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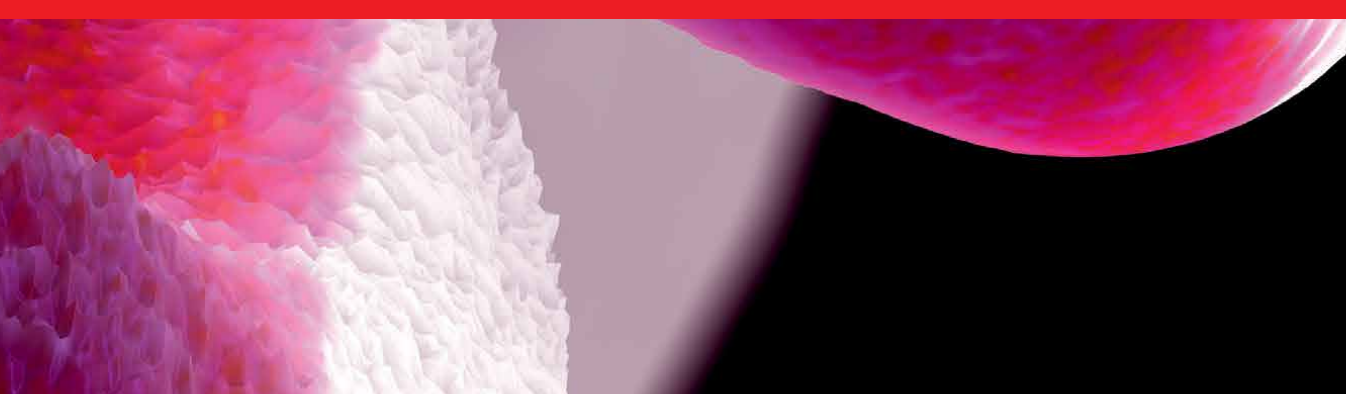
Thirty Years since the Discovery of Toll-Like Receptors

Edited by Vijay Kumar



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Meet the editor

Dr. Vijay Kumar has more than 14 years of academic research experience in the field of infection, immunity, and inflammation. He is the recipient of the prestigious Indian Council of Medical Research junior and senior research fellowships. He received the Piero Periti Review Article Award from the *Journal of Chemotherapy* in 2008 for his article "Innate immunity in sepsis pathogenesis and its modulation: new immunomodulatory targets revealed." Currently, he leads the Laboratory of Tumor Immunology and Immunotherapy in the Department of Surgery, Morehouse School of Medicine, Georgia, USA. He has more than eighty publications to his credit, including editorials, book chapters, and journal articles. He has also edited eight books. He is an associate editor for *Frontiers in Immunology* and an editorial board member and peer reviewer for several other immunology journals.

Contents

Preface

The story of toll-like receptors (TLRs) began with the discovery of the involvement of the toll gene in embryonic development, specifically dorsoventral body patterning in *Drosophila melanogaster* (the common fruit fly) in 1985. However, Dr. Jules Hoffmann from Strasburg, Germany revealed its antimicrobial or immunological function in the common fruit fly in 1996. Thus, Jules Hoffmann and Bruno Lemaitre were the first to describe the antimicrobial, specifically antifungal, function of toll in *D. melanogaster* by regulating the antifungal peptide drosomycin. Thereafter, in 1998, Bruce A. Beutler at the University of Texas Southwestern Medical Center, USA, identified that TLR4 serves as a receptor for bacterial lipopolysaccharide (LPS) in mice. However, the human analog of Drosophila toll was discovered in the laboratory of Charles A. Janeway Junior in 1997 who, along with his team, identified the immunoregulatory function of human TLRs via activating NF-κB and controlling adaptive immune response. This discovery revolutionized immunology research, and now we know that humans have 10 functional TLRs (TLR1–TLR10), which recognize different microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) and death/damage-associated molecular patterns (DAMPs) extra and intracellularly to maintain immune homeostasis.

From their first discovery as pattern recognition receptors (PRRs) recognizing bacterial LPS until today, different PRRs, such as NOD-like receptors comprising inflammasomes, absent in melanoma-2-like receptors (ALRs), RIG-1-like receptors, C-type lectin receptors (CLRs), and cGAS/STING signaling or cGLRs have been discovered, but TLRs still rule the PRR family. This may be due to their diversity, ubiquitous presence (as almost every cell expresses at least one or more TLRs), and ability to identify diverse ligands (external as well as host-derived endogenous PAMPs) to exert a protective immune response. Furthermore, TLR activation also has a regulatory effect on the aforementioned PRRs.

This book discusses the role of TLRs in immunity, inflammation, infection, and cancer (**Figure 1**). The introductory chapter discusses the evolution of TLRs and the forces behind the evolution of different TLRs in the animal phylum. Chapter 2 discusses the role of TLRs in inflammation and immunity. We all know that dysregulated or uncontrolled inflammatory events are associated with different autoinflammatory and autoimmune diseases as well as different cancers. Therefore, understanding the role of TLRs in inflammation and immunity is critical for researchers in this area. Chapter 3 discusses the role of TLRs in emerging viral infections, including COVID-19, which still pose a threat. Chapter 4 discusses the interaction of TLRs with different immune molecules generated during inflammatory cascades associated with different diseases.

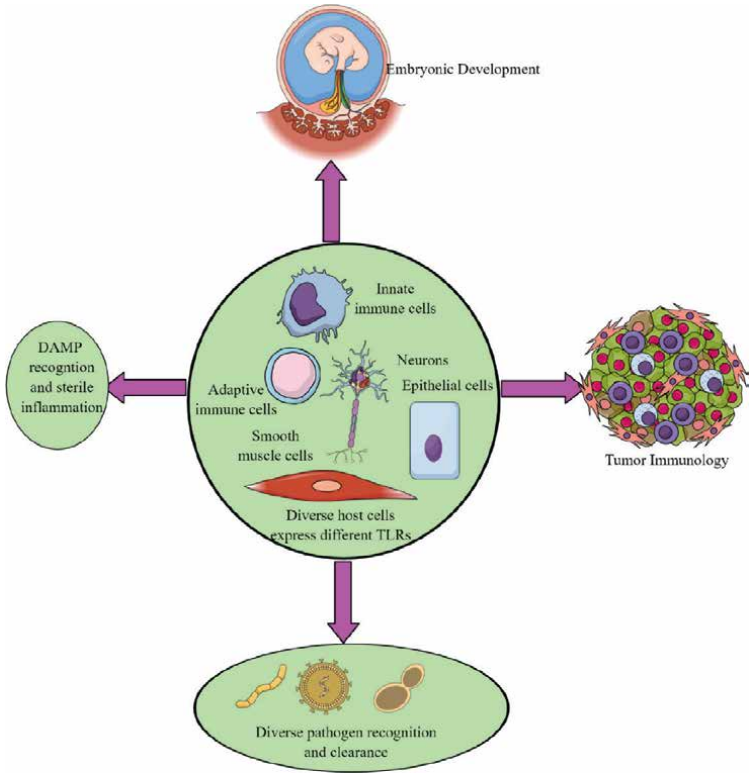

Figure 1.
Almost every eukaryotic or mammalian cell type such as epithelial cells, endothelial cells, immune cells, smooth muscle cells, and neurons express TLR. Therefore, they provide innate immune function to recognize pathogens and DAMPs to maintain immune homeostasis. Failure to do this or their functional dysregulation induces disturbances in the immune homeostasis that may cause chronic inflammation, cancers, and even autoimmunity or autoinflammatory diseases. In addition, TLRs are also critical in mammalian embryonic development.

Studying this interaction/crosstalk is critical to designing better immunomodulatory therapeutics for several chronic inflammatory conditions, including cancers. Finally, Chapter 5 discusses the role of TLRs in cancers.

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Chapter 1

Introductory Chapter: Evolution of Toll-Like Receptors

Vijay Kumar and John H. Stewart IV

1. Introduction

The story of TLRs started with the discovery of Toll protein in the common fruit fly or *Drosophila melanogaster* (*D. melanogaster*) controlling the dorsoventral body patterning during embryonic development [1, 2]. Later studies identified that the Toll protein in Drosophila also provides antifungal immunity [3]. Furthermore, serine protease network/cluster plays a critical role in the Drosophila Toll protein activation, like cysteine clusters present in the leucine-rich repeats (LRRs) comprising TLR ectodomains (ECDs) [4, 5]. Nineteen-to-Twenty-five tandem copies of LRR motifs form TLR ECDs [6]. The LRR motifs of TLRs form a horseshoe-shaped solenoid to directly interact with their corresponding ligands [5, 7–9]. The Drosophila Toll protein is like human IL-1 receptor (IL-1R), which, upon recognizing IL-1, activates NF-κB (a human homolog of Drosophila dorsal protein) and generates associated pro-inflammatory molecules [10–12]. Human TLR4, which recognizes LPS, was identified as a human homolog of the Drosophila Toll protein to generate signaling events activating adaptive immune response [13]. This discovery revolutionized immunology research. Now, we have identified thirteen TLRs in the laboratory mice and ten functional TLRs in humans (**Figure 1**), which are expressed on the outer cell surface (TLR1, 2, 4, 5, and 6) and intracellularly (TLR2, 7, 8, 9, and TLR13 (in mice only) recognize cytosolic nucleic acids) in cytosolic organelles, such as endosomes [14–17]. These TLRs recognize different bacteria, viruses, fungi, parasite-derived microbe, or pathogen-associated molecular patterns (MAMPs or PAMPs) and host-derived damage or death-associate molecular patterns (DAMPs), which have been mentioned elsewhere [14, 18–20]. Thus, the discovery of TLR4 in humans strengthened Janeway's PRR-PAMP theory [13, 21].

Evolution has played a critical role in the immune system's development and function [22, 23]. TLRs are critical mediators of innate immune response against diverse pathogens and host-derived DAMPs. Their activation generates a pro-inflammatory immune response to central immune homeostasis and critically regulates adaptive immune response [18, 24–27]. Hence, the current chapter introduces the evolution of TLRs, which is essential to understand as their dysregulation is associated with different inflammatory diseases and developmental defects.

2. Evolution of TLRs

Placozoans (*Trichoplax* sp.), the simplest animals on earth with no body part or organ, lack canonical TLRs (**Figure 1**) and have only TIR domain proteins with LRR motifs [28]. However, they have orthologs of downstream mediators of canonical

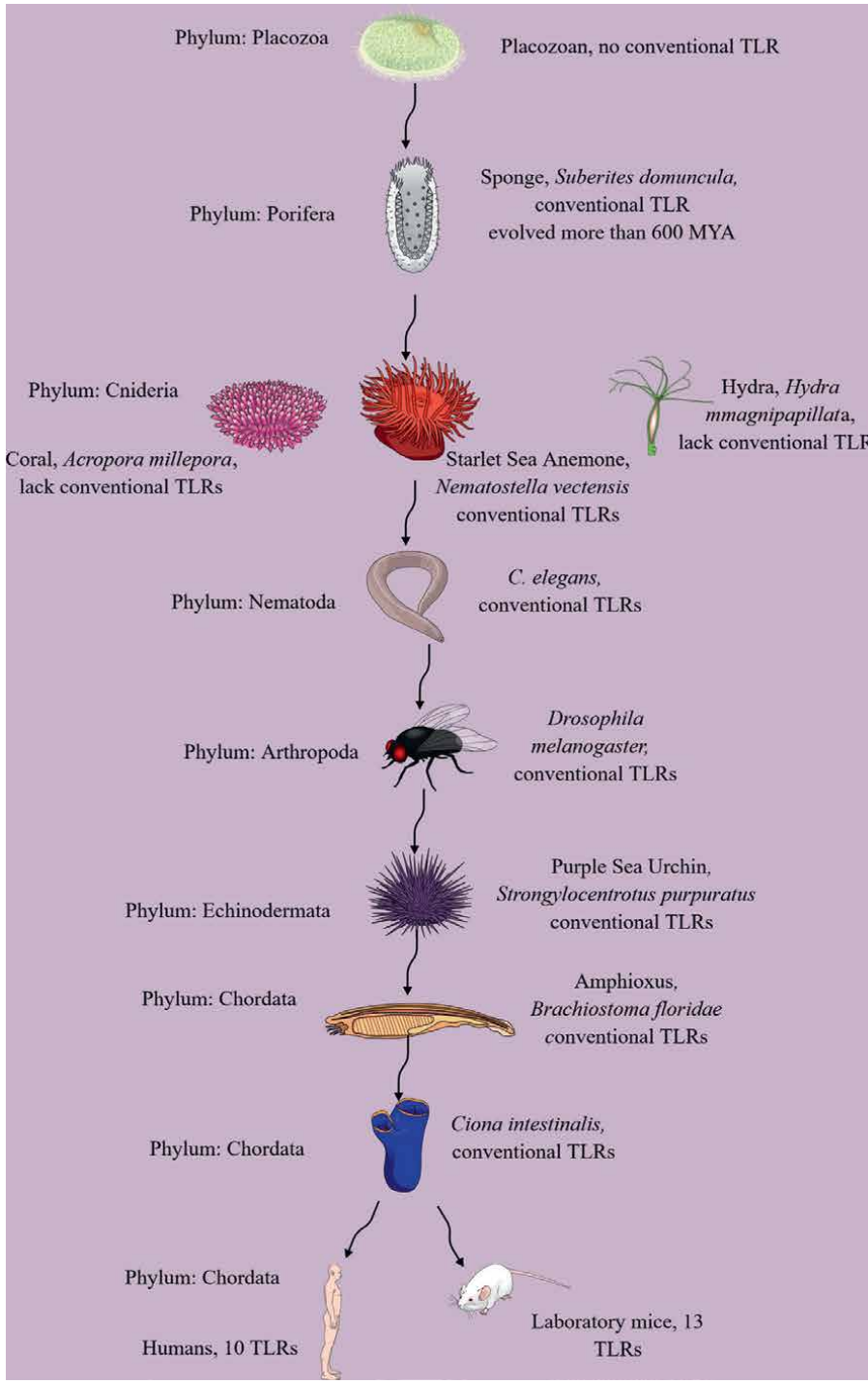

Figure 1.
Evolution of TLRs in the animal kingdom. Conventional TLRs are not present in placozoans, the smallest living animals. They first evolved in porifers or sponges more than 600 MYA. Thereafter, cnidarians, except Hydra and coral, express TLRs involved in host defense and development. Nematodes (C. elegans), arthropods (D. melanogaster), echinoderms (Purple Sea urchin), chordates, including amphioxus, sea squirts (Ciona intestinalis), and mammals, such as humans and mice, express different numbers of TLRs. Please see the text for details.

TLR signaling, indicating the presence of components of primordial TLR signaling in placozoans. For example, they have complete activator protein-1 (AP-1) and mitogen-activated protein kinase (MAPK) pathways working downstream of TLR signaling in higher animals but lack IκBα of the NF-κB pathway. Their NF-κB called NF-κB-like protein (p1000-subunit-like) lacks death domain (DD), indicating the absence of canonical TLR signaling. Placozoan (*Trichoplax* sp.) also lacks TBK1 and TRIF, indicating the absence of myeloid differentiation factor 88 (MyD88)-independent TLR signaling [28]. Earlier studies have indicated that evolutionarily, TLRs existed during protostome (develop mouth first during embryogenesis) development [29]. However, a study has indicated the existence of TLRs as a part of innate immune defense in sponges (Demospongiae: Phylum Porifera) (**Figure 1**), which are lower to protostomes and deuterostomes (develop anus first and mouth develops later) [30]. Along with the TLR, demosponge *Suberites* homunculus (*SD)* also express IL-1receptor-associated kinase-4-like protein (IRAK-4 L) and a novel effector caspase, which shares significant sequence similarity with their homologs in higher metazoans. The *SD*TLR and *SD*IRAK-4 L are expressed constitutively [30]. However, *SD*CASPL (caspase) expression is highly artificial triacyl lipopeptide Pam(3)Cys-Ser-(Lys)(4) inducible. Additionally, sponges or porifers also express MyD88 with two protein interaction domains, TIR (Toll/IL-1 receptor) and DD (Death domain), like a higher animal, including human's MyD88, an adaptor protein working downstream to TLRs [31–33]. Evolutionarily, sponges (Phylum Porifera) are among the most ancient of the metazoans, dating back to more than 600 million years ago (MYA) (**Figure 1**) or at least 60 million years before the Cambrian period (between 541 and 485 MYA) [34]. Thus, evolutionarily TLRs are more ancient PRRs than expected earlier.

Starlet Sea anemones or *Nematostella vectensis* have at least one TLR belonging to the Phylum Cnidaria, Class Anthozoa, and Order Actinaria (**Figure 1**) [5, 35, 36]. Cnidarians are early diverged metazoans descended from a common ancestor of protostomes and deuterostomes [37]. Another deuterostome invertebrate, the ascidian *Ciona intestinalis*, expresses only two TLR genes (Ci-TLR1 and Ci-TLR2), which possess a hybrid functionality of human TLRs, which cannot be predicted by the sequence comparison of vertebrate TLRs [38]. This indicates the confounding evolutionary lineages of deuterostome invertebrate TLRs. *N. vectensis* has separate NF-κB and IκB genes. The *N. vectensis* NF-κB lacks the C-terminal IκB-like sequences present in all other NF-κB proteins, and two IκB-like genes are present on the loci different from the Nv-NF-κB gene [35]. A gene fusion event has created the NF-κB gene in *D. melanogaster* and humans. *N. vectensis* also expresses genes involved in up (Toll- and tumor necrosis-like receptors and ligands, adaptor proteins (Trafs, Myd88), caspases, and a TBK-like kinase) and downstream (NF-κB coactivator protein Bcl-3 and several NF-κB target genes) signaling components of vertebrate NF-κB signaling. Thus, *N. vectensis* in their cnidocytes have a functional TLR to detect pathogens, such as *Vibrio coralliilyticus*, and activate NF-κB signaling to mount an effective immune response and their development [36, 39].

Notably, genomes of other cnidarians, such as Hydra (*Hydra magnipapillata*) or the coral species *Acropora millepora,* lack TLR genes and conventional TLRs (**Figure 1**) [35, 40, 41]. However, they (Hydra and *Acropora millepora*) have receptors with the TIR domain with ECDs lacking LRR motifs, which cluster with other higher animal TIRs, suggesting their relation as TLR-related molecules [5, 40]. They produce antimicrobial peptides (AMPs) by interacting with the LRRs domain-containing proteins with the TIR domain with ECDs lack LRR motifs [41]. Furthermore, they express MyD88 and NF-κβ to exhibit ancestral TLR signaling as MyD88 deficiency decreases

their ancient TLR or TIR-dependent antibacterial immunity [42]. *Caenorhabditis elegans* (*C. elegans*, a Nematode) has only one TLR called TOL-1 that provides innate immunity by supporting the correct expression of antibacterial factor-2 (ABF-2), a defensin-like molecule, and development and function of chemosensory BAG neurons activated by carbon dioxide (CO_2) for their pathogen-avoidance behavior [43, 44].

The TOL-1 also regulates chemosensory Amphid Wing B (AWB) neurons sensing the cyclic lipodepsipentapeptide, Serrawettin W2, produced by *Serratia marcescens* [45]. Thus, TOL-1 in *C. elegans* is critical for the innate immune response against infection and behavioral response to avoid the pathogenic environment. The TOL-1 downstream signaling in *C. elegans* occurs in the absence of MyD88 and NF-κB as their homologs are absent but involve trf-1, pik-1, and ikb-1, which are homologs of Drosophila Traf, pelle, and cactus genes [44, 46, 47]. The purple sea urchins (*Strongylocentrotus purpuratus*) have maximum numbers of TLRs (222), which is followed by annelids expressing 105 TLR homologs in Capitella and 16 in Helobdella, *Brachiostoma floridae* (Amphioxus) expressing 42 TLRs, *Xenopus tropicalis* (Xenopus) having 19 TLRs, and *Danio rerio* (Zebrafish) expressing 17 TLRs [14, 48–50]. Humans have functional TLR10, which is inactive in laboratory mice. Most sea urchin and amphioxus TLR genes are paralogues, indicating these animals have expanded their TLR genes in a species-specific manner [38]. The details of TLRs in different animal phyla have been discussed elsewhere [38, 51–53].

TLRs have not evolved due to coincidental evolution; instead, they originated due to multigene evolution, except for TLR5 and TLRS5, which may have evolved due to coincidental evolution [49]. Furthermore, they have evolved at a constant and conserved rate. Significant TLR families have diverged during or before the Cambrian period, which lasted for 53.4 million years from the end of the preceding Ediacaran Period 538.8 MYA to the beginning of the Ordovician Period 485.4 MYA and produced the most intense burst of evolution ever known [49]. Synonymous/non-synonymous substitution ratio evaluation has further not supported the positive selection pressure in the vertebrate phylogeny. The coding sequence, function, and signaling pathways initiated by vertebrate TLRs are highly conserved upon recognizing their corresponding ligands [49, 54, 55]. Hence, the TLRs are an example of the evolutionary conservation of a biological system at multiple levels, such as genes, proteins, and networks.

Further studies have indicated that the rapid speciation and adaptation to freezing water temperatures are not critical for the evolution of TLR numbers in Nototheniidae (Perciformes order, Notothenioidei sub-order). This stenothermal monophyletic teleost clade evolved relatively recently in the cold-stable waters of Antarctica). However, it induces a shift in the LRR pathogen recognition domain common to all the Nototheniidae analyzed, and of the six subfamilies of TLR in Nototheniidae fishes, TLR22 was most affected [56].

