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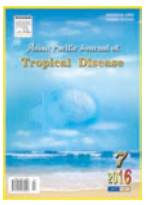

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Bridging the importance of Toll like receptors in human viral infections

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ABSTRACT

Toll-like receptors (TLRs) have important role in innate immunity, since they detect pathogen-associated molecular patterns on a wide range of microbes, leading towards activation of innate immune responses and orchestration of adaptive immune response. Most of the viruses have evolved mechanisms to subvert for the benefit of virus and to evade immune system. Literature search was performed from Pubmed and Google Scholar search engines. Among thirteen different types of TLRs, TLR1, TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9 are involved in responses towards viral infection. In this review, we will discuss earlier evidence, mainly from knockout mice studies, implicating TLRs in the innate immune response to viruses, in light of more recent clinical data demonstrating that TLRs are important for anti-viral immunity in humans.

1. Introduction

The Toll like receptors (TLRs) are principal modulators of innate immune system performing central role in identification of foreign infection in mammals. These type 1 membrane-spanning, pattern recognition receptors detect conserved products (pathogen associated molecular patterns) unique to microbial metabolism. TLRs are not only significant for innate immune activation but also important for adaptive immune responses. It has been reported that identification of various microbial products by TLRs which are expressed on the dendritic cells (DCs) usually activates functional maturation leading towards antigen-specific activation of adaptive immune responses[1]. Multiple viruses have been reported to activate innate immune system through TLRs which suggests that these molecules are likely to be associated to outcome of viral infection. Most of the viruses evolved not only to get escape from the innate immune system, but also to confound it for the benefit of viruses[2]. Viral infections are propagating silently worldwide[3]. The nature contains hidden remedies against viruses and there is a strong need to identify therapeutic potentials of natural entities[4-6].

TLRs result into activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, which ultimately promotes cytokine production, through multiple adaptor molecules like TIR domain-containing adaptor inducing IFN-β (TRIF), myeloid differentiation primary response gene 88 (MyD88) and TIRAP/Mal. Along with the production of various inflammatory cytokines [such as tumor necrosis factor alpha (TNF alpha), interleukin 12 (IL-12), IL-8, IL-6, IL-1], chemokines and induction of co-stimulatory molecules (such as CD40, CD86 and CD80), the activation of NF-κB pathway tends to link both primary and secondary immune response. MyD88 tends to bind FAS-associated via death domain, thereby promoting apoptosis via caspase cascade. Therefore, TLRs mediated activation of apoptosis tends to contribute towards defense strategies utilized by the innate immune response.

Among thirteen different types of TLRs, TLR1, TLR2, TLR3, TLR4, TLR7, TLR8 and TLR9 are involved in responses towards viral infection[7,8]. TLR1 gets associated with TLR2 in order to develop heterodimers. The TLR3 tends to recognize dsRNA, which acts as universal viral molecular pattern. TLR4 was identified as the first human homologue of *Drosophila* Toll and was initially explained to induce gene expression of inflammatory responses[1]. The TLR4 expresses on the surface of many immune cells. The main ligand of TLR4 is Gram negative bacteria's lipopolysaccharide (LPS) and

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responsible for activation of MyD88 dependent and independent pathways[9]. TLR7 and TLR8 are phylogenetically and functionally related forms of TLRs that are expressed mainly in the endosomal compartments and are specialized for the recognition of single stranded ribonucleic acid (ssRNA) form a number of viruses[10-12]. Upon activation by specific RNA ligands, these TLRs initiate a series of signaling event that ultimately leads to production of a number of antiviral and pro-inflammatory cytokines[13]. TLR9 also designated as CD289 recognizes CpG sequences of bacterial genome and viral DNA present in DNA molecules and activates anti-viral immune responses[14]. Viruses have certain proteins that inhibit TLR signaling thus playing the role in immuno-pathogenesis and virulence. Elevated activation of various cellular proteins may cause cancer proliferation, which can be further inhibited by potential inhibitors[15-19]. Rapidly increasing viral epidemics could bring havoc in poor countries and can potentially wipe out even the entire nations[20]. Tremendous efforts are being put forward by many scientists, from whole world, for prevention and control of viral infection[16]. In the light of most recent clinical and experimental investigations on human, this review illustrates importance of TLRs in the innate immune response and elucidates potential therapeutic interventions.

