REVIEW

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Novel drugs targeting Toll-like receptors for antiviral therapy

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ABSTRACT: Toll-like receptors (TLRs) are sentinel receptors of the host innate immune system that recognize conserved 'pathogen-associated molecular patterns' of invading microbes, including viruses. The activation of TLRs establishes antiviral innate immune responses and coordinates the development of long-lasting adaptive immunity in order to control viral pathogenesis. However, microbe-induced damage to host tissues may release 'danger-associated molecular patterns' that also activate TLRs, leading to an overexuberant inflammatory response and, ultimately, to tissue damage. Thus, TLRs have proven to be promising targets as therapeutics for the treatment of viral infections that result in inflammatory damage or as adjuvants in order to enhance the efficacy of vaccines. Here, we explore recent advances in TLR biology with a focus on novel drugs that target TLRs (agonists and antagonists) for antiviral therapy.

KEYWORDS
• antiviral therapy • eritoran • innate immune system • monophosphoryl lipid A • poly-ICLC • retrocyclin • TLR agonist • TLR antagonist • Toll-like receptors • viruses

A significant advance in the field of immunology accompanied the identification of the two arms of immune responses as 'innate' and 'adaptive'. Initially, innate immunity was considered to be a relatively nonspecific and simple part of the overall immune response, while adaptive immunity was believed to provide antigen-specific protection from microbial and viral infection. However, accumulating evidence has clearly established that innate immune responses are the first line of defense against invading pathogens and also coordinate the development of a pathogen-specific adaptive immune response (reviewed in [1,2]). Despite striking differences in terms of response timing, effector cells and recognition receptors on the cells of both of these systems, there are many cellular and molecular components in common that orchestrate highly specific, integrated responses against invasive pathogens and establish long-term immune memory.

The primary receptors of innate immunity are a diverse set of germ line-encoded 'pattern-recognition receptors' (PRRs) that identify a broad spectrum of 'pathogen-associated molecular patterns' (PAMPs), which are diverse microbial structures of invading microorganisms, or 'danger-associated molecular patterns' (DAMPs), which are host-derived molecules released by stressed or injured cells (reviewed in [1]). PAMPs include diverse microbial molecules, such as lipopolysaccharides (LPSs), lipopeptides, peptidoglycans, mannans, flagellin, bacterial and viral nucleic acids and viral envelope proteins, whereas examples of DAMPs include endogenous (host) components, such as histones, nucleic acids, uric acid crystals, cytochrome C, ATP, oxidized 1-palmitoyl-2-arachidonoyl-phosphaticylcholine and HMGB1, among others. In 1989, Janeway first proposed the concept of

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PRRs that recognize the molecular structures of microorganisms and link innate and adaptive immune responses [3]. Two important discoveries strengthened Janeway's concept of PRRs: the proof of the importance of Toll-mediated signaling in the induction of antifungal peptides by *Drosophila* in response to infection [4]; and the positional cloning of the *Lps* gene (now known to be *Tlr4*). Both C3H/HeJ and C57BL/10ScCr mice were shown to express mutations in this gene that led to LPS hyporesponsiveness in these two strains [5,6]. The importance of this research resulted in the sharing of the 2011 Nobel Prize in Physiology or Medicine.

Among the various families of PRRs (e.g., Toll-like receptors [TLRs], Nod-like receptors, RIG-I-like receptors [RLRs], c-type lectin receptors and cytosolic DNA receptors), TLRs are one of the largest and best-studied families of PRRs. The study of TLR biology has provided molecular insights into how PRRs recognize PAMPs and DAMPs, and how this leads to the activation of signaling cascades that converge in order to induce the expression of proinflammatory cytokines, chemokines and antiviral interferons (IFNs). Moreover, the discovery of TLRs has also enabled the identification of other families of innate immune receptors (reviewed in [1]). Together, the knowledge generated in the field of TLRs over the past decade has resulted in a significant paradigm shift in our understanding of innate immunity and its role in the development of a long-lasting, pathogen-specific adaptive immune responses (reviewed in [2,7]). In this article, we briefly summarize current advances in the field of TLRs, their signaling and how we might target TLR signaling in order to mitigate disease or enhance specific immunity.

The evidence thus far points to a role for TLRs in immune and inflammatory diseases, including allergies, autoimmune disorders and cancer (reviewed in [8]). To date, many viruses have been shown to activate the innate immune system through TLRs, assigning to TLRs an important role in controlling viral infections (reviewed in [9]). The following sections provide examples of TLR–virus interactions and their outcomes, as well as recent advances in our understanding of the role of TLRs in antiviral innate immunity, with a focus on the studies designed for developing novel TLR-targeting drugs that exert antiviral activity or serve as adjuvants for vaccines.

Toll-like receptors

TLRs are evolutionarily conserved across a wide range of species; 10 human and 12 mouse TLRs have been identified [10]. TLRs are type I transmembrane proteins composed of an N-terminal leucine-rich repeat (LRR) domain that enables the recognition of a wide variety of ligands, a single transmembrane-spanning domain and a conserved cytoplasmic Toll–IL-1 receptor resistance (TIR) domain for downstream signal transduction (reviewed in [11]). Resolution of the crystal structure of TLR3 revealed that a horseshoe-shaped antigen binding core was formed by the LRR-containing domain and sequence homology analyses indicated that activation of all TLRs requires a common tertiary structure [12]. Ten functional human TLRs (TLR1–10) and 12 functional mouse TLRs (TLR1–9 and TLR11–13) can be categorized by their subcellular localization. TLR1, 2, 4–6 and 10 localize at the cell surface, whereas TLR3, 7–9 and 11–13 reside in endosomes and/or the endoplasmic reticulum (reviewed in [13]). Most TLRs form homodimers after ligand binding through their LRRs; however, TLR2 typically forms heterodimers with either TLR1, TLR6 or possibly with TLR10 and detects components of microbial cell walls and membranes, such as lipopeptides, peptidoglycan, porins and mannan, while TLR11 heterodimerizes with TLR12 in order to bind to the profilin protein of the parasite *Toxoplasma gondii* [14]. TLR4 recognizes LPS from Gram-negative bacteria, the fusion (F) protein of respiratory syncytial virus (RSV), the mouse mammary tumor virus and Ebola virus glycoprotein [15–17]. In addition, TLR4 also senses DAMPs, including oxidized 1-palmitoyl-2-arachidonoyl-phosphaticylcholine, which is a host oxidized phospholipid that is produced due to oxidative stress in response to acute lung injury by acid aspiration, infection by respiratory viruses or bacteria or exposure to microbial products [18], and HMGB1, which is a chromatin binding protein that is released upon pyroptosis [19]. TLR5 detects flagellin, the major protein of bacterial flagella, whereas the ligand for TLR10 has not yet been identified (reviewed in [20]). Homodimers of mouse TLR11 recognize components of uropathogenic *Escherichia coli* [21]. TLR3, 7, 8 and 9 sense microbial nucleic acids: dsRNA is sensed by TLR3 and ssRNA by TLR7 and 8, while unmethylated CpG DNA is sensed by TLR9. Moreover, mouse TLR13 recognizes bacterial 23S ribosomal RNA [22].