Furthermore, MyD88-dependent and MyD88-independent downstream TLR signaling pathways have evolved separately with common ancestors for vertebrate and invertebrate orthologs of the MyD88 adaptor molecule [57]. Thus, the MyD88 signaling pathway is very ancient as it originated in sponges), like TLRs, and early duplication events generated different adaptor molecules and their corresponding TLRs.

3. Conclusion

TLRs exist in sponges (Phylum Porifera), which have evolved at least 600 MYA. TLRs protected sponges from infections and critically regulated cellular (cnidocytes)

development in cnidarians. They have carried over this ancestry to the hierarchy of the animal kingdom (humans). Hence, TLRs evolved in lower animals (Poriferans) to protect them from invading microbes.

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Chapter 2

Toll-Like Receptors in Immunity and Inflammation

Vijay Kumar and John H. Stewart IV

Abstract

Toll-like receptors (TLRs) are critical components of innate immunity and serve as pattern recognition receptors (PRRs). These PRRs recognize different microbe or pathogen-associated molecular patterns (MAMPs or PAMPs) and death/danger-associated molecular patterns to initiate the pro-inflammatory immune reaction in response to foreign and internal dangers. PRRs, including TLRs, also connects innate immunity to adaptive immunity. Furthermore, TLRs expressed on both innate and adaptive (T and B cells) immune cells regulate their functions. TLRs were first discovered in the common fruit fly or *Drosophila melanogaster* as genes controlling dorso-ventral body patterning during embryonic development. Immunological and scientific advances have led to the discovery of different TLRs (extra and intracellular) with diverse functions. The present chapter introduces the role of TLRs in immunity and inflammation and their expansion to mammalian reproduction and embryonic development, maintenance of immune homeostasis, health, and disease, specifically neurological disorders, including neurodegeneration and cancers.

Keywords: TLRs, immunity, inflammation, immune homeostasis, neurodegeneration, cancer

1. Introduction

The discovery of toll-like receptors (TLRs) revolutionized the field of immunology by filling the gap that the immune system recognizes and clears pathogens to maintain immunohomeostasis or immune homeostasis. For example, the first discovery of TLR4 in human spleen, intestinal epithelial cells (IECs), and peripheral blood leukocytes (PBLs), including monocytes, macrophages, dendritic cells (DCs), T and B cells, and its downstream signaling via NF-κB upon recognizing the corresponding pathogen/microbe-associated molecular patterns (PAMPs or MAMPs) such as lipopolysaccharide (LPS) showed its involvement in the activation and regulation of the adaptive immunity [1–3]. This groundbreaking research led to the reemergence of innate immunity in mainstream immunology research and the subsequent discovery of many other TLRs. For example, 13 TLRs in laboratory mice (TLR1-TLR13) and 10 TLRs in humans (TLR1-TLR10) recognizing different ligands are known today (**Table 1**) [2, 5].

TLRs are expressed extracellularly (on the plasma membrane, TLR1, TLR2, TLR4, TLR5, TLR6, TLR10 (in humans) and TLR11, and TLR12 in mice) and intracellularly

TLRs	TLR Localization	Ligands (PAMPs and DAMPs)	Origin of Ligands
TLR1	Plasma membrane	Triacyl lipopeptide soluble factors	Bacteria and mycobacteria
TLR2	Plasma membrane and endosomes	Peptidoglycan (PGN), lipoteichoic acid (LTA), Lipoproteins or lipopeptides, lipoarabinomannan, glycolipids, porins, zymosan, atypical LPS, heat shock protein 70 (Hsp70), eosinophil-derived neurotoxin (EDN) acts an alarmin	Gram +ve bacteria, mycobacteria, *S. epidermidis, Trypanosoma cruzi, Treponema maltophilum, Neisseria, Fungi, Leptospira interrogans, Porphyromonas gingivalis, host*
TLR3	Endoplasmic reticulum (ER), endosomes, multivesicular bodies, lysosomes, and Endolysosomes	dsRNA and ssRNA and synthetic analog polyinosinic-polycytidylic acid (poly I:C)	dsRNA, ssRNA, and dsDNA Viruses
TLR4	Plasma membrane and endosome	LPS, Taxol, Fusion protein, Envelope proteins, high mobility group box 1 protein (HMG-B1), Hsp60, Hsp70, Hsp22, Hsp96 Type III repeat extra domain A of fibronectin, hyaluronic acid, heparin sulfate, Fibrinogen, Saturated fatty-acids and Fetuin-A	Gram negative bacteria, Plant, respiratory syncytial virus (RSV), mouse mammary tumor virus (MMTV), *Chlamydia pneumoniae*, *Chlamydia trachomatis,* host
TLR5	Plasma membrane	Flagellin	Bacteria
TLR6	Plasma membrane	Di-acyl lipopeptides, Zymosan	Mycoplasma
TLR7	ER, endosomes, multivesicular bodies, lysosomes, and Endolysosomes	guanosine- and uridine-rich ssRNA, Loxoribine (a guanine analog), Bropirimine, ribonucleoproteins (RNPs), siRNAs, and imidazoquinoline derivatives such as resiquimod (R848) and imiquimod	Viruses (human immunodeficiency virus-1 or HIV-1, Influenza virus and vesicular stomatitis virus or VSV), synthetic compounds
TLR8	ER, endosomes, multivesicular bodies, lysosomes, and Endolysosomes	ssRNA, RNPs	Viruses
TLR9	ER, endosomes, multivesicular bodies, lysosomes, and Endolysosomes	CpG oligodeoxyneucleotide (ODN), viral double stranded DNA (dsDNA), Hemozoin pigment	Bacteria and viruses (Herpes Simplex Virus-1 and -2 or HSV-1 and -2, mouse cytomegalovirus or MCMV), Malaria, and protozoa genome (*T. cruzi*)
TLR10 (Humans)	ER, endosomes, multivesicular bodies, lysosomes, and Endolysosomes	dsRNA [4]	Viruses

TLRs	TLR Localization	Ligands (PAMPs and DAMPs)	Origin of Ligands
TLR11	ER, endosomes, multivesicular bodies, lysosomes, and Endolysosomes	Profilin-like protein	*Toxoplasma gondii*
TLR12	ER, endosomes, multivesicular bodies, lysosomes, and Endolysosomes	Profilin-like protein	*Toxoplasma gondii*
TLR13 (Mouse)	ER, endosomes, multivesicular bodies, lysosomes, and Endolysosomes	23 s ribosomal RNA	Bacteria

Table 1.
Different TLRs in laboratory mice and humans, their localization, ligands, and their origin.

in endosomes, endolysosomes, and lysosomes (TLR3, TLR7, TLR8, and TLR9) (**Table 1**) [5, 6]. Mouse TLR13 resides in endosomes, but its ligand is unknown [6, 7]. However, some immune cells, such as dendritic cells (DCs), epithelial cells, and endothelial cells, also have intracellular TLR2 and TLR4 [8–10]. TLRs are members of the pattern recognition receptor (PRR) family, which frequently recognize molecules expressed and released by microbes and damaged or dying cells, called damage/death-associated molecular patterns (DAMPs), to initiate pro-inflammatory immune response for their clearance. The clearance of the invading agent proceeds to the resolution phase of the inflammation for maintaining immune homeostasis that otherwise activates the adaptive immune system later. The combined protective action of innate and adaptive immunity fights back to take care of the outer or endogenous threat, failure of which leads to chronic inflammatory diseases and many cancers.

It is important to note that TLR homolog (called *Toll*) was first discovered in the *Drosophila melanogaster* (*D. melanogaster*) or fruit fly as a gene responsible for dorsoventral body patterning during embryonic development in 1980 [3, 11]. Hence, the story of TLRs' discovery is fascinating, starting with embryonic development and ending with their immunological functions and immune homeostasis maintenance. The current introductory chapter discusses TLRs in the context of human immunity, their impact on immune-mediated diseases, and their use in drug discovery and immunotherapeutic approaches. We will not discuss the myeloid differentiation primary-response protein 88 (MyD88)-dependent and -independent or TIR-domain-containing adapter-inducing interferon-β (TRIF) or TIR-domain-containing adapter molecule 1 (TICAM-1)-dependent TLR signaling pathways activating NF-κB and interferon regulatory factors (IRFs)-dependent cytokines, chemokines, and type 1 interferons (IFNs) to initiate pro-inflammatory immune response as it is discussed elsewhere in detail [6, 12–16]. TIR-domain-containing adaptor protein (TIRAP) and TRIF-related adaptor molecule (TRAM) are other TIR-domain-containing adaptor molecules involved in MyD88 and TRIF-dependent signaling pathways [16]. However, we have included **Figure 1** for MyD88- and TRIF-dependent TLR signaling activating NF-κB- and IRF-3 and IRF-7-mediated inflammatory immune response.

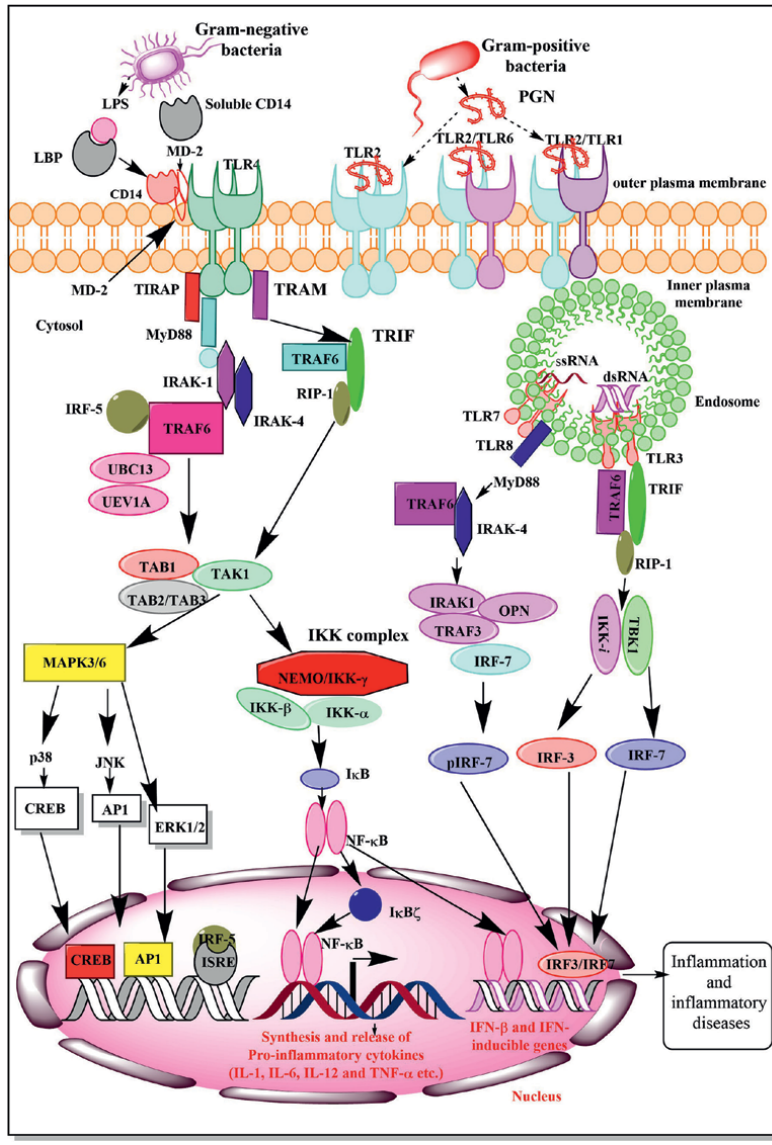

Figure 1.
TLR (MyD88-dependent and -independent or TRIF-dependent) signaling. The recognition of Gram-negative bacteria or LPS by TLR4 leads to the activation of downstream signaling pathways through the activation MyD88-dependent and -independent (TRIF-dependent) manner to activate NF-κB causing the transcription and translation of pro-inflammatory genes (cytokines and chemokines) and well as the generation of type 1 IFNs. The TRIF and MyD88-depedent signaling pathways downstream to TLR4 activation converge at transforming growth factor (TGF)-β-activated kinase 1 (TAB1), 2, and/or 3 and transforming growth factor beta-activated kinase 1 (TAK1) complex. TAK1 activation stimulates MAPK3/6 that activates AP1 and CREB along with activating NF-κB signaling via NF-κB essential modulator (NEMO). Activation of intracellular TLRs (TLR3, TLR7, TLR8, and TLR9) induces the generation of type 1 interferons (IFNs) that regulate immune response, including adaptive immunity. The activation TLR3 signaling via TRIF-dependent signaling activates TNAK-binding kinase 1 (TBK1) and IKK-1 activates interferon regulatory factor 3 (IRF3) and IRF7 to produce type 1 IFNs. On the other hand, TLR7/8 activation involves MyD88-dependent downstream signaling activating TRAF6 and IRAK4 complex formation that further activates IRAK1/ TNF receptor associated factor 3 (TRAF3)/osteopontin (OPN) complex formation to phosphorylate IRF7, which enters to the nucleus for inducing IRF7-dependent type 1 IFN production. Thus, TLR activation induces NF-κB and IRF-dependent pro-inflammatory immune response to clear the external or internal danger, but its overactivation causes exaggerated inflammation and predisposition to several inflammatory conditions, such as sepsis, cytokine storm, autoimmunity, and cancers.

2. TLRs are critical to maintain immune homeostasis

Homeostasis maintenance and immunological well-being, called immune homeostasis, is critical for healthy life and longevity. For example, TLR-mediated commensal microbes' recognition maintains intestinal epithelial homeostasis and protects the host from gut injury and associated mortality [17]. Furthermore, TLR3-mediated macrophage priming for subsequent TLR7 activation involves the Janus-associated kinase (JAK) and signal transducer and activator of transcription (STAT) pathway activation that controls synergistic production of cytokines, innate immune memory generation, and immune homeostasis maintenance during temporally separated subsequent infection [18]. Thus, TLR3 and TLR7 crosstalk are critical for immune homeostasis and innate immune memory generation.

Immune homeostasis disruption causes several auto-immune, auto-inflammatory, and immunodeficiency diseases. Furthermore, foreign invasion from microbes, allergens, and xenobiotics disrupts the immune homeostasis by activating different PRRs, including TLRs, which becomes detrimental to the host once it persists longer. For example, several pro-inflammatory diseases, including sepsis-associated cytokine storm, coronavirus disease 2019 (COVID-19), and neuroinflammation involve TLR overactivation [5, 12, 13, 19].

Several endogenously expressed TLR signaling negative regulators (**Table 2**) prevent exaggerated TLR signaling responsible for inflammation and inflammatory diseases. The details of endogenous negative regulators of TLR signaling are beyond the scope of this introductory chapter and are discussed elsewhere [13, 16, 20–23]. Hence, dysregulated TLR signaling in response to external and endogenous threats (PAMPs, MAMPs, or DAMPs) impairs immune homeostasis, causing cytokine syndrome or cytokine release syndrome (CRS) seen during sepsis, acute or severe COVID-19, and other inflammatory conditions [13, 24, 25]. In addition to overactivated TLR signaling-induced inflammatory conditions, the deficiency of TLR signaling molecules causes different primary immunodeficiency diseases (PIDs) [26, 27]. For example, TLR3 signaling defect correlates well with herpes simplex virus-1 (HSV-1) encephalitis, and a dampened TLR2 and TLR4 signaling has been observed in chronic granulomatosis disease (CGD) and X-linked agammaglobulinemia (XLA) [26, 27]. **Table 3** shows different TLRs and their signaling pathway molecules' deficiency and impact on immunity or PIDs. Therefore, balanced TLR signaling is critical for immune regulation or immune homeostasis [14, 25, 28–34].

Endogenous TLR signaling negative regulators	Classification	Mode of action
TRAM (Translocating chain-associated membrane protein) adaptor with Golgi dynamics (GOLD) domain) or TAG	Splice variant of TRAM	Competes with TRAM for binding to TRIF
Sterile alpha- and armadillo-motif-containing protein or SARM	TIR-domain containing adaptor molecule	Binds and inhibits TRIF-dependent TLR signaling
Interferon regulatory factor 4 or IRF4	IRF family of transcription factors	Inhibits IRF5 binding to MyD88 and TRAF6 and its translocation to nucleus

Endogenous TLR signaling negative regulators	**Classification**	**Mode of action**
Tumor necrosis factor-α-induced protein 8-like 2 or TIPE2	Member of TNF-α-induced protein-8 (TNFAIP8) family	Inhibits TLR signaling via binding to CASP8 and inhibiting NF-κB and AP-1 activation
Bruton's tyrosine kinase or Btk	Tec family tyrosine kinase	Phosphorylates MAL that activates SOCS-1
DNAX activation protein of 12 kDa or DAP12, also called KARAP	Transmembrane adaptor protein	DAP12 mediated inhibition of TLR signaling involves another adaptor protein called DOK-3
Downstream of kinase 1 and 2 (DOK1 and DOK2)	Adaptor Proteins	Inhibit Ras-Erk dependent signaling
Axl	TAM family of receptor kinases	Inhibits NF-κB mediated production of TNF-α
Interlukin-1 receptor-associated kinase-M (IRAK-M) or IRAK-3	Member of serine/threonine kinase family	Inhibits dissociation of IRAK-1 and IRAK-4 form MyD88 and formation of IRAK-TRAF6 complex
TOLL-interacting protein (TOLLIP)	Adaptor protein that interacts with cytoplasmic TIR domain of IL-1Rs	Inhibits phosphorylation and kinase activity of IRAK1
Src homology 2 domain-containing protein tyrosine phosphatase-1 (SHP-1)	Intracellular tyrosine phosphatase	Inhibits MAP kinase and NF-κB activation
Calcineurin	A serine/threonine phosphatase	Specific pathway unknown
Protein tyrosine phosphatase-1 B (PTP1B)	Intracellular tyrosine phosphatase	Inhibits MAPKs, NF-κB and IRF3
A20 or TNF-α-induced protein 3 (TNFAIP3)	Ubiquitin modifying enzyme	Inhibits NF-κB signaling as negative feedback by removing ubiquitin moieties from TRAF6
Cylindromatosis or CYLD	Tumor suppressor deubiquitinase	Inhibits TLR2 signaling via inhibiting MyD88, TRAF2, TRAF6 TRAF7 and NEMO
Ubiquitin-specific protease 4 or USP4	Deubiquitinase	Inhibits TRL4 signaling via deubiquinating TRAF6 and inhibiting its adaptor function
USP18 or UBP43 or ISG15 isopeptidase	Isopeptidase	It cleaves the K63-linked polyubiquitin chains of TAK1 and also targets NEMO
Deubiquitinating enzyme A (DUBA)	Cysteine protease	Removes K63-ubiquitin chain from TRAF3 to inhibit NF-κB and IRF3 activation
Nuclear receptor 4A1 or NR4A1 (Nur77)	Member of nuclear receptor 4A receptor subfamily	Prevent auto-ubiquitination of TRAF6 via binding to TRAF6
NR4A2 or Nurr1	Member of nuclear receptor 4A receptor subfamily	Inhibits NF-κB activation downstream to TLR4 signaling
Small heterodimer partner (SHP) or NR0B2	Orphan nuclear receptor	Prevents Lys63-linked polyubiquitination of TRAF6 and subsequent activation of NF-κB
Mitogen and stress-activated kinase 1 and 2 (MSK1 and 2)	Nuclear kinase sharing homology with ribosomal S6 kinase ($p90^{rsk}$) family	Phosphorylate histone H3 and CREB that negatively regulates TLR signaling and induces several anti-inflammatory genes

Endogenous TLR signaling negative regulators	Classification	Mode of action
TRAF-associated NF-κB activator (TANK)	TRAF binding protein	Binding of TANK to TRAF6 inhibits NF-κB and AP-1 activation
PDZ and LIM domain containing protein or PDLIM-2 (Mystique and SLIM in mice)	PDZ and LIM domain containing Alkaline Phosphatase	Suppresses TLR signaling by acting as a nuclear E3 ubiquitin ligase and inhibits NF-κB activation
Mankorin ring finger protein 2 (MKRN2 or RNF62)	Zinc finger and RING finger domain containing nuclear protein	Binds to PDLIM2 and inhibits activation of NF-κB downstream to TLR signaling
PDLIM1 or CLP36 or Elfin	PDZ and LIM domain containing protein of APL subfamily	PDLIM1 inhibits NF-κB activation by sequestering p65 into cytosol
Tripartite motif-containing protein 30 A (TRIM30α)	A member of tripartite-motif (TRIM) protein family	Blocks TRAF6 autoubiquitination by degrading TAB2 and TAB3and suppresses NF-κB activation
TRIM8	Acts as ubiquitin E3 ligase	Polyubiquitinates TRF and inhibits TRIF-TBK1 interaction
Triad3A or Ring finger protein 216 (RNF216)	RING finger type E3 ubiquitin ligase	Degrades TLR proteins
NOD-like receptor family member X1 (NLRX1)	Member of NLR family	NLRX1 binds to IKK complex causing an inhibition of IKKα and IKKβ phosphorylation and NF-κB activation
NLRC3 or NOD3	Member of NLR family	NLRC3 interacts with TRAF6 to attenuate its K63-linked ubiquitination to inhibit NF-κB activation
NLRC5	Member of NLR family	Blocks IKK complexes to inhibit NF-κB activation and type 1 IFN signaling pathways
Serum stimulation 2 factor (ST2)	Serves as a part of IL-33 receptor	ST2 binds to sequesters MyD88 and MAL without affecting TRIF and IRAK to inhibit TLR-induced NF-κB activation
Single immunoglobulin IL-1R-relate receptor (SIGIRR or TIR-8)	Member of TLR/IL-1R superfamily	Blocks TLR-mediated NF-κB and JNK activation via stopping the recruitment of IRAK and TRAF6 towards MyD88
TLR10	Member of TLR family	Stimulates PI3K/Akt/IL-1R antagonist pathway and inhibits MyD88 and TRIF-dependent signaling pathways
Activating transcription factor 3 (ATF3)	Member of activating transcription factor/cAMP response element family of bZip transcription factors	Bind to consensus c-AMP response element (CRE) sequences
Interleukin-37 or IL-37	Member of IL-1 cytokine family	Blocks NF-κB and MAPK activation

Table 2.
Endogenous negative regulators of TLR signaling.

