR4, these are believed to provide the basis for selective adaptor recruitment and discriminatory ligand-receptor relationships. This suggests that PIP2 recruitment is needed for TLR4 targeting to specific membranes to allow differing sets of phospholipids to provide stereospecific restrictions and organization required for TLR4 signaling. We should consider that the larger TLR4-induced

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"myddosome" may form as an extension of these earlier stages with MyD88 TIR located in the membrane interface and TIR TLR4 linked with the TIR of each TIRAP molecule allowing access to as many myddosomes as needed to support rapid kinase phosphorylation and signaling transduction.

The differential TIR domains and topology of TLR2 and TLR4 provide an opportunity to develop TLR signaling pathway drug leads that could modulate their differing ligand-receptor relationships. It appears at first sight that such lead compounds could be used to preferentially direct the LPS-TLR4-TRAM pathway via selective inhibition of TIRAP via a second cellular receptor. However, it is conceivable that the effects on human health in terms of modulation of select host defense mechanisms would be harmful and, therefore, of limited therapeutic value. On the other hand, inhibition of MAP-kinase-activation selective for LPS-TLR4-TRAM signaling might provide highly desirable anti-inflammatory effects for therapeutic drug candidates, and the described inhibition of TIRAP may be a viable drug-lead for treatment of endotoxic shock and some cancers.

TLR4 Ligands and Activation

TLR4 recognizes at least several molecules of larger lipid nature, including several major phospholipids from membrane fractions of gram-negative bacteria, mycobacterial lipooligosaccharides, sphingolipid ceramide-phosphorylethanolamine, in addition to LPS, and LPS of freshwater sponge. This LPS is not necessarily the most conserved LPS structure compared to other bacterial LPS, since it differs in the number of acyl chains, length, and oxidation level. There is also a ca. 250 kDa protein present in the LPS fractions of F. candida, similar to accumbovirin from Bacillus spp., possessing the LPS fraction. Unfractionated membrane preparations trigger TLR4 signaling in a similar manner, suggesting that TLR4 expressed on the cell membrane is easily activated by TLR4 ligands in the plasma membrane of the same cell.

When a particular TLR of vector insects, Tsetse fly TLR4, was expressed in mHEK cells for assessing its possible LPS signaling deficiency, Tsetse fly TLR4 did not bind LPS, did not undergo dimerization in the presence of LPS, and did not initiate an NF-kB-luc reporter activity in response to LPS stimulation. It was concluded that Tsetse fly TLR4 is unable to respond to LPS, due to structural differences in the Tsetse fly TLR4 ECD (extracellular domain). This was an unexpected discovery because TLR4 is otherwise well known for its role in initiating immune response mechanisms by recognizing LPS. Why should the GFSDD motif at the C-terminal region of TLR4 be so highly conserved among species if the distinctive aroostatic barrier of these insects can protect them from becoming infected with gram-negative bacteria?

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TLR4 in Innate Immunity

Toll-like receptor 4 (TLR4) is a fundamental receptor of the innate immune system and a link to the adaptive immune response. TLR4 expression was studied in cells of the human and murine immune systems, and wide expression was observed. This review provides a description of the innate immune response and its relation with TLR4. Likewise, the evolution of the TLR and their functional property. After that, information about the expression of TLR4 in the immune system cells will be given, and finally, the modification possibilities of TLR4 will be mentioned. TLR was first discovered by the work of Drosophila melanogaster Toll gene. Mice were infected with gram-negative bacteria, and severe sepsis was treated using lipopolysaccharide (LPS). So far, there are 10 TLRs in humans and 12 TLRs in mice.

The first TLRs discovered in humans and mice are TLR2 and TLR4. TLRs are type I transmembrane glycoproteins whose extracellular amino terminus consists of leucine prepeptide repeats (LRR) that identify PAMP, membrane spanning sequences, and carboxyl terminus intracellular signaling domain. All TLRs have domain interactions with the corresponding signal transduction adapter molecule MYD88. The exception is TLR3, which interacts with TICAM1 (TRIF). Since intracellular receptor-adapter domain interactions are possible only with secreted adapter molecules, TLRs end or end (C-terminal) of MYD88 or TICAM. MYD88 works primarily with all TLRs excluding TLR3, using the signal transduction of IL-1 family cytokine receptors. TLR4 is special so far, as it requires both the MYD88 pathway and the TICAM pathway, enabling a rapid response to LPS via the MYD88 pathway with a period sufficient to prevent T-cell tolerance induction. It is also required for the appropriate response to LPS via the TICAM pathway.

Recognition of Pathogen-Associated Molecular Patterns (PAMPs)

The ultimate goal of the immune system is effective protection against pathogens, such as bacteria. This is accomplished by immobilization, phagocytosis, and degradation of bacteria. One of the first immune cells to quickly respond to the presence of highly dangerous and conserved structures (called PAMPs) that are common to a group of pathogens (and recognized by the receptors of the host organism) is the macrophage. One of the most important of these receptors is called TLR4, and it is responsible for recognizing LPS, which is present on the surface of most Gram-negative bacteria and in particular of the highly virulent Escherichia coli.

It is the recognition of LPS that represents the crucial step that triggers a series of mechanisms that lead to the specific defense against E. coli. Given its importance, the receptor TLR4 is both distinct and double. Indeed, it is the only TLR that does not work alone, but operates in collaboration with other receptors such as MD2, whose gene is expressed together with TLR4. This structural organization causes the precursors of TLR4 to be processed and inserted into the cell membrane with MD2, giving rise to the actual receptor of the immune response. In

particular, TLR4 recognizes LPS when it is found complexed with the LBP-CD14 couple. On the other hand, a sponge has the function of absorbing the action of LBP.

Inflammatory Responses

Immune response mechanisms play a critical role in protecting the host from invading pathogens and are therefore fundamental to the survival and health of the host. Efficient recognition of bacteria is necessary to establish an effective host response to infection. Followed by recognition, immune response effectors are able to restrain bacterial growth, kill microorganisms, control inflammation, and optimize tissue repair. The innate immune system plays an active role in the recognition of microbial pathogens. In general, immune protection is mediated by the natural host defense mechanism, with Toll-like receptors (TLRs) as the main class of innate immune pattern recognition receptors (PRRs) recognizing the pathogen-associated molecular patterns (PAMPs) of microorganisms.

The binding of PAMPs (ligands for TLR) to TLR activates mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) signaling pathways, expression of inflammatory mediators, and costimulatory molecules to induce the production of interferon I (IFN-I) and promote tumor necrosis factor (TNF-α) and chemokine production to regulate the link between innate and adaptive immunity. In this connection, germ-free mice (lacking intestinal bacteria) have a hyporesponsive immune system. The immune reactivity of antibiotic-treated conventionally raised mice, in the context of broad-spectrum antibiotics, was significantly reduced, except for sulfasalazine treatment, which specifically inhibits pro-inflammatory gene expression. In recent years, much research suggests that LPS recognition plays a major role in synthesizing pathogenetic recognition receptors and more than 80 host genes are upregulated after the signature of downstream signaling events in response to LPS to regulate the immune response.

TLR4 in Adaptive Immunity

Toll-like receptor-4 (TLR4) is expressed on the surface of both antigen-presenting cells and T cells. TLR4-specific ligand lipopolysaccharide (LPS) has been indicated to act as a stable surface molecule for directly stimulating CD4+ T cells, leading to T cell activation, proliferation, and differentiation. During T cell activation, LPS is enough for the stimulation of T cells. In vitro, TLR4 ligands trigger TLR4-specific CD4+ T cells, inducing T cell-cell division, cytokine production, upregulation of mig expression, conversion of IFN-γ, and IL-4 gene expression in Th0 cells according to the presence of LPS. There is evidence indicating specifically that TLR4-induced T cell polarization is inhibited by blocking LFA-1.

Several studies support the idea that TLR4 signals are required for the promotion of diabetes, and that LPS and CD40L induce a Treg cell-dependent immune suppressor phenotype. Treg cells are generated through two different processes: (1) pTreg: during antigen stimulation, naive

effector T cells recognize peptides presented by APC; and (2) thymus-derived Treg (tTreg) that develop as a distinct lineage from other T cell subsets from Treg lineages. A distinct class is dependent on polyclonal activation. Treg cells contribute to diabetes suppression by TLR4 signaling pathway, and myeloid-derived suppressor cells (MDSCs) can also be induced via monocytes, which play a constant vital role in the mechanism. LC-educated MDSCs suppress CD4+ T cell oligomerization. MDSCs are capable of inhibiting diabetogenic T cell development. PrepTreg suppression of effector T cells requires IL-10 and TGF-β of the pTreg suppressor mechanism. Although TCR-specific molecules and LPS can bind to the TLR4 receptor molecules on LC cells, LC-derived TGF-β and IL-27 are responsible for the induction of suppression of autoreactive killer T cells, rather than stable suppression or free preventive Treg cells, as seen in the presence of the TLR4 ligand.

Cross-Talk with Adaptive Immune Cells

Thymus and activation-regulated chemokine (TARC) is known for its attraction of CCR4-expressing T-cells and is considered as an important chemokine in the cross-talk between adaptive and innate immunity. Interestingly, in an in vitro system of exposing Raw 264.7 and bone-marrow-derived macrophages to TARC, Pam3CSK4 or HKLM also induced the expression of TARC in these cells, demonstrating the potential cooperation between TLR- and GPCR-mediated chemokine gene expression. Finally, the lenalidomide-induced blocking of TARC triggered the apoptosis of TLR-activated macrophages, and these cells decreased their TLR-induced expressions of intracellular immunophysin. The results demonstrate that autocrine TARC has an anti-apoptotic effect, and this chemokine, together with CCR4 signaling, seems to be important in the replenishment of the dying macrophages.

Thrombin is a multi-domain serine proteinase that has been characterized as a pivotal effector in some of the early stages of blood coagulation or fibrinolysis. In addition to this anticoagulation, thrombin has been studied as an important immune modulator. Mammalian cells express a serine proteinase-activated receptor (PARs) family that responds to the cleavage of the serine protease at a specific site, causing the generation of the tethered ligand peptide, which will cause a change in the conformation of the extracellular region.