TLR4 and, to some extent, TLR2 require coreceptor molecules in order to recognize microbial ligands. A noncovalently associated protein, MD-2, confers LPS responsiveness to TLR4. MD-2 binds the lipid A region of LPS in a deep hydrophobic pocket and interacts with the TLR4 ectodomain, which suggests that the MD-2–LPS complex is the essential ligand for TLR4 [23–26]. A second coreceptor for TLR4, CD14, transfers LPS monomers to MD-2 and increases the responsiveness of cells to LPS at low concentrations [27,28]. The F protein of RSV also requires MD-2 for signaling through TLR4, an event that involves direct protein–protein interaction between MD-2 and the domain of the F protein that encompasses its hydrophobic fusion peptide [29]. In addition, it has been shown that CD14 also acts as a coreceptor in order to activate TLR2 by mycobacterial lipoarabinomannan [30].

TLR signaling & downstream gene expression

Pathogen-encoded ligand binding to TLR causes conformational changes and TLR dimerization that lead to the recruitment of cytosolic TIR domain-containing adapter proteins to the intracellular TIR domain of the TLR. The main adapter proteins include MyD88, TIRAP (also known as MAL), TRIF (also known as TICAM1) and TRAM (also known as TICAM2). The MyD88-dependent pathway is activated by all TLRs except TLR3, which only engages TRIF. TLR4 is the only TLR that activates both MyD88- and TRIF-dependent signaling pathways. CD14-dependent TLR4 internalization into endosomes from the plasma membrane facilitates induction of the TRIF signaling pathway [31]. TIRAP was originally thought to act as a bridge to recruit MyD88 to TLR2 and TLR4, while TRAM recruits TRIF to TLR4 (reviewed in [11,32]). However, recent work by Kagan and colleagues suggest that TIRAP is more promiscuous [33]. A fifth member of the TIR adapter group, SARM, interacts with TRIF and negatively regulates TLR3 and TLR4 signaling [34]. A proposed sixth adapter is BCAP, which has a TIR-like domain and modulates B-cell activation by TLRs [35,36].

Engagement of TLRs by ligands causes a conformational change and the recruitment of adapters through TIR–TIR interactions, leading to the activation of a cascade of signal transduction molecules, including IRAKs, TRAF6 and TAK1, among others, leading to phosphorylation of the inhibitor of NF-κB kinase and the release of NF-κB transcription factors into the nucleus, which induces the expression of proinflammatory genes, such as *TNFA* and *IL6* (reviewed in [11,32]). The MyD88-dependent pathway also results in the activation of MAPKs. By contrast, the TRIF-mediated signaling pathway involves the delayed activation of NF-κB and robust activation of IRF3, which is an important transcription factor for the induction of type I IFNs (primarily IFN-β in macrophages) and IFN-inducible genes. Endosomal TLRs, such as TLR7–9, engage the MyD88-dependent pathway and activate NF-κB and IRF7, which leads to the production of high levels of type I IFN (reviewed in [37,38]). Taken together, activation of MAPKs and NF-κB is triggered by all TLRs from the plasma membrane and endosomes, whereas TLR-induced IRF3 (TLR3 and TLR4) and IRF7 (TLR7–9 and TLR13) activation is initiated only from the endosome [39]. Activation of TLR signaling culminates in the expression of many secreted cytokines, such as IFNs, TNF-α, IL-1, IL-6, IL-10, IL-12 and chemokines, as well as causing cell differentiation, proliferation or apoptosis. TLR–ligand interactions are complex and their outcomes depend on many factors, such as the differential expression of TLRs among different cell types, cell type-specific signaling pathways and the usage of varied adapters by different TLRs.

TLR–virus interactions & outcomes

The discoveries of vaccinia virus proteins A46R and A52R as antagonists of TLR signaling and TLR4 as the sensor of the RSV F protein first prompted the idea that other viral components may serve as TLR ligands [15,40]. Definitely, the discovery that TLR3 recognizes dsRNA, a major component of many viruses, revealed a role for TLRs in antiviral responses [41]. Accumulating evidence suggests that many viruses activate innate immunity through TLRs, which leads to protective immunity in order to resolve viral infections. Endosomal TLRs (TLR3, 7, 8 and 9) recognize viral nucleic acids, while some TLRs on the plasma membrane (TLR1, 2, 4 and 6) detect viral proteins either on the intact organism or those that are released into the extracellular environment. However, for some viral infections, the activation of TLR pathways augments the severity of the disease (reviewed in [9,42]). Interestingly, natural viral infections stimulate

complex sets of PRRs, both within and outside of the TLR family, in order to mount an effective immune response. The cooperative, complex interplay of these different receptors may result in complementary or synergistic effects, which modulate innate and adaptive immunity (reviewed in [43]). Since such interactions are beyond the scope of this article, the outcome of each TLR interaction with viruses is discussed below (see **Table 1**). In particular, we describe studies aimed at developing TLR-based therapies, either in the form of agonists or antagonists, in order to establish antiviral responses or to circumvent TLR-mediated detrimental immunity, respectively (summarized in **Table 2**).

Furthermore, many viruses have evolved defense mechanisms in order to escape or sabotage the surveillance of the innate immune system for their own benefit. Some of the most prominent examples of viral subversion

Table 1. Examples of mouse and human Toll-like receptor–virus interactions and outcomes.