PIDs	Impact on TLR functions on immune cells
Common variable immunodeficiency (CVID)	Reduced expression and function of TLR7 and 9 on B cells, reduced activity of TLR7 and 9 on plasmacytoid DCs (pDCs)
Chronic granulomatous disease (CGD)	Increased TL 4 activity (increased TNF-α and IL-18 production) on neutrophils, peripheral blood monocytes (PBMCs), decreased TLR5 and TLR9 activity, TL 9 activity on B cells reduces as indicated by a decreased memory response
X-linked agammaglobulinemia (XLA)	Decreased TLR2 and 4 responses by monocytes and macrophages, monocyte-derived DCs show decreased TLR2, 4, 3, and 7/8 responses, including decreased DC maturation
Hyper Immunoglobulin E (IgE) syndrome	TLR2 and 4 response increases in PBMCs as indicated by increased TNF-α and IL-12 production
Adenosine deaminase (ADA) deficiency	Decreased TLR7 and 9-meidated immune response by B cells
Interleukin-receptor-associated kinase 4 (IRAK4) deficiency	Abolishes TLR7, 8, and 9-mediated type 1 IFN production, leaving TLR3 and TLR4-dependent IFN production intact

Table 3.
TLRs in different PIDs.

3. TLRs in human health, including embryonic development and disease

TLRs are responsible for dorso-ventral body patterning during the embryonic development of *D. melanogaster*. TLRs' role in mammalian development is also emerging, for example, TLR 4 and 7 expression has been observed during murine embryonic development, and TLR7 expression has been observed in embryonic day 12 (E12) in the dorsal root ganglia (DRG) and nodose ganglion [35]. At E14, sympathetic ganglia, vagus nerve, and nerve fibers and ganglia in the respiratory apparatus express TLR7. Also, TLR4 expression increases at later embryonic days (E17 onwards). Furthermore, murine E10.5 macrophages co-express TLRs and CD14, which phagocytose apoptotic cells and bacteria, and secrete several cytokines and chemokines [36]. Hence, TLRs regulate embryonic homeostasis via iron metabolism.

The preimplantation human embryos also express TLRs [37]. Furthermore, TLR3 in the brain cells of mice in the early embryonic stages and neural stem/progenitor cells (NPCs) control the neurosphere formation. For example, NPCs from TLR3-deficient murine embryos proliferate highly and form higher numbers of neurospheres than wild-type (WT) embryos [38]. Therefore, TLR3 is a negative regulator of NPC proliferation and neurosphere formation in mice, which needs exploration in humans. TLR2 and TLR4 expression increases throughout gestation in sheep lungs, and LPS exposure increases their expression in fetal sheep lungs [39]. This study is critical as ovine and human TLR2, 3, and 4 share 83–88% homology. Human blastocysts highly express TLRs 9 and 5, and TLRs 9, 5, 2, 6, and 7 are expressed throughout embryonic development, and their stimulation *in vitro* produce different cytokines and chemokines such as IL-8 and monocyte-chemoattractant protein-1 (MCP-1) [37]. Therefore, it will be novel to observe the impact of TLRs in human embryonic development and TLR deficiency on reproduction or reproductive health. For example, TLRs are critical in reproductive tract inflammation, which can impair reproductive potential [40, 41]. Furthermore, human sperms express TLR2 and 4, and their activation induces apoptosis that can impair male fertility [42]. Hence, it is critical to understand TLRs' biology in human reproduction and development.

Plenty of data are available for TLRs in a vast array of infectious (bacterial, viral, fungal, and parasitic) and inflammatory diseases [5, 13, 43–48]. We have previously mentioned TLRs' role in autoimmune and PIDs. Therefore, this section will introduce their role in neurodegenerative diseases (NDs) and cancers. For example, Alzheimer's and Parkinson's disease (AD and PD) incidence has increased worldwide since their first report. PD patients' number has more than doubled from 1999 (2.5 million) to 2016 (6.1 million) worldwide, which is further increasing [49]. For example, in the United States, 6.1 million people may have AD as per data collected by the Alzheimer's Disease Association (ADA) in 2022, and according to PD foundation, over a million people have PD.

Inflammation plays a significant role in ND pathogenesis so as TLRs do [12, 50–56]. A novel variant p.E317D in the TLR 9 gene, co-segregating with early-onset AD (EOAD) in an autosomal dominant manner, identified in a Flander-Belgian family, increases AD risk by compromising innate immunity-mediated protection [57]. The p.E317D TLR9 variant reduces its potential to activate NF-κB by 50%, indicating that p.E317D is a loss of function mutation. The protective role of TLR9 in AD comprises the release of anti-inflammatory cytokines and upregulation of Axl (a negative regulator of TLR-mediated pro-inflammatory signaling), Run domain Beclin-1 interacting and cysteine-rich containing protein (RUBICON, an autophagy suppressor), and associated signaling pathways regulation microglial phagocytic function and inflammatory signaling [57]. For example, microglia and myeloid cell-specific deletion of RUBICON in mouse genetic models of AD induce early onset of neurotoxic amyloid-beta (Aβ) plaques, microgliosis, and increase in pro-inflammatory cytokines in cortex and hippocampus [58, 59]. AD patients' brains express lower RUBICON, Atg16L, and Atg5 levels than non-AD brain samples [60]. Furthermore, TLR9 activation ameliorates vascular amyloid pathology in mice and provides cognitive benefits [61]. The increased TLR4 expression and its co-localization with pSer129 αSyn and Iba-1in glial cells of substantia nigra (SN) and medial temporal gyrus (GTM) in PD patients can be targeted to prevent inflammatory neuronal damage [62, 63].

Autophagy is critical for Aβ secretion and plaque formation, and TLRs are known to control autophagy [64–66]. For example, TLR7-mediated autophagy increases epileptic susceptibility by reducing kinesin family member 5 A (KIF5A)-dependent gamma-aminobutyric acid (GABA)A receptor transport in mice [67]. Furthermore, TLRs are critical players in non-NDs of the brain called neurological diseases due to their role in central nervous system (CNS) homeostasis and neurogenesis, including neuronal pruning, learning, and memory [68]. The recognition of extracellular Tau protein (one of the causal factors for PD and associated dementia) stimulates microglia to phagocytose live neurons via the TLR4/NLRP3/Caspase 1 axis and NADPH oxidase activation [69, 70]. The burden of neurological diseases is also increasing world-wide [71]. For example, neurological diseases ranked third after cardiovascular diseases and cancers in Europe's total death [71]. Therefore, exploring TLRs' role in NDs and other neurological diseases has the potential for designing novel therapeutics. For example, targeting TLRs may serve a promising therapeutic strategy against AD and PD [62, 63, 69, 72–74]. Furthermore, *Clostridium butyricum* improves the cognitive decline in mice with intraventricular injection of streptozotocin (ICV-STZ)-induced AD by suppressing TLR4 signaling through gut-brain axis [74]. However, *C. butyricum use in humans may* cause serious adverse events [75].

Cancer is another critical human health issue. The immune system and inflammation play significant roles in cancer pathogenesis, and TLRs are relevant regulators of inflammation and immunity [5, 76–78]. Thus, chronic inflammation due to prolonged TLR

signaling activation may predispose to cancer development later in life. However, TLR signaling in cancer cells may exert tumor-suppressive or promoting effects, depending on the type of TLR signaling [79]. For example, TLR 2, 4, and 7/8 activation in tumor cells promotes tumor progression via creating and supporting immunosuppressive tumor microenvironment (TME) or tumor immune microenvironment (TIME), supporting resistance to apoptosis among cancer cells and their proliferation and metastasis. On the other hand, TLR2, 3, 4, 5, 7/8, and 9 activation on cancer cells in conjunction with chemotherapy and immunotherapy exerts antitumor action [79]. For example, TLR5 agonists enhance antitumor immunity and increase the efficacy of immune checkpoint inhibitors (ICI) by overcoming their resistance [80]. Furthermore, TLR activation on cytotoxic T and natural killer (NK) cells and antigen-presenting cells (APCs, macrophages, and DCs) has anticancer action [79]. Therefore, TLRs are critical in cancer immunotherapy depending on the cancer and targeted cell type [81–83]. Hence, TLRs are expanding their boundaries to cancers and neurological diseases, including epilepsy and NDs.

4. Conclusion

The story of TLRs began in 1997 with the discovery of TLR4 in human spleen, immune, epithelial, and endothelial cells as a homolog of Drosophila Toll protein that filled the long-standing gap of the microbial recognition, their phagocytosis and clearance by immune cells. After almost 30 years of TLR4 discovery and revolutionizing immunology research, TLRs are still at the top as critical PRRs and immune regulators. However, in the last 30 years, they have expanded their territory from infection, immunity, and inflammation to neurosciences, mammalian reproductive biology, and cancer. Even TLRs and DAMPs are critical in heart transplant rejection [84]. TLR agonists and antagonists have a wide range of applications in different inflammatory and infectious diseases and cancers (as adjuvants). TLR-based emerging therapeutics and vaccine candidates are under clinical trials for several diseases [85–87]. Furthermore, harnessing innate immunity for cancer and ND therapy is a critical research area, and TLRs are relevant components of innate immunity [4, 88, 89]. Hence, TLRs control innate immunity as crucial members of the PRR family with diverse immune and non-immune functions.

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Chapter 3

Toll-Like Receptors and Emerging Viral Infections

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Abstract

Emerging viral infections are considered a global threat, and they have gained more importance after the coronavirus outbreak in 2019, which affected the whole world. The innate immune system recognizes invading pathogens via pattern recognition receptors (PRRs) expressed on different immune cells extracellularly and intracellularly. Out of several PRRs, Toll-like receptors (TLRs) are one of the critical PRRs recognizing diverse pathogen-associated molecular patterns (PAMPs) varying from viruses, bacteria, and fungi. Viral pathogens possess specific molecular signatures such as dsRNA and high CpG content that differentiate them from mammalian cells. TLRs play their role in innate immunity against pathogenic viruses by producing antiviral cytokines and chemokines. Most emerging viral pathogens are RNA viruses including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These viruses are recognized by TLR 2, TLR 3, TLR 4, TLR7, and TLR8 with the coordination of other PRR members resulting in the activation of costimulatory molecules that initiate immune response. This chapter provides insights into the TLRs' role in developing and regulating the immune response against emerging viral pathogens. It exploits their roles in innate immunity to develop treatment strategies against deadly emerging viral pathogens.

Keywords: emerging viral infection, TLRs, innate immunity, viral pathogens, SARS CoV

1. Introduction

It has been established that the recurrence and emergence of viral diseases unquestionably play a significant role in influencing the human world, from Walter Reed's discovery of the first human virus, known as the yellow fever virus, in 1901 to the coronavirus disease-2019 (COVID-19) pandemic today caused by SARS-CoV-2. They not only have an immense effect on the economy and society in today's connected world, but they also have the potential to cause high mortality as they spread.

Despite significant advancements in medical research, newly emerging and reemerging viral infections continue to have catastrophic effects on human populations all over the world. Unfortunately, there is currently no treatment for most of these viral infections.

A developing infective condition is brought on by novel disease-causing microbes that have either never before arisen in a population and are now affecting it, or that have previously existed but are now quickly spreading to new geographic areas [1]. Either locally or globally, they are to blame for serious public health issues. Rarely can it be determined whether an illness is new to humans or if it has always existed but gone unnoticed by the world of science. However, many newly emerging illnesses are assumed to be the result of a rise in the frequency of close interaction among people and pathological repositories found in nature. This proximity makes it easier for an agent to successfully "jump" from an animal or arthropod to a person and pass the interspecies border [2].

Additionally, the past 50 years have seen exceptional pandemic outbreaks, including HIV in early 1990 [3]. H1N1 "swine flu" in 2009, Zika in 2015, chikungunya in 2014, and as well as Ebola fever outbreaks that resembled pandemics in wide areas of Africa from 2014 to the present [4]. In light of the fact that there are four endemic coronaviruses that afflict people globally, coronaviruses must have originally emerged and circulated extensively during the era when viruses were first acknowledged as causes of human diseases. The SARS-CoV caused a near-pandemic that afflicted 26 nations between 2002 and 2003 before going away in accordance with public health control measures. The animal host was most likely a domestic cat at the time.

The associated Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) first developed in people in 2012 after emerging from dromedary camels [5]. By the end of 2019, COVID-19, which was identified, as just the most recent instance of unforeseen, innovative, and fatal global epidemic illness. SARS CoV-2 has been linked to about 6.96 million fatalities and around 771 million cases as of 04 October 2023 [6]. The root reasons for this novel and risky scenario are varied, and intricate, and deserve careful consideration [7].

The innate immune system serves as the first line of defense against invading pathogens. In viral infection, innate immunity plays a vital role in inducing adaptive immunity. Upon entry immune cells recognized the conserved regions of invading pathogens named PAMPs, that are not present in the host cells. Immune cell receptors known as PRRs engage with pathogen PAMPs to activate a number of intracellular signaling pathways, that trigger the production of pro-inflammatory cytokines and type I interferons (IFNs), that ultimately initiate the antiviral immune response to clear the invading agent [8]. The clearance of viral cells is accomplished within a few weeks in typical infections however, certain viruses learn how to evade or circumvent the host's immunological defenses, which helps them replicate and infect the host persistently.

2. TLRs in pro-inflammatory immune response

The host's innate immune system serves as its first line of defense contrary to infectious diseases caused by microbes. The innate immune system is essential and plays an important part in viral infections by locating and eliminating contaminated cells as well as organizing an adaptive immune reaction. The innate immune system comprises different innate immune cells (basophils, eosinophils, mast cells,

neutrophils, monocytes, dendritic cells (DCs), natural killer cells) and secreted humoral components such as complement proteins, cytokines, IFNs, and chemokines. These innate immune cells recognize pathogens via different PRRs including nucleotide-binding oligomerization domain (NOD)-like receptor family proteins (NLRs), Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) [9].

PAMPs are persistent architectural motifs that may trigger the body's natural defenses against infections by identifying characteristics of bacteria or viruses, such as nucleic acids, peptides, and lipoproteins, that differentiate the pathogen from the host. Host PRRs expressed in epithelial cells and first-line immune cells such as DCs, macrophages, and natural killer cells were able to recognize PAMPs during viral infection [10]. Antigen-presenting cells (APCs) including macrophages and DCs as well as certain kinds of T cells express the transmembrane receptors known as TLRs.

The innate immune system is also an initial defense mechanism in emerging viral infections including SARS-CoV. It also prevents the entry, translation, replication, and assembly of the virus. They also assist in the identification and elimination of infected cells along with coordinating and hastening the progression of adaptive immunity. In response to PAMPs, PRRs in the cytosol and on cell surfaces, in endosomes, trigger inflammation and promote apoptosis that stops infections caused by viruses and helps with the removal of viral debris [9–11].

TLRs were the first to be identified and have been the subject of the most in-depth research, even though vertebrate hosts also express many other classes of PRRs, including the NLRs, RLRs, and C-type lectin receptors [12, 13]. Initially discovered in Drosophila, the TLRs have since been demonstrated to be crucial for the host's defense against fungus [14]. Researchers identified 10 homologs in humans (TLR1–TLR10) and 12 in mice (TLR1–TLR9 and TLR11–TLR13). These toll receptor homologs were named as TRLs [15, 16]. TLRs are characterized as type I transmembrane proteins consisting of a Toll-interleukin-1 receptor (IL-1R) homology (TIR) domain in the cytoplasmic carboxy-terminal region, which triggers downstream signal transduction. They also possess a transmembrane domain and an amino-terminal ectodomain that is rich in repeats of the amino acid leucine, which makes it easier to recognize PAMPs [17, 18].

The six major families of TLRs found in vertebrates are TLR (1, 3, 4, 5, 7, and 11) [19]. The members of the TLR1 family are TLR (1, 2, 6, and 10). These are found on cell membranes and can identify peptidoglycans and lipoproteins that are found in microbial cell walls and membranes. Furthermore, TLR4 and 5 (located extracellularly on the plasma membrane) recognize LPS and flagellin to initiate TLR-dependent pro-inflammatory immune response [16]. On the other hand, endosomes and lysosomes express TLRs from the TLR (3, 7, and 11) families, which are cytoplasmic TLRs. Transportation of these TLRs from the endoplasmic reticulum to endolysosomal spaces, where they are modified by proteases to turn into functional receptors, is facilitated by UNC93B1, a polytopic membrane protein [20]. Double-stranded RNA (dsRNA) is detected by TLR3. The TLR7 family contains TLR (7, 8, and 9). Single-stranded RNA (ssRNA) is detected by TLR (7 and 8), while TLR9 interacts with unmethylated CpG DNA TLR (7 and TLR8 detect single-stranded RNA (ssRNA), while TLR9 interacts with unmethylated CpG DNA [16]. TLRs in humans with their respective ligands and adaptor molecules are summarized in **Table 1**. The *Toxoplasma gondii* parasite's profilin is sensed by TLR (11 and 12) forming a heterodimer and belongs to the TLR11 family [30], whereas bacterial 23S ribosomal RNA is recognized by TLR13 [31, 32].

Localization	TLRs	Ligands	Adaptor molecules	References
Plasma membrane	TLR1/ TLR2	Diacyl/triacyl lipopeptides	MyD88	[19, 21–23]
Plasma membrane	TLR2	peptidoglycan, lipoteichoic acid, Lipoproteins, Zymosan	MyD88	[21–23]
Endosome	TLR3	DsRNA	TRIF	[24]
Plasma membrane	TLR4	LPS, Envelope glycoproteins, endogenous HSP, HMGB1, β-defensin 2	MyD88/TRIF	[25]
Plasma membrane	TLR5	Flagellin	MyD88	[26]
Plasma membrane	TLR6/ TLR2	lipoproteins, zymosan, lipoteichoic acid	MyD88	[21–23]
Endosome	TLR7	SsRNA	MyD88	[16, 27]
Endosome	TLR8	SsRNA	MyD88	[16, 27]
Endosome	TLR9	Unmethylated CpG DNA, MtDNA	MyD88	[16, 28]
Plasma membrane	TLR10	HIV-1 gp41, H1N1/H5N1	MyD88	[29]

Table 1.
Toll-like receptors (TLRs) in human respective natural ligands.

Human TLRs that can recognize viral nucleic acids, such as TLR (3, 7, 8, and 9), are mostly expressed in the endosomal compartment, in contrast to the TLRs that recognize bacteria and are primarily expressed on the cell membrane [33]. Viral dsRNA taken up into sentinel cell endosomes is detected by TLR3, leading to the activation of downstream signaling pathways dependent on the cytoplasmic adaptor protein TRIF. Downstream signaling pathways for TLR (7 and 8), and ssRNAs, rely on MyD88. TLR9, the sole known DNA sensor, specifically detects the unmethylated CpG DNA of DNA viruses [34]. Furthermore, viral structural and nonstructural proteins have been associated with the production of inflammatory cytokines, mediated by TLR (2 and 4) within the TLR family.

TLR signaling is carried out through two mechanisms: the (TRIF)-mediated route, which involves the Toll-interleukin-1 receptor (TIR)-domain-containing adaptor inducing IFN-β, and the (MyD88)-mediated pathway, which relies on the myeloid differentiation factor 88 [35, 36]. The former induces IFNs, which when stimulated, put cells into an antiviral state. The latter MyD88-mediated pathway triggers the activation of the tumor necrosis factors (TNF) such as NF-κB, which subsequently initiates the activation of multiple genes involved in inflammatory reactions. Only the TRIF-mediated pathway is activated by TLR3. IRF-3, a crucial for TF for IFN- β, is activated by TLR3 signaling, inducing the synthesis of IFN. TLR2 exclusively activates the MyD88-mediated pathway, whereas TLR4 activates both the MyD88-mediated pathway and the TRIF-mediated pathway. As a result, TLR4 agonists have the dual effect of activating NF-κB and promoting the production of IFN [37].