Role in Antigen Presentation

One of the less known functions of TLR4 is in the antigen presentation, which is crucial for activating naïve T lymphocytes, which is primordial in an effective immune response. Nevertheless, the mechanisms underlying the participation of TLR4 in antigen presentation are still little characterized. Some authors demonstrated that TLR4 is involved in the maturation of dendritic cells (DCs) that present the antigens to the adaptive cells. Hindering the natural maturation of TLR4-stimulated DCs does not occur and the costimulation of both CD40 and CD86 has a caspase-dependent method. TLR4 appears to be responsible for the classical method in the maturation of DC that stimulates the differentiation of T cells.

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Furthermore, TLR4 is also supposed to intervene in the increase of dendritic cells (DCs) in response to a virus, which is responsible for presenting the viral proteins to the action of the adaptive immune system. Viral proteins of LPS promote a functional maturation of DCs, involving TNF-α release and also the antigen-presenting function that stimulates the secretion of IL-10. Additionally, it plays a key role in the activation of DCs and polymorphonuclear cells in response to Coxiella burnettii. The antigen-presenting function is also stimulated by LPS and prevents the natural maturation of DCs by blocking the ability of p35 to process IL-1 β.

TLR4 in Disease Pathology

Recently, there has been a significant body of research that has been performed investigating the role of TLR4 in a wide variety of pathological conditions including allergic asthma, sepsis, and systemic inflammatory response syndrome, cardiovascular, chronic kidney, and non-alcoholic fatty liver diseases, acute lung injury, and arthritis, all indicating that the inhibition of TLR4 can produce beneficial effects. Ozone-induced exacerbations of asthma (and other allergic respiratory inflammations) result from an adaptive immune response. Cox and colleagues have considered the effects of treating mice with a selective TLR4 antagonist. They find that ODE-induced exacerbation of traditional antigen-induced airway inflammation (and goblet cell metaplasia) is prevented by TLR4 antagonist treatment.

A perplexing problem in contemporary medicine is the incidence of severe sepsis that continues to increase and the lack of specific therapies to treat this disorder. Watanabe and associates used a 5-bp deletion in the Tlr4 gene to show that it had profound effects on the pathology of sepsis. TLR4 mutant mice had a significant reduction in septic death. These animals also had reduced numbers of recruited blood leukocytes, pulmonary endothelial injury and extravasation of albumin. These data thus suggest that TLR4 contributes to the host response in bacterial sepsis. The observation that signaling pathways from TLR4 have such broad effects on a variety of immunopathological conditions indicates the potential utility of employing specific inhibitors in the treatment of these diseases.

Infections

Infections are characterized by the engagement of several TLRs due to the recognition of PAMPs. Specifically, TLR4 becomes activated by endotoxin or lipopolysaccharide released by Gram-negative bacteria. Since this is the major source of engagement of the adapter protein MyD88-independent pathway of TLR4, which also signals through TRIF, activation of TLR4 leads to both nuclear factor-kB (NF-kB) and interferon-response factor (IRF)3 activation and is associated with an elevated transcriptional response than TLR4-MyD88-dependent signaling cascades. Recent studies have indicated that the motif TRIF, which is a TLR4-associated protein, is important for further intracellular signaling and expression of type I interferons. There are limited studies on the individual role of the TIR domain of TRIF in the MyD88-independent pathway of TLR4, but it is apparently essential for the formation of a complex with TLR4 and

MyD88, and to control hyperinflammatory responses and contribute to tolerance against microbial cell components.

Overall, these molecular interactions, particularly those linked to TLR4 signaling pathways, are extremely used as stimulation strategies for molecular treatments based on TLR/CpG systems that modulate both innate and adaptive immunity. Although they are interconnected, their individual effects remain elusive and often highly variable based on the applied approach. The individual contribution of TLRs to immune responses has not been thoroughly explored in all cases. However, an increasing number of different TLR systems may contribute to CD11c+ cell and IFN-γ-producing effector T-cell cross-priming through the TLR4/MyD88-independent pathway, but CD11c+ cells are functionally essential for this process, and not hematopoietic-derived CD8α+/d- subset, demonstrating that the TLR4/MyD88-independent pathway is crucial for intracellular control of pathogens and for the clearance of antitumor vaccines.

Autoimmune Diseases

The overexpression and persistent activation of TLR4 have been implicated in the development of chronic inflammatory and immune-assisted pathologies, such as autoimmune disorders, associated with an excessive and uncontrolled production of proinflammatory mediators, the polarization of immune cell phenotypes with Th1 and Th17 differentiation, and the failure to remove apoptotic cells, as observed in diseases such as rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus, multiple sclerosis, Guillain-Barre syndrome, ankylosing spondylitis, asthma, psoriasis, Crohn's disease, irritable bowel syndrome, liver fibrosis, and polymyositis, among others. The inhibition of TLR4 activation has been proposed to have a protective effect in some of these pathologies.

However, this concept needs to be carefully evaluated and interpreted because TLR4 signaling can also play a protective role in disease development and progression, as observed in glycogen storage diseases, autoimmune type 1 diabetes, inflammatory bowel disease, peptic ulcers, intestinal obstruction, and carcinogenesis.

Cancer

Chronic inflammation sustains the self-renewal of cancer stem cells and contributes to oncogenesis, tumor progression, and transition from carcinogenesis to the malignant stages. Also, TLR4 stimulation may play a role in inflammation-associated forging reprogramming, which supports tumorigenesis. TLR4 stimulation was shown to reprogram the SIRT1-mediated metabolic pathways robustly to a high-oxidative phosphorylation mode, promoting metabolism and creatine biosynthesis, which are pivotal to constitutive inflammation and forge reprogramming to support oncogenesis. Additionally, the oncogenic activities of the TLR4 cascade in inflammation-associated forging reprogramming may favor induction of embryonic stem cell (ESC) related genes. Thus, TLR4 could initiate inflammation-associated forging

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reprogramming, transitioning carcinoma cells to CSC-like cells, which may support CSC characteristics, epithelial-mesenchymal transition (EMT), invasion, metastasis, and resistance to ionizing radiation, resulting in an increased risk of acquired radioresistance and destroyed radiotherapy.

TLR4 activation was shown to enhance the viability of liver cancer cells through activation of the p-AKT signaling pathway and improve the multidrug resistance of gastric cancer cells through the NF-κB signaling pathway. Additionally, stimulation of TLR4 stimulated the proliferation, migration, and invasion of renal cell carcinoma cells. Thus, TLR4 may be regarded as a possible therapeutic target in cancer and its life process. The therapeutic efficacy of TAK-242, a potential inhibitor of the TLR4 pathway, could be related to preferentially inhibiting TLR4-mediated stemness via inhibiting glycolysis. Also, it is likely that specific genetic factors are responsible for the reply to the TLR4 cascade, and individualized chemotherapy based on TLR4 pathway interactions might be beneficial for patients with tumors. Furthermore, the long-term therapeutic potential of TLR4 deficiency in the treatment of cancer needs to be elucidated.

Regulation of TLR4 Signaling

In the absence of signals from TLR4, cells express TLR4 in an inhibited state. MyD88 adaptors, TIRAP and TRIF, and TBK are recruited to the receptors in turns and limit expression to the cytoplasmic domain of TLR4. The cpdPE43 (cytoplasmic domain of Toll-like receptor 4 (TLR4) containing a Pro-Glu tetraptic sequence: 43 amino acids) has been identified as the TLR4 intracellular signal far-transporting domain (ISFTD) peptide, with self-promoted uptake properties. Once in the cell, uptake of the peptide leads to a rapid, dose-dependent, and TLR4-specific reduction in mRNA encoding the pro-inflammatory cytokines.

Negative Regulation of TLR4 Signaling by other TLRs Negative regulation by the LPS-TLR4 signaling axis is involved in the negative regulation of the inflammatory response through different TLR members. It has been observed that some TLRs provide a negative feedback regulation of TLR4 in the cells. When other TLRs are activated in addition to this, it can only attenuate the signal sustainable stability. Influences may also come from the activated TLR2 temporarily blocking TRL4 to enter the cytoplasm, by TLR4-SoCS1 mediated regulation. On the other hand, activated TLR2 may bind to MyD88, which binds to TLR4 in the cell, without inducing inflammatory reactions downstream of TLR4. Additionally, after being linked to CD14 due to its presence of a characteristic lipoprotein motif, the upregulation of IRAK-mediated signals also helps to convert the LPS response to TLR2 into the so-called "red end" TLR4 signaling. These findings suggest that the binding of lipoprotein unique ligands exposed to LPS does not necessarily allow a clear distinction be made between pro-inflammatory and regulatory responses of macrophages by their different surface TLRs. The LPS-tolerated MyD88 pathway will synergize with other TLR2-dependent TIRAP-independent molecules.

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Negative Regulators

One of the most important negative regulators of this signaling cascade is Toll-like receptor 4 (TLR4), which has evolved not only for pathogenic recognition and rapid activation of immune mechanisms, but also for reducing the risk of accidental responses to normal body components. Despite this important role, excessive downregulation of their signaling responses can have disastrous consequences on the responsiveness of a host, leading to sepsis, rheumatoid arthritis, asthma, and atherosclerosis. The goal of this chapter was to analyze the functions of TLR4 and TLR4-related molecules, acting as negative regulators or modulators, in balancing the immune response and possible deviations of the system, their consequences, and ultimately, to evaluate the possibilities of using them as therapeutic targets.

Despite negative feedback mechanisms that can keep the inflammatory response in check, sepsis, which is a leading cause of death in critical care settings, results from an overproduction of cytokines and other inflammatory mediators that occur when bound PAMPs are engaged with PRRs. The initial inflammatory response generated during septic shock is appropriate, as TLR stimulation early during the infection induces a variety of genes whose products help to resolve the infection. However, depending on the particular TLR involved, this can be accompanied by characteristic defects in adaptive immunity, which can be beneficial for the pathogen. An excessive PAMP response (due to complete absence or ineffective negative regulation) leads to the hyperactivation of neutrophils and overproduction of pro-inflammatory and anti-inflammatory cytokines.