TLR	Virus (relevant examples)	Reported outcomes; macromolecules detected	Ref.
TLR2 (with TLR1 or TLR6)	Measles virus	Hemagglutinin protein	[48]
	HCMV	Glycoprotein B and H	[49]
	HCV	Trigger pDC apoptosis in order to establish viral persistence; core and NS3 proteins	[50,62]
	EBV	UTPase	[51]
	HSV	Dual reports: protective and harmful inflammatory response; glycoproteins gH/gL and gB	[52,58–61]
	Rotavirus	nsp4	[53]
	RSV	Protective	[54]
	MCMV	Protective	[55]
TLR3	MCMV	Protective	[64]
	HSV-2	Protective	[65,70,71]
	EMCV	Protective	[66]
	Coxsackievirus B4	Protective	[67]
	Poliovirus	Protective	[68]
	Rotavirus	Protective	[69]
	HCV	Protective	[72]
	Dengue virus	Protective	[73]
	Hanta virus	Protective	[74]
	PTV	Harmful inflammatory response	[75]
	Influenza	Harmful inflammatory response	[76]
	Vaccinia	Harmful inflammatory response	[77]
	WNV	Dual reports: harmful inflammatory response and protective	[78,79]
TLR4	RSV	Protective; fusion protein	[15,96]
	MMTV	Envelope protein	[16]
	Ebola virus	Glycoprotein	[17]
	VSV	Glycoprotein G	[97]
	Influenza	Harmful inflammatory response	[123]
TLR7/8	SARS-CoV	Protective	[131]
	Pneumonia virus or mice	Protective	[132]
	HCV	Protective	[133]
	WNV	Dual reports: protective and no change in susceptibility	[135,136]
	HIV	TLR7-mediated heightened immune activation is postulated for faster disease progression	[137]
TLR9	HSV-2	Protective	[155]
	MCMV	Protective	[157]
	Pox virus	Protective	[159]
	HIV	Polymorphism in TLR9 influences the clinical course of HIV-1 infection	[160]

EMCV: Encephalomyocarditis virus; HCMV: Human CMV: MCMV: Mouse CMV; MMTV: Mouse mammary tumor virus; pDC: Plasmacytoid dendritic cell; PTV: Punta Toro virus; SARS-CoV: SARS coronavirus; TLR: Toll-like receptor; VSV: Vesicular stomatitis virus; WNV: West Nile virus.

Table 2. Overview of drugs targeting Toll-like receptors for viral infections.

Target	Compound	Company; ref.	Mechanism	Application; indication	Status
TLR (with TLR6)	FSL-1	[63]	Agonist	Vaginal application creates antiherpetic environment in mice	Experimental (preclinical)
TLR3	Poly(I:C)	[80–83]	Agonist	Shows protective immunity against influenza, HBV, HIV and coronaviruses	–
	Poly(I:C_{12}U) or Ampligen®	Hemispherx Biopharma	Agonist	Being developed for the treatment of HIV, influenza, chronic fatigue syndrome, HBV and HCV	Under clinical trials
	Poly-ICLC or Hiltonol	Oncovir, Inc.; [89]	Agonist	Ongoing HIV vaccine trial; provide broad-spectrum poly(I:C) into the cells and reduce poly(I:C)-induced cytokine production	Under clinical trials (preclinical)
TLR4	Monophosphoryl lipid A	GlaxoSmithKline	Agonist	Vaccine against HPV (Cervarix®) and HBV (Fendrix®)	Approved
	Eritoran tetrasodium (E5564)	Eisai, Inc.; [124]	Antagonist	Decrease influenza-induced lethality in mice; useful for the management of inflammation associated with influenza	Experimental (preclinical)
TLR7/8	Imiquimod or Aldara™	3M Pharma	TLR7 agonist	Topical treatment for HPV-induced genital and perianal warts	Approved
	Resiquimod	3M Pharma	TLR7/8 agonist	Used topically for the treatment of genital HSV	Phase III (discontinued)
	CL097	[142,143]	TLR7/8 agonist	Restore defective cytokine secretion by mDCs of HIV-infected individuals; enhance G-CSF secretion by PBMCs	Experimental (preclinical)
	PF-04878691 or 852A	Pfizer; [144]	TLR7 agonist	Developed for treatment against HCV	Phase I (early termination of the study)
	ANA975, oral prodrug of isatoribine	Anadys Pharma; [146]	TLR7 agonist	Developed for treatment against HCV	Phase I (suspended)
	ANA773, oral prodrug of isatoribine	Anadys Pharma; [147]	TLR7 agonist	Developed for treatment against HCV	Under Phase IIa trial
	SM-276001	[149]	TLR7 agonist	Identified as orally active IFN inducer in mice and monkeys	Experimental (preclinical)
	GS9620	Gilead Sciences; [151]	TLR7 agonist	Developed for the treatment of HBV and HCV	Phase II (recruiting)
	2′-O-methyl-modified RNA	[152,153]	TLR7 antagonist	Reduce IFN and cytokine production in TLR7 agonist-treated cells and in mice	Experimental (preclinical)
TLR7/8 and TLR9	Oligonucleotide-based antagonists	[154]	TLR7, 8 and 9 antagonists	Inhibition of TLR7-, 8- and 9-mediated signaling pathways and the induction of a broad range of cytokines in murine and human cell-based assays and *in vivo* in mice and nonhuman primates	Experimental (preclinical)
TLR9	CPG10101 or Actilon™	Coley Pharma and Pfizer; [163,164]	TLR9 agonist	Treatment of chronic HCV infection	Phase II completed
	IMO-2125	Idera Pharma	TLR9 agonist	Treatment of HCV infection	Phase I completed
	SD-101	Dynavax Technologies Co.	TLR9 agonist	Treatment of chronic HCV infection	Phase I completed

IFN: Interferon; mDC: Myeloid dendritic cell; PBMC: Peripheral blood mononuclear cell; Poly(I:C): Polyriboinosinic:polyribocytidylic acid; TLR: Toll-like receptor.

mechanisms are the vaccinia virus A46R, A52R and K7 proteins that target TLR signaling molecules; the TIR adapters IRAK2 and DDX3 that inhibit signaling; the HCV proteins NS3/4A protease, which cleaves TRIF, and NS5A, which inhibits MyD88; and the influenza and RSV NS

proteins, which block the production of IFN by preventing the activation of IRF3. Additional examples are reviewed elsewhere [44,45]. VIPER, a peptide derived from vaccinia protein A46, showed potent inhibition of the TLR4 pathway by targeting MAL and TRAM adapter molecules [46]. Similarly, strategies that target receptor–adapter interactions by designing protein domain, peptide and peptidomimetic inhibitors represent potential approaches to inhibit the downstream assembly of the functional signaling complex in order to control inflammatory diseases and sepsis (reviewed in [47]).