The activation of transcription factors (TFs) like NF-κB, AP-1, and interferon regulatory factors (IRFs), occurs as a result of signaling pathways initiated by PRRs. These TFs play an important part in stimulating the expression of different genes that have a role in antiviral defense such as type I and III interferons (IFNs),

pro-inflammatory cytokines, and chemokines [8]. These genes of focus can increase the adaptive immune reaction, prevent the spread of pathogens, and help the host cells to adapt to changes in their environment. Macrophages and DCs primarily produce type I IFNs, causing an antiviral defense in the surrounding cells which start expressing the several genes collectively called IFN-stimulated genes (ISGs). Neutrophil, monocyte, and NK cell recruitment may be aided by the chemokines released at the site of infection.

3. Emerging viral pathogens and Toll-like receptors

Members of the Coronaviridae family that are phylogenetically and antigenically unique include SARS-CoV and MERS-CoV [38, 39]. Similar classes of cellular sensors may be able to detect PAMPs, as they are likely to move through similar spaces in the host cells infected by coronaviruses. Virus and other invading pathogen-specific PAMPs are recognized by innate immune sensors, which then cause transcriptional modifications in host cell signaling pathways that make an antiviral state and reduce the effectiveness of viral reproduction.

The emergence of highly virulent 1918 and 2009 H1N1, H5N1, and H7N9 and influenza A viruses (IAV), as well as the SARS, MERS, and most recent SARS-CoV-2 epidemics, demonstrate the potential danger that respiratory virus infections provide to world health [40]. Essential roles in gas exchange are performed by the human lung, which serves as an intricately complex and highly delicate mucosal surface that interacts with numerous environmental bacteria. The lung's ciliated cells and type II pneumocytes of the airway epithelium are the primary targets of SARS-CoV and IAV infection [40, 41]. To ensure maximum detection of viruses at different stages of the replication cycle, including virus entrance and genome replication, PPRs which contain various classes of cellular sensors, are allocated at cellular membranes and within the cytosol. When exposed to infectious microbes, innate immune signaling cascades are initiated by these cells [42].

3.1 SARS CoV

The SARS outbreak that occurred in 2003 had a disastrous impact globally as it rapidly spread across the continent, causing over 8000 ailments, and resulting in a mortality rate of 10% [43]. It had severe economic consequences for local and regional areas.

SARS CoV genome comprises linear, single-stranded, and positive sense RNA. They are membrane-enveloped viruses [44]. The innate immune system reacts quickly to viral invasion and is crucial in triggering the adaptive immune system. When PAMPs engage with host PRRs in innate immunity, numerous signaling mechanisms are activated in immune cells. This results in the release of pro-inflammatory cytokines and IFNs, which in turn trigger an antiviral response. The primary innate immune cells with antiviral activity are macrophages, DCs, and natural killer cells [8].

Extracellular membrane-bound TLRs recognize extracellular pathogens. TLR3 has been linked to recognizing changes in the pathogenesis of airway disease brought on by infections with respiratory viruses such as IAV, rhinovirus, and respiratory syncytial virus (RSV) as well as recognition of the range of different RNA viruses [45–47]. Basal levels of TLR3 expression can be found in the lung tissues including human bronchial epithelial cells and alveolar cells, as well as different immune cells

(19). TLR3 is attached to the endosomal membrane in cells, where it detects dsRNA patterns produced by invasive pathogens [48].

TLR 3 dimerizes and draws in the TRIF adaptor protein after engaging the dsRNA motif [49, 50]. TFs such as interferon regulatory transcription factor (IRF3) and NF-κB are signaled to be activated as a result of TRIF recruitment to the endosome [51]. TRIF has been defined as an adapter for TLR4 signaling in addition to TLR3-specific signaling pathways [50, 52]. The basal level of TLR4 expression in alveolar cells and bronchial epithelial cells rises in response to inflammatory cell infiltration brought on by insults like viral infections [53, 54]. TLR4 communicates with TRIF or MyD88 via two sorting adaptors: TRAM for TRIF-dependent signaling, and MAL for MyD88-dependent signaling pathways [55]. An increase in the severity of acute respiratory distress syndrome (ARDS) brought on by influenza virus infections and acid damage models has been connected to the TLR4/TRAM/TRIF signaling pathways in earlier investigations [56]. The involvement of TLR4 in the immunopathogenesis of the influenza virus has been a subject of controversy, and the TLR4 antagonist Eritoran has been suggested as a potential immunomodulatory treatment for influenza viral illnesses [57, 58].

The involvement of TLR4 in the highly infectious SARS-CoV remains uncertain. The activation of proinflammatory cytokines (IL-6, TNF, IFN-, and CCL5), type 1 interferons (IFN-α and IFN-β), and interferon-stimulated genes (ISGs) (RSAD2, IFIT1, and CXCL10) is induced by TLR signaling through TRIF [48, 59]. In the setting of ARDS and respiratory viral illnesses, these effector molecules are of definite importance [40, 60]. In the setting of vaccine adjuvants and antiviral medication, TLR antagonists and agonists have been postulated as substances having wide-range efficacy over diverse respiratory illnesses [57, 61–63]. Lipopolysaccharide (LPS), a TLR4 agonist, and poly(I:C), a TLR3 agonist, have been proven in mouse experiments to give prophylactic protection against SARS-CoV infection, with poly(I:C) displaying better effectiveness than LPS [61]. Additionally, administration of poly(I:C), a TLR3 agonist that initiates signaling regardless of MyD88, demonstrates antiviral effects in mouse models infected with coronavirus species that are extremely contagious, such as group 2c MERS like coronaviruses [64]. Given the wide range of zoonotic precursors that have the potential to spread into human and cattle populations, it becomes crucial to comprehend how TLR signaling pathways and effector networks could affect coronavirus pathophysiology. Previous studies in SARS-CoV infection in the mouse model have suggested a shielding role for the TLR adapter protein MyD88, which facilitates downstream signaling via multiple TLRs [65]. Research has demonstrated that a powerful cell-intrinsic defense network is activated in response to SARS-CoV illness through MyD88-independent signaling via TLR (3 and 4), facilitated by the TRIF adaptor protein.

3.2 MERS-CoV

Severe respiratory disease with a 30% death rate was first linked to the MERS-CoV in 2012. Human individuals who encounter MERS-CoV develop a severe respiratory illness with a high mortality rate [66]. The most fatal human coronavirus infection to date is MERS [67]. Even though it has a lower rate of human-to-human transmission, almost all MERS-CoV infections can cause severe symptoms, making clinical care difficult. Similar to SARS-CoV, MERS-CoV manifests in humans with serious pneumonia with ARDS, lymphopenia, leukopenia, septic shock, and multi-organ failure [67].

Innate immunity is significantly aided by DCs, which can also significantly increase cytokine and chemokine output. These cells can move from lymphoid tissue to peripheral organs, where they can activate the T cell population [68]. As a result of their role as intermediaries between innate and adaptive immunity, DCs are seen as possible targets for pathogen invasion.

Innate immunity relies on PRRs, such as TLRs and retinoic acid-inducible gene-I- (RIG-I-) like receptors, which play a crucial role [69–71]. One of the two distinct adaptor molecules MyD88 or TRIF—becomes active following TLR virus identification. Various pathways such as MAPK and NF-κB, which are in charge of encouraging the synthesis of proinflammatory cytokines and IFNs, are further activated by these molecules [72–74].

The interaction between MERS-CoV and the host cell's DPP4 receptor via the S protein results in the localization of genomic RNA within the cytoplasm. MERS-CoV replication has the potential to partially trigger an immunological response to dsRNA. When dsRNA sensitizes TLR-3 and triggers a series of reactions involving IRFs and NF-κB activation through TRAF3 and TRAF6, respectively, it leads to the generation of type I IFNs and proinflammatory cytokines. The generation of type I IFNs is crucial for enhancing the secretion of antiviral proteins and protecting cells that are not yet infected.

TLR-3 activates IRF7 and IRF3 following attaching to its specific ligand, irrespective of MyD88 [73]. A possible treatment for MERS-CoV disease in a mouse model has recently been revealed using the TLR-3 agonist poly IC [65]. Type 1 IFN-α and IFN- β expression gets increased after poly IC injection [75]. Consequently, the activation of various effectors such as macrophages, CD8 T cells, and NK cells is initiated, leading to their antiviral effects [76, 77]. The host experiences various detrimental effects due to the proinflammatory cytokine response, including the production of cytokines like TNF-α and IL-6, which can lead to pathological tissue damage during any infection [78]. On the other hand, inflammatory cytokine reactions can restrain the spread of viruses. By comprehending the TLR signaling events in a better way and effectively controlling viral infections, the risk of viral dissemination can be minimized in the picture of infection of MERS-CoV.

TLR-3 activation inhibition and evasion of the immune reaction can occur when auxiliary proteins of the MERS-CoV occasionally bind to the viral dsRNA during replication, leading to interference with TLR-3 signaling. Proinflammatory cytokines may be activated by TLR-4 upon recognition of S protein via the MyD88-dependent signaling pathway. Strong synthesis of immunological mediators results from virus-cell interactions. Following MERS-CoV infection, infected cells are stimulated to release substantial quantities of chemokines (MCP-1, CXCL10) and cytokines (IL-10), which in turn attract lymphocytes and leukocytes to the infection site [79].

3.3 SARS-CoV-2

In the detection of PAMPs from SARS-CoV-2, several TLRs have been implicated, as indicated by recent studies. It has been indicated that an antiviral response to SARS-CoV-2 disease may be triggered by TLR (2, 3, 4, 7/8, and 9) [80]. While TLRs are found all over the human respiratory system, their expression varies among different innate immune cells. For instance, NK cells exhibit a higher abundance of TLR3, while macrophages predominantly express TLR4.

3.3.1 TLR2

In murine macrophages lacking TLR2 and in human macrophages administered with a TLR2 inhibitor exhibit reduced activation of pro-inflammatory signaling events and decreased cytokine production upon the stimulus of the SARS-CoV-2 E protein [81, 82]. This indicates that TLR2 plays a role in detecting the E protein of SARS-CoV-2 and initiating inflammatory responses. Moreover, in vivo, experiments demonstrate that the SARS-CoV-2 E protein promotes inflammation through TLR2-dependency, as evidenced by the decreased levels of IL-6 in the serum of TLR2−/− mice following administration of the E protein [81]. TLR2 plays a role in the activation of innate immunity, according to an independent single-cell computational approach that sought to anticipate potential sites for modification to lessen the dysfunctional innate immune reaction brought on by the virus [83]. These results are supported by the observation that TLR2 inhibitor therapy of K18-hACE2 transgenic mice lowers inflammatory cytokine levels in the blood and increases survivability after this viral infection [81]. To find out whether TLR2 directly interacts with the E protein or other ligands of this virus, more research is needed. Apart from TLR2, the extent of research on the involvement of other TLRs in SARS-CoV-2 infection is not as definitive in both in vitro and in vivo studies.

3.3.2 TLR4

In silico studies indicate that TLR (1, 4, and 6) may be potential binding partners for the SARS-CoV-2 S protein, with TLR4 exhibiting the maximum affinity [84]. The activation of TLR4 in reaction to the S protein is evidenced by the observation that the gene expression of Il1b is reduced in Tlr4−/− murine macrophages stimulated with the S protein compared to wild-type cells in vitro [85].

TLR4 exhibits a remarkable ability to identify various molecular patterns from disease-causing agents and initiates downstream signaling events that involve either of these (MyD88-dependent and MyD88-independent) signaling. These pathways activate TFs such as NF-κB, which govern the expression of the proinflammatory cytokine [86]. Recent findings indicate that the spike glycoprotein, a major infection-causing surface protein of SARS-CoV-2, acts as a ligand for human TLR4 [84], as a result of this protein-to-protein interaction, TFs, including NF-κB, IRFs, and activator protein 1 (AP-1), resulting in the expression of proinflammatory cytokines and IFNs [85]. Intense local inflammation caused by the activation of TLR4 in the alveolar macrophages eventually leads to an accumulation of inflammatory substances that disrupt gas exchange in the respiratory system, respiratory gas, induce issues in breathing, and sometimes result in mortality [84, 87]. The presence of inflammatory agents has the ability to stimulate the immune system including the innate and adaptive immune system, amplifying the inflammation and causing a "cytokine storm", or overt immunopathology. The major organs such as the heart, kidney, pancreas, *etc.* are directly damaged by cytokine storm [88].

3.3.3 Intracellular Toll-like receptors

Intracellular TLRs (TLR3, TLR7, and TLR8) that detect nucleic acids are found in endosomes to avoid the detection of self-genetic material. Like other coronaviruses, SARS-CoV-2 is also a ssRNA virus and after replication in their host cells generates double-stranded RNA [89, 90]. Intracellular RNA sensors are likely to play a key

role in SARS-CoV-2 infection identification. TLR3 recognizes dsRNA via the TRIF signaling pathway in endosomes and stimulates the production of type I IFNs and proinflammatory cytokines. While TLR7 and TLR8 use MyD88 downstream signaling pathways to recognize ssRNA of SARS-CoV-2. Recently three-dimensional lung multicellular spheroids were used to identify the antiviral role of TLR3 and TLR7 following COVID infection [90]. In SARS-CoV-2-infected multicellular spheroids, NF-κB and IRF3 were found to be involved in the TLR3 and TLR7-mediated downstream signaling. Further, the relative expression levels of both TLRs (TLR3 and TLR7) as well as the production of type I IFNs and proinflammatory cytokines were also high.

In vivo, studies have shown that the TLR3 signaling pathway plays a protective role in the context of SARS-CoV infection [42, 91–93]. TLR3-deficient mice (Tlr3−/−) exhibit higher viral load and reduced function when exposed to mouse-adapted SARS-CoV [42]. Furthermore, the activation of TLR3 with an agonist leads to a reduction in SARS-CoV load in human alveolar epithelial cells [92]. TLR3 has not been particularly connected with SARS-CoV-2 sensing in any investigations to date, though. Although a genomic investigation of individuals with severe COVID-19 discovered a link between innate defects in TLR3 and the severity of infectious illness, subsequent research was unable to confirm this link. As a result, it is unknown what role TLR3 plays in SARS-CoV-2 infection.

Another study revealed that genomic ssRNA fragments of SARS-CoV-2 infection activated the expression of TLR7 and TLR8 along with the MyD88 downstream signaling pathway in humans [94]. Because it was found that uridine and guanosine-rich RNA fragments activated the expression of TLR7 and TLR8, and to test their hypothesis this group searched the ligand of TLR7/8 and chose two UG-rich regions within the genome of SARS-CoV-2 and named as SC2-RNA. After treating human monocytes-derived DCs (MoDCs) with SC2-RNA the authors discovered that SCV2-RNA therapy promoted the release of the CXCL9 (T cell-recruiting chemokine) and pro-inflammatory cytokines expression including IL-6, IL-12, and TNF-α. After stimulation with SCV2-RNA, MoDCs matured and induced IFN-γ production in cocultured CD4 and CD8 T cells, indicating that SCV2-RNA may regulate DC activation. In pDCs, similar results were obtained as the expression of CD86 and production of TNF-α and IFN-α increased. The authors hypothesized that ssRNA-induced activation in MoDCs is mediated by the TLR8/MyD88/NF-Kb pathway and TLR7 in pDCs. These findings suggest that endosomal RNA sensors are responsible for recognizing the ssRNA and dsRNA produced by SARS-CoV-2.

4. Therapeutic interventions based on TLRs

TLR's vital role in the viral recognition adaptive immunity regulation makes them strong candidates in vaccine formulation as vaccine adjuvants. TLR agonists are generally accepted because they do not cause side effects while offering dose-saving effects by increasing vaccination effectiveness. TLR4 and TLR9 agonists have been most efficient and have been approved for clinical usage while other TLR agonists including TLR3, TLR5, TLR7, and TLR8 require more research. Increased understanding of TLR expression and function, as well as their involvement in viral infection response, may lead to the creation of vaccination platforms including intrinsic TLR PAMPs TLR agonists to elicit efficient and lasting responses from the immune system [95].

TLR agonist adjuvants are more efficient in producing a humoral immune response as compared to first-generation adjuvants like aluminum adjuvants. Recently

TLR agonists such as CpG oligonucleotide (ODN) Pam3CSK4, resiquimod (R848), poly(I:C), and monophosphoryl lipid A (MPLA) are being studied as vaccine adjuvants against SARS-CoV-2 infection. Pam3CSK4, a synthetic triacylated lipopeptide TLR1 & TLR2 ligand that can activate the proinflammatory transcription factor NF-B. XS15, a novel water-soluble synthetic Pam3CSK4-derivative, is being used in conjunction with spike protein peptides of SARS-CoV-2 to create a vaccine format that promotes CD4+ T-cell responses against peptides anticipated to bind to human leukocyte antigen – DR isotype (HLA-DR) [96].

LPS, a TLR4 ligand, can regulate the effector T-cell differentiation and influence inflammation. MPLA is a modified version of LPS that stimulates the immune system while avoiding most inflammatory effects. It is found most effective against hepatitis B and human papillomavirus as an adjuvant vaccine and is licensed for use against these viruses. In the previous study fusion of human IgG FC region (S377-588-Fc) and Alum-adjuvanted spike protein of Middle East respiratory syndrome coronavirus produced a significantly higher titer of specific IgG antibodies against this virus as compared to MPLA-adjuvanted protein. Similarly, a higher Th2 (IgG1)-biased response was evoked followed by MPLA-adjuvanted S377-588-Fc protein [97]. A biomaterial-based COVID-19 vaccine also showed a strong adaptive immune response. Biomaterial mesoporous silica rods loaded with SARS-CoV-2 viral protein, a growth factor (granulocyte-macrophage colony-stimulating factor) and MPLA showed an effective immune response by delayed releases after making subcutaneous scaffoldings. These biomaterials recruited the antigen-presenting cells on the local site and produced a strong adaptive immune response [98].

SARS-CoV-2 continues to mutate; however, MPLA-adjuvanted antigens such as S-trimer/MPLA, RBD/MPLA, and S1/MPLA continue to elicit significant cellular and humoral and cellular immune responses against spike variants (alpha, beta, gamma, delta, and omicron) [99].

TLR7 and TLR8 activation could be examined as a new technique for anti-SARS-CoV-2 vaccinations to solve the present problem of viral escape from the immune system. TLR7 activation exerts an antiviral immune response through Th1 while also exerting favorable broncho-vasodilatory effects [100]. Iquimod, a synthetic TLR7 ligand, has been licensed against perianal and genital wart infection induced by human papillomavirus.

A synthetic agonist of TLR7 and TLR8 named resiquimod has been developed that elicits an effective immune response by IL-6, IFN-α, IFN-β, and proliferation of B cell and T cells. Cytokine profiles induced by the mRNA COVID-19 vaccine and resiquimod are the same [101]. The complex of SARS-CoV-2 spike protein S1 subunit, resiquimod, and nanoparticle-based SARS-CoV-2 virus vaccine exert a greater immune response by activation and maturation of antigen-presenting cells, systemic T cells, and specific B cells response as compared to nanoparticles alone [102].

Anti-coronavirus immunity is most likely provided by CD8+ T cells and neutralizing antibodies. TLR3 and TLR9 ligands Poly(I:C) and CpG ODN and Poly(I:C) significantly increase CD8+ T-cell responses more than other adjuvants among TLR agonists. These ligands CpG ODN and poly(I:C) have therefore been used as adjuvants in vaccines against the influenza virus. Inactivated SARS-CoV-2 vaccine combined with CpG ODN has exponential effectiveness by improving the humoral immune response when administered in animals [103, 104]. Boosting with nanoparticles formulated with CpG 1018 and poly(I:C) results in enhanced T cells and B cell activation and IFN- production, as compared to the alum adjuvanted SARS-CoV-2 S1 vaccination [105].

5. Conclusion

TLRs play a vital role in the innate immune system and recognize a wide range of PAMPs. This chapter has described the role of TRLs in the immunity against emerging viral infections including SARC CoV, MERS CoV, and SARC CoV-2. TLRs (2, 3, 4, 7, 8, and 9) are considered very effective against these emerging viral pathogens. TLR 3 is TRIF dependent while TLRs 7 and 8 are MyD88 dependent for the downstream signaling. Viral components including E protein, S protein, and viral genetic material (ssRNA and dsRNA intermediate) act as ligands for these TLRs. Upon viral invasion, TLRs recognize the infectious agent trigger the downstream signaling, and ultimately initiate the immune response. As a result, the production of pro-inflammatory cytokines and type I interferons (IFNs) starts that ultimately initiate the antiviral immune response to clear the invading agent. However overactive immune system activity may lead to detrimental production of pro-inflammatory cytokines and chemokines that can lead to severe illness and widespread inflammation. TLR antagonists are found satisfactory against these severe infections and most of these therapeutics clinical trials are under different phases.

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Chapter 4

TLRs and Other Molecules Signaling Crosstalk in Diseases

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Abstract

Inflammatory diseases affect human health and the quality of life, causing heavy medical burdens in our society. Multiple pathogen-related molecular patterns, risk-related molecular patterns, and inflammatory cytokines exist in the inflammatory environment; these molecules activate immune cells and trigger inflammatory responses through pattern recognition receptors and cytokine receptors. Inflammatory molecules can activate immune cells alone or together through signaling crosstalk. For example, macrophages pretreated with interferon γ enhance Toll-like receptor 4 signal-induced gene expression through epigenetic remodeling. However, there are multiple forms of interactions between inflammatory molecules, including synergistic effects and antagonistic effects. At present, the forms of crosstalk between inflammatory molecules and TLRs that participate in immune cell activation and inflammatory disease progression and their detailed mechanisms are not fully discovered yet. In this chapter, we will enumerate the interaction between different immune molecules and TLRs and discuss how the interactions affect the process of inflammatory disease development and progression.