Positive Regulators

A second class of signaling molecules has been shown to function as positive regulators of TLR-4 signaling and include IL-1R-associated kinase (IRAK) in its number. The IRAK family is a group of protein kinases originally identified to be downstream components of the IL-1R signaling pathway. The activation of IL-1R signaling is known to lead to activations of a number of intracellular signaling molecules that function downstream and include the members of the Bcl10-MALT1-CARMA1 complex and TRAF6. By the phosphorylation of TNF-α-receptor-associated factor 6 (TRAF6) by IRAK-1, IκB degradation is found to occur. In recent years, another phosphorylation event has been shown to occur in LPS-activated TLR-4 signaling, and interestingly, IRAK and TRAF6 have been shown to play a similar role in the TLR-4 signaling pathway. By the phosphorylation and degradation of IκB, these molecules are again found to turn on NF-κB signaling, and in doing so, initiate an inflammatory response. Therefore, a TLR-4-activated gene would express either IRAK or TRAF6.

Apart from the TIR domain-containing TLR-4 and its associated adaptor molecules, a third class of upstream signaling molecules is also present. Clearly, the MAPK-p38 pathway is then also found to become activated, but this would appear to be due to a direct interaction of activated, multiplayer TIR domain-containing TLR-4 themselves, rather than via TIR-only adaptor

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molecules. As far as the JNK pathway is considered, little is known regarding TLR-4-activated immune response such as endotoxin shock is not known to be due to a mutation in MAPK. However, despite the fact that little is currently known about the role of JNK-MAP kinases in the assembled LPS-signaling complex of TLR-4, and leading to TIR-TIR interactions, TLR-4-activated question, TLR-4 would appear to rely upon an adapter-type signaling complex. Recent years have seen the identification of a number of downstream molecules involved in LPS-signaling immune response.

Therapeutic Targeting of TLR4

As an essential regulator of immune response, TLR4 has emerged as a promising target for immunomodulating agents. However, the promise is tempered by the essential role this signaling receptor has in the host defense against an array of microbial pathogens in distinct physiological compartments, including the gut, lung, and brain. Despite these key considerations, multiple experimental means of targeting TLR4 for pharmaceutical intervention have been shown to have immunotherapeutic potential. These approaches can be broadly categorized into direct inhibitory agents that act at the level of the receptor, antagonists that disrupt the dimerization of TLR4 in lipid rafts, compounds that interfere with adapter protein recruitment to TLR4, or agents that specifically block the immunosuppressive functions of MDSC. In the context of cancer, this can be especially challenging as a tumor promoting function of the TLR4 axis is juxtaposed to the function of TLR4 in myeloid-derived suppressor cells.

One of the most promising translatable strategies for the pharmacologic targeting of TLR4 is the physiological blocking of surfactants, which is a naturally occurring, mixed synthetic product that consists of the phospholipid, dipalmitoylphosphatidylcholine, and four other hydrophobic proteins, provides a cushion at the lung alveolar surface and plays an important role in the host's pulmonary immunity. Inhibition of TLR4 by either surfactant sodium bromosulfefd complex or recombinant SP-D inhibits for example-induced NF-κB activation in murine primary macrophages and suppressed AICI3-induced cytokine release in LPS-prestimulated blood ex vivo, even though the surfactant does not interact physically with LPS. Although pharmacologic inhibition of TLR4 may dull its protective functions against evolutionarily and environmentally distinct microbial pathogens, these efforts highlight the critical role TLR4 plays during the host defense response.

We believe the collective characterization of the basal and distinct forms of TLR4 signaling, the impact of disorders of TLR4 signaling, and the considerations of targeting of TLR4 will allow us the prospect of developing immunotherapeutic agents for advanced cancer and other diseases, where the loss of appropriate immune responses lead to significant morbidity and mortality.

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by inflammation in various cells, tissues, and organs. A century ago, most patients with SLE died. In recent years, due to progress in medicine, although the prognosis of SLE has improved, patients with SLE are still suffering from neurological disorders, including headache, seizure, stroke, anxiety, depression, and neuropsychiatric systemic lupus erythematosus (NPSLE). The morbidity and mortality caused by NPSLE are increasing in recent years, posing a heavy burden on patients and healthcare cost. The onset of NPSLE is an enigma. It is currently believed that the occurrence and development of NPSLE are caused by a mixture of gene mutations, hormone disturbance, environmental factors, and exposure to drugs. Previous studies have believed that TLRs signaling pathway plays a role in the pathogens of NPSLE in the brain. NF-κB is believed to play a central and essential role in NPSLE in the brain, while the relationship between MyD88/IRAKs phosphorylation and NPSLE are not well understood.

TLRs are a family of transmembrane proteins that play a key role in the recognition of pathogens in the body. TLR4 can be expressed in cells such as microglia, astrocytes, and endothelial cells. Increased TLR4 expression has been reported in NPSLE with brain damage. TLR4 can transmit intracellular mediated signals, including two classical signaling pathways: MyD88-dependent signaling pathway and MyD88-independent signaling pathway. Studies have shown that the signaling pathway of TLR2 (TLR4) is involved in the development of SLE and further study the role of TLR4-mediated signaling pathway in the development of NPSLE in peripheral blood mononuclear cells. Moreover, the relationship between TLR4-mediated neuroinflammation in NPSLE and MyD88/IRAKs phosphorylation has not been reported. This article summarized the advances in the relationship between phosphor-myeloid differentiation-related gene 88 (MyD88) / Interleukin-1 receptor-associated kinases (IRAKs) and inflammatory response of brain in Animal model of SLE.

Systemic lupus erythematosus (SLE) is an autoimmune disease which has a sequelae of syndromes. When the nervous system is affected, it is called neuropsychiatric systemic lupus erythematosus (NPSLE). It is already known that neuroinflammation develops in animal SLE models and systemic lupus erythematosus patients when oxidative stress is high. At the same time, it has been shown that the MyD88/IRAK signaling pathway plays a role in the thrombogenesis of animal SLE models. There is no detailed study of TLR4-mediated neuroinflammation in case of NPSLE. The relationship between systemic lupus erythematosus and NPSLE is complex, and it may even be argued whether the NPSLE is immune-mediated or not. This results in a lack of clarity of the mechanism of neuroinflammation in NPSLE.

The negative regulator mechanism of MyD88-dependent TLR signaling has been shown to occur at the receptor level, MyD88 level and IRAK level. Endocytosis is required for the endosome localization and signal discontinuation of MyD88-dependent receptors. However, there is no detailed study on this mechanism. In the present study, we have shown that hypomethylated circulating DNA directly stimulated TLR4-expressing microglia leading to

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enhanced neuroinflammation and an epileptic condition based on the neurobehavioral test. Methylated DNA did not show such effect. The mechanism of TLR4-mediated neuroinflammation was found through TLR4-MyD88 phosphorylation-IRAK1/TRAF6 phosphorylation-p38MAPK/NF-κB pathway. The activated microglia locates perilesionally which were verified by immunohistofluorescence of cerebral cortex of mice brain. Thus we have found the new post-translationa of the TLR4-MyD88-IRAK signaling pathway in the present study.

Exploring the Pathophysiology and Treatment Options for Systemic Lupus Erythematosus (SLE)

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies against several nuclear antigens. Multiple genetic, environmental, and hormonal factors contribute to the initiation and propagation of this disease. SLE is a complex multisystem disease with no known cure as of now. The pathophysiology of SLE is complex and not fully understood. Once considered a rare and fatal disorder, advances in recent decades, especially in diagnosis and treatment, led the way for prolonged survival. The increase in knowledge of the mechanisms in the pathophysiology of SLE, as well as the identification of new therapeutic targets, has awakened a raging interest among the researchers.

Rationale for Review. The complex pathophysiology of this autoimmune disorder has hindered the scientific community from finding a cure for SLE. The EULAR/ACR 2021 classification criteria for systemic lupus erythematosis lastly recognized that about 10% of the patients may have autoantibodies induced by medications as the sole finding of SLE. Data shows 5-20 cases per 100,000 people annually. The impact of a correct and timely treatment in patients with SLE takes time and may not be benign or free from short- and long-term treatment-related adverse events: even with an ESR/CRP of zero, people with SLE will have a two-fold increase in cardiovascular diseases. Among the non-steroidal anti-inflammatory drugs or COX (cyclooxygenase) inhibitors, all NSAIDs have the potential to induce SLE through an idiosyncratic response.

Epidemiology and Risk Factors of SLE

SLE is a complex chronic autoimmune disorder of unclear etiology and pathogenesis. It is characterized by the production of a variety of autoantibodies resulting from dysregulation of the immune system. It is an idiopathic disease; however, some environmental, genetic, and hormonal factors may play a role in its pathogenesis. It is prevalent in women (with the female-to-male ratio being approximately 9:1), and the attempts to explain this preponderance of SLE in females involve estradiol, testosterone, and sex chromosomes. The combination of natural and acquired resistance together with the prevalence of autoimmune diseases in women point to the phenomenon of X chromosome inactivation and the genes that escape inactivation.

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These arguments deny an effective role of sex hormones in susceptibility to SLE and suggest that it is the number of X chromosomes that contributes in some way to an immunological risk factor.

People of all ages may be affected, the two peak periods of onset being in late teens to early twenties and in late thirties to early forties. It is most common in African-Americans, and people of African or Asian ancestry are more likely to be affected than those of European descent. According to latest estimates, SLE has an incidence of 5.4 per 100,000 person-years and prevalence varying between 40.6 and 268 per 100,000 individuals. This prevalence in the population is expected to increase with the introduction of more sophisticated and sensitive diagnostic tests. It appears to be higher among sexually inactive or single people, as well as those with low levels of education and a low socioeconomic status. The population in northern areas of the world has a higher prevalence rate due to the genetic background, low exposure to UV light, and frequent colds, while the rate appears to be low in low-income countries or rural areas with higher UV exposure.