2. TLR1

In 1994, TLR1 was initially recognized as Toll in *Drosophila* (TIL), possibly involved in mammalian development. Prior to TIL discovery, a molecule possessing significant association with mammalian immune system was recognized and termed IL-1 receptor. On the basis of cytoplasmic portions, it was identified that the molecule had approximate homology towards *Drosophila* Toll[21]. TLR1 (of approximately 786 amino acid protein) plays an important role in innate immune response and cooperates with TLR2 to mediate innate immune responses against bacterial lipopeptides or lipoproteins. TLR1 utilizes MyD88/Mal and TNF receptor-associated factor 6 (TRAF6) adapter molecules for ligand specific signal transduction. TLR1 signal transduction usually leads to NF-κB activation, generation of inflammatory response and production of cytokines. It has been reported that TLR1 is ubiquitous and its high expression is found in spleen, thymus peripheral blood leukocytes, small intestine and ovary[22]. Some of the alternative names associated with TLR1 include: TLR1, Toll/IL-1 receptor-like protein, TIL, rsc786, CD281, DKFZp547I0610, KIAA0012, DKFZp564I0682, MGC126312, MGC126311 and MGC104956.

TLR1 gets associated with TLR2 in order to develop heterodimers. TLR1s are located at cellular surface of macrophages/monocytes, B lymphocytes and a subset of DCs. According to Jin *et al.*, TLR1-TLR2 heterodimer formation promotes intracellular cytoplasmic Toll/IL-1 receptor-resistance (TIR) domains to come close thereby promoting dimerization and initiation of intracellular signaling. Extensive hydrophobic interactions and hydrogen-bonding between TLR1 and TLR2 tends to further stabilize heterodimer[23]. It has been reported that TLR1 usually recognizes multiple triacyl lipopeptides[24]. It was reported that TLR1, TLR2 and TLR2, TLR6 exist as heterodimers at surface of cell and recognize multiple diacylated lipoproteins and triacylated lipopeptides of bacterial origin[25]. According to Daley *et al.*, TLR1 variant (rs4543123) was found to be associated with multiple viruses such as parainfluenza virus and respiratory syncytial virus (RSV)[26]. It has been reported that TLR1 and TLR2 heterodimer recognizes envelop proteins of human cytomegalovirus (HCMV) thereby leading towards release of proinflammatory cytokines[2]. Chang *et al.* reported that TLR1 and TLR6 are associated with TLR2-mediated macrophage activation via hepatitis C virus (HCV) core and nonstructural protein 3 (NS3) proteins[27]. It was further investigated in human cells that TLR1 or TLR6 deficient cells result into significant cytokine reduction upon HCV protein stimulation. This suggests that in case of macrophages, TLR2 tends to exploit TLR1 or TLR6 in innate immune recognition of HCV proteins. A decreased expression of TLR1 and TLR2 was identified in carotid plaques, after 4 weeks of lentiviral transfection[28].

It has been reported that the activation of antiviral TLR1 dependent signaling cascade would ultimately lead towards activation of the key transcription factors such as NF-κB which would ultimately promote various antiviral responses via induction of specific genes[29]. TLR1/TLR2 dimer generates intracellular signaling via IL-1 receptor-associated kinase4 (IRAK4) mediated activation of IRAK1/2 which causes stimulation of NF-κB, p38 and JNK proteins in cytoplasm. These proteins (NF-κB, p38 and JNK) enter into nucleus thereby causing activation of various proinflammatory cytokines such as TNF alpha, IL-1 beta, IL-6, IL-8 and IL-18. Abnormal TLR1/TLR2 signaling may contribute towards the enhancement of infection-related morbidity and mortality.