Keywords: TLRs, signaling transduction, inflammasome, cell death, inflammation, diseases

1. Introduction

During infection and sterile inflammatory conditions, the host's immune response is triggered by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which are recognized by pattern recognition receptors (PRRs). Therefore, PRRs are critical during infection and noninfection-associated inflammatory diseases. There are four major families of PRRs involved in this process: Toll-like receptors (TLRs), RIG-like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs). These PRRs have overlapping ligand specificities and converge on common downstream signaling pathways, such as the NF-κB pathway [1, 2]. TLRs are membrane-bound receptors, which can travel between cell surface membranes and intracellular compartments, including endosomes, endolysosomes, phagosomes, and phagolysosomes [2, 3]. TLR1, 2, 4, 5, 6, and TLR10 are located in the cell surface membrane, while TLR3, 7, 8, 9, 11, 12, and 13 are located in the intracellular compartments [2, 4–6]. TLRs recognize a wide range of PAMPs, including bacterial and viral components; RLRs are cytoplasmic receptors

that primarily detect viral RNA, while NLRs detect various intracellular PAMPs and DAMPs. CLRs are expressed on the surface of immune cells and recognize carbohydrates in pathogens [2, 7–9].

Upon activation, PRRs initiate signaling cascades that activate transcription factors, such as nuclear factor-kappa B (NF-κB), thereby inducing the production of proinflammatory cytokines and other immune mediators [2]. This coordinated immune response helps to eliminate the invading pathogens and restore tissue homeostasis. While there are numerous examples of positive and synergistic interactions between different PRRs and inflammatory molecules, it is also essential for the host to have mechanisms in place to prevent excessive immune activation and inflammation. Notably, PRRs can also exhibit negative regulation of each other, especially in the context of different pathogen infections. For instance, prior viral infection has been shown to increase susceptibility to subsequent bacterial infection [10, 11]. To maintain immune balance, various mechanisms come into play, including upregulation of inhibitory pathways, induction of anti-inflammatory mediators, and internalization and degradation of receptors. Additionally, PRRs and inflammatory molecules can directly antagonize each other at the signaling level. These regulatory mechanisms collectively contribute to preventing excessive immune responses and maintaining immune homeostasis [12]. In this chapter, we summarized the positive and synergistic interactions between TLRs and certain molecules, but also reviewed the negative regulatory or antagonistic effects of signaling crosstalk between TLRs and certain molecules. These signaling crosstalks are involved in and contribute to various pathogenic conditions. The understanding of the mechanisms of various paradigms of signaling crosstalks between TLRs and other molecules would benefit to develop new therapeutic strategies for disease control.

2. Signaling crosstalk between TLRs and other molecules in diseases

2.1 IFN signaling training to augment TLR response

Interferon γ (IFN-γ) is a very important cytokine for host defense against pathogen invasion and tumorigenesis, with the ability to modulate the immunological status in both infected cells and tumor cells [13–15]. IFN-γ is also involved in inflammatory diseases, where it activates inflammatory immune cells, especially monocytes and macrophages. IFN-γ combined with lipopolysaccharide (LPS) activate IFN-γ receptor- and TLR4-mediated signaling crosstalk to polarize macrophages toward an 'M1-like' state, characterized by increased proinflammatory responses and resistance to anti-inflammatory factors [16, 17]. In addition, cells pretreated with IFN-γ for 24 hours (hrs) or 48 hrs are very sensitive to TLRs signaling for inflammatory gene expression [18, 19]. In vivo, IFN-γ pretreatment increases LPS and TLR9 ligand CpG-induced responses and improves the capability of host cells to control pathogen infection [20]. Type I IFN (IFN-1) signaling modulates monocyte fate from tumor-associated macrophages to active monocytes, which can activate $CD8^+$ T cells for tumor immunity [21]. Similar to IFN-γ, IFN-1 can also train monocytes/macrophages, but only under a concentration higher than 10 ng/ml in vitro [22]. Monocytes/macrophages primed with high concentration of IFN-1 are highly sensitive to TLR ligands, such as LPS (TLR4 ligand), ORN8L (TLR8 ligand), Pam3CSK4 (TLR2 ligand) and Poly (I:C) (TLR3 ligand), thereby inducing massive inflammatory response and cytokine storm responsible for tissue damage and dysfunction during viral infection,

such as coronavirus disease 2019 (COVID-19), influenza pneumonia, etc. [22, 23]. IFN-1 signaling is the hallmark of and contributes to the development of autoimmune diseases such as systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) [24–26]. Signaling crosstalk between IFN-1 and self-DAMPs-mediated TLR activation underlies the pathogenesis and severity of inflammatory disorders [27].

Through the mechanism study of the model of IFN and TLR4 signaling crosstalk, we now know that both IFN-1 and IFN-γ-mediated training seem to be not through modulating TLR signaling pathway activities but to be through epigenetic chromatin remodeling of monocyte/macrophages to decrease the threshold of the cell sensitivity to TLR ligands for inflammatory gene expression [19, 22]. Epigenetic remodeling is a sophisticated process. How does IFN signaling initiates the epigenetic remodeling process and changes the chromatin accessibility of inflammatory genes is still under heavy study. In addition, IFN signaling also selectively alters macrophage metabolic pathways by suppressing TLR4-mediated gene activation [18, 22]. While IFN-γ abrogates the LPS-induced IL-10 negative feedback loop by completely suppressing IL-10 expression [18, 19], IFN-1 does not suppress LPS-induced IL-10 expression [22]. But, whether IFN-1 affects IL-10 auto-loop to augment inflammatory gene expression induced by TLRs is unclear yet. These findings suggest that similar but different mechanisms underpin IFN-1 and IFN-γ training.

2.2 IFN-1, TNF, and TLR signaling crosstalk

Tumor Necrosis Factor (TNF) plays an important role in host defense and inflammation and contributes to various inflammatory diseases and tumors. However, pretreatment of the human macrophages with TNF for a minimum of 12 h suppresses TLR4-mediated inflammatory gene expression and diminishes protection from LPS-induced cell death [28, 29]. This phenomenon is referred to as TNF cross-tolerance, which resembles classical endotoxin tolerance (which will be discussed in the other part of this chapter). The TNF cross-tolerance is mediated by the inactivation of glycogen synthase kinase 3β (GSK-3β) and the subsequent accumulation of NF-κB inhibitor alpha (IκBα), which prevents the translocation of active NF-κB to the nucleus and the induction of proinflammatory cytokines. TNF-induced tolerance may contribute to the regulation of sterile inflammation in conditions such as surgery, ischemia/reperfusion, and acute coronary syndrome, as well as to the control of chronic inflammation in autoimmune diseases [30–32]. A subgroup of genes substantially expressed by TNF treatment can be superinduced by secondary LPS challenge, referred to as the synergistic effect of TNF and TLR signaling crosstalk [29]. In the context of the TNF and TLR4 signaling crosstalk example, we believe that, in a substantial signaling crosstalk situation, synergistic effect and tolerizing effect co-exist for different subgroups of gene expression. The synergistic genes induced by TNF and TLR4 signaling crosstalk are TNF late response genes, which are epigenetic primed by TNF and are mainly involved in IFN signaling and lipid-metabolic processes [29]. Individuals with inflammatory diseases such as sepsis and SLE are exposed to TNF and TLR ligands. Strikingly, monocytes from septic patients mimic what happens during TNF and TLR4 signaling crosstalk for gene expression patterns [29]. However, the gene expression patterns in SLE differ from those regulated by the combination of TNF plus TLR4. IFN-1 s drive the pathogenesis of SLE and contribute to training immunity [22, 33]. Thus, the altered gene expression profile in SLE is modified by IFN-1 s in the presence of TNF and TLR signaling. Indeed, IFN-1 s block TNF-mediated tolerization of TLR4-induced inflammatory gene expression through

epigenetic regulatory mechanisms [29]. In addition, the efficacy of TNF-induced feedback inhibitory mechanisms may be compromised by other cytokines like IFN-γ or genetic variants in genes involved in tolerance regulation, for example, TNF alpha-induced protein 3 (TNFAIP3, encodes A20) [34, 35].

2.3 Heme and TLR signaling crosstalk activates PANoptosis and pathology

During infection or inflammatory disorders, cell death occurs, and multiple DAMPs are released in the process of inflammatory cell death. The presence of PAMPs and DAMPs mimics infection and cell lytic diseases. The signaling crosstalk between PAMPs and DAMPs may affect the progress of the disease [36]. Heme is one important DAMP that is released into the bloodstream when red blood cells are damaged or lysed during infectious and inflammatory diseases [37, 38]. Heme can activate TLR4 signaling and inflammatory responses [38, 39]. Recently, it has been demonstrated that heme can coordinate with TLR2, TLR4, and TLR7 signaling, but not TLR3 signaling to activate a late inflammatory cell death-PANoptosis [36], which is a type of cell death caused by the combination of either apoptosis, pyroptosis or necroptosis [40]. The late PANoptosis induced by heme and PAMPs might be attributed to the need for time for the activated cells to produce enough amounts of critical protein interferon regulatory factor 1 (IRF1) and NLR family pyrin domain containing 12 (NLRP12). Although how does the combination of heme with certain TLRs initiates the signaling cascades to induce the late PANoptosis is unclear, heme and TLRs costimulation induced IRF1 and the generation of reactive oxygen species (ROS), which were responsible for NLRP12 expression; NLRP12 thereby interacted with and activated Caspase-8, RIPK3, and NRLP3 for apoptosis, necroptosis and pyroptosis [36]. Injection of phenylhydrazine (PHZ) to mice can induce hemolysis, which increases the level of heme in serum. The combination of PHZ and a nonlethal dose of LPS-induced kidney damage in mice, while the deficiency of *Nlrp12* could significantly protect mice from lethality and kidney damage [36]. High levels of free heme exist in various pathological conditions [38, 41]. The signaling crosstalk between heme and TLRs is likely implicated in numerous inflammatory and infectious diseases. Targeting the critical signaling nodes such as NLRP12 will be beneficial to the control of disease progression.

2.4 Chemokines participate in regulating cytosolic TLR signaling

The natural ligands of cytosolic TLR3/7/8/9 are nucleic acids (NAs), which are derived from microbial DNA/RNA or extracellular NAs that are released by dying or netting cells. These NAs are negatively charged molecules, which can form nanoparticles with cationic proteins via charge:charge interactions, such as LL37 and HMGB1. The formed nanoparticles facilitate the internalization of NAs into the endosome for cytosolic TLR recognition [42–47]. Thus, some of the cationic proteins are involved in and amplify cytosolic TLR responses to trigger and to exacerbate inflammatory diseases [48–51]. Chemokine (C-X-C Motif) ligand 4 (CXCL4), CXCL10, CXCL12, CXCL14, and C-C motif chemokine ligand 5 (CCL5) are such cationic proteins that can form nanoparticles with TLR9 NA ligand CpG to increase the internalization of CpG in plasmacytoid dendritic cells (pDCs), thereby superinducing type I IFN, independently of their known chemokine receptors [27, 52]. CXCL4, for example, is the most studied chemokine that physically interacts with CpG to form nanoparticles. The nanoparticles can be taken up by pDCs through clathrin-mediated endocytosis. Besides, the

nanoparticles formed between CXCL4 and CpG mediate transcriptional and epigenetic changes in pDCs that are responsible for the superinduction of IFN-1 [27].

CXCL4, activating a C-X-C motif chemokine receptor 3 (CXCR3)-independent inflammatory response in human monocytes, also forms nanoparticles with TLR8 oligoribonucleotides ligand (ORN8L) and promotes the internalization of ORN8L into the endosome of human monocytes [51]. The signals induced by both CXCL4 and ORN8L lead to a massive TBK1/IKKε activation, which then activates IRF5 and IRF3 to induce strong inflammatory gene and IFN-1 expression. The signaling crosstalk between CXCL4 and TLR8 also triggers NLRP3 inflammasome activation, IL-1β secretion, and pyroptosis. In addition, both CXCL4 and TLR8 signaling themselves can induce epigenetic remodeling; the combination of CXCL4 and TLR8 signaling mounts the epigenetic remodeling in human monocytes, which increases the accessibilities of NF-κB, AP-1, and IRFs to the opened chromatins of inflammatory genes [51, 53]. In addition, the signaling crosstalk between CXCL4 and TLR8 activates RIPK3 to modulate inflammatory gene expression and IFN response through activating PI3K-AKT serine/threonine kinase (AKT)-X-box binding protein 1 (XBP1)/NFE2 Like BZIP transcription factor 2 (NRF2) axis and through regulating signal transducer and activator of transcription 1 (STAT1) activation [54]. Both TLR8 and CXCL4 are highly expressed and associated with various inflammatory diseases including SSc and rheumatoid arthritis (RA) [33, 55]. Thus, the signaling crosstalk between CXCL4 and TLR8 ought to contribute to inflammatory disorders. Except for CXCL4, NET-associated RNA in complex with cationic LL37 amplifies TLR8 response in peripheral blood mononuclear cells (PBMCs) [56, 57]. With these examples and the evidence of charge:charge interactions between cationic chemokines and NAs, it is reasonable to deduce that chemokine CXCL10, CXCL12, CXCL14, and CCL5 might likewise contribute to TLR8 response.

2.5 TLR7/9 and B cell receptor-Signaling crosstalk in autoimmune diseases

B cells are lymphocytes that play an important role in adaptive immunity by producing antibodies against pathogens and tumor cells. In autoimmune diseases such as SLE, aberrant activation of B cells by sensing DNA and RNA self-antigens produces autoantibodies that cause inflammation, tissue damage, and dysfunction. B cell activation relies on B cell receptor (BCR)-mediated signaling, co-stimulatory molecules-mediated signaling, and cytokines. In autoimmune conditions, the ligands of TLR7 and TLR9 are endogenous NAs, which are more likely to form complexes with cationic protein or antibodies, facilitating the internalization of NAs for TLR7 and TLR9 activation. It has been shown that such BCR as rheumatoid factor (RF) AM14 BCR specifically can bind with low affinity to IgG2a to facilitate the internalization of its bound endogenous or synthetic, highly purified NA [58]. Similar to myeloid cells, B cells also experience tolerance after TLR signaling [59]. Nevertheless, simultaneous activation of BCR signaling and TLR signaling overcome TLR tolerized B cells [59]. TLR7 and TLR9 can activate B cells and cooperate with BCR signaling to induce substantial high amounts of cytokine expression and RNA-associated and DNA-associated autoantibody production, respectively [58]. TLR9 activation of atypical memory B cells from hepatitis C virus (HCV) infected patients triggers TNF secretion and rheumatoid factor-type IgMs; in addition, the TLR9 activated atypical memory B cells promote type 1 effector T cell activation and reduce regulatory T cells proliferation, which are associated with HCV infection caused with autoimmune disorder cryoglobulinemia vasculitis [60]. In lupus-susceptible mice, TLR7 is responsible for the generation of

antibodies to RNA-containing antigens [58, 61]. In these mice, however, loss of TLR9 exacerbates autoimmune diseases with increased serum IgG and IFN-1 [58, 61]. Further, it has been demonstrated that TLR7 and TLR9 in B cells play pathogenic and protective roles in the balance of the autoimmune disease progression, respectively [62]. The opposite function of TLR7 and TLR9 in autoimmune diseases might be explained by the fact that TLR9 can directly suppress TLR7 signaling through direct or indirect physical interactions between the TLRs [63, 64].

2.6 TLRs interactions affect their signaling in immune response

Heme and TLRs signaling crosstalk to induce PANoptosis; the signaling crosstalk also exists in the combination of different TLRs to activate PANoptosis. Intriguingly, the combinations of TLR2 signaling with TLR7 signaling fail to induce PANoptosis [36]. The reason for this is still elusive but could basically exclude the contribution of IFN-1 signaling in the system, as TLR7 is professionally responsible for IFN-1 expression [22]. However, TLR7 and TLR3 or TLR4 signaling crosstalk could induce PANoptosis [36]. The concurrent activation of various TLRs, such as TLR2 and TLR4 sensing bacterial wall components, along with TLR3 and TLR7 detecting bacterial RNAs, closely mimics the effects of a live bacterial infection. This resemblance suggests that PANoptosis occurs during infectious diseases and contributes to host defense or severe inflammation in conditions like sepsis.

TLR8 and TLR2 signaling crosstalk affects cell activation. During *Borrelia burgdorferi* infection, TLR8 and TLR2 cooperatively activate NF-κB-mediated cytokine production in human monocytes, whereas TLR8 alone is solely responsible for the transcription of IFN-β. The activation of TLR2, TLR4, or TLR5 suppresses TLR8-IRF5 signaling and IFN-β expression partly through regulating Interleukin 1 Receptor Associated Kinase 1 (IRAK1) [65–68]. During *Mycobacterium tuberculosis* (Mtb) infection, however, TLR4 and TLR8 form heterodimers in endosomes to increase the activation of TLR8, which thereby activates Th1 responses against Mtb [69]. The loss and instability of TLR8 contribute to TLR7 activation and spontaneous autoimmune disorders [70, 71]. TLR8 can inhibit TLR7 and TLR9 functions, and TLR9 can suppress TLR7 function in B cells, but not vice versa [63, 71, 72]. These inhibitory effects seem not to rely on direct competition for downstream signaling proteins but not on the direct or indirect physical interactions between TLR8 and TLR7/9 (probably by forming heterodimers) [63, 73].

TLR tolerance refers to a modified responsiveness (unresponsiveness or low responsiveness) of cells following repeated or chronic activation of TLRs. The phenomenon of LPS-induced tolerance, involving TLR4 and endotoxin, was the first to be described and was observed primarily in monocytes, macrophages, and dendritic cells. LPS tolerance drives comprehensive transcriptional reprogramming, shifting the inflammatory response toward the expression of anti-inflammatory and pro-resolution factors while maintaining innate immune protection. Further studies revealed gene-specific regulatory mechanisms and epigenetic changes involved in TLR-tolerance development [74]. It is worth noticing that TLR tolerance is a reversible phenomenon, and these changes can be reversed over time or in response to competing signals [74–76].

Heterologous tolerance, or cross-tolerance, which refers to the reduced responsiveness to TLR stimulation after initial exposure to a specific ligand, may not be as effective as auto tolerance induced by repeated stimulation of the same TLR type. For instance, cells initially treated with synthetic lipopeptide TLR2 agonist MALP-2 fail to

respond to subsequent stimulation with TLR4 ligand LPS [77]. This MALP-2-induced cross-tolerance to LPS is not attributed to the decrease in TLR4 surface expression. Instead, it is believed that TLR2-induced tolerance specifically affects the signaling of TLR4 and TLR7 ligands, while leaving TLR3 and TLR5 signaling unaffected. This effect is achieved through the inhibition of paracrine type I interferon amplification, leading to the suppression of IL-12 production [78]. Similarly, treatment with a low concentration (1 μg/ml) of Pam3CSK4 induced a state of tolerance, leading to reduced production of TNF-α and IL-6 upon secondary stimulation with LPS [79]. On the other hand, TLR9 triggers the production of IL-12 family members in response to intact Gram-positive bacteria. However, in the absence of TLR2 signaling, this response becomes exaggerated in microglia [80]. Interestingly, when cells are treated with a combination of TLR2, TLR4, and TLR9 ligands, auto tolerance is induced for each TLR toward these ligands, while cross-tolerance is specifically induced by lipoteichoic acid and LPS, but not by CpG (TLR9 ligand). Besides LPS, TLR7/8 agonist (R848) has also been shown to induce homologous and heterologous tolerance to various TLR ligands in macrophages [81]. These findings suggest that TLR cross-tolerance is mediated by distinct mechanisms depending on the specific ligands involved [82].

2.7 TLRs and NLRs signaling crosstalk

NOD-like receptors (NLRs) work distinctly from TLR that function as cytoplasmic receptors to recognize PAMPs and DAMPs. Most NLRs play important roles during inflammatory diseases by driving inflammation, while some NLRs serve as negative regulators of inflammation. The best-characterized members of the NOD subfamily are Nucleotide Binding Oligomerization Domain Containing 1 (NOD1) and NOD2, which detect distinct subunits of bacterial peptidoglycans. The activation of NOD1 was found to inhibit the activation of TLR1/2, leading to a decrease in the secretion of IL-6 and IL-10 and a reduction in the percentage of $CD11b^+$ $F4/80^+$ macrophages. These findings suggest that NOD1 exhibits antagonistic effects of TLR1/2 response in macrophages [83]. While there is evidence supporting a cooperative interaction between TLR2/4 and NOD2 in cytokine production [84–86], NOD2 has the ability to inhibit the induction of inflammatory cytokines mediated by TLR2/4 and promote immune tolerance and homeostasis [87–89]. In mice, intact NOD2 signaling inhibits the NF-κB activation driven by TLR2, primarily through the inhibition of c-Rel [89].