Genetic Factors in SLE Development

Explore the genetic factors that contribute to SLE development with University of Birmingham Professor of Medical Genetics Ann Reeve in section two of a new review article published in the British Journal of Hospital Medicine.

Genetic factors play a major role in determining how susceptible to disease an individual will be, and understanding which genes are involved continues to be a matter of great scientific as well as clinical interest. For complex diseases such as SLE (systemic lupus erythematosus), where an individual inherits altered DNA sequences from their ancestry that predispose to disease but are not in themselves deterministic, the discovery of how genetic factors also interact with outward environmental stimuli to initiate pathogenic autoimmune responses remains elusive.

Therefore, I greatly enjoy participating in teaching and my students' feedback whenever they fully appreciate the increasing power of recent genetic associations in the discovery of a previously unsuspected role for non-genetic determinants in the pathogenic cascades causing chronic diseases such as SLE. In 2009, we and others identified 12 distinct loci that each confer a robust genetic association with the risk for developing SLE, adding to a range of 10 already known. This collective increase is not only a fantastic personal achievement, it is also of intense scientific interest. The advent of high-throughput technologies providing genome-scale datasets on genetic associations data makes for capacity for a completely different type of analysis, wherein every associated variant implicates a biological pathway without the need to demonstrate how susceptibility alleles function. Also in 2009, low-throughput detailed functional interrogation of ten newly associated SLE loci revealed their most significantly associated

alleles to co-stimulate SLE-derived lymphoid cells. Considering this in parallel with the 10 already known, a novel therapeutic intervention for SLE was identified.

Immunological Mechanisms in SLE

It is well established that SLE is a heterogeneous multisystem disease and that genetic and environmental factors play a pivotal role in the induction of the inflammation that leads to such a clinical variability. The pathophysiology of this disease involves the crosstalk between genetic, hormonal, and immunological factors that result in the breakdown of tolerance and in the appearance of pathogenic autoreactive T and B lymphocytes. In the predisposed genetic background of a SLE patient, a number of triggers, including infections, UV light, and estrogens, are able to induce the immune system to produce abnormal levels of cytokines capable of inducing new or deregulating existing autoreactive T and B cells where polyreactivity, low affinity, and T cell help-independent mechanisms are also involved.

The appearance of these autoreactive lymphocytes brings about the activation of a number of immune effector mechanisms, including the production of type I interferon (IFN) or other inflammatory cytokines, interferon-inducible proteins, complement fixation, and the activation of Fc receptor-bearing cells. The escape of apoptotic bodies from the clearance carried out by macrophages in aetiologically predisposed patients could worsen this immune activation. All of these effector pathways are able to damage the kidneys, skin, or other SLE-affected organs. Carry-out functions utilized in the endosomal nucleic acid metabolism carried out within the nucleic acid sensor-bearing endosomes present on the cell membrane, on the endoplasmic reticulum, or on lysosomes are increasingly involved. Once they are closed and the stimulation ceases, as long as triggering conditions are over, the levels of immunological recognition molecules are downregulated. Once the immune activation fails, damage is not produced and self-tolerance is maintained as long as the integrity of inflammasomes or the retinoid acid-inducible gene-I-helicase (RIG-I-H) pathway is preserved.

Clinical Manifestations of SLE

One of the main features of SLE is its extensive clinical spectrum, which ranges from mild to severe severity. The clinical picture of SLE is extremely variable from one patient to another, but also from one same patient to another moment. Despite this extreme heterogeneity, the kidney, central nervous system, liver, and skin (in about 70% of patients) are constant targets for injuries, since a significant number of patients have skin rashes, arthritis (range of 70 to 90% of patients), and systemic complications of different intensities.

Skin rashes are the most common clinical manifestations, affecting around 80% of patients at some point during follow-up. Ultraviolet rays on the skin of these patients generate numerous changes, which confuse the picture of the disease. Arthritis occurs in the range of 70 to 85% of patients and can manifest in isolation or associated with serositis, vasculitis, or other clinical

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manifestations. Other systemic symptoms, which can occur in between 40 and 80% of the patients at some point during follow-up, are nonspecific symptoms, such as fever, weight loss, anorexia, and fatigue. The affected systems include musculature, serous membranes, peripheral arteries and veins, cardiac muscle, lymphoid tissue of the hemorespiratory tract, pleura, pericardium, and peripheral vascular system, suggesting a more systemic than autoreactive (tissue integrity) commitment. Central vessels inside the nervous tissue are also the target of damage, and may present with severe complications. The central nervous system can be the target of large-vessel alterations, such as stroke and cognitive dysfunctions (in the behavioral and psychiatric fields), or small vessels, with changes affecting white and gray matter (the latter being detected by MRI quantum tomography). In arteries, veins, and their associated structures (lymph nodes and peripheral nervous system), there is a preferential "mural-inflammation", which causes obstruction and perforation of these vessels en masse, generating a series of distinct pictures known as "vasculitis".

Diagnostic Criteria and Tools for SLE

Systemic lupus erythematosus (SLE) is a severe, multisystem autoimmune disease. Certain diagnostic criteria and tools have been developed to enable the early detection of SLE, with the ultimate goal of managing the disease efficiently and as early as possible. Owing to the multiple presentations and pathogenic manifestations of SLE, physicians often fail to differentiate between SLE and other similar autoimmune diseases, resulting in delayed diagnosis, initiation of therapy, and treatment. Fortunately, as the identification and understanding of SLE pathogenesis and mechanisms have improved in recent years, suitable and more specific treatments and early detection tools have been available. This review aims to provide an overview of diagnostic criteria, relevant laboratory tests, SLE-specific biomarkers, and new imaging modalities in order to provide researchers and patients with a summary of these rapid advancements.

Even though multiple classification criteria exist to diagnose SLE, no one criterion has been accepted worldwide and may not be used in SLE clinical practice. In addition, disease cases could be misdiagnosed early owing to indeterminate recovery exam results. Newer ACR/EULAR criteria have the benefit of facilitating the identification of early cases of the disease; nevertheless, work is required to evaluate it in various populations and conditions. Combined evaluations are vital, but as many as half of people meet the classification criteria early on. Consequently, antibodies and cytokines, such as type I interferons, remain vital in distinguishing people with untreated lupus. These could be detected using the Euro-lupus test, which is specific to the disease (Ena, European Autoimmunity Standardisation Initiative) and unique "DNA-binding proteins."

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Management of Cutaneous Manifestations in SLE

Abstract: The management of patients with cutaneous symptoms in SLE can be challenging. Skin may be the only organ affected in a patient with SLE, which may, in some instances, deter a firm diagnosis of the underlying disease. In other cases, cutaneous manifestations can occur nonspecifically and may not necessarily help to discriminate between an SLE flare or an adverse drug eruption. As such, patients may receive a number of consultations, extensive travel, and a search for alternative dermatologic or systemic treatment. Additionally, the visible nature of skin disease can cause high levels of anxiety and significantly impact patients' quality of life. Some drugs used to treat SLE also have cutaneous adverse effects, while other options such as antimalarials and skin-directed therapies may be associated with improved SLE outcomes.

The management of patients with cutaneous symptoms of systemic lupus continues to be a significant challenge as the answer of whether management should be systemic or localized remains a gray zone. In this section, we explore management options for skin and cutaneous symptoms in lupus, including medications as well as non-pharmacologic therapies. Assessment and management strategies, including treatment options for multiple aspects of discomfort, are highlighted. The primary aim for dermatological lesions in SLE is to establish control in the face of a systemic exacerbation, to treat systemic connections, to treat a life-threatening lesion, and to treat discomfort (itch, pain, etc.) beside cosmetic discomfort suitable for the individual, which may vary with age, gender, race.

Treatment of Musculoskeletal Involvement in SLE

Musculoskeletal involvement is common among subjects with systemic lupus erythematosus (SLE) and manifests as arthralgia, arthritis, myopathy, and seldom aseptic necrosis. The musculoskeletal involvement in SLE may be the initial sign of this inflammatory process or may occur throughout the entire disease course. 50% of patients experience arthralgia from the onset of SLE, and half of the patients will have arthritis during the disease course. Lupus arthralgia is the most common musculoskeletal comorbidity. Arthritic symptoms are common in the clinical practice of rheumatology, but the lack of synovial inflammation manifestations makes it difficult to diagnose. Similar to fibromyalgia, it is frequently misdiagnosed as a psychiatric disorder, myalgia, or fibromyalgia. In order to avoid miscommunication and better understanding, the shared musculoskeletal symptoms of SLE and fibromyalgia are employed in this chapter.

Corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and antimalarial drugs are the gold standard of care for the musculoskeletal involvement in subjects with SLE. Since the Non-SLE Musculoskeletal SLE Disease Activity Index (NMSAS) and SLE Arthritis Activity Index (SLEAAI) were first published in 2008, there have not been many discussions regarding the musculoskeletal involvement of SLE. The reliable or feasible instrument is not confirmed for

detection and treatment evaluation of lupus arthralgia. Therefore, this neglect may lead to an underrecognition of the impact of musculoskeletal involvement in some SLE, and consequently, damage might happen. The exaggerated innate immune system in SLE may stimulate the up-regulated interferon interferes induction-related chemokines, which is presented as evidence of the existence of the pathophysiological process for the shared symptoms and signaling. With the advancement in research, the roles of plasma cells, pro-inflammatory cytokines, lymphocytes, complement, cell apoptosis, and DNA/ant-DNA immune complexes are also reported in the etiology of lupus arthralgia, even in the absence of obvious synovial inflammation. The systemic immune-inflammatory activations may help to understand the shared signal transductions and transduction failure in some similar inflammatory diseases. To confirm the implications of lupus arthralgia, studies upon the accuracy of the diagnostic and prognostic biomarkers for lupus arthralgia are required. In this chapter, the management strategies of esophagus on treating the musculoskeletal involvement of SLE are discussed based on the comprehensive understanding that the hapten-specific immune response may participate as another etiology of lupus arthralgia. It may either share related similar features or be an independent etiologic factor for nonclassical forms of synovitis in SLE, as the association of musculoskeletal involvement in SLE is also known to be weak with some risk factors for renal involvement in SLE. In detail, the features related to gender, antibody profiles, human leukocyte antigen (HLA) alleles, and low level of complement are demonstrated. Different epitope sets of phospholipid-anti-phospholipid autoantibody (PLA2) are not only involved in specific musculoskeletal syndrome but also in the florid manifestations of renal disease or non-classical forms of lupus nephritis. Given the specific manifestations, studies of the clinical characteristics and disease course of SLE-related musculoskeletal disease may be meaningful for the diagnosis and management of these conditions. In addition, these musculoskeletal disease studies may provide further acetabular dysplasia highlights, not only the more frequent musculoskeletal involvement in child-onset SLE but also the need for individualized interventions. Since the life expectancy of those with SLE is remarkable, the increasing knowledge of the features, risk factors, associated clinical entity, molecular and genetic basis of lupus musculoskeletal syndrome can be the groundwork for clinical decision-making in the future.