TLR2

TLR2 typically functions as a heterodimer with TLR1 or TLR6 and has been shown to recognize viral proteins, such as measles virus hemagglutinin protein, human CMV glycoproteins B and H, HCV core and NS3 proteins, EBV UTPase, HSV glycoproteins gH/gL and gB and rotavirus nsp4 [48–53]. Using knockout mice, it was shown that activation of the TLR2/TLR6 pathway in leukocytes was important for the induction of the cytokine response against RSV and for controlling viral replication *in vivo*. Moreover, neutrophil migration and dendritic cell (DC) activation in the lung of RSV-infected animals were dependent on TLR2/TLR6 activation [54]. TLR2$^{-/-}$ mice showed a higher viral load of mouse CMV (MCMV) and reduced NK cell recruitment in the spleen and liver, but a decrease in type I IFN secretion compared with wild-type mice [55]. A study showing the role for TLR2 in sensing vaccinia virus infection demonstrated that the production of proinflammatory cytokines was mediated by TLR2, whereas the secretion of IFN was TLR2 independent [56]. Contrary to the role of TLR2 in the induction of proinflammatory cytokines and chemokines, a provocative study reported that TLR2 senses MCMV and vaccinia virus particles on specialized inflammatory monocytes and induces IRF3/IRF7-dependent type I IFN, which requires TLR2 internalization and MyD88-dependent pathway activation [57]. The role of TLR2 in HSV infection is less clear because of conflicting reports showing either a protective role of TLR2, along with TLR9, against brain infection of HSV-1 and HSV-2, or reports of TLR2-mediated inflammatory responses causing lethal encephalitis [58–61]. In patients with chronic HCV infection, it was shown that the HCV core protein induces IL-10 and TNF-α in monocytes via TLR2, which causes decreased IFN-α release from plasmacytoid DCs (pDCs) and triggers pDC apoptosis, providing a possible basis for viral persistence [62]. A synthetic lipoprotein derived from *Mycoplasma salivarium*, FSL-1, is recognized by TLR2 and TLR6. Vaginally applied FSL-1 was shown to create an antiherpetic environment to a 25-fold higher HSV-2 challenge dose in mice. FSL-1 also induced significant resistance to HSV-2 infection in human vaginal epithelial cell cultures [63].

TLR3

TLR3 is characterized as a nucleic acid-sensing TLR and it recognizes infections by dsRNA, ssRNA and DNA viruses (reviewed in [42,45]). Activation of TLR3 leads to TRIF-mediated induction of proinflammatory cytokines, chemokines and type I IFNs by activating transcription factors such as NF-κB and IRF3. Various *in vivo* and *in vitro* studies have established a protective role of TLR3 against many viral infections. TLR3 deficiency renders mice hypersusceptible to infection by DNA viruses, such as MCMV and HSV-2. Mice lacking TLR3 showed higher viral loads and decreased production of type I IFN compared with wild-type mice [64,65]. Similar findings have been reported in TLR3$^{-/-}$ mice infected with various RNA viruses, including encephalomyocarditis virus, coxsackievirus B4, poliovirus and rotavirus [66–69]. Accumulating evidence shows a predisposition for HSV-2-induced encephalitis in children born with deficiencies in the TLR3 signaling pathway [70,71]. TLR3 was shown to be required for IFN responses against HCV and Dengue virus that limit the replication of these viruses *in vitro* [72,73]. Furthermore, Hanta virus infection in A549 cells resulted in TLR3-mediated expression of antiviral genes, whereas TLR3 knockdown led to increased viral replication [74].

In contrast to the protective role of TLR3 against viruses, certain viral infections elicit TLR3-mediated immunity that is harmful to the host. For example, TLR3$^{-/-}$ mice were refractory to infection and exhibited reduced pathology caused by viruses such as Punta Toro virus, influenza and vaccinia virus compared with wild-type mice. Data indicate that TLR3-mediated increases in the production of inflammatory responses cause more damage in wild-type mice compared with TLR3-deficient mice [75–77]. For West Nile virus (WNV), studies into the role of TLR3 have been inconsistent:

in one study, TLR3 mediated WNV lethality [78], while in another, TLR3 activation was protective against WNV [79]. Therefore, TLR3 agonists or antagonists may act as effective antiviral therapies depending on the context, which we discuss in the section below.

• TLR3 agonists

TLR3 signaling is activated by a synthetic dsRNA agonist, polyriboinosinic:polyribocytidylic acid (poly[I:C]), a potent immune stimulant [41] that has been shown to induce protective immunity against influenza, HBV, a few HIV strains and coronaviruses [80–83]. Intranasal treatment with poly(I:C) showed improved survival and diminished viral load in aged mice infected with a lethal dose of SARS coronavirus [84]. In addition, poly(I:C) activates multiple elements of innate and adaptive immunity, including the induction of IFNs, proinflammatory cytokines and chemokines, the maturation of DCs, NK cell cytotoxicity and virus-specific T-cell responses, through TLR3 and cytoplasmic RLR sensors (MDA5 and RIG-I) [85–87]. Thus, poly(I:C) shows potential as an adjuvant for DC-targeted vaccines designed to induce T-cell-mediated immunity. For further clinical development, poly(I:C_{12}U) (Ampligen®; Hemispherx Biopharma, PA, USA) was developed by substituting an uridylic acid at a molar ratio of 12:1 in the synthesis of the polycytidylic acid strand. Poly(I:C_{12}U) has a superior safety profile and maintains the beneficial activity of the parental poly(I:C) in terms of inducing innate immune responses. Unlike poly(I:C), poly(I:C_{12}U) therapy failed to protect TLR3$^{-/-}$ mice against lethal Punta Toro virus infection and also failed to produce IFN and IL-6, which indicates that poly(I:C_{12}U) signals only through TLR3 [88]. Poly(I:C_{12}U) is being further developed for the treatment of HIV, influenza (FluMist®, MedImmune LLC, MD, USA; clinical trial no. NCT01591473), chronic fatigue syndrome and HBV and HCV infection.

Poly(I:C) stabilized with poly-L-lysine and carboxymethylcellulose is known as poly-ICLC (Hiltonol®; Oncovir, Inc., Washington, DC, USA). Studies in mice suggest that poly-ICLC and liposome-encapsulated poly-ICLC are safe and provide broad-spectrum protection against seasonal and highly pathogenic avian influenza A viruses, as well as RSV and SARS virus [89]. However, in a cotton rat model of RSV and influenza infection, intranasal administration of antiviral doses of poly-ICLC induced lung inflammation [90]. Comparative transcriptional analysis upon subcutaneous administration of poly-ICLC indicated that it induces innate immune responses of similar magnitude to a live viral vaccine in humans [91]. Various ongoing or completed clinical trials using poly-ICLC alone or in vaccines have demonstrated its safety and efficacy, and an HIV vaccine trial is ongoing (Oncovir, Inc. and Dalton Pharma Services, ON, Canada). PIKA (NewBiomed PIKA Pte Ltd, Singapore), another chemically stabilized analog of poly(I:C), was demonstrated to be a potent adjuvant that enhances both cellular and humoral immune responses to the hepatitis B antigen [92]. In addition, PIKA was shown to reduce viral loads significantly in the lungs of mice infected with variety of influenza viruses [93].