3. TLR2

TLR2 of approximately 784 amino acids normally exist at surface of cell. TLR2s are mostly found on monocytes/macrophages, myeloid DCs and mast cells. These TLRs generally utilize MyD88/Mal adapter molecules. TLR2 ligands include multiple ligands such as glycolipids, lipopeptides, lipoproteins, lipoteichoic acid, heat shock proteins (Hsp70) and zymosan (beta glucan)[30]. TLR2s are highly expressed in monocytes, peripheral blood leukocytes, lymph nodes bone marrow and spleen. These TLRs are also found in fetal liver and lungs. While in other tissues, TLR2 levels are comparatively low. TLRs, after their expression, experience post-translational modification. It has been observed that Asn-442 glycosylation is important for secretion of TLR2 N-terminal ectodomain. Literature review illustrated that TLR2 coordinates with LY96 to mediate innate immune response. It also coordinates with TLR1 to mediate innate immune response against bacterial lipopeptides or lipoproteins. Some of the alternative names of TLR2 include Toll-like receptor 2, Toll/interleukin-1 receptor-like protein 4, TIL4 and CD282.

TLR2 usually dimerizes with TLR1 and TLR6 for recognition of various ligands such as envelop glycoproteins of various viruses, GPI-mucin of protozoa, beta-glucan and zymosan of different fungi, porins and peptidoglycans and lipoproteins of multiple pathogens. It has been reported that when TLR1/TLR2 dimeric complex gets assembled with TLR2/TLR6 complex, then two signaling pathways are generated which meet at single point of proinflammatory cytokines activation. Structurally, the receptor TLR2/TLR6 complex, compared to TLR1/TLR2 complex, contains an additional CD36 surface protein molecule which tends to stabilize the complex. In both types of complexes, the intracellular signaling cascade follows the same pattern. Cytoplasmic IRAK4 protein causes activation of IRAK1/2. The TRAF6 after interacting to TAK1 and TAB1/2 complex generates IKK and MKK proteins which further result into activation of NF-κB, p38 and JNK mediated cellular pathway. NF-κB, p38 and JNK enter into the nucleus, thereby resulting into transcription of various important proinflammatory cytokines including TNF alpha, IL-1 beta, IL-6, IL-8 and IL-18.

The TLR2 acts via TRAF6 and MyD88 thereby leading towards activation of NF-κB, inflammatory responses and cytokine secretion.

In response to lipoproteins, the TLR2 may also promote phenomenon of apoptosis. DNA viruses such as HCMV, vaccinia virus (VACV), lymphocytic choriomeningitis virus, RSV, HCV and herpes simplex virus are at least partially dependent on TLR2[31-34]. Heggelund *et al.* has identified that among HIV-infected patients, freshly isolated monocytes expressed significant level of TLR2 compared to TLR4[35]. Further investigation revealed that elevated surface expression level of TLR2 was associated with stimulation via HIV type 1 envelope protein gp120. It was further confirmed that TLR2 stimulation in such patients, tends to promote TNF alpha responses and viral replication[35]. Hernández *et al.* reported that HIV type 1 infection causes upregulation of TLR2 expression in both *in vitro* and *in vivo* conditions[36]. Hepatitis B virus (HBV) is considered to be 10 and 100 times more infectious, compared to HCV and HIV[16]. Worldwide, on average, HBV infection causes the death every 30–45 s[37]. Interplay between HBV and TLR2-mediated innate immune responses proposed that restoration of TLR2 functions could possibly be used as a new therapeutic option[38].

In absence of hepatitis B envelop antigen, the viral replication was significantly associated with increased activation of TLR2 mediated pathway which leads towards upregulation of TNF alpha. It was further concluded that there exists an important interaction between host immune responses, HBV and hepatitis B envelop antigen. It has been reported that when TLR2 agonist Pam2Cys was intranasally administered, it triggered a cascade of innate immune signals and inflammatory cytokines which resulted into macrophages and neutrophils attraction, further leading towards secretion of IL-2, IL-6, IL-10, TNF-α, IFN-γ and MCP-1. Such events significantly reduced the potential for transmission of infection via promoting resistant strategies against influenza A virus. It did not affect host generated adaptive immune response which was measured by identifying virus-specific $CD8^+$ T cells after exposure made by influenza A[39]. Cuevas *et al.* while working on Junín virus induced innate immune responses reported that TLR2 is a cellular sensor of both the Candid 1 and Parodi viral glycoproteins[40]. A study conducted on RSV reveled possible anti-viral responses generated via TLR2. Anti-RSV cytokine production was dependent upon TLR2/6 heterodimerization. While TLR2-RSV interactions were involved in DCs activation and neutrophil migration[41]. Initially TLR2 were found to be responsible for sensing VACV infection on bone marrow derived DCs. The release of pro-inflammatory cytokine was found TLR2 dependent, whereas the type I interferon production was found TLR2 independent[42].