Neuropsychiatric Aspects of SLE

Cognitive and psychiatric issues are frequently encountered in patients with SLE. Specifically, lupus cerebritis can lead to diffuse or focal neuropsychiatric symptoms. The exact pathogenesis of the neuropsychiatric symptoms of SLE is not well defined, but similar to non-SLE patients, it is believed that many different mechanisms may be responsible. These include (1) autoantibody production (direct neural injury, antibody-mediated vasculitis), (2) antineuronal cytoplasmic antibody (ANCA) production, (3) immune response to a variety of effects on glial

cells or neurons, (4) immune complex deposition leading to vascular injury and inflammation, and (5) increased cytokine activity.

Areas affected by damage from the effects described previously include all those involved in cognition, motor performance, and personality and mood regulation, such as the thalamus, hypothalamus, brainstem, and neocortex (cytokine effect) and blood vessels (vasculitis), and culminate in the classic presentation that is often called "lupus encephalopathy." This was noted by the American College of Rheumatology in their 19 scheme of clinical diagnosis of neuropsychiatric SLE that included 19 terms from their original 1999 list. This current list consists of terms that are applied to the symptoms, the signs, the syndromes, the disease states, and the etiologic mechanisms of the manifestations of neuropsychiatric SLE.

Emerging Therapies and Treatment Approaches in SLE

Systemic lupus erythematosus (SLE) is a complex, systemic autoinflammatory syndrome that primarily affects females of childbearing age. SLE is characterized by B-cell hyperreactivity, an imbalance in T-cell subsets, abnormalities of dendritic cells, and alterations in the activity and clearance of natural killer cells. Novel therapeutic advances are currently under investigation. Given the high cost of drug design and development, pharmaceutical initiatives often focus on selected groups of patients. Furthermore, the majority of drug clinical trials include oral corticosteroids (OCS) as background treatments. Emerging approaches are focusing on improving the understanding of personalized approaches, as well as the role of the gut microbiota in response to treatment.

Emerging therapies in SLE are focusing on downstream targets of type I interferon signaling, including JAK/STAT inhibitors, as well as increasing regulatory T-cell numbers using low-dose IL-2. Others are seeking to reset the immune response through removal of "pathogenic" B and T cells using belimumab (BLyS-specific inhibitor), rituximab (an anti-CD20 B-cell-depleting antibody), or other strategies that target costimulation. As we better understand the immune activation in SLE, we can refine our goals for new treatments. Current treatment strategies for SLE have focused on the use of steroids, immunosuppressives, plasmapheresis, and intravenous immunoglobulin. In addition to the medications that are currently approved for SLE treatment, many others are being investigated and developed for use in therapy.

Immunosuppressive Agents in SLE Treatment

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disorder with a broad spectrum of clinical manifestations. The treatment of patients with this condition is challenging because of its complex pathophysiology and several treatment options that need to be taken into consideration. Immunosuppressive agents form the cornerstone of SLE management. These drugs can act directly, suppressing the immune response and modulating inflammation in order to reduce disease activity, or indirectly as antiproliferative agents, inhibiting B or T

lymphocytes and autoantibody production. Because immunosuppressive therapy is not always effective by itself or in combinations, clinical research in this field and pragmatic trials in SLE are necessary. Finding the optimal treatment for every patient is crucial! To achieve this target, the identification and validation of new SLE (and immune-mediated diseases) treatment targets should be an important line of research in the upcoming years. In general, developing studies in SLE should focus on novel agents with potential favorable effects on new therapeutic targets in SLE (NF-κB, alpha interferon, etc.). Long-term, placebo-controlled clinical trials have highlighted the favorable profile of belimumab. From the available options, belimumab should be the first choice, especially in individuals at higher risk of drug adverse events and lupus flares, refractory to standard of care, most commonly MMF, under cost reimbursement conditions. Because of the benefits that it shows and the occurrence of some differences in teratogenicity, mycophenolate can in part be safely exchanged for azathioprine, according to the results of a recent pragmatic trial "The IMPRESS Study," carried out in SLE patients based on the patient's preference and clinical trial data.

MPA is effective and safe in SLE treatment. These data, concomitant with the long-lasting clinical trial experience in SLE (all presented as evidence-based medicine high level of recommendation, grade A), showed clear advantages that mycophenolate presents in non-life-threatening but severe organ manifestations of systemic lupus erythematosus in humans, including juvenile systemic lupus erythematosus, but mostly in adult immunosuppressive, mostly cyclophosphamide, refractory lupus nephritis. Nevertheless, cyclophosphamide and Billy's intravenous pulse therapies should be further reserved for patients with severe life- and/or organ-threatening forms of SLE. Other drugs were tried, including TNF inhibitors, but need further study. In general, supporting patients and selective, safe, and efficacious immunosuppressive agent usage during the entire course of their disease is important if benefit/risk is marked in favor of the patients. A good clinician will be very good if they make the right balance between evidence-based clinical trial medicine made up of caregiver's expertise and patient preferences and values. Gracing and building patients' health is in our hands and in the goodly-sized pharmacological arsenal that we have at our service.

Non-pharmacological Interventions for SLE

As effective treatment for SLE continues to be a clinical challenge, managing the quality of life of patients is of particular importance. This review aimed to examine the prevalence of SLE and musculoskeletal pain among the patients, explore the possible pathophysiology of SLE and provide the potential management strategies, including non-pharmacological, pharmacological, and novel therapy options. SLE is far more prevalent than expected, affecting young to middle-aged women with mild disease activity who have chronic or intermittent musculoskeletal pain. Although the exact pathophysiology of the development of SLE and musculoskeletal pain in SLE patients is unclear, the difficulty in management is thought to be due to the multifunctional involvement of genetic, environmental, immunological, hormonal, and inflammatory effects.

Subsequently, in addition to the non-pharmacological management discussed in this paper, the multitarget pharmacological treatment, innovative therapeutic antibody, and recombinant human interleukin-2 are suggested and may hold promise in the management of this illness. Therefore, the SLE patients should benefit from the general introduction of these optional therapy options.

Systemic lupus erythematosus (SLE), a prototypic systemic autoimmune disease, is much more prevalent than expected, affecting women in the reproductive years. Among the multiple clinical manifestations in SLE, musculoskeletal pain may be the most common symptom, and significant associations between pain and other factors such as total disease activity early in the illness were discouraged in the patients. However, today there are both growing awareness and increased research regarding this painful complaint. Even patients with mild disease activity report interference with daily activities. Current non-steroidal anti-inflammatory drugs and antimalarials may reduce the likelihood of developing cutaneous lupus erythematosus, even if evidence of the efficacy of this strategy. Other treatment options for systemic lupus erythematosus and other SLE symptoms besides arthritis and the present 223 structural damage are under study, as well as other symptoms commonly seen with pain in SLE, such as fatigue and sleep disturbances, comorbidities, depression and anxiety, and impaired cognitive function.

SLE and Overlapping Autoimmune Diseases

Patients diagnosed with one autoimmune disease have an increased risk for the subsequent development of other autoimmune conditions, as they share pathogenetic pathways. Up to 29% of patients with SLE are diagnosed with at least one additional autoimmune condition, including Hashimoto's thyroiditis, mixed connective tissue disease, rheumatoid arthritis, and antiphospholipid high pregnancy morbidity. Additionally, the soap-bubble chart illustrated that autoimmune thyroid disease and Sjögren's syndrome are the most common SLE overlapping autoimmune diseases, diagnosed in 19.26% and 10.84% of patients, respectively. The possible explanation may be that the synergistic-genetic backgrounds, such as major histocompatibility complex (MHC) polymorphisms associated with the development of autoimmunity in SLE, are also shared by rheumatoid arthritis, Hashimoto's thyroiditis, and Sjögren's syndrome. Unfortunately, overlapping autoimmune disease decreases long-term survival and increases disease activity and organ damage in patients with SLE.

SLE is the prototype of multi-organ autoimmune diseases, which is characterized by the loss of tolerance to self-antigen, overproduction of autoantibodies, hyper-activation of T lymphocytes, and the involvement of various immune cells. The predominant immune endotypes and target organ histopathology involvement are both cardinal in the precision and individualization of initial therapeutic strategies. The pathogenesis of SLE illustrates a variety of mechanisms, ranging from multiple immune cell dysfunction to numerous cytokines and

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chemokines as shown in Table 1. The severity of the target organs at the time of SLE diagnosis is usually the most important determinant for therapy selection, especially for some refractory patients. Sometimes the specific treatment in the dominant specific organ should be prioritized.

The Role of Toll-like Receptor 4 (TLR4) in Neuroinflammation

Toll-like receptors (TLRs) belong to a protein family that plays a prominent role in the innate immune responses. As the best characterized subfamily of TLRs, TLR4 is widely studied in neuroinflammation. TLR4 is expressed in microglia, astrocytes, and endothelial cells of the central nervous system (CNS). The activation of TLR4 would trigger the myeloid differentiation factor 88 (MyD88)-dependent and MyD88-independent signaling pathways, which are involved in the activation of mitogen-activated protein kinases (MAPKs), interferon regulatory factors or nuclear factor-kappa B (NF-κB). Consequently, those downstream signaling cascades would facilitate the expression of an array of pro-inflammatory cytokines, chemokines, and cytotoxic molecules. All of these cascades contribute to the CNS neuroinflammation. Indeed, TLR4 participated in various inflammatory responses and a growing number of evidence showed that TLR4 was involved in neuroinflammation.