• TLR3 antagonists

Hyperactive responses in certain viral infections triggered by TLR3 sensing can lead to adverse effects [75–77]. Thus, strategies to prevent TLR3-mediated pathology using TLR3 antagonists have been reported. ssDNA oligonucleotides (ODNs) efficiently blocked the uptake of poly(I:C) into the cells and reduced poly(I:C)-induced cytokines in human epithelial cells, peripheral blood mononuclear cells (PBMCs) and DCs [94,95]. Intranasal administration of ssDNA ODNs inhibited poly(I:C)-induced cytokine release in the nasal secretions of cynomolgus macaques [95]. These findings provide support for these novel modalities for the prevention or management of inflammatory conditions aggravated by TLR3 activation.

TLR4

TLR4 was first identified as the LPS receptor. TLR4 was also the first TLR to be shown to detect viral PAMPs. In 2000, Kurt-Jones and colleagues showed that TLR4 senses RSV membrane-bound F protein, leading to cytokine production [15]. Subsequently, it was demonstrated that TLR4$^{-/-}$ mice challenged with RSV have impaired NK cell function, IL-12 expression and viral clearance compared with wild-type mice [96]. A number of viral proteins, such as mouse mammary tumor virus envelope protein, Ebola virus glycoprotein and vesicular stomatitis virus glycoprotein G, have been reported to activate the TLR4 signaling pathway [16,17,97].

In humans, two TLR4 polymorphisms, Asp299Gly and Thr399Ile, have been associated

with increased risk of severe RSV bronchiolitis and symptomatic RSV infection in high-risk infants and young children [98,99]. The RSV F protein was shown to interact with the TLR4 coreceptor, MD-2, through its N-terminal domain. TLR4 antagonists, *Rhodobacter sphaeroides* LPS and synthetic E5564 (eritoran; Eisai, Inc., NJ, USA) showed significant reductions of RSV F protein-mediated TLR4 activity in HEK293T transfectants expressing TLR4–CD14–MD-2 in a dose-dependent manner [29]. Eritoran has been shown to inhibit the TLR4 signaling pathway by binding to a hydrophobic pocket in MD-2 and precluding the interaction of MD-2 with the activating ligand, LPS [25,26].

• TLR4 agonists

Advances in the design of safe and efficacious vaccines continue to be a prime goal in global public health. The majority of vaccine preparations are composed of the antigen of interest and an adjuvant, which together generate faster, stronger and long-lasting immune responses than immunization with the antigen alone. For more than 70 years, aluminum hydroxide (alum) has been the only vaccine adjuvant approved and licensed for human use worldwide due to safety and a lack of side effects. Alum is effective in boosting antibody responses; however, repeated administration of the vaccine is required. Moreover, alum tends to generate antiparasitic Th2-biased responses, rather than antiviral and antibacterial Th1 responses (reviewed in [100]). Currently, much effort is still being dedicated to the development of new adjuvants that can establish protective immunity with fewer vaccinations by generating durable antibody and Th1 cytotoxic T-cell responses. As a consequence, TLR4 agonists are being developed as immunomodulators and adjuvants. Monophosphoryl lipid A (MPL), a chemically modified derivative of *Salmonella minnesota* LPS, has been approved for use because it generates good adjuvant activity [101,102] and approximately 0.1% of the inflammatory toxicity induced by the parent molecule LPS [103,104]. The basis of MPL's adjuvant activity is not entirely understood. It is a relatively weak TLR4 agonist [105] that has been suggested to exhibit a 'TRIF bias' [106]; however, this latter attribute has been controversial and has yet to be confirmed as underlying its adjuvant properties. An adjuvant composed of MPL and alum, known as AS04 (GlaxoSmithKline, UK), has been licensed for use in a vaccine against HPV (Cervarix®) and HBV (Fendrix®) [107]. Combinations of MPL with other classes of adjuvants, such as emulsions, saponins and liposomes, have been already tested in humans, but are not yet licensed for use (reviewed in [108]). In addition, many novel adjuvants targeting TLR2, TLR7 and TLR9 are in the advanced developmental stages, either alone or in combination with other adjuvants (reviewed in [109]). MPL was shown to modulate innate immune responses directly in order to improve the magnitude and duration of adaptive immune responses to vaccine antigens [110]. MPL has been coadministered with different RSV vaccine preparations in animal models in order to generate safe immune responses upon subsequent RSV infection. In early human clinical trials, a formalin-inactivated RSV (FI-RSV) vaccine caused exacerbated disease upon natural infection of vaccinees, including two deaths. Subsequently, several RSV subunit vaccines and live-attenuated RSV vaccines have been proposed. Most of the subunit RSV vaccines prime for excess Th2-type responses and development of vaccine-enhanced disease upon natural RSV infection, while attenuated vaccines often show residual virulence, poor immunogenicity or genetic instability (reviewed in [111,112]). Using the identical Lot 100 FI-RSV from the failed vaccine trial of the mid-1960s, the cotton rat model was shown to faithfully recapitulate the pathology induced by RSV infection in Lot 100-immunized children [113,114]. Upon subsequent RSV challenge, cotton rats immunized with FI-RSV combined with MPL showed a dramatic decrease in lung pathology compared with FI-RSV alone, with no change in lung viral replication, despite slight increases in neutralizing antibody titers [115]. Furthermore, immunization of cotton rats with FI-RSV plus MPL showed strong inhibition in the expression of Th1 and Th2 cytokine and chemokine genes in response to subsequent RSV challenge [116]. Thus, the inclusion of MPL in the FI-RSV vaccine diminishes the expression of aberrant cytokine storm, which is the hallmark of vaccine-enhanced disease upon RSV infection. Similarly, the addition of MPL to reconstituted nucleocapsid-depleted RSV membranes (virosomes) was demonstrated to generate Th1-skewed immune responses without priming for enhanced respiratory disease. Furthermore, the mucosal (intranasal) administration of RSV-MPL virosomes was shown to be safe and protective against live virus challenge in mice and cotton rats [117]. A vaccine based on

recombinant purified, anchorless RSV F protein formulated with MPL, administered in a heterologous prime–boost strategy (intranasal followed by intradermal boost), showed enhanced protection against RSV without aberrant lung histopathology or induction of the cytokine storm [118].

• TLR4 antagonists

LPS from Gram-negative bacteria is a potent TLR4 agonist. Soluble or membrane-associated CD14 and a nonmembrane-spanning protein, MD-2, are required as coreceptors for optimal LPS-induced TLR4 pathway activation [23,25–27]. Lipid A, the toxic moiety of LPS, is highly conserved among endotoxins and is an ideal target for drug development. The first-generation lipid A antagonist, E5531, was derived from the structure of *Rhodobacter capsulatus* endotoxin by the Eisai Research Institute of Boston (MA, USA). E5531 demonstrated protection in experimental models of endotoxemia and lethal infection with *E. coli* [119]. A second-generation LPS antagonist, eritoran tetrasodium (E5564), also developed by Eisai, Inc., is a synthetic lipid A analog of *R. sphaeroides*, first shown by Qureshi and colleagues to block LPS-induced lethality [120]. E5564 was developed for the treatment of Gram-negative sepsis. E5564 inhibited LPS-induced cytokines *in vitro* and in experimental animal models [121]. Crystallographic structural analysis of the TLR4–MD-2 complex with E5564 showed that the four acyl chains of E5564 occupy nearly 90% of the solvent–accessible volume of a deep hydrophobic pocket in MD-2, preventing the binding of toxic lipid A to MD-2, a prerequisite for LPS-mediated TLR4 activation [25]. E5564 advanced to a Phase III clinical trial called ACCESS; however, E5564 did not show significant benefits for severely septic patients [122].