NLRX1 is a unique member of the NLR family which is localized in the mitochondria. Xia et al. demonstrated that NLRX1 acts as a negative regulator of TLR-mediated NF-κB signaling [90]. Upon LPS stimulation, NLRX1 is rapidly ubiquitinated, disassociates from TRAF6, and then binds to the IKK complex, leading to inhibition of IKKα and IKKβ phosphorylation and NF-κB activation [90, 91]. The NLRX1-dependent suppression has also been tested with a number of NLRX1 binding molecules, e.g. punicic acid (PUA) in both in vitro as well as an in vivo DSS model of colitis [92]. Similarly, additional NLRs, including NLRC3, NLRC5, NLRC6, and NLRP12 have been shown to play a role in regulating TLR-induced canonical and noncanonical NF-κB activation and MAPK signaling pathways [93–99].

2.8 TLR and CLR crosstalk

C-type lectin receptors (CLRs) are intracellular receptors expressed on antigen-presenting cells (APCs) and function as PAMP recognition and antigen-uptaking,

playing a crucial role in the immune system. When activated, CLRs recognize specific carbohydrate structures on pathogens and initiate immune responses. In the context of APCs, such as dendritic cells (DCs), triggering the dendritic cell immunoreceptor (DCIR), a specific CLR, does not affect the upregulation of neither TLR4- nor TLR8-mediated co-stimulatory molecules CD80 and CD86. Interestingly, DCIR activation inhibits the production of IL-12 and TNF-α during TLR8 activation. This suggests that DCIR can modulate specific cytokine responses induced by TLR8 signaling. However, the production of cytokines induced by TLR2, TLR3, and TLR4 is not affected by DCIR triggering [100]. Mannose Receptor, an important CLR that interacts with a number of products generated by a variety of helminths, is able to downregulate TLR4-mediated IL-12 production in DCs to favor Th2 cell responses. The engagement of macrophage galactose-type lectin (MGL) enhances TLR-induced IL-10 expression, which in turn promotes the generation and activation of regulatory T cells. This regulatory T cell response contributes to immune tolerance and the control of immune reactions, helping to maintain a balanced immune system.

2.9 Crosstalk between complement receptor and TLRs

The complement system and TLRs are two crucial components of the innate defense system that rapidly respond to infection. In recent years, there has been increasing evidence indicating the existence of crosstalk between the complement system and TLR signaling pathways [101]. The crosstalk between C5a receptor (C5aR) and TLR4 signaling leads to the downregulation of primarily proinflammatory mediators, such as IL12B, IL2RA, and jagged canonical notch ligand 1 (JAG1), and the upregulation of anti-inflammatory factors, including Sphingosine kinase 1 (SPHK1), adrenoceptor beta 2 (ADRB2), and four and a half LIM domains 2 (FHL2), in monocyte-derived dendritic cells (moDCs). This crosstalk is mediated, at least in part, by the transcription factors Forkhead Box O1 (FOXO1), FOXO3, and serum/glucocorticoid regulated kinase 1 (SGK1) [102, 103]. C5a-C5aR1 pathway activated by *Porphyromonas gingivalis* infection can induce ubiquitination and proteasomal degradation of the TLR2 adaptor myeloid differentiation primary response 88 (MyD88), leading to suppression of the antimicrobial effect of neutrophils [104]. While C5aR1-TLR2 crosstalk also dampens intracellular killing of *P. gingivalis* in macrophages with a mechanism that is different to what happens in neutrophils. C5aR1-TLR2 crosstalk induces massive Cyclic adenosine monophosphate (cAMP) production to activate protein kinase A (PKA), which inactivates GSK-3β and inhibits the expression of inducible nitric oxide synthase (iNOS). The consequence of this causes the reduction of nitric oxide-dependent killing of *P. gingivalis* in macrophage [105].

Complement receptor 3 (CR3; CD11b/CD18) plays a controversial role in regulating TLR response by either promoting TLR response through membrane-bound phosphatidylinositol-(4,5)-bisphosphate (PIP2) to recruit Mal/MyD88 for initiation of MyD88-dependent signaling or suppressing TLR response through activating Syk and promoting the degradation of MyD88 and TIR domain-containing adaptor molecule 1 (TRIF) via the E3 ubiquitin ligase Cbl-b [106–108]. Activation of C5aR1 and CR3 signaling by *P. gingivalis* selectively downregulates TLR2-induced IL-12 production through activation of the phosphoinositide 3-kinase (PI3K) and ERK1/2 pathways in macrophages. In addition, gC1qR, a complement receptor for C1q, also suppresses TLR-induced IL-12 but no other inflammatory genes in human monocytes [105]. The selective inhibition of IL-12 production by complement receptor causes less IFN-γ production, ultimately leading to enhanced survival of the pathogen during infection [106].

2.10 GPCR and TLR signaling crosstalk

Recent studies have shed light on the intricate interplay between G-protein coupled receptor (GPCR) and TLR signaling pathways, revealing a fascinating crosstalk that significantly influences immune responses and cellular functions. Protease activated receptors (PARs) are a family of GPCRs that mediate serine proteases triggered cellular effects. Activation of PAR2 by PAR2-AP reduced TLR3-mediated STAT1 activation and TLR3/IRF3-induced IFN-β expression [109]. However, using a global PAR1 deficient mouse model, it has been revealed that PAR1 promoted polyinosinic-polycytidylic acid (poly I:C)-triggered CXCL10 expression while suppressed CXCL1 induction [110]. In addition, PAR1 activation was found to suppress TLR4-mediated NF-κB activation in murine embryonic fibroblasts [111].

A2A adenosine receptor (A2AR), another member of the GPCR family, plays a critical role in a physiological immunosuppressive pathway. By utilizing A2AR-deficient mice, Lukashev et al. uncovered the role of A2AR in inhibiting NF-κB activity and the transcription of proinflammatory cytokines induced by TLR in vivo [112]. Chemokine receptors, belonging to the large family of GPCRs, play a crucial role in facilitating cell migration through binding to their specific chemokine ligands. These receptors can be categorized into four main subtypes: CXC chemokine receptors, CC chemokine receptors, CX3C chemokine receptors, and XC chemokine receptors. Among CCR8 has been implicated in the progression of various diseases such as sepsis, type I diabetes, and experimental autoimmune encephalomyelitis [113]. CCL1 per se, as a ligand of CCR8, can induce TNF-α and IL-6 production in macrophages; however, CCL1 also exerts a suppressive effect on LPS-mediated cytokine production [113].

2.11 Crosstalk between immunosuppressive mediators and TLRs

TLR signaling pathways were also being regulated via immunosuppressive mediators. IL-10, known for its potent inhibitory effects on innate immune cells, is the most effective regulator of TLR-induced inflammatory cytokine production [114, 115]. Some pathogens take advantage of the regulatory effects of IL-10 to dampen immune responses and evade host immune defenses. This manipulation allows the pathogens to establish infectious niches within the host, leading to immune evasion and disease progression. These studies offer mechanistic insights into the immunoregulation of IL-10. IL-10 distinctly affects LPS-induced IP-10 and IL-12B expression—by inhibiting type I interferon production and restraining RNA polymerase II recruitment [116, 117]. Conversely, IL-10-responsive microRNA, miR-146b, emerges through an IL-10-mediated loop, adeptly targeting various TLR4 signaling components [118]. It achieves this not only through direct inhibition of cytokine transcription but also by destabilizing the coding RNA [116, 117] and upregulation of miRNA by and IL-10-mediated STAT3-dependent loop [118]. Furthermore, it has been observed that IL-10 activates the PI3K-Akt-GSK signaling pathway, leading to the suppression of inflammatory gene expression [119]. These findings collectively highlight the multifaceted role of IL-10 in quelling immune responses by intervening at various points within TLR4 signaling pathways.

IL-4 and IL-13, both significant immunoregulatory cytokines, prompt alternative macrophage polarization. IL-4 exhibits inhibitory effects on IFN-β and IFN-responsive gene expression upon TLR7 and TLR9 stimulation in conventional dendritic cells (cDCs), achieved by curbing IFN-dependent and NF-κB-dependent signaling pathways [120]. These inhibitory actions of IL-4, along with the suppression

of proinflammatory cytokine production, operate through the STAT6 pathway. Recent research emphasizes the critical role of IL-4-activated STAT6 transcription factor in directly repressing the LPS-induced inflammatory program of macrophages. This repression results in reduced lineage-determining transcription factors, p300, and RNA polymerase II binding, coupled with diminished enhancer RNA expression, H3K27 acetylation, and chromatin accessibility. Notably, the repressed enhancers influenced by STAT6 significantly overlap with the NF-κB p65 cistrome, resulting in reduced responsiveness to LPS post-IL-4 stimulation. This cascade ultimately leads to decreased inflammasome activation, IL-1β production, and pyroptosis [121].

In addition to the interleukin signaling pathways that suppress TLR activation, two orphan receptors, SIGIRR and ST2, also play significant roles in modulating immune responses. SIGIRR, also known as TIR8, possesses a unique architecture with a single extracellular immunoglobulin domain, a transmembrane domain, a cytoplasmic TIR domain, and an unusually long tail [122–124]. It selectively inhibits NF-κB activation induced by TLRs and IL1R1, which are TIR domain-containing receptors. Interestingly, SIGIRR also modulates the TRIF-dependent pathway and inhibits TLR3 signaling. Its inhibitory mechanism involves blocking interactions between TRAM and TLR4, as well as TRIF and TRAM. ST2, or IL1RL1, is another orphan receptor with a cytoplasmic TIR domain. It inhibits NF-κB activation in response to IL-1R1 and TLR stimulation as well. ST2 interacts with Mal and MyD88, suggesting its inhibitory roles are specific to the MyD88-dependent pathway of TLRs. Thus, overexpression of ST2 specifically prevents NF-κB activation induced by TLR4, but not TLR3, which utilizes a TRIF-dependent downstream pathway. Notably, ST2 can form homodimers with TLR4, MyD88, and TRAM, while its interactions with Mal and TRIF disrupt Mal homodimerization and TRAM-TRIF interaction, respectively. Although the physiological relevance of ST2's interaction with TRAM and TRIF may require further investigation, computational analysis predicts these interactions.

Transcription factor aryl hydrocarbon receptor (AhR) is a cytosolic sensor. AhR has been suggested as an immunosuppressive effector on various types of immune cells, playing a crucial role in modulating immune responses [125, 126]. Under TLR activation, AhR is induced in both macrophages [127] and DCs [128]. In AhR-deficient macrophages, the production of IL-6 and TNF-α in response to LPS was notably increased compared to wild-type (WT) cells. Interestingly, activation of AhR increased the expression of IL-1β but decreased the expression of IL-12A in TLR-activated MoDCs through a NF-κB RelB-dependent manner.

Tryptanthrin, a natural alkaloid, exhibits anti-inflammatory effects by modulating the immune response. In PMA-differentiated THP-1 cells, tryptanthrin reduces the phosphorylation of STAT1 in response to TLR3 ligand poly I:C. This results in the suppression of interferon-stimulated gene expression and the inhibition of IFN-β induction [129].

2.12 Crosstalk between nuclear receptor and TLRs

Ligand-activated nuclear receptor (NR) transcription factors, such as the glucocorticoid receptor (GR), peroxisome proliferator-activated receptor gamma (PPARγ), and liver X receptor (LXR), exhibit robust inhibitory effects on TLR-induced inflammatory gene expression. In a recent study, it was discovered that Nuclear Receptor Related 1 (NURR1) was found to decrease the production of TNF-α by interacting with NF-κB p65 and preventing its translocation during LPS stimulation. The interaction between NURR1 and NF-κB p65 contributes to the regulation of the TLR4 NF-κB

signaling process [130]. Furthermore, treatment with NR ligands also inhibited the association of AP-1 and NF-κB subunits, repressing both basal and TLR-modulated HIV-1 replication in macrophages [131]. Crosstalk between GR and TLR has been well summarized in previous reviews briefly; while glucocorticoids have long been recognized as negative regulators of NF-κB and AP-1 transcriptional activity, they also play a broader role in modulating immune and inflammatory responses through a mechanism involving competition with the transcription factor IRF3 for binding to the Rel domain of the p65 subunit of NF-κB [132–134]. Similarly, PPAR agonists serve as potential therapeutic targets in neuroinflammation, CNS disorders, and cancer due to their ability to inhibit TLR-induced proinflammatory cytokine production in different cell types. They achieve anti-inflammatory effects by targeting MyD88, NF-κB, MAP kinase pathways, and IRF3, thereby offering a promising approach for managing these diseases and reducing inflammation-associated pathology [135, 136]. LXRs function as transcriptional regulators of lipid homeostasis and possess strong anti-inflammatory properties. Upon activation, LXRs effectively suppress signaling from TLRs 2, 4, and 9 to downstream NF-κB and MAPK. This inhibition is achieved through Abca1-dependent modifications in membrane lipid organization, which disrupt the recruitment of MyD88 and TRAF6, essential components of TLR signaling pathways [91, 137], leading to the suppression of proinflammatory cytokine production and promotion of cholesterol efflux, and preventing foam cell formation and plaque progression during atherosclerosis progression.

2.13 Lipid metabolism-TLR crosstalk: bridging immunity and metabolism

In addition to nuclear receptor LXR, lipid metabolism pathways have been implicated in the regulation of TLR responses. Oxidized PAPC (oxPAPC), derived from phospholipid PAPC and lipoproteins, exerts modulatory effects on TLR4-induced inflammatory responses through a GPCR. At low concentrations, oxPAPC acts as an antagonist to counteract TLR4-induced inflammation, while higher doses of oxPAPC enhance the proinflammatory response. The precise mechanism underlying the anti-inflammatory function of oxPAPC remains unclear, but it is known that oxPAPC inhibits NF-κB transcription factor activity by generating cAMP and by reducing TLR4 sensitivity to LPS through binding to CD14 and LPS binding protein (LBP) [138, 139]. However, it is worth noting that the inhibitory effects of OxPAPC on proinflammatory products, such as TNF-α, were specifically observed in the signaling pathways downstream of TLR2 and TLR4 but no other TLRs.

GPR120 serves as a receptor/sensor for omega-3 fatty acids. When stimulated by omega-3 or specific chemical agonists, GPR120 exerts broad anti-inflammatory effects in various immune cells, including monocytic RAW 264.7 cells and primary intraperitoneal macrophages. Activation of GPR120 by omega-3 fatty acids can inhibit TLR4 signaling, resulting in a decrease in the production of proinflammatory cytokines such as IL-6 and TNF-α [140]. Prostaglandin E2 (PGE2), a lipid mediator, has been found to suppress alveolar macrophages (AMs) immune responses by inhibiting signaling events downstream of PRRs. In a study on rat AMs, it was observed that while PGE2 did not reduce TLR4 mRNA levels, it decreased TLR4 protein levels [141], or through induction of IL-1R–associated kinase-M (IRAKM), which blocks the scavenger receptor-mediated phagocytosis and the TLR-dependent activation of TNF-a [142]. PGE2 also inhibits the production of IL-23 by monocytes stimulated with LPS. The inhibitory effects of PGE2 on IL-23 production were mediated through the cAMP signaling pathway, as the cAMP enhancer forskolin significantly reduced

IL-23 and IL-12 production by monocytes [143]. However, in the case of DCs, the addition of PGE2 has been shown to enhance their migratory capacity in TLR ligands (poly(I:C) and/or R848) matured DCs, while maintaining the production of IL-12 by DCs when they encounter T cells [144]. These findings suggest that the effects of PGE2 on TLR signaling can vary depending on the cell type.

3. Conclusions

Signaling crosstalk occurs consistently in a sophisticated milieu and determines whether a host can successfully tackle an infection, tumorigenesis, and inflammatory response or not. TLRs play vital roles during the whole life of a host. The signaling crosstalk between TLRs and other molecules affect our bodies in tackling sophisticated situations. Such signaling crosstalks include synergistic effect, training/priming, antagonist effect, and tolerance. Mechanistically, these signaling crosstalks influence immune responses by manipulating the downstream signaling pathways and epigenetic status of immune cells. In this chapter, we enumerate multiple examples of signaling crosstalk between TLR and other molecules contributing to host defense or inflammatory diseases. There is still more evidence for signaling crosstalk between TLR and other molecules. In the natural environment, coinfection with viruses and bacteria is a prevalent occurrence, and it is probable that concurrent activation of both RIG-I-like receptors (RLRs), which serve as receptors for viral pathogens, and TLRs takes place. Negishi et al. found that virus-induced activation of cytosolic RLRs led to the selective suppression of transcription of the gene encoding the p40 subunit of interleukin 12 (Il12b) that was effectively induced by the activation of TLRs [12]. The transcription factor IRF3, activated by RLRs, prominently bound to the promoter region of Il12b and interfered with the assembly of a functional transcription-factor complex induced by TLR activation. TLR4 and CD40 costimulation synergistically increased the frequency of IL-10-producing but not proinflammatory cytokine-producing B cells at multiple sclerosis relapse [145]. Platelets-enriched serum suppresses TLR-induced inflammation and has been used to treat joint inflammation such as rheumatoid arthritis [146–149]. However, the detailed mechanisms of these signaling crosstalks are not fully understood yet. Discovering and depicting the detailed mechanisms of TLR signaling crosstalk with other molecules are important for better understanding the sophisticated situation of disease and for helping to develop new therapeutic strategies to tackle the disease.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

A2AR	A2A adenosine receptor
ADRB2	adrenoceptor beta 2
AhR	aryl hydrocarbon receptor
AKT	AKT serine/threonine kinase

APCs	antigen-presenting cells
BCR	B cell receptor
C5aR	C5a receptor
cAMP	cyclic adenosine monophosphate
CCL	C-C Motif Chemokine Ligand
CLRs	C-type lectin receptors
COVID-19	coronavirus disease 2019
CR	complement receptor
CXCL	chemokine (C-X-C Motif) ligand
CXCR	C-X-C motif chemokine receptor
DAMPs	damage-associated molecular
DCIR	dendritic cell immunoreceptor
DCs	dendritic cells
FHL2	four and a half LIM domains 2
FOXO	Forkhead box O1
GPCR	G-protein coupled receptor
GR	glucocorticoid receptor
GSK-3β	glycogen synthase kinase
HCV	hepatitis C virus
Hrs	hours
IFN	interferon
iNOS	nitric oxide synthase
IRAK	interleukin 1 receptor associated kinase
IRAKM	IL-1R-associated kinase-M
IRF	interferon regulatory factor
IRGs	IFN-responsive genes
IκBα	NF-κB inhibitor alpha
JAG1	jagged canonical notch ligand 1
LBP	LPS binding protein
LPS	lipopolysaccharide
LXR	liver X receptor
MGL	macrophage galactose-type lectin
moDCs	monocyte-derived dendritic cells
Mtb	*Mycobacterium tuberculosis*
MyD88	myeloid differentiation primary response 88
NA	nucleic acid
NF-κB	nuclear factor-kappa B
Nlrp	NLR family pyrin domain containing
NLRs	NOD-like receptors
NOD	nucleotide binding oligomerization domain containing
NR	ligand-activated nuclear receptor
NRF2	NFE2 like BZIP transcription factor 2
NURR1	nuclear receptor related 1
ORN8L	TLR8 oligoribonucleotides ligand
oxPAPC	oxidized PAPC
PAMPs	pathogen-associated molecular patterns
PARs	protease activated receptors
PBMCs	peripheral blood mononuclear cells
pDCs	plasmacytoid dendritic cells
PGE2	prostaglandin E2

PHZ	phenylhydrazine
PI3K	phosphoinositide 3-kinase
PIP2	phosphatidylinositol-(4,5)-bisphosphate
PKA	protein kinase A
poly I:C	polyinosinic-polycytidylic acid
PPARγ	peroxisome proliferator-activated receptor gamma
PRRs	pattern recognition receptors
PUA	punicic acid
RA	rheumatoid arthritis
RF	rheumatoid factor
RLRs	RIG-like receptors
ROS	reactive oxygen species
SGK1	serum/glucocorticoid regulated kinase 1
SLE	systemic lupus erythematosus
SPHK1	sphingosine kinase 1
SSc	systemic sclerosis
STAT	signal transducer and activator of transcription
TLRs	toll-like receptors
TNF	tumor necrosis factor
TNFAIP3	TNF alpha-induced protein 3
TRIF	TIR domain-containing adaptor molecule 1
XBP1	X-box binding protein 1

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Chapter 5

TLR and Cancer: The Enigmatic Cross Talk

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Abstract

The connection between inflammation and cancer has been well recognized at the epidemiological, biological, and pharmacological levels. Unresolved chronic inflammation is implicated in most stages of cancer development and thus can induce certain solid tumors. The molecular regulators of these linkages are emerging and should be well-decorticated. Toll-like receptors (TLRs) recognize pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) and death–associated molecular patterns (DAMPs) secreted from dying or damaged cells of the host. TLRs can be pro and anti-tumorigenic depending on the type of TLR signaling, cancer, and its stage. Therefore, comprehensive studies are required in this direction. The current chapter supplies a concise schematic concerning the biology and the characteristics of TLRs and summarizes the major findings of the enigmatic role of TLRs and their associated signaling in the pathogenesis of human cancers. On one hand and in some neoplastic contexts, TLR activation mediates proliferation invasion, migration and correlates with poor prognosis and metastasis, and inhibits apoptosis, leading to cancer progression. On the other hand and depending on other neoplastic context, TLRs agonists enhance radiosensitivity and chemotherapy, apoptosis, immune cell infiltration, and raise the antitumor effect of T cells.