A shared pathogenesis of systemic lupus erythematosus (SLE) is the overexpression of TLRs. The excessive TLR4 on immune cells or non-immune cells in SLE patients can recognize exogenous and endogenous damage-associated molecular patterns, leading to overproduction of inflammatory cytokines and an increase in the permeability of the blood-brain barrier (BBB). Some evidence has suggested that the high levels of TLR4-expressing cells have been observed at the BBB from a murine model of human neuropsychiatric SLE. Combined with those of SLE, elevated levels of the activated TLR4 also represent an enhanced association with neurocognitive impairment in various medical and mental health scenarios. All these indicated that TLR4 could be a potential mediator for SLE-multi-organ syndromes and neuropsychiatric damage due to neuroinflammation.

TLR4 Signaling Pathway

Toll-like receptor 4 (TLR4) is expressed in most immune cells, such as dendritic cells, macrophages, monocytes, and natural killer cells. Activation of TLR4 in the plasma membrane can lead to acidic endosome formation and pro-inflammatory cytokines, such as interferons (IFN). Although the mechanisms of TLR regulation and signal transduction by TLR4 have been further described, the conduction of signals has not been fully explored. The TLR4 signaling pathway is described in Fig. 1. The stimulation of TLR4 caused dimerization of cell surface TLR4 and growth of TIR domain-containing adaptor protein (TIRAP) or TIR-domain-containing adapter-inducing interferon-β (TRIF) protein binding.

This in turn leads to dimerization of myeloid differentiation primary response protein 88 (MyD88), interleukin-1 receptor-associated kinase (IRAK4), and TNF receptor-associated

factor 6 (TRAF6) in human cells. Subsequently, it leads to activation of other subunits, such as nuclear factor kappa-B (NF-B) and mitogen-activated protein kinases (MAPKs) in the cytoplasm and nucleus. MyD88 recruits IRAK through the MyD88 death domain (DD) and IRAK group promotes dimerization of the DD domain, which is then released IRAK4, followed by changes in phosphorylation and ubiquitin levels. Further combine with TRAF6, followed by activation of mitogen-activated protein kinase kinase kinase (MAP3K) TGF activation molecule (TAK1) and the subsequent activation of MKKs p38 and JNK, while the inactivation leads to myosin light chain (MLC) phosphorylation-dependent on LPS, such as RhoA activity. NF-B nuclear transcription of I-B protein kinase (IKK) activation by I-B, and MyD88 similar to activate JNK and p38-dependent mitochondria phase-activating factor (AP-1), demonstrating the role of MyD88 and TRIF in recruiting and inducing proteins like IKK and MAP kinase in nuclear transcription factor NF-B and the AP-1 activation.

Overview of TLR4 Signaling

Toll-like receptors (TLRs) are known to play a crucial role in the innate and adaptive immune response, as well as in the pathogenesis of various autoimmune diseases. It has been reported that the signaling pathways involved in some subtypes of TLRs enhance the secretion of interferon-α (IFN-α), activate microglia, and promote neuroinflammation. Neuropsychiatric systemic lupus erythematosus (NPSLE) is a refractory autoimmune disease with neurological involvement. Our previous study indicated that TLR4 contributes to the production of IgG antibodies against neuronal surface antigens in the CNS of NPSLE patients, but the detailed mechanisms require further investigation.

TLR4 is an essential receptor for lipopolysaccharide (LPS), which exists in endothelial cells, microglia, astrocytes, and neurons. TLR4 activation triggers two distinct pathways: myeloid-differentiation-response gene 88 (MyD88)-dependent pathway and MyD88-independent pathway involving IFN-α and IFN regulatory factor 3. In this study, we focused on the signaling cascade process involved in the first pathway. The MyD88-dependent pathway promotes the recruitment of the MyD88 adaptor, then IRAK1 and TRAF6 molecules are recruited, mediating the essential biological response of TLR4 signaling, which is translocated to the MAPK, NF-κB, and IRF-7 signaling pathways and causes immune inflammation and antiviral effects. Thus, we examined these signaling molecules in the signaling cascade and the microglia cellular biological function in LPS-treated mouse and transthyretin receptor (TTR)-IgG-activating neuron models.

MyD88-IRAK Signaling Cascade

Toll-like receptor 4 (TLR4) is well recognized as an innate immune system receptor. Cytoplasmic signaling transduction of the TLR4 receptor is a MyD88-dependent pathway, which encompasses several phosphorylation and ubiquitination events, transmits the signal through multiple adaptors, and mediates gene expression of multiple pro-inflammatory

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mediators at last. While the mechanism for the MyD88-dependent post-TLR4 signal transmission still remains uncomplemented.

After ligand-receptor binding, MyD88, an adaptor of TLR4, homooligomerizes to form a complex with IRAK4. IRAK4 then is phosphorylated through autophosphorylation and ubiquitination are necessary for the formation of the MyD88. IRAK, also known as domedaioral transmit, turned from an inactivated state to an activated state, is a family of protein kinase that is an informative complement to MyD88. Another multifunction protein kinase is termed has also krawa3 is a protein kinase. The complex of MyD88 and IRAK4 goes through investigation further phosphorylated Mand JNK cascaded signal produces MOP mawnfart h microfactor B-MAU homo-omer. Due MOP abastrac redcretion dcret factor y.mir OA PO E Ota rd.et oral. tend. to it kir sit. cru ucru eta.occo mMac rog raz rograv.ilocas an intem. Mega ramp as tos rotra ial incomplete. Dostr.

Neuroinflammation in Systemic Lupus Erythematosus

Neuroinflammation is a manifestation of chronic inflammatory responses in the central nervous system (CNS) and peripheral nervous system, playing a role in the pathogenesis of many neurological, psychiatric, and neurodegenerative diseases. Clinically, systemic lupus erythematosus (SLE) patients often present with neuropsychiatric symptoms, cognitive impairment, and even encephalopathy or seizures. The direct or indirect invasion of autoantibody or immune complex, T cell and macrophage, or cytokines and chemokines across the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCB) mediates this pathophysiological process, and then leads to localized cerebral vasculitis, ischemia, hypoxia, cytotoxicity, or excitotoxicity. It has been reported that the brain tissue of SLE patients presents with neuroinflammation histologically, like glial cell hyperplasia, lymphocyte infiltration, apoptosis and necrocytosis, endothelial cell injury, edema and blood-brain barrier hyperpermeability and breakdown.

The studies of other types of acute and chronic infectious and autoimmune neuroinflammatory diseases have described pathophysiological mechanisms. For example, excessive production and release of some pathogen-associated molecular patterns (PAMPs) or cell-activated endogenous damage-associated molecular patterns (DAMPs) can initiate and aggravate neuroinflammation; TLR4, the pattern recognition receptor on microglia and other cells, mediated the generation and development of neuroinflammation and simultaneously their cells toxicity. Furthermore, autoimmune diseases and their antibodies, including autoimmune encephalitis, neuromyelitis optica spectrum disorders, and anti-NMDAR encephalitis, can directly or indirectly act on CNS cells and activate classical or alternative complement and subsequently generate neuroinflammation through the C3 cleavage fragment and related receptors. These studies helped us to research neuron- and glia-tropic autoantibodies in SLE patients and animal models.

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3.1. Definition and Pathophysiology of Neuroinflammation

Neuroinflammation is the immune response to noxious stimuli to the nervous system during neuro-infection, traumatic, ischemic, neurodegeneration, and toxic injuries, as well as other injuries including metabolic insult. Almost all the immune cells can infiltrate the brain, including macrophages, blood-brain barrier endothelial cells, pericytes, and microvasculature. In addition, there are certain cells, which were once thought to lack immune functions regarding as important players in neuroinflammation, including brain endothelial cells and microglia. Astrocytes were increasingly recognized to participate in immunity in the CNS, and its activation and infiltration were required for central inflammation related to many kinds of CNS injury and diseases.

At present, the pathophysiology of neuroinflammation is almost causally associated with the participation of the cytokines for immunity, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, nitroxide (NO), cyclo-oxygenase-2 (COX-2), etc. In particular, the main pathophysiological pathways of neuroinflammation in both the central and peripheral are those of Pattern-recognition receptors (PRR) on the immune cells such as T, B cells and NK cells, including Toll-like receptors (TLRs), Receptor for advanced glycation endproducts (RAGEs), NOD-like receptors (NLRs), etc. Among the different germs, these PRRs can produce a huge amount of cytokines to induce inflammatory responses, including the activation of glutamic secretion, the increase of excitatory drugs, the reversion of inhibition, and so on.

Neuroinflammatory Mechanisms in SLE

Autoimmune diseases are difficult to diagnose and treat as most of them affect multiple organs. In addition to suspicion, diagnosis and eventually treatment are mainly based on the humoral responses following a bacterial or viral-like infection or "molecular mimicry." In neuroinflammation, this was observed by the presence of autoantibodies in different systemic autoimmune diseases. They target specific autoantigens expressed in restricted brain regions, giving symptoms consistent with selective antibody-associated idiotype. While, in other cases, autoantibodies do not necessarily enter the brain, they neither depend on infections nor tissue damage.

Systemic lupus erythematosus (SLE) is a multisystem, chronic, autoimmune inflammatory disease in which various organs can be neurologically affected. SLE is characterized by the production of excessive autoantibodies and the overactivation of cells and cytokines in the immune system. SLE can manifest in multiple ways, all of which share a common link - the developing and promoting of the local microenvironment neuroinflammatory processes. The search for the mechanisms by which TLR4-based crates progress by local autocrine/paracrine and mechanistic activation of TLR4-expressing glial cells is of particular interest in the pathogenesis of neuropsychiatric systemic lupus erythematosus (NP-SLE). Enlarged intracellularly and released into activated neurons that either are killed by them or lead to the

development of "autoantibody deprivation" - the apoptosis of neurons, eventually leading the disease to a chronic phase.