TLR2s are found to be associated with HCV immune evasion mechanism. It has been reported that among chronic HCV infection, the HCV core protein induces cytokine production (TNF-α and IL-10) from macrophages. Production of such cytokines causes reduction in interferon alpha releasing from plasmacytoid DCs or may induce apoptosis of plasmacytoid DCs. In patients' clinical aspects, administration of interferon alpha could provide protection against HCV infection[43]. In humans monocytes and HEK-293S human fibroblasts, experiments were performed to identify TLR association with viruses. It was found that HCV, Epstein Barr virus and HCMV have association with human TLR2 via core protein NS3, unknown and envelop proteins B and H respectively. Similarly, when experiments were performed on HEK293, it was found that HCMV associates with TLR1/TLR2 possibly via envelop proteins B and H[44].

It has been reported that HCV three proteins including core (C), NS3/4 and non structural 5A (NS5A) have close association with TLRs related signaling pathway and RNA helicase retinoic acid inducible gene-I associated signaling pathway. The core protein of HCV (only in its monomeric form) has found to be associated with TLR2 signaling pathway. This interaction facilitates HCV innate immune evasion. HCV NS3/4A has found to be associated with disruption of RIG-1 and TLR3 signaling pathways. HCV NS5A downregulates NKG2D expressions in natural killer (NK) cells thereby resulting into functional disability. It has been reported that when HCV core protein was expressed in Mono Mac 6 human monocytic cell line and HEK293, there was significant increase in IL-6 and IL-8 levels via TLR2 pathway. It was further revealed that after expression of HCV core protein, the MyD88 deficient spleen cells were unable to produce IL-6. These findings illustrated TLR2 associated signaling defects as MyD88 is an important downstream effect or molecule of TLR2 signaling pathway[45].

4. TLR3

TLR3 is a member of the Toll-like receptors family. It is also known as CD283 and is encoded by the TLR gene. This gene is present on the human chromosome 4[9]. TLR3 recognizes universal viral molecular pattern dsRNA. Thus TLR3 is involved in host immune responses against viruses. Poly(I:C), a synthetic ligand, has been reported to mediate immune responses through TLR3. TLR3 can induce the production of TNF-alpha, IFN-beta, IL-12 and IL-6 by responding to dsRNA as it specifically recognizes poly(I:C) and Lang reovirus purified genomic dsRNA[46]. However, certain studies have also illustrated that TLR3 do not play significant role in the antiviral immune responses of the host to dsRNA of reovirus. In one of the studies, it was shown that both TLR-deficient and -sufficient mice were equally susceptible to infection and an equivalent T cell immune response was generated against reovirus[47]. The TLR3 structure has been recently investigated by scientists at the Scripps Research Institute. According to this study, TLR3 makes a large horseshoe shape that associates with a neighboring horseshoe. So by this way it forms a dimer of two horseshoes. A greater portion of the surface of TLR3 is covered with sugar molecules making it a glycoprotein. There exists a large sugar-free surface on one of its faces including the proposed interface between the two horseshoes. Two regions of positively charged amino acids are distributed on TLR3 surface. It has been suggested that these distinctive regions of amino acids may provide binding sites for negatively charged double-stranded RNA. So the structure might play a crucial role in identification and attachment to its target molecule. Although TLR3 is a glycoprotein, it can crystallize for analysis of its structure by X-ray crystallography[48].