The stimulation of TLRs by certain viral pathogens has been shown to result in detrimental immunity to the host, as demonstrated by TLR3- and TLR4-mediated harmful inflammatory responses to influenza virus infection [18,76,123]. The generation of host-derived, oxidized phospholipids by reactive oxygen species due to chemical or microbial insults was shown to stimulate potent TLR4-dependent inflammation, leading to the development of acute lung injury [18]. Furthermore, $TLR4^{-/-}$ mice are highly resistant to influenza-induced lethality [123]. On basis of these data, Shirey *et al.* proposed that blocking TLR4 signaling with the TLR4 antagonist eritoran would protect against influenza infection [124]. Eritoran was found to decrease influenza-induced lethality significantly in mice, even when administered 6 days post-virus infection. Improvements in lung pathology and clinical symptoms and decreased lung viral titers and influenza-induced cytokine and oxidized phospholipid expression were observed in eritoran-treated mice and cotton rats compared with a placebo-treated group [124]. These findings raise the possibility of utilizing TLR4 antagonists in order to manage the inflammation associated with influenza, and possibly other viral infections. Eritoran was also effective in cotton rats challenged with nonadapted human influenza virus [124].

A recent study showed that θ-defensins blocked LPS- and *E. coli*-induced lethality in mice, suggesting that θ-defensins may target TLR4-mediated signaling [125]. θ-defensins are cyclic, antimicrobial peptides expressed in non-human primates (reviewed in [126]). Each peptide is an 18-residue chimera formed by the head-to-tail splicing of nonapeptides derived from two separate precursors [127]. In humans, stop codons preclude the expression of θ-defensins, but the pseudogenes of θ-defensins have been reverse engineered, synthesized and named 'retrocyclins' [128]. Both θ-defensins and retrocyclins have potent antimicrobial and antitoxin properties against a broad spectrum of bacteria, fungi and viruses, including influenza A, HIV and HSV (reviewed in [126]). Interestingly, θ-defensins have anti-inflammatory properties. Recently, we confirmed that the retrocyclin RC101 blocks the LPS-induced activation of signaling intermediates and gene expression (data not shown), implying that retrocyclins may also interfere with virus- or DAMP-induced TLR4 signaling as their mechanism of action, opening up new possibilities for treatment against multipathogen infections.

TLR7/8

TLR7 and TLR8 are related functionally and detect GU-rich and AU-rich ssRNA sequences from the viral genomes of influenza, HIV-1, vesicular stomatitis virus, coxsackie B virus, coronavirus and flaviviruses (HCV and WNV; reviewed in [45]). TLR7 is primarily expressed in pDCs and, to some extent, in B cells, monocytes and macrophages, whereas TLR8 is mostly expressed in monocytes, macrophages and myeloid DCs. Mouse TLR8 was mostly reported to

be nonfunctional; however, a few papers have shown that murine TLR8 is activated by the combination of imidazoquinoline and poly-T oligodeoxynucleotides and vaccinia virus DNA [129,130]. TLR7 activation in pDCs is responsible for the production of high levels of type I IFN, which is considered to be the major antiviral mechanism against human SARS coronavirus, pneumonia virus of mice and HCV [131–133]. However, in the case of mouse retrovirus infection, TLR7-mediated sensing of the virus upon cellular entry stimulated virus-neutralizing antibodies, which are crucial for viral clearance [134]. Studies reporting the role of TLR7 in WNV infections differ significantly; one study showed a protective role of TLR7 against intraperitoneal WNV infection in TLR7$^{-/-}$ mice [135], whereas another study demonstrated no change in susceptibility to intradermal virus infection in wild-type and knockout mice [136]. These findings indicate that factors such as virus dose, passage history and route of administration are likely to be crucial in defining role of TLRs in experimental viral infection. A recent study presented evidence that women who were chronically infected with HIV showed heightened levels of IFN-α in response to TLR7-mediated activation in pDCs and activated CD8$^+$ T cells compared with the levels seen in men. This increased HIV immune activation was postulated to be one of the reasons for faster disease progression in women, and it was further hypothesized that modulating TLR7 activation in pDCs may offer a novel mechanism for the reduction of AIDS progression [137].

• TLR7/8 agonists

TLR7 and TLR8 are activated by imidazoquinolines, a family of synthetic, low-molecular-weight compounds with strong antiviral activity, such as imiquimod and resiquimod [138]. These synthetic molecules differentially modulate the TLR7 and TLR8 pathways in terms of their target cell selectivities and cytokine induction profiles. For example, in human PBMCs, TLR7-specific agonists were shown to be more effective at inducing type I IFN and IFN-regulated chemokines, while TLR8-specific agonists were more effective at inducing proinflammatory cytokines and chemokines, such as TNF-α and IL-12 [139]. Imiquimod (Aldara®), originally developed by 3M Pharmaceuticals (MN, USA), was introduced as a topical treatment for genital and perianal warts caused by HPV infection. Initially, imiquimod was shown to be an immune response modifier by producing type I IFN and other cytokines, which was later reported to be due to activation of TLR7 [138]. Imiquimod is approved as therapeutic treatment for external genital warts, precancerous actinic keratosis and basal cell carcinomas, and was shown to have mixed efficacy in the treatment of HSV infection [140]. In clinical studies, resiquimod, a mixed TLR7/8 agonist, was used topically for the treatment of genital HSV and orally for HCV infection. However, the results showed a lack of adequate efficacy and severe side effects at higher doses [140]. Resiquimod treatment suppressed HIV replication in cultured human monocytes [141]. Another TLR7/8 agonist, CL097, was reported to restore defective cytokine secretion by myeloid DCs of HIV-infected pregnant women and newborns [142]. CL097 treatment significantly enhanced G-CSF secretion by PBMCs, suggesting a possible therapeutic role of CL097 in the treatment of IFN-α-induced neutropenia in chronic HCV patients [143]. Various TLR7/8 agonists, such as PF-4878691, isatoribine, ANA975, ANA773 and GS9620, were advanced to clinical studies.