Keywords: TLRs, tumor progression, tumor regression, dichotomic role, innate immunity

1. Introduction

The innate immune mechanisms are the first distinct defense fighting pathogen attack. They implicated many immune cell types such as monocytes, macrophages, dendritic cells (DCs), neutrophils, and natural killer (NK) cells [1]. They also comprise innate humoral components produced by the innate immune cells such as complement system, cytokines, chemokines, and antimicrobial peptides (AMPs; LL37 and Bactericidal/permeability-increasing protein (BPI), etc.) [2–5]. These innate immune cells contain various intracellular or membrane-associated pattern recognition receptors (PRRs). The PRR family germline-encoded receptors include several forms of recognition receptors: nucleotide-binding oligomerization domain-like receptors (NOD)-like receptors and retinoic acid inducible gene I (RIG-I)-like receptors (RLR), C-type lectin

receptors (CLR), Aim2- like receptors (ALR) and Toll-like receptors (TLRs) and intracellular DNA sensors such as cyclic GPM-AMP synthase cGAS [6, 7]. These receptors could discriminate between self and non-self-molecules. The well-known are Toll-like receptors (TLRs) to acknowledge highly conserved molecules expressed by pathogens: pathogen-associated molecular pattern (PAMP) or microbial-associated molecular pattern MAMPs (expressed/released by pathogens) or endogenous ligands released from dying, stressed and damaged cells: damaged-associated molecular pattern (DAMP) molecules released from dying cells Hsp60, Hsp70, fibronectin Host DNA from dying cells, mitochondrial DNA etc. [8, 9]. They produce inflammatory cytokines and type I interferons (IFNs) to ascertain a highly effective defense system [10]. TLRs are expressed not only by immune cells but also by epithelial cells, for defense against pathogens invading the body either through skin or mucous membranes.

Despite the host's protective role against foreign molecules and fighting diseases, including cancer, chronic inflammation has been referred to as one of the new hallmarks of cancer since 2011 [11], besides the above settled in 2000 as proposed by Hanahan and Weinberg [11]. Further proof of the link between chronic inflammation and carcinogenesis is revealed by the diminished cancer rates in patients receiving non-steroidal anti-inflammatory drugs and elevated rate in obese patients exhibiting high adipose tissue inflammation [6]. Chronic inflammation activates constitutively signaling pathways, such as Nuclear factor kappa B (NF-kB) or mitogen-activated protein kinase (MAPK) whose pro-tumoral effect is well documented [12]. In hepatocellular carcinoma (HCC) [13] and colitis-associated cancer, the activation of NF-kB prevented tumor apoptosis and stimulated the production of pro-inflammatory cytokines in the tumor microenvironment, thereby enhancing tumor progression. In this context, NF-kB activation is implicated in both tumor initiation and progression in liver cancer [14]. Therefore, the inflammatory response can lead to carcinogenesis after NF-kB activation, by the induction of anti-apoptotic molecules [15, 16]. TLR stimulation enhances NF-kB activation and thus leads by consequence to pro-inflammatory cytokines and chemokines, growth factors and anti-apoptotic proteins release leading to tumor progression and chemoresistance. TLR overstimulation may bring about strong inflammation since it plays a part in the recruitment of inflammatory cells in the tumor microenvironment.

2. TLR structure and signaling pathway

All TLRs, type I integral membrane receptors, have a characteristic structural organization with an extracellular recognition domain (ECD), a single transmembrane (TM) helix, and cytoplasmic signal domains (**Figure 1**) [17].

The ectodomain (ECD) is a terminal extracellular ligand binding domain made up of leucine-rich repeat (LRR), TLR-ECD shares a typically standard structural framework adopting horseshoe-shaped structures built from leucine-rich repeat (LRR) motif. The N-terminal extracellular ligand binding domain comprises varying numbers (19–26) hydrophobic leucine-rich repeat (LRR) modules (residues) every one of 20–30 Aa in length containing the consensus LxxLxLxx sequence in charge of ligand recognition and PAMPs and DAMPS recognition," The LRR-NTs are disulfide linked β-hairpins, LRR-CTs are globular structures that have two α helices and are stabilized by two disulfide bonds [18, 19].

The intracellular C-terminal signaling domain of TLRs (150 Aa) is one of numerous evolutionarily conserved foundations of the immune system' It's named Toll

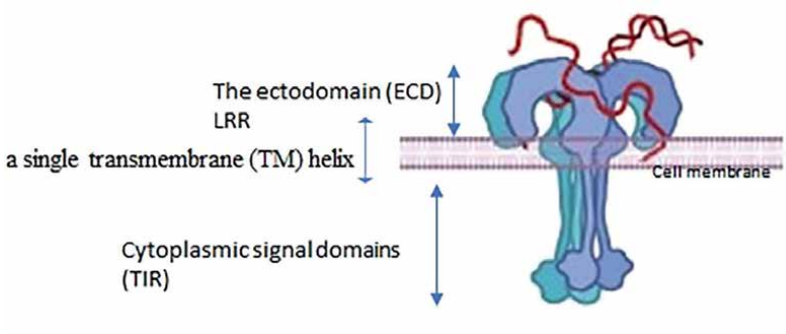

Figure 1.
Full-length TLR structure. The extracellular domain is composed of the N-terminal (LRRNT), LRRs and the C-terminal (LRR-CT) regions, a single transmembrane segment (TM), and an intracellular domain (TIR).

IL-1 Receptor (TIR) domain since it shares homology with the signaling domain of IL-1R family members and is carboxyl-terminal to LRR [20]. TIR domain initiates downstream signaling cascades by interacting making use of its adaptor proteins

TLR	Localization	Ligands	Signaling adaptator	References
TLR1/6	cell membrane	lipoproteins, lipopeptides	MyD88 and TIRAP/MAL	[22]
TLR2/6	cell membrane	lipopeptides peptidoglycan and lipoteichoic acid lipoarabinomannan, zymosan tGPI-mucin, hemagglutinin protein	MyD88 and TIRAP/MAL	[22]
TLR3	endoplasmic reticulum, lysosomal membrane	polyinosinic-polycytidylic acid (poly(I:C)), ds RNA derived from viruses	TRIF	[23]
TLR4	cell membrane, endoplasmic reticulum, lysosomal membrane	lipopolysaccharides (LPS), Mannans, Taxol	MyD88, TIRAP/MAL, Trif and TRAM	[22]
TLR5	cell membrane	flagellin	MyD88	[22, 24]
TLR7	endoplsmic reticulum, lysosomal membrane	imiquimod, resiquimod (R-848), loxoribine, ssRNA (RNA viruses) and synthetic poly(U) RNA	MyD88	[22]
TLR8	endoplasmic reticulum, lysosomal membrane	R-848 and viral ssRNA	MyD88	[24]
TLR9	endoplasmic reticulum, lysosomal membrane	unmethylated 2′-deoxyribo(cytidine-phosphate-guanosine) (CpG) DNA motifs, crystal hemozoin	MyD88	[22, 25]
TLR10	Endolysosomes	HIV-1 gp41	MyD88	[26–28]

Table 1.
Human TLRs, their localization, ligands, and signaling adaptors.

such as myeloid differentiation response protein 88 (MyD88) that will be the absolute most commonly used adapter, MyD88 adaptor-like (MAL), TIR-domain-containing adapter-inducing interferon-β (TRIF), and TRIF-related adaptor molecule (TRAM). The signaling pathway activates transcription factors such as NF-κBs and interferon regulatory factors (IRFs), ultimately causing the release of proinflammatory cytokines and type I interferons, various anti-viral and anti-pathogen proteins, and initiation of the adaptive immune response [21].

Upon PAMPs or DAMPs sensing, TLRs undergo conformational changes, dimerize and reorient their TIR domains, to communicate with TIR-containing proteins, MyD88 and MAL, TRIF, and TRAM. This interaction results in undergoing a series of intracellular cascade signal transduction involving interleukin-1 receptor (IL-1R)-associated kinase 4 (IRAK4), IRAK2/1 phosphorylation resulting in the activation and dimerization of tumor necrosis factor receptor-associated factor 6 (TRAF6). TRIF interacts with TRAF3 and TRAF6 limited to TLR3 and TLR4. For endosomal TLR4, TRAM interplays with TRIF contributes to TRAF3-dependent stimulation of the kinase TBK1. These pathways promote inflammation and host defense and drive the IFN pathway activation with interferon-stimulated gene expression **Table 1** [29, 30].

3. TLR localization

TLRs are classified into two subfamilies depending on their localization. TLRs localize on cell/surface membrane and/or reside within intracellular compartments such as endosomes, multivesicular bodied, lysosomes, and endolysosomes. TLR1, 2, 4, 5, and 6 are located in cell. However, TLR3, 7, 8, 9 are located on endosome membrane within the cell. Their different location is ultimately linked to the type of ligand they recognize. Thus, TLRs located on the cell membrane mainly bind microbial membrane lipids and proteins, and TLR4 binds bacterial lipopolysaccharides (LPS). TLR5 recognizes bacterial flagellin, TLR 2,1 and 6 recognize peptidoglycans and zymosan, whereas TLRs located on the endosomal membranes bind nucleic acids derived from virus and bacteria but also from self-nucleic acid. Thus, compartmentalization of nucleic acids sensing TLRs in the endolysosome is primordial to control their stimulation by self-derived nucleic acids and minimizes the risk of autoimmune reactions. TLR3 recognizes viral double stranded RNA (dsRNA), whereas TLR7 recognizes single-stranded RNA (ssRNA), TLR9 binds to bacterial and viral DNA (CpG-DNA motifs) [31, 32].

4. TLR expression on immune cells

TLRs are expressed in varied cell types. Mainly they are located in innate immune cells Such as monocytes/macrophages, mast cells (MCs), neutrophils, eosinophils, basophils, natural killer (NK) cells, γδ T cells, innate lymphoid cells, DCs, platelets; brain innate immune cells like microglia and astrocytes. They are also expressed in adaptive immunity such as T and B cells. Non-immune cells, such as endothelial cells/ECs could express TLRs. TLR1 is detected in DCs and B Cells [33]. TLR2 is expressed in peripheral mononuclear leucocytes, DCs, monocytes, and T Cells [34–36]. TLR3 is mainly expressed in DCs, NK cells, and T cells [37, 38]. TLR4 in macrophages, DCs, and T Cells [36, 37, 39]. TLR5 in monocytes, DCs, and NK cells [37, 40]. TLR6 is highly expressed in B cells and DCs, but low in monocytes and NK cells [37, 41]. TLR7

is expressed in B cells, DCs, monocytes, and T cells [38, 42]. TLR8 is expressed in monocytes, DCs, and low in NK and T cells [43, 44]. TLR9 is present in DCs, B cells, macrophages, NK, and microglial cells [33, 43].

5. TLR expression and roles on cancer cells

TLRs are expressed in certain types of cancers. Their expression could be closely associated with cell proliferation invasion and the probability of metastasis promoting then cancer progression or enhancing tumor regression. In this regard, the conclusions from *in vitro* or mouse model studies differ significantly (**Table 2**).

Cancer type	Tissue or cell line	Role of TLR in cancer	References
Cervical	Human cancer tissue	TLR1 and 3 in early carcinogenesis	[45]
	HeLa cell line	TLR4 promotes tumor progression	[46]
	Human cancer tissue	TLR9 promotes tumor progression	[47, 48]
	Human cancer tissue, Hela cell line	TLR8 promotes tumor progression	[49]
	Human cancer tissue	TLR2 and 4 promote tumor progression	[50]
	Human cancer tissue	TLR3,7,8 and 9 promote tumor regression	[51]
	Human cancer tissue	TLR2,7 and 8 promote tumor regression	[52]
	Human cancer tissue	TLR3,4 and 5 promote tumor regression	[53]
Ovarian	Human cancer tissue and cell line	TLR4 promotes tumor progression	[54–56]
	Human cancer tissue	TLR9 promotes tumor progression	[57]
	fisher 344 rats	TLR2 and 4 promote tumor regression	[58, 59]
	SKOV3 cell line	TLR8 promotes tumor regression	[60]
Endometrial	Human cancer tissue	TLR3 and 4 involved in adenocarcinoma	[61]
	Human cancer cell	TLR2 and 6 promote tumor progression	[62]
	Human cancer cell	TLR1,2,3 and 4 promote tumor progression	[63]
Melanoma	Mice	TLR7 and 8 promote tumor regression	[64]
	Human, mice and B16 cell	TLR1 and 2 tumor regression	[65]
	cell line	TLR4 promotes tumor regression	[66]
Hepatocellular	HUH7 HCC cells, Human samples	TLR2 promotes tumor progression	[67]
	In vivo and in vitro	TLR9 promotes tumor progression	[68]
	Human samples	TLR5 predictor of poor prognostic	[69]
	Human samples, cell line	TLR4 promtes tumor progression	[67, 70–72]
	cell line	TLR2 promotes tumor regression	[73]

Cancer type	Tissue or cell line	Role of TLR in cancer	References
Lung	In vivo and in vitro	TLR1/2 inhibits tumor growth	[74]
	In vivo and in vitro	TLR2/6 promotes tumor growth	[75]
	In vitro	TLR3 inhibits tumor growth	[76, 77]
	In vivo and in vitro	TLR3 promotes tumor growth	[78]
	In vitro	TLR4 promotes tumor growth	[79]
	In vitro	TLR4 inhibits tumor growth	[80]
	In vitro	TLR5 has a protective role	[81]
	In vitro	TLR7/8 inhibits tumor growth	[82, 83]
	In vitro	TLR9 promotes tumor progression	[84]
	Human	TLR9 promotes tumor regression	[85]
Breast	In vitro, Human, in vivo	TLR4,2,9 promote tumor progression	[86, 87]
	In vitro, in vivo	TLR3,7 promotes tumor regression	[88, 89]
Colorectal	In vitro, vivo, Human	TLR4 promotes tumor growth	[90–92]
	In vivo and in vitro, Human	TLR9 promotes tumor regression	[93, 94]
	In vitro	TLR9 promotes tumor growth	[95–97]
	In vivo	TLR2 has protective role	[98]
	in vitro	TLR2 promotes tumor progression	[99]
	In vitro	TLR3 promotes tumor regression	[100]
	Human	TLR5 is linked to better prognostic	[101]
Glioma	In vivo and in vitro	TLR2 promotes tumor progression	[102–105]
	In vitro	TLR4 promotes tumor progression	[106–108]
	In vitro	TLR7/8 inhibits tumor growth	[109]
	In vitro	TLR9 promotes tumor progression	[110]
	In vitro, in vivo	TLR9 promotes tumor regression	[111–113]

Table 2.
TLR expression in human cancers.

5.1 Cervical cancer

Cervical cancer is caused essentially by High-risk human papillomavirus (HR-HPV) types. A study highlighted a down expression of TLR3 and an overexpression of TLR1 at transcriptional level in dysplastic and carcinoma epithelium. in stroma, TLR 1, 2, 5, 6, and 9 were overexpressed in association with disease severity. Thus, TLR3 and TLR1 are proposed as implicated in early and late stages of cervical carcinogenesis thus their usefulness for diagnostic and prognostic. Moreover, the stromal overexpression of TLRs may play a crucial role in cervical cancer progression [45].

TLR4 was over-expressed in cervical cancer, and its activation by LPS can accelerate the proliferation and promote anti-apoptosis in Hela cells in vitro in a dose-dependent manner. The mechanism causing this overexpression is regulated by the NF-kB/IL-6/TGF-β1 secretion [46]. Patients with persistent HR HPV exhibit over-expressed TLR9

for more than 1 year in comparison to women who cleared HPV infection and to those re-infected with low-risk LR HPV. These results speculate that overexpression of TLR9 in persistently infected women could lead to chronic inflammation thus contributing to cervical cancer risk [47]. Furthermore, TLR9 may play a role in progression of cervical neoplasia in Tunisian patients and could therefore be considered a convenient biomarker for malignant transformation of cervical cells [48]. TLR8 may be an interesting therapeutic target in cervical cancer. In this regard, overexpression of TLR8 in cervical cancer patients and Hela cells was observed. Furthermore, a strong correlation with increased expression of TLR8, VEGF, and Bcl-2 in cervical cancer patients was demonstrated. Continually, upon binding to its agonist CL075, TLR8 is able to remarkably increase the percentage of cells in G2/M + S of Hela cells, accompanied by increased COX-2, BCL-2, and VEGF mRNA levels [49]. TLR2 and TLR4 are strongly associated with TNF-α and TNF-β in cervical cancer with gradually increased expression from premalignant lesions to cervical cancer compared to normal controls [50], However, higher TLR expression is associated with HPV16 clearance revealing an important link between innate and adaptive immunity in the control of HPV infections after a persistent period [51]. It is speculated that high levels of TLR2, TLR7, and potentially TLR8 in cervical mucosa are important for CIN2 regression, suggesting their role in the clearance of HPV [52]. These studies highlight the important protective role of the innate immune system at different stages of HPV infection. A deeper understanding of host immune response toward virus factors would affect if a lesion progresses or regresses. This finding is crucial to drive the proper choice of today's available immunotherapeutic for HPV-associated disease. Down-expression of TLR4 during the progression of cervical neoplasia is linked to P (16INK4A) expression, the crucial marker of HPV integration into host cells. These results demonstrate the crucial link between HPV infection and TLR signaling during the carcinogenesis of cervical cancer [114]. Remarkable diminution of mRNA level of TLRs 3, 4, and 5 and overexpression of TLR1 was noted in cervical squamous cell carcinoma (CSCC) as compared to controls. These results point toward TLRs 3, 4, and 5 agonists exploration as therapeutic targets to treat cervical cancer [53]. The chimeric molecule including the extracellular domain of CD200 and a murine IgG2a Fc region CD200Fc seemed to repress TLR4/NF-κB and NLRP3 inflammasome inflammatory effects in SiHa cells and Caski cells treated with LPS. It provided a novel mechanistic understanding toward the conceivable therapeutic usefulness of CD200Fc for cervical cancer [115].

Regarding TLR gene polymorphism and cervical cancer association, several studies in several ethnicities have been conducted, but results vary greatly. In like manner, Oliveira and al concluded there was not always a noteworthy association between TLR9 polymorphism and HPV clearance or persistence. Accordingly, polymorphism in the promoter region of the TLR9 gene does not appear to be primordial in the natural history of the HPV infection [116]. These authors concluded the usefulness of TLR 3 and 9 gene polymorphisms in cervical cancer susceptibility in North India [117].

In the same context, a Tunisian study searching for TLR2 (−196 to −174 del) and the TLR 9 (2848 G > A) polymorphisms and the susceptibility of cervical cancer among Tunisian women show no association. Whereas, in the same cohort study, TLR3 (c.1377 C > T) and (Asp299Gly) TLR4 polymorphism has been shown to be associated with a higher risk of cervical cancer [118]. TLR9 (−1486 T/C, rs187084)-but not TLR9 (2848G/A, rs352140)-may be a threat factor for cervical cancer [119]. Furthermore, Chinese Han patients with TLR9 rs352140-GA + AA genotype and infected with HPV have the highest cervical cancer risk, compared to no HPV-infected patients holding the rs352140-GG genotype. Furthermore, the minor alleles of TLR2-rs3775290,

TLR4-rs7873784, and TLR9-rs352140, and interaction with HPV infection were linked to a high cervical cancer risk in Chinese Han populations [120]. In the Indian population, TLR4 haplotype ACAC is highly linked to multiple HR-HPV infections. In otherwise, TLR9 SNPs rs187084, rs352140, and rs352139 were linked to diminished risk of high HPV16 viral load [121]. The study gene polymorphisms in cervical cancer susceptibility in North Indian pointed toward the implication of TLR 2 (−196 to −174 del) and TLR 4 (Thr399Ile) in cervical cancer susceptibility. The TLR gene polymorphisms, may be a good tool in setting out mechanisms related to innate immunity in cervical cancer susceptibility [122]. The later Indian study points toward considering TLR4 haplotype GCAG and TLR9 haplotype GATC as threat to hrHPV infection. Further evaluation of a larger sample size covering diverse ethnic populations globally is warranted [123].

5.2 Ovarian cancer

Ovarian cancer (OC) is the deadliest in gynecological cancer. TLR4 expression was linked to ovarian cancer progression, treatment resistance, and poor prognosis. Kelly et al. demonstrated that TLR4 is overexpressed in various ovarian epithelial tumors. The high TLR4 expression correlates with increased tumor progression and leads to chemo-resistance to Paclitaxel. Thus, blocking TLR4 is proposed to be beneficial to the OC patient if targeted specifically in cancer cells that overexpress the molecule [54].