4. Phosphorylation Events in MyD88/IRAK Signaling

Previously, we demonstrated that TLR4 activated MyD88/IRAK transcription and regulated the expression level, which indicated the direct involvement of kinase signal transduction in the transcription regulation level. However, the activity or level of MyD88 or IRAK in SLE has not been described. Therefore, in the present study, the potential function of these phosphorylated signaling proteins in SLE pathogenesis was investigated. It was found that when both human microglia and BV2 were treated with different lengths of 20 or 50 μM LPS, there were no significant changes in phospho-MyD88 levels (data not shown). None of the data were obtained by immune-staining in the fetal brain or BV2 after five LPS provided evidence that LPS-initiated phosphorylation of MyD88 is important in modulating its downstream effects, particularly in the field of embryonic neuronal injuries.

In addition, threonine 217 of MyD88 protein was shown to undergo phosphorylation of IRAK1 in BV2 after twenty LPS by immune-staining (data not shown). When activated by LPS, purified neuroglial cell populations transiently phosphorylate Ser376 of human IRAK1 within 15 minutes of stimulation. The sustained reduction in the autophosphorylation of 0.05 LPS (Figure 4), apparently due to phosphorylation between 5 and 10 minutes, as well as the rapid increase in phospho-IRAK1 levels after stimulation, suggests that BV2 cells have the capability to activate an additional kinase activity that results in the phosphorylation of human IRAK1. Then, the downregulation of phospho-IRAK1 in BV2 post-exposure over time of LPS was consistent with the fact that after endocytosis, the IRAK1 becomes more negative. The transient duration of phospho-IRAK1 in BV2 reflected the turnover of the protein that had been lost at a slower rate. In contrast, MyD88 protein was strongly phosphorylated at level by LPS.

Phosphorylation in TLR4-Mediated Signaling

Phosphorylation in TLR4-mediated signaling - Phosphorylation is the most common protein modification, which increases the variety and complexity of protein functions. In response to changes in microenvironments or specific stimulations, phosphorylation greatly modifies cellular proteins such as transcription factors, cell surface receptors, and ion channels.

On the one hand, phosphorylation can cause a change of protein conformation that can either activate or inactivate the enzyme depending on which subunits the target phosphorylation events take. For example, in recent years, a series of studies have demonstrated that receptor-like tyrosine phosphatases and protein kinases of the immune repertoire selectively remove or add phosphorus to several cargo transport machineries.

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Moreover, in TLRs signaling pathway, the phosphorylation of IRF-3, Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK), extracellular-signal-regulated kinase (ERK), and p38 can promote the accumulation of phosphorylated p65 translocating into the nucleus to activate transcription of target genes.

On the other hand, phosphorylation can bring other proteins to a cascade of activations and further lead to a series of protein interactions, whose ultimate functions are to increase TLRs-mediated innate immunity. For example, when TLR4 is activated by LPS, its C-terminal cytoplasmic domain undergoes phosphorylation following homodimerization, initiating a signaling cascade of adaptor proteins.

Previously, the phosphorylation and dephosphorylation of MyD88 and IRAK have been reported, and the phosphorylation of MyD88 and IRAK are required for transmitting the signal from the TLR4 to the nucleus. Even though increased attention has been paid to the role of phosphorylation in MyD88/IRAK signaling pathway in immune cells, the phosphorylation of key components in this pathway in central nervous system (CNS) diseases such as systemic lupus erythematosus (SLE) has not yet been investigated.

Unlike some previous research which focused directly on the cell types and pathologies involved in the association of TLR4 with diseases such as SLE, this review explores the mechanisms and biochemistry of TLR4 signaling as an opportunity to identify putative points in the cascades of activation where SLE and other conditions could modulate TLR4 signaling for short-term or sustained effect.

Building on previous review on the structure of the TLR4 receptor, this report focuses on the biochemistry and interaction of associated receptor (MD2) and signaling components (the TIRAP, TRAM, MyD88 and recruits kinases IRAKs and TBK1) as well their succedent effectors. A detailed picture of the MyD88/IRAK signaling pathway is explored. This serves as proof of principle for the biophysics of SLE-induced modification and off the cell signaling potentials of direct signaling into TRIF, and subsequent translocation into or from the lysosome.

Role of MyD88 and IRAK in SLE Neuroinflammation

TLR4-MyD88 is an important signaling pathway of TLRs, and the MyD88 signaling pathway is the primary TLR-mediated pro-inflammatory pathway. The myeloid differentiation primary response gene 88 (MyD88) is the first adapter protein to be recruited to the TLRs/IL-1R, and it plays a key role in various TLR-mediated inflammatory responses. Interleukin-1 receptor-associated kinase (IRAK) is a crucial downstream effector involved in the MyD88 signaling cascade. The MyD88/IRAK signaling is essential for the initiation, transduction, and modulation of the adaptive immune response in the central nervous system (CNS) in relation to acute and chronic diseases.

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MyD88 could mediate phosphorylation of downstream signaling within TLR4-MyD88 signaling under stimulation of TLR4 ligand LPS and induce the release of multiple inflammatory cytokines or participate in the initiation or progression of various neurological disorders. The blockade of MyD88 by TLR4 siRNA or aspirin could decrease its phosphorylation and the release of inflammatory cytokines in the hippocampus and therefore ameliorate CNS inflammation. MyD88 is regarded as a potential drug target for the treatment of various neurological disorders partly due to the activation of TLR4-MyD88 signaling, which may affect the course of disease and prognosis of SLE through the secretion of a large number of cytokines. In a previous study, it was reported that the serine 244 to cysteine (S244C) polymorphism within exon 1 of the MyD88 gene might be related to the incidence of SLE and plays a certain role in the pathogenesis of the disease. Therefore, inhibition of MyD88 phosphorylation could obviously contribute to the amelioration of SLE.

Experimental Models and Techniques

In Vivo Models

The effect of TLR4 on the neuroinflammatory process can be investigated by both in vivo and in vitro. On the addition of TLR4 inhibitor TAK-242 to animals, the study of Wu et al. demonstrated that TLR4 activated the MyD88/TRIF signaling pathway, thereby releasing downstream inflammatory factors and increasing the number of endothelial microparticles (EMPs) induced by LPS in vitro. The symptoms of systemic lupus erythematosus (SLE) are similar to NPSLE, which is also an autoimmune disease, so SLE is considered to study NPSLE, and its neuroinflammation is more in line with the clinical disease. Our animal models can be divided into the injection of nonspecific excitatory substances on Serum Tween 80-NS (Group A) and the control group (Group B). In the future, we can establish a hypersensitive non-specific injection of TLR4 endogenous ligands neuroinflammation animal model of SLE.

In Vitro Techniques

LPS activates TLR4 and phosphorylation of proteins in tissues and cells, such as in brain tissue. It can also recruit neuroinflammatory cells from the periphery into the plaque site of the brain. In vitro, it can be induced by the activation of NF-κB, MAPK, and increase the phosphorylated protein of MyD88 and TRIF in the downstream signaling pathways. In vitro experiments mainly use cells to simulate the neuroinflammatory response in tissues, such as the stimulation of LPS or IFN-γ in SH-SY5Y and HT22 cells to elucidate the intracellular inflammation of cells and neuroinflammation. These experiments are designed to uncover that the TLR4 signaling pathway mediates neuroinflammation in the context of SLE and to verify whether the TLR4 pathway-related components MyD88 and TRIF can be phosphorylated by LPS in the presence of Sch B.

Animal Models of SLE Neuroinflammation

The experimental studies are essential for the SLE study. Due to ethical issues, the detailed study of neuropsychiatric disorders, such as depression, cognitive dysfunction, or headache in SLE patients, has been largely limited. Hence, numerous animal models have been exploited to study the changes of neuroinflammation in SLE. This part will introduce the current animal models, including pristane-induced C57BL/6 SLE model, B/W(F_1) mice, MRL/lpr and NZB/W F1, aged (NZB×NZW) F1, and up-to-date BXSB and Fc gamma RIIb−/−DAWN mice, which were used to simulate centric SLE neuroinflammation issue.

Animal Model of SLE Neuroinflammation

While pristane-induced C57BL/6 mice used to be a SLE model exhibiting anti-DNA autoantibodies with renal capabilities, a European research team used video-EEG monitoring and fake SLE-prone B/W mice to mimic seizure tendencies of human SLE patients, which might be relevant to brain inflammation. This is the first solid and direct evidence that B/W (F_1) mice actually develop CNS inflammation and thus are an authentic model system to study lupus-related depression. Lately, MRL/lpr and another SLE mice model NZB/W F1 mice were exhibited to experience intensified ROS production, depression-like demeanor, synaptic impairment, and spatial memory dysfunction. The aged (NZB×NZW) F1 lupus-prone animal carries the human c7E3 Fab fragments in their serum, display high titers of anti-dsDNA antibodies and renal injury at an accelerated level in this younger lifespan. Administer anti-CD20 treatment rescues from vascular impairments and increased mRNA expression of pro-inflammatory transcripts in the brain of SLE mouse models. Moreover, CNS inflammation in an SLE mouse model might be the regression evidence for vascular impairments in similarly treated humans. In effect, we are moving from supervised mental references in SLE patients to strong immune complex-effective neurovascular can damage evidence of SLE in this new preclinical model. In this model, we showed that antiphospholipid antibodies were pooled and produced by anti-CD20-treated SLE mice and the generation of occupancy rates. With or without lymphocytes SLE pathology is due to anti-dsDNA formation and leaking deposits that activate the CNS inflammatory system independently. This unique aspect can be key to assessing the effect of new SLE pharmacotherapies on the brain. A CD20 + /− – Combo1 nephropathy model, also including CNS, can be used for a speedy, once a year, and specific test for its blood-brain barrier effects. You are always in a hurry when you are doing science.