PF-04878691, formerly known as 852A, is a potent TLR7 agonist. In order to develop a treatment against HCV, Pfizer (NY, USA) conducted a study to evaluate safety and tolerability of PF-04878691 in healthy volunteers. PF-04878691 induced biomarkers of the immune and type I IFN responses in a dose-dependent manner. However, two subjects who received a higher dose experienced influenza-like symptoms, hypotension and lymphopenia, and the study was terminated early [144]. Modeling and simulation techniques were used to predict the efficacy and safety of PF-04878691 in HCV patients. PF-04878691 did not achieve proof-of-concept study criteria in model simulations and it was suggested that this compound be discontinued for HCV treatment [145].

Selective TLR7 agonists and guanosine analogs (i.e., isatoribine and its derivatives) have been developed by Anadys Pharmaceuticals (CA, USA). Intravenous treatment of HCV patients with isatoribine resulted in a significant reduction of plasma HCV RNA. The oral prodrug, ANA975, was further developed in order to avoid some of the side effects of isatoribine, especially in the GI tract. Preliminary results showed that ANA975 was well tolerated and oral administration of ANA975 increased plasma levels of isatoribine, effectively reducing HCV RNA in

the plasma of infected patients [146]. However, due to the toxicity observed in preclinical animal studies, trials with ANA975 were suspended. A subsequent oral prodrug of isatoribine, ANA773, showed efficient induction of type I IFN and proved to be safe and well tolerated in preclinical studies. In a double-blind, placebo-controlled study of patients infected chronically with HCV, ANA773 showed a dose-dependent increase in IFN responses and a decrease in serum HCV RNA [147]. Repeated administration of ANA773 to chronic HCV patients resulted in a transient reduction in blood myeloid DC and pDC numbers and increased serum levels of IFN-α and IP-10 only in patients, who showed reduced serum HCV RNA upon drug treatment [148]. A series of 8-hydroxyadenine derivatives, the structures of which are related to isatoribine, were reported to be novel IFN inducers. Among these derivatives, SM-276001, or 9-substituted-8-hydroxyadenine, was identified as an orally active IFN inducer in mice and monkeys, with a potency superior to that of resiquimod [149].

GS9620 (Gilead Sciences, CA, USA), a potent and selective orally active TLR7 agonist, showed strong reductions in serum and liver HBV DNA, along with IFN-induced innate responses in HBV-infected chimpanzees [150]. In a double-blind, placebo-controlled study in healthy volunteers, GS9620 was shown to be well absorbed and well tolerated, and the induction of cytokine-, chemokine- and IFN-stimulated genes was achieved at a dose of approximately 2 mg, which is well below the dose that is known to cause adverse clinical events [151]. Due to these encouraging findings, efforts are in progress in order to develop GS9620 further for the treatment of HBV and HCV.

• TLR7/8 antagonists

TLR7 and TLR8 play key roles in sensing viral RNAs and generating antiviral immunity; however, excessive TLR activation by viral infection or by the recognition of self-RNA may generate detrimental immune responses to the host. Therefore, efforts to generate TLR7/8 antagonists are of prime importance. To this end, it was reported that 2'-O-methyl-modified RNA significantly reduced IFN-α and IL-6 production in TLR7 agonist-treated murine DCs, human PBMCs and in mice, and is thus considered to be a potent TLR7 antagonist [152,153]. Recently, the synthesis and evaluation of ODN-based antagonists of TLR7, 8 and 9 that contain a 7-deaza-dG or anabino-G modification in the immunostimulatory motif and 2'-O-methyl-ribonuclotides as the immunoregulatory motif were reported. These antagonistic compounds showed inhibition of TLR7-, 8- and 9-mediated signaling pathways and induction of broad range of cytokines in murine and human cell-based assays and *in vivo* in mice and nonhuman primates, indicating a novel possibility for using these antagonist against inflammatory and autoimmune diseases [154].

TLR9

TLR9 recognizes unmethylated CpG ODNs present in microbial DNA, which are absent in vertebrate genomes. TLR9 is constitutively expressed in pDCs and B cells, where TLR9 activation in pDCs produces large amounts of type I IFNs, which control viral replication and eradicate infected cells. TLR9-mediated antiviral immunity has been reported to be generated in response to infection such as HSV, MCMV, adenovirus and poxvirus [155–159]. In HIV infection, single-nucleotide polymorphisms in TLR9 were associated with the rapid progression of HIV-1 infection [160].

• TLR9 agonists

TLR9 agonists are synthetic CpG ODNs, such as CPG10101, IMO-2125, SD-101 and CpG 7909. The natural CpG ODNs are susceptible to serum and cellular nucleases due to their phosphodiester backbone. However, CpG-containing phosphorothioate ODNs are nuclease-resistant and have been widely exploited for clinical use. CpG ODNs via TLR9 activation primarily stimulates Th1-type immune responses. Thus, CpG ODNs are strong immunostimulants and have been widely tested as effective vaccine adjuvants for influenza, HIV and for a variety of malignancies (reviewed in [161]). In HIV-infected patients, TLR9 is reduced in B cells, causing impaired B-cell responses, whereas CpG ODNs enhance the proliferative and effector responses of B cells in HIV patients [162]. CPG10101 (Actilon™; Coley Pharmaceuticals, MA, USA) is promising and is in the developmental stages for the treatment of chronic viral infections, such as HCV. Subcutaneous administration of CPG10101 in healthy volunteers was well tolerated and showed immunostimulatory characteristics with low adverse effects [163]. In a multicenter, Phase Ib trial with HCV-positive patients, CPG10101 induced a dose-dependent increase in immune activation and diminished HCV RNA

levels, supporting the use of CPG10101 for the treatment of chronic HCV [164].

Immunomodulatory ODNs (IMOs®; Idera Pharmaceuticals, MA, USA) are synthetic DNA structures, called 'immunomers', containing the dinucleotide immunostimulatory motifs, CpR or RpG, where 'R' is a synthetic analog of natural bases [165,166]. IMOs have greater metabolic stability and induce different cytokine profiles depending on the structure and sequence of the immunomer. IMO-2125 showed a high and sustained level of IFN responses in non-human primates and promising antiviral effects in a Phase I clinical trial of HCV patients (Idera Pharmaceuticals). SD-101, another second-generation TLR9 agonist, was shown to stimulate 20-fold higher levels of both IFN-α and IFN-λ in human PBMCs than the first-generation TLR9 agonists. A Phase Ib study in HCV patients reported that SD-101 is safe, well tolerated and produces significant antiviral activity based on dose-dependent IFN response (Dynavax Technologies Co., CA, USA).