In several types of OC cells, TLR4 inhibition in synergy with standard chemotherapy may be therapeutically beneficial to decrease drug resistance. In this regard, osteopontin (OPN), brought by LPS activation, leads to the proliferation and metastasis of ovarian cancer cells. These results pave the way to decorticate novel mechanistic pathways promoting cancer progression, invasion, and metastasis. Furthermore, TLR4 combined with OPN may be considered a promising target for ovarian cancer therapy [55]. In the same issue, the TLR4/MyD88/NF-κB axis is associated with the survival of patients with ovarian epithelial cancers (OECs). MyD88 was demonstrated to be an independent prognostic predictor in patients with OECs. The TLR4/MyD88 axis may be a possible mechanism for poor prognosis in patients with clear cell types of OEC in association with drug resistance [56]. Moreover, overexpression of TLR9 is correlated with tumor-grade severity and within poorly differentiated tumors. OC patients with metastatic disease had in their serum an over level of TLR9 ligand (hypo-methylated DNA). Furthermore, the TLR/PI3K signaling axis modulated the invasion and metastasis through the production of galectin 1, suggesting that inhibition of the p110, the PI3K isoform, is a promising therapeutic approach against metastatic ovarian cancer [57]. Protein aggregate magnesium-ammonium phospholinoleate-palmitoleate anhydride (P-MAPA) upregulated TLR2 and TLR4 signaling pathway. Thus, the combination of (P-MAPA) with IL-12 improves the antitumor immunoresponse, paving the way to a novel therapeutic approach for fighting OC [58]. Another way, the association with (P-MAPA) and cisplatin (CIS) is thought to be a promising target therapy against OC cells through TLR4 signaling pathways activation [59]. Stimulation of the TLR8 abolishes glucose metabolism in CD4+ Tregs through mTOR signaling reduction, thus reverting the immunosuppressive function of the previous cells in an OC cell growth microenvironment [60].

5.3 Endometrial cancer

Uterine cancer is the second most common gynecologic cancer worldwide (globocan, 2020). Endometrial cancer (EC), originating in the epithelium is about 90%

of uterine cancers. TLR3 and TLR4 protein expression level was studied during the menstrual cycle and in postmenopausal endometrium taking into account different grades of cancer from grade 1 to grade 3 (peritoneal endometriosis, hyperplasia, and endometrial adenocarcinoma specimens). The lowest TLR expression levels were shown in poorly differentiated carcinoma (grade 3). These findings propose the implication of TLR3 and TLR4 in endometrial diseases as shown by changed expression levels in endometriosis and endometrial cancer [61]. EC and endometrial hyperplasia cells overexpress TLR2 and TLR6. In addition, the expression of TLR6 marked an advanced stage of EC [62]. Wojcik-Krowiranda et al. study demonstrated a significant correlation between the expression of TLR1, TLR2, TLR3, and TLR4 and VEGFR1, VEGFR2, VEGF-A, and HIF1α on human neoplastic endometrial samples at the clinical stage and pathological grading of EC [63]. Ashton et al. reported that different alleles of TLR9 polymorphisms (The rs5743836 and rs187084 alleles of TLR9 polymorphisms in Caucasian women) were protective against EC [124]. In contrast to previous thoughts, the overexpression of TLR showed that the use of TLR agonists could be harmful in some types and stages of cancers. Indeed, TLR agonists are not only not useful in the late stages of the tumor but also may cause tumor progression. Considering this hypothesis, scientists lead various types of research using natural or synthetic inhibitors of TLRs. In the case of EC, the results showed that the use of TLR inhibitors, such as TLR4 inhibitors, could inhibit chronic inflammation. However, clinical trial research should be conducted to prove the usefulness of this therapeutic approach in comparison to other approaches in EC [125].

5.4 Melanoma

Cutaneous melanoma is one of the foremost aggressive tumors and a life-threatening skin cancer. Within the setting of rising incidence and mortality, there is a necessity to discover new prognostic markers and early diagnosis is the big challenge to improve its prognosis. When searching their transcriptional and protein expression level, TLR2, 3, 4, 7, and 9 exhibit a high amount of expression in tumor tissue and melanoma cell lines. The results are more disparate at the protein level. TLR3 and 8 *ex vivo* are down-expressed in invaded LNs and however over expressed by melanoma cell lines extracted from the same LNs. Moreover, TLR2 and 4 proteins were absent in melanoma cell lines, whereas they were expressed in invaded LNs. This study gives evidence for a broad range of TLR expression in melanoma cells both *ex vivo* and *in vitro*. On one side, TLR2 and TLR4 are highly expressed in melanoma cells *ex vivo* and down expressed *in vitro*. On the other side, TLR3 and 8 expression are weak or absent *ex vivo* in invaded LNs however overexpressed in cell lines. However, more research should be conducted to reveal the functional and mechanistic aspects of these receptors on melanoma cell lines and therefore discover the therapeutic potential of targeting these receptors to treat melanoma [126]. TLR7/8 agonists are Resiquimod (RSQ), an imidazoquinolinamine derivative. The immune checkpoint blockage, Anti-PD-1 in association with RSQ can significantly extend the survival of melanoma-challenged mice, compared to untreated mice and mice treated with only anti-PD-1 [64]. TLR2/6 agonists +IFNY treatment synergically enhances CXCL10 production from melanoma cells when compared to IFNY alone. CXCL10 promotes the migration of CD4+ and CD8 + T cells, thus suggesting new therapeutic way of enhancing immune cell infiltration through CXCL10 production [127].

More recently, vaccination with MelQbG10, an innovative vaccine that integrates three components essential for successful immunotherapy consisting of virus-like

nanoparticle (VLP), A-type CpG-ODN, and a Melan-A/MART-1 peptide, was tolerated and used safely in melanoma patients by the induction of tumor-specific CD8+ T cells after MelQbG10 vaccination [128]. To further improve immune responses and increase MelQbG10 immunogenicity, additional immune stimulatory agents, such as imiquimod, a TLR7 agonist lead to enhance Melan-A-specific T-cell frequencies. Furthermore, MelQbG10 can be considered safe and well tolerated when given in combination with imiquimod [129].

Immunization against a genetically engineered tumor-specific antigen, ovalbumin, when adjuvanted with Diprovocim, which targets the innate immune receptor TLR1/TLR2 in mice and humans, inhibited the growth of B16 melanoma and prolonged survival in the presence of immune checkpoint blockade by anti–PD-L1; all mice responded to treatment. These data propose Diprovocim improving the benefit of anti–PD-L1 therapy by raising the number and activation of tumor-specific CTLs able to reply to this checkpoint inhibitor [65].

Activation of PPARγ by its agonist, pioglitazone, reduces tumor volume through LPS/TLR4/MyD-88/Nf-kb1/TNF-α axis in melanoma tumor. Moreover, treatment of melanoma cells with pioglitazone has a beneficial protective effect against melanoma through LPS-TLR4-dependent signaling pathways inhibition [66]. TLR3 polymorphism L412F was linked to an elevated mitotic index. Whereas TLR4 D299G and T399I polymorphisms were associated with melanoma severity, nodal metastases, and advanced stage III and could be then potential markers targeting the survival and prognostic of melanoma patients. TLR4 T399I polymorphism was highly correlated with worse survival [130]. However, no association between TLR7 single nucleotide G pp. ln11Leu polymorphism and susceptibility to develop melanoma was found. Further, other studies on TLR polymorphisms and their susceptibility to malignant melanoma are needed [131].

5.5 Hepatocellular carcinoma

In 2020, approximately, 830,200 people died from liver cancer globally. Despite some progress, hepatocellular carcinoma (HCC) remains a major cause of death often detected at inoperable stage. In 80%, HCC occurs often in the setting of chronic liver disease and cirrhosis [132]. New biomarkers to identify patients who could benefit from more aggressive treatment are needed. HCC cells exhibit cytoplasmic and nuclear TLR2 expression which are associated with proliferative index, Caspase-3 expression, and vascularization. Furthermore, HCC shows notably nuclear TLR2 expression; however, hepatitis and cirrhosis cell patients demonstrate a predominant cytoplasmic expression. At a functional level, upon TLR2 activation by its agonist, HUH7, HCC expressed cellular proliferation and vascularization markers CD34 and VEGF. These results propose a plausible role for TLR-2 in HCC pathogenesis [67]. The interaction of mtDNA-HMGB1 and TLR9 might contribute to tumor growth signaling pathways in response to hypoxia *in vivo* and *in vitro* in hepatocellular carcinoma [68]. TLR5 is suggested to be an independent predictor of poor prognosis in HCC. However, TLR8 was not associated with patient survival [69].

Jing et al. detected positive TLR4 expression in 86% of cases of HCC patients. Other studies confirmed that LPS/TLR4/NF-κB signaling is involved in the invasion and metastasis of HCC patients [70]. In the same context, another study demonstrated that HCC patients whose tumors expressed high levels of both TLR4 and TLR9 had a poor prognosis. TLR4–MyD88 signaling pathway appears to be primordial for hepatocarcinogenesis. LPS-induced TLR4 signaling also promotes cancer cell survival and proliferation in HCC [71].

Activation of TLR4 could enhance the proliferation of HCC cells. Furthermore, TLR4/COX-2/PGE2/STAT3 axis is activated in HCC cells. Abolition of TLR4 or COX-2/PGE2/STAT3 pathway reduces LPS-induced inflammation and proliferation of HCC cells and improves the perceptivity of HCC cells to chemotherapeutics in vitro. These findings shed light on the precise molecular mechanism involved in the TLR4 signaling pathway leading to HCC progression and then pointing toward TLR4 as a promising target for HCC treatment [72].

To further investigate the expression and function of TLR2 in hepatocarcinoma proliferation recombinant plasmids expressing one of three forms of TLR2 siRNA (sh-TLR2 RNAi (A, B, and C) were transfected into BLE-7402. sh-TLR2 RNAi (B) had the most important knockdown effect. TLR2 abolition with sh-TLR2 RNAi (B) reduced cell proliferation and secretion of IL-6 and IL-8. Also, mice treated with sh-TLR2 RNAi (B) demonstrate a severe reduction in tumor volume, suggesting that TLR2 knockdown inhibits proliferation of cultured hepatocarcinoma cells and decreases the secretion of cytokines. Thus, TLR2 silencing could be proposed for siRNA-based gene therapy to treat of hepatocarcinoma patients [133]. Abolition of TLR2 by siRNA exhibited a decrease in proliferation, invasion, migration, and NF-κB/P65 expression but an increase in apoptotic ratio. In other way, rHMGB1 led to proliferation, invasion, and migration, enhanced NF-κB/P65 expression, and abolished cells apoptosis. Moreover, TLR2 reduced the role of rHMGB1. This finding proposes TLR2 and HMGB1 potential therapeutic targets against HCC [73].

Overexpression of miR-122 significantly diminished TLR4 expression in hepatoma cells. However, knocking down miR-122 overexpressed TLR4. It was found a putative miR-122 target in TLR4 3'UTR. Over-expression/down-expression of miR-122 could impact the proliferation and the expression of natural immune factors [134]. NADPH oxidase 4 (Nox4) enhanced LPS/TLR4/NF-κB/AP-1 signaling pathways in hepatocytes. Moreover, the effect of Nox4 abolition was time-dependent as proved in the mice model. Therefore, these data suggest LPS/TLR4/Nox4 axis is a tumor-promoting pathway as demonstrated both in human hepatoma cells and murine hepatocytes [135].

Genetic polymorphisms of TLRs are suggested to influence susceptibility to HCV infection and progression to end-stage liver disease. One haplotype (GCCCTTAG) of TLR4 was associated significantly with a decrease in the occurrence of hepatocellular carcinoma [136]. In another study, a strong association between allele C of rs3804099 of TLR2 and C allele of rs10116253 TLR4 and the risk of HCC was observed. Furthermore, A powerful link between allele T of rs1816702 of TLR2 and allele A of rs5030728 of TLR4 and the non-responder group was found. Haplotypes CAGT of TLR4 and ATAC of TLR2 showed significant association with CH and HCC groups in comparison to haplotype TGAC for TLR4 and haplotype GCGT for TLR2 [137].

5.6 Lung cancer

Lung cancer is a leading cause of cancer-related deaths with 18% of several deaths and 11.4% of new cases diagnosed in 2020 (Globocan, 2020). A better understanding of innate immunity and its related molecular mechanism in the lung could lead to the improvement and development of novel immunotherapy. TLR2 uses TLR1 and TLR6 [138]. TLR2 has been proposed to be a good target for lung cancer as the complex TLR1/2 promotes inhibition of tumor growth, decreases monocytic MDSC, and shifts macrophage toward M1 profile through JNK pathway [74]. However, the TLR2: TLR6 complex has been associated with promoting lung cancer metastasis [75].

TLR3 expression in lung cancer is a double-edged sword. Indeed, TLR3 activated by exosomal dsRN leads to chemokine liberation-inducing metastasis *via* SLIT2 gene [78]. However, an *in vitro* study demonstrates that activating TLR3 by its agonist could enhance apoptosis [76], and induce a protective immune response by activating DCs [76]. TLR4 activation in lung cancer exerts tumor progression by enhancing the immune escape through immunosuppressive cytokine liberation such as transforming growth factor beta (TGF-b), VEGF, and IL-8 [77]. and PDL1 [79]. TLR4 contributes to tumor regression through (calreticulin) CALR-TLR4-MyD88 axis promoting the maturation of DCs and boosting immunity [80]. TLR5 has a rather protective role in lung cancer, upon NSCLC cell treatment by flagellin (TLR5 agonists), inhibition of proliferation, migration, and invasion was observed [82]. TLR7/8 agonist, R848, combined with nanoemulsion (NE) exerts antitumor activity in lung cancer models through tumoral T cell activation [81]. In NSCLC cells, TLR7 induces inhibition of angiogenesis and survival [83]. However, on one side TLR9 activation by its agonist CpG, enhances lung tumor progression, proliferation, and metastasis [84]. On the other side, Vidutolimod (ODN-A), TLR9 agonist is used to treat patients with resistance to PD-1 blockade thus priming T-cell responses to fight lung cancer cells (clinic phase Ib trial) [85].

5.7 Breast cancer

Breast cancer remains a major cause of death in women in the developed world. Further to their expression on immune cells, TLRs are also expressed in tumor cells. Recent evidences hold up the connection between breast cancer and inflammation. TLR4 promotes breast cancer progression and metastasis. *In vitro* analysis demonstrates that MDA-MB-231 expresses TLR4 and abolishing this receptor induces a severe diminution of cell viability [86].

TLR2 and TLR9 activation in MDA-MB-231 cell lines promotes proinflammatory cytokines secretion leading to invasiveness and then metastasis. A high level of TLR9 was strongly associated with triple-negative subtypes. However, stimulation of TLR7 in combination with radiotherapy inhibits tumor growth, enhances T-cell memory, and is correlated with significant regression of spontaneous breast cancer in mice, suggesting a better prognosis [88]. TLR2 enhances breast cancer survival. Thus, preclinical *in vivo* breast cancer models demonstrate the efficiency of combining doxorubicin with CU-CPT22, the inhibitor of TLR2, to improve its anticancer effects and therefore attenuate breast cancer progression. This finding points toward considering TLR2 as a novel target therapy when associated with doxorubicin [87]. In vitro analysis showed that synthetic TLR3 agonist, polyI: C induces apoptosis either through caspase 3,8 and TRAIL pathway [89].

5.8 Colorectal cancer

Colorectal carcinoma (CC) is the third in cancer prevalence in both men and women and is a major leading cause of cancer morbidity and mortality worldwide. CC is among solid cancers in which inflammation is crucial in mechanistic oncogenesis. TLR4 is overexpressed in inflammatory-related colorectal cancer both in human and *in vivo* models. TLR4 induces colorectal through TLR4 high level of prostaglandin E2 and Cox-2 production. Inhibition of TLR4 by siRNA diminished tumor growth and abolished cell proliferation, migration, and invasion in colorectal cancer cell lines through suppressing ACAT1 expression [90]. A recent finding shows that TLR4

activity is inhibited through the direct binding of baicalein to TLR4 then abolishing HIF-1α and VEGF expressions and as a consequence inhibits CRC growth, angiogenesis, and metastasis [91]. Moreover, an *in vitro* study showed that Curcumae longae Rhizoma extract (CLRE) could oppose 5-Fu resistance in colon cancer by inhibiting TLR4/PI3K/AKT/mTORC1 pathway [92]. A synthetic agonist of TLR9, immune modulatory oligonucleotide (IMO), acts by impairing epidermal growth factor receptor (EGFR) expression and function and exhibited a synergistic antitumor effect with anti-EGFR antibody cetuximab or the tyrosin kinase inhibitor gefitinib in GEO human colon cancer xenografts, whereas it is ineffective in VEGF-overexpressing cetuximab-resistant GEO cetuximab-resistant (GEO-CR) tumors [93]. MGN1703, other synthetic TLR9 agonist show antitumor activity and III trial in colorectal cancer patients is conducted [94]. However, circulating cell-free DNA (cfDNA), when recognized by TLR9, could enhance colorectal cancer cell proliferation through TLR9/MyD88/IL8 signaling pathway [95]. Moreover, in vitro study shows that cellular migration and invasion is mediated through TLR9/MMP13suggesting inhibition of MMP13 as a therapeutic target to treat colorectal cancer [96]. In addition, chloroquine, an antagonist of TLR9, inhibited cell viability, proliferation, and migration of the CRC cell line HT29, the mechanism through this inhibition is downregulation of NF-κB, and Bcl-xl [97]. TLR2 agonist, Pam3Cys (P3C), exhibited cellular proliferation signaling in the human CRC cell line upon PI3K/Akt and NF-κB associated anti-apoptotic genes BCL2A1, WISP1, and BIRC3 suggesting a primordial role of TLR2 in colorectal carcinogenesis [99]. putative tumor suppressor, miR-143regulates negatively TLR2 pathway in colorectal cancer [139]. However, other studies using mice model of colitis cancer demonstrate a protective role of TLR2 in colitis against developing colorectal cancer [98]. TLR3 agonist polyI: C is able to boost colorectal cancer cell resistance to chemotherapy by promoting IFN-β production, which induces apoptosis and impairs proliferation in some cancer cells [100]. Furthermore, overexpression of TLR5 in colorectal tumor cells is linked to better prognosis in CRC patients [101].

5.9 Glioma

Gliomas are the deadliest type of brain tumor with a median survival time of 15 months. The current treatments are complex, offensive, and far from satisfactory. Thus, the urgent need for alternative efficient therapy. Chronic inflammation is largely implicated in glioma carcinogenesis and progression. TLR2 was found to be overexpressed in Glioma-associated microglia (GAM). This expression leads to tumor immune evasion through abolishing MCHII transcription via activation of MAPK/ERK1/2 signaling pathway thus altering the efficiency of T-cell–dependent tumor elimination in *in vivo* and *in vitro* models [103]. Moreover, in human and *in vitro* studies show that TLR2 promotes glioma carcinogenesis through autophagy [104]. Invasion of glioma stem cells (GSCs) is promoted *via* TLR2/MMP axis [105]. However previous studies focus on the crucial role of HMGB1/TLR2 signaling to enhance tumor regression [106]. TLR4 is implicated in the proliferation of glioma cells and inhibition of TLR4 by short hairpin (sh) RNA-induced abolition of cell proliferation and induction of apoptosis [107]. *In vitro* experiments suggested that the neurotrophic factor, prosaposin (PSAP) Highly expressed in glioma could lead to tumor progression through TLR4/NF-κB pathway [108]. Another study showed that impaired TLR4 function is associated with immune invasion at the site of glioma [109]. Parenteral treatment with the TLR7/8 agonist, resiquimod (R848), eventually

induced complete tumor regression of CNS-1 glioblastoma tumors in Lewis rats [110]. The most crucial findings on TLR9-signaling in the pathogenesis of glioma are largely detailed in previous studies. TLR9 promotes brain cancer progression by inducing invasion-induced hypoxia *via* the matrix metalloproteinases (2, 9, and 13) [111]. However when combined with radiotherapy (RT), TLR9 agonist could enhance tumor regression through inhibition of angiogenesis [112], shift immune response to CD8 cytotoxic immunity or macrophage M1 [113, 140].

6. Conclusion

TLRs are involved in several carcinogenic processes to promote cancer progression in some kinds of tumors and some individuals by inducing cell proliferation, invasion, and metastasis. The mechanisms behind this implicate many hallmarks in the setting of tumor cells such as inhibition of apoptosis, and stimulation of angiogenesis. On the other side, TLRs are expressed mainly on macrophages and dendritic cells (DCs). Stimulation of TLRs fundamentally starts not only innate immune response but can also induce adaptive immune response boosting the immune system to fight cancer. In this respect, TLR ligands are used as immunostimulatory molecules as anticancer treatment in numerous preclinical and clinical studies. From both points of view, one should be aware when choosing for adjuvants to treat cancer patients. In related TLR clinical trials, the dual role of TLR should be considered and further mechanistic investigations on the dual roles of TLR in tumor biology are needed [140–142]. Thus, conducting a deeper understanding of each TLR on each cancer is of crucial need. It is worth knowing whether to design a TLR inducer or inhibitor when considering TLR-targeted therapeutic. Furthermore, with the era of novel pharmacology and personalized medicine, the use of nanotechnology in combination with conventional chemotherapy and radiotherapy is of great interest as it impacts efficiently cancer treatment trials.

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Conflict of interest

The authors declare no conflict of interest.

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Toll-like receptors (TLRs) have attracted interest due to their role in detecting pathogens and regulating the pro-inflammatory immune response. To date, 10 functional TLRs (TLR1–10) have been detected in humans. These TLRs control diverse immunological and embryonic development processes to maintain immune homeostasis and healthy fetal growth and development. They recognize diverse pathogen-associated molecular patterns (PAMPs) and death/damage-associated molecular patterns (DAMPs) to maintain immune homeostasis via regulating diverse immunological processes. This book discusses TLRs from their evolution to immunological functions governing infection and inflammation pathogenesis, cancer immunology, and their interactions with other pro-inflammatory molecules generated during inflammation pathogenesis. It is a must-read for researchers interested in TLR biology and the role of TLRs in infection, immunity, and cancer.

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