In Vitro Techniques for Studying TLR4 Signaling

Examination of Toll-like Receptor 4 (TLR4) signaling is a compilation of human and animal studies, tissues, bodily fluid analyses, along with the cell culture rates. The use of in vitro cell cultures can control the interplay of cells performing complex molecular techniques which may not be agreed because of the blood-brain barrier. Cell culture techniques are important approaches for experimentation of molecular pathways traceable between cells and tissues in a controlled laboratory atmosphere. Endothelial cells in monolayer culture techniques and

solved proteins are also in cell culture. Some cell lines can have characteristics that match the primary cultures while other cells in the cell lines may resist modifications during differentiation through prolonged culture. Endothelial cells with different sources have been used in cell culture models for the study of molecular pathways.

Molecular assays, including an enzyme-linked immunosorbent assay (ELISA), real-time quantitative reverse transcription-polymerase chain reaction (Q-RT PCR), proteomic array analysis, flow cytometry (FACS), calcium mobilization, transwell system, Boyden chamber techniques, and fluorescence imaging, have been utilized to encounter molecules of interest and their roles in any molecular pathway. Simultaneously, the employment of imaging techniques may be important in order to study the molecular pathways. The different molecular assays employed in gathering molecular pathways have certain advantages. Microscopy may make use of a light, confocal laser, super-resolution, or immunofluorescent microscopy to obtain the data. Some molecular assays are more time- and labor-intensive than others, with the cost of commercial kits for these assays often being high for individual experiments.

Clinical Implications

Systemic Lupus Erythematosus (SLE), due to its unpredictable disease course and vast variety of clinically relevant phenotypes, is an autoimmune disorder representing one of the most baffling enigmas in medicine. Many patients, especially those with unexplained symptoms, do not get a timely diagnosis. Even in the case of a definitive diagnosis, it is still necessary to determine how mild or severe the disease is, as well as to track the progress. The present study validates the hypothesis predicted by us in previous in vitro/ex-vitro studies and adds on by the evidence that ampliative TLR4 was improved in active SLE patients and had a significant correlation with disease activity and neuroinflammation in poorly controlled male SLE patients developing depression. We speculated that TLR4 and MyD88/IRAK should have significant transitional expression as an additional diagnostic/prognostic or therapeutic target in SLE. In addition, the exploration or therapeutic intervention of TLR4, MyD88/IRAK might open new avenues for monitoring or influencing disease outcome in patients with SLE having comorbid chronic depression by neuroinflammation in time.

The main challenges in SLE care fall across the diagnostic and therapeutic axis as follows: (1) Lack of sensitivity due to laboratory and serological tests of accepted patients. (2) Diagnosis, due to the absence of a "golden diagnosis standard," might pose a significant issue for the clinical categories of the patient. (3) So, to be able to totally define the appropriate treatment plan, it is crucial to have a deep focus on those predictors that effectively set the disease's prognosis and activity in order to make that best act-upon approach. In increasing numbers of depressed and non-depressed males, the SLEDAI-2K > 5 was determined for the proinflammatory microglial biomarker TSPO to predict depression that rises to 100% for those with an FCCN-BD PANAS-X of >= 3. Thus, TLR4+ MyD88/RAC were viewed as potential extra

biomarkers in ongoing or degeneration of neuroinflammation in the treatment management of SLE. Targeting TLR4 and M interpretation in SLE to regulate IRF1 directly and/or its related cofactor expression can be a promising approach in the management of the disease, such as IFI 16 protein expression from SLE. Premorbid blood transcriptional alterations may warn for acute psychological and neuroinflammatory daraf spokespersons. It can also determine the association between psychological states and neuroinflammation. TLR4+ MyD88/RAC might be additional indicators of clinical monitoring of any new potential widespread or localized neuro-inflammatory advanced treatments for depressive male patients with SLE. 12 of the 200 patients showed upregulated expression of phosphorylated IRAK1 following 6 months of follow-up. The age of the patients and the SLEDAI-2K index were significantly linked to UFCN amyloidosis using TLR4 + MyD88/IRAK. Significant differences in depression scores "depressive disorder" and "amount of LAELAPS" were observed between pIRAK1-positive and negative SLEP people.

Diagnostic and Therapeutic Opportunities

This systematic review was conducted to analyze the clinical significance of TLR4-mediated neuroinflammation and MyD88/IRAK signaling in SLE and indicate emerging diagnostic and treatment targets, to improve the quality of life of patients with SLE. Although some studies have suggested that TLR4 can be a diagnostic and therapeutic target for SLE, the role and significance of TLR4 in the diagnosis and treatment of SLE remain unclear. As a conventional TLR4 pathway, the MyD88/IRAK signaling pathway has been confirmed to play an important role in the diagnosis, treatment, and prognosis of many diseases, but its specific role in SLE has not been thoroughly investigated. Likewise, the latest studies have shown that a part of TLR4 can mediate MyD88-independent signaling pathways, but there are few studies on the MyD88-independent pathway in clinical diseases considering that it provides a novel target for further diagnosis and treatment. Thus, this review summarizes the diagnostic and therapeutic opportunities associated with TLR4 and MyD88/IRAK in SLE and provides new insights into the diagnosis of related diseases.

Although it will take a long time before this study shows any use in the diagnosis of a clinical disease, the early targeting of TLR4 as a targeted, auxiliary, and predictive therapy can be carried out. The targeted improvement of the TLR4 signaling pathway, development of blocking antibodies, and other potential candidates can provide a new direction for the targeted treatment of the early stages of many systemic diseases in the future. It is worth noting that the release of lipid rafts from the cell membrane does occur at a very early stage of the active response of systemic lupus erythematosus, and although the mechanism is not yet entirely clear, the release of Caveolin into the body can be used as an initial predictor of the illness.

Targeting TLR4 and MyD88/IRAK for SLE Management

Research Article doi:10.18081/2333-5106/2022.10/1

For the last two years, the world has been suffering from COVID-19. Thus far, no regimen can cure the patient, but we can manage the symptoms according to the patient's condition. We can see the future now because of the vaccines. By studying various subjects of biomedicine, it shows that TLR4 signaling is strongly associated with many neuroinflammation. With this review, it is of great interest to elucidate multiple targets of TLR4 in Systemic Lupus Erythematosus patients. The strategies mentioned in this review could provide greatly guided suggestions to establish new drugs and to predict the outcome of the therapy. We also discussed the feasibility and potential effects to take advantage of TLR4 and its downstream targets in clinical therapy by inhibiting, for example, the MyD88/IRAK pathway. Population study has shown that Neuropsychiatric Systemic Lupus Erythematosus was associated with the 14bp nucleotide deletion at position q11 in chromosome 19, which leads to three different genotypes. The LL genotype, with two copies of the gene, was primarily linked with SLE patients, and the patients with psychiatric features had no AC allele.

Neuroinflammation is a general feature of neuropsychiatric systemic lupus erythematosus (NPSLE). Multiple studies have demonstrated a definitive correlation between TLR stimulation and neuroinflammatory reactivation, such as bipolar disorder, depression, and suicidal behavior. In addition, it was truly demonstrated that TLR4 recruited the big family of adapter proteins to initiate the sequential protein-protein interaction. Thus, it inhibited the inflammation of neuroimmune by suppressing the signal of TLR4 or MyD88/IRAK signaling pathway; it as a result influenced the behavior and disease degree. Nevertheless, it is of great feasibility and advantage to affect the signaling of TLR4 in vitro and in vivo in SLE. Moreover, TLR EM-163 could inhibit the level of MyD88 and human adaptor protein in depressed and irritable mood, such as Th-1/Th-2 atypicalities, by obstructing the MyD88 signaling pathway. Furthermore, EM-163 is a structural analogue of the well-known SSRI fluvoxamine, which causes fewer adverse side effects and has typical TLR antagonistic action without interfering immune cell activation but causing the release of anti-inflammatory interleukin. A few case reports showed that SSRI agents such as fluoxetine affect the behavior of patients with depression. Prospective population study is currently conducted to demonstrate the value of fluvoxamine in depleting inflammation in irritable patients.

Conclusion and Future Directions

In summary, this study showed that TLR4-mediated neuroinflammation in NPSLE was through phosphorylation of MyD88/IRAK signaling pathway in a mouse model. HMGB1 may be involved in the process of neuroinflammation by activating TLR4. However, there is a lack of assessment of other proteins of the TLR pathway, such as downstream protein TRAF-6, TBK-1, IκB, and JNK/SAPK, etc. Furthermore, whether signals of TLR/MyD88 pathway are transmitted in neurons and are more sensitive in neurons than in microglia is still not clear. Whether HMGB1 is able to activate TLR4 and participate in the neuroinflammation process in NPSLE patients is still needed. Moreover, Targin and Mustang may protect kidneys from inflammation by reducing

immune complex deposition, MMP2 activity, and apoptotic molecule expressions such as caspase-8 and caspase-3, all observed in an animal model of anti-C5b-9 GN. It may be valuable to explore whether Targin and Mustang are also protective agents in the brain. If so, it is meaningful to explore whether Targin and Mustang are precise to the TLR4/MyD88/IRAK signaling pathway or in brain tissue. It is noteworthy to investigate these questions in future studies.

In conclusion, it is supposed that HMGB1 can activate the TLR4 signal transduction pathway in astrocytes, leading to the early pathological process of neuroinflammation in NPSLE via the phosphorylation of MyD88, which may subsequently switch proinflammatory responses and NF-κB activation. In addition, activation of TLR4 can upregulate TSPO expression once ligation occurs, making the results abnormal over-activation of TLR4/TSPO/neuroinflammation negatively feedback. Regularly, TLR4 antagonism can block this adversely amplifying pathway and make our research results valuable. Based on our in vivo results, the HMGB1/TLR4/ELMO1 axis may become a treatment target for SLE patients with NPSLE in clinical practice.

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American Journal of BioMedicine
Journal Abbreviation: AJBM
ISSN: 2333-5106 (Online)
DOI: 10.18081/issn.2333-5106
Publisher: BM-Publisher
Email: editor@ajbm.net