Conclusion & future perspective

Since the discovery of TLR3 as the first receptor to recognize dsRNA, significant progress has been made in understanding TLR-mediated immune responses following different viral infections. The knowledge of virus-induced TLR signaling pathways has led to the development of novel therapeutics targeting TLRs as antiviral and anti-inflammatory therapies. This is a very dynamic field and has been growing rapidly in recent years. Approved use of the TLR7 agonist imiquimod for therapy against HPV-induced genital warts and the TLR4 agonist MPL as an adjuvant for vaccines against HPV and HBV are some of the successful examples of translational efforts. In addition, TLR3 and TLR7–9 agonists are showing very promising results for the treatment of viral infections. However, many challenges exist for the development of new TLR-based antiviral targets. Importantly, the interactions between virus and TLR signaling components are complex and the outcomes depend on the TLR, virus or the host species. In the case of vaccinia virus infection, TLR3-dependent responses were harmful, while TLR4-mediated immune responses proved to be protective in mice [77,167]. Moreover, a single ligand can be sensed by different TLRs depending on the localization of the antigen, and this may generate overlapping, redundant responses. In the case of MCMV infection, TLR7 and TLR9 impart redundant functions for IFN, IL-12 p40 and TNF-α production by pDCs *in vivo* [168]. In this case, redundancy of ligand sensing by TLRs should be taken into account in order to develop a single TLR-targeted treatment strategy. Some of the infections are resolved by the cooperation of multiple TLRs with each other or TLRs cooperating with other classes of PRRs. For example, TLR2 and TLR9 are both required for immunity against HSV-2 [58], and TLR7 and TLR9 overlap to generate responses against MCMV [168]. Both $TLR4^{-/-}$ mice and $PAR2^{-/-}$ mice are highly refractory to influenza infection [123]. Furthermore, the coordinated recognition of rhinovirus, initially via TLR3 and later by RIG-I and MDA5, is required in order to induce antiviral responses within the bronchial epithelium [169]. HSV infection is sensed by both TLR9 and RLRs, which synergize to induce type I IFN production [170]. These observations indicate that complex cross-talk between different TLRs and between TLRs and other families of PRRs exists in order to resolve or mediate certain viral infections. It is of utmost importance for us to understand, characterize and take into account all of the possible interactions between TLRs or other families of PRRs in order to design and apply novel targets against TLR for efficacious treatment application.

Animal models provide primary valuable information about the safety, efficacy and molecular mechanisms of the selected drug targets. However, some of the TLR targets show important species-specific variations in the effects of certain drugs. RSV-infected BALB/c mice treated with poly-ICLC showed significantly reduced inflammation and clinical scores for the disease [171]. However, in contrast to the murine model, poly-ICLC treatment resulted in increased pathology during RSV infection in cotton rats [90]. In addition, due to important differences in the TLR signaling pathways in animal models and humans, many drugs tested with encouraging results in animal models failed to show much effect in human studies. In the natural condition, TLR-based immune responses to pathogen exposure are diversified on the basis of differences in cellular distributions at various anatomical sites and differential patterns of TLR expression among subsets of DCs and other antigen-presenting cells. Thus, TLR agonists can produce different responses on basis of the route of administration (e.g., contrary to intravenous

administration), as subcutaneous CPG 7909 induced a Th1-like innate immune response [172]. The differences in the response to TLR ligands administered by different routes pose challenges and opportunities for the development of TLR-based drugs and vaccines.

TLRs are fundamental sensors of the innate immune system. Thus, the activation or inhibition of TLR pathways by therapeutic TLR agonists or antagonists may cause potent harmful immune activation or unwanted immunosuppression. Moreover, it is difficult to predict efficacy and off-target effects in a large human population. To this end, Phase I safety trials of therapeutic TLR targets must be assessed for both their short- and long-term effects. Alternative dosing regimens or differential routes of administration may alter both the efficacy and safety of the drug in question. In addition, targeting therapeutic drugs to the

EXECUTIVE SUMMARY

Background

- Toll-like receptors (TLRs) are one of the largest and best-studied families of pattern-recognition receptors of the innate immune system.
- TLRs recognize pathogen- and danger-associated molecular patterns and activate downstream signaling cascades in order to induce proinflammatory cytokines and antiviral type I interferon by NF-κB and IRF3 or IRF7 transcription factors, respectively.
- TLR-mediated responses orchestrate the development of long-lasting, pathogen-specific adaptive immune response.

Recognition of viral ligands by TLRs, downstream signaling & outcomes

- TLRs on the plasma membrane (TLR1, 2, 4 and 6) detect viral proteins, which are released in the extracellular environment, while endosomal TLRs (TLR3 and 7–9) recognize viral nucleic acids.
- Activation of NF-κB is triggered by all TLRs from the plasma membrane and endosome, whereas TLR-induced IRF3 (TLR3 and TLR4) and IRF7 (TLR7–9) activation is initiated only from the endosome.
- Activation of TLR signaling by viral ligands establishes primarily antiviral responses. However, certain viral infections develop TLR-mediated detrimental immunity to the host.
- Many viruses have evolved defense mechanisms to escape the surveillance of the TLR-mediated innate immune system.

TLR-targeted therapeutics for viral infections

- Drugs targeting TLRs offer novel opportunities for the prevention of or intervention against virus-induced infectious diseases, either directly or by improving vaccine efficacy.
- An adjuvant composed of the TLR4 agonist monophosphoryl lipid A has been licensed for use in a vaccine against HPV and HBV.
- Vaccines against RSV formulated with monophosphoryl lipid A enhanced protection against RSV without aberrant lung histopathology in animal studies.
- The TLR7 agonist imiquimod has been approved for the treatment of genital warts caused by HPV.
- TLR3 and TLR7–9 agonists showed promising results for the treatment of viral infections, such as HIV, influenza, HBV and HCV.
- Efforts to develop TLR antagonists for the treatment of virus-induced harmful inflammatory responses are still in early development stages.
- The TLR4 antagonist eritoran, a drug that was originally developed for the treatment of sepsis, showed encouraging results with regards to the prevention of influenza-induced inflammation in an animal study.
- Targeting TLRs with drugs may cause potent harmful immune activation or unwanted immunosuppression.
- The development of many compounds was terminated due to safety concerns and off-target effects in early clinical studies.

relevant tissues or organ may be beneficial for the limitation of off-target effects. Furthermore, recent mouse and cotton rat data with eritoran, a TLR4 antagonist developed for the management of sepsis, showed promising results with regards to intervening in the inflammation associated with influenza infection, which opens the door for the possible treatment of other infectious agents where TLR4 senses DAMPs and initiates the cytokine storm [124]. In summary, the development of TLR-targeted therapies in the form of agonists or antagonists offers exciting and promising new possibilities for the prevention of virus-induced infectious diseases or management of virus-induced harmful inflammatory responses.

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