A two-hybrid screening has resulted in the identification of TRIF/TICAM-1 as a molecule that works in association with TLR3. In contrast to other signaling molecules like TIRAP/Mal and MyD88, the TRIF is a large protein that is made up of 712 amino acids in humans. The attachment of TLR3 to dsRNA leads to over expression of TRIF as well as TIRAP and MyD88 subsequently causing the activation of the NF-κB dependent promoter in human cells. But the activation of promoter for IFN can only be brought about by overexpression of TRIF and not MyD88 or TIRAP. The TLR3 ligand induced IFN promoter activation can be inhibited by dominant negative TRIF. Also if TRIF is knocked down by RNAi, it can result in aberration in expression of TLR3 ligand induced IFN. TLR3 and TLR4 mediated responses have been found defective in TRIF mutant mice. This was revealed by a random germline mutagenesis study which used alkylating agent *N*-ethyl-*N*-nitrosourea. TRIF has been demonstrated to be crucial molecule for TLR3 and TLR4-

mediated MyD88-independent pathway. The signaling pathway of TLR4 is likely to require activation of both MyD88-dependent and -independent pathways to induce inflammatory cytokines.

The rhinoviruses cause common cold and were studied for understanding the role of TLR3. It has been found that TLR3 mRNA expression and surface protein expression induce rhinoviral replication. TLR3 tends to mediate antiviral activity in the rhinovirus infected human bronchial epithelial cells. The increase in rhinovirus replication is the result of the blockage of TLR3 during infections[49]. West Nile virus is ssRNA flavivirus. TLR3 plays a significant role in pathogenesis. This virus causes a human disease with different levels severity by replicating through a dsRNA. The West Nile virus infection causes TLR3-dependent inflammatory response which tends to promote viral penetration into brain tissues leading towards encephalitis which may prove lethal[13]. Cytokines such as interferons, TNF-alpha and IL-6 are secreted when virus replicates in peripheral tissues. This is because of the inflammation. The West Nile virus infection and TLR3 stimulation cause TNFR1 mediated signaling which participates in blood-brain barrier breakdown[50]. Another amazing characteristic of TLR3 is that it accelerates the cross-priming to virus-infected cells. It is hypothesized that TLR3 may have originated to permit cross-priming of cytotoxic T cells against those viruses which are directly unable to infect DCs[51]. Evidence till now have shown that TLR3 is not universally required for the antiviral responses, which proposes a potential role of other pattern recognition receptors. In this regard, the RNA helicases represent an alternative major cellular sensor for various viral infections associated with dsRNA[52].

5. TLR4

TLR4 is a protein of approximately 835 amino acids that is encoded by TLR4 gene in humans[9]. TLR4 has been reported to express on the cellular surface of variety of immune cells that migrate to phagosomes after activation. TLR4 was identified as the first human homologue of *Drosophila* Toll, and was shown to induce the expression of genes involved in inflammatory responses[1]. Mutation in *TLR4* gene was identified in mouse strains, which were hyporesponsive to LPS[53]. The mechanism of activation of TLR4 is quite complex. A co-receptor myeloid differentiation factor 2 (MD-2) is a soluble protein which recognizes and binds to endotoxin LPS that is outer membrane component of Gram negative bacteria[54,55]. MD-2 binds to the five acyl chains of lipid A component of LPS and sixth chain interacts with TLR4 receptor. This causes the dimerization of the MD-2/TLR4 and ultimately activation of the TLR4 signaling cascade *i.e.*, MyD88 dependent pathway triggers transcription of inflammatory responses and MyD88 independent pathway triggers type I interferon[11,56,57]. As LPS is the central ligand for TLR4 in mammals it can activate over thousands of genes. So TLR4 is involved in not only the immune responses against Gram negative bacteria but in viral infections, chronic inflammation, malignancies, cancers and autoimmune diseases.

TLR4 is involved in immune responses against viral infections. Fusion protein of the RSV is ligand for TLR4 and CD14. This gives antiviral response via induction of cytokines. The mice deficient in TLR4 have viral infection persisted longer. TLR4 polymorphisms show increased susceptibility to respiratory infection where high risk infants show heterozygosity for D299G and T399I polymorphisms. In TLR4 knock-out mice, the RSV infection showed impaired IL-12 expression, reduced NK cell function and impaired virus clearance as compared to wild-type mice[58]. Coxsackie virus B4 cause insulin dependent diabetes mellitus via viral induced pancreatic damage. CBV4 infection induces the production of proinflammatory cytokines via TLR4 that are involved in damage to insulin producing cells[59]. Vaccinia viral ligand is recognized by TLR4 and induces protective immune responses and the mice deficient in TLR4 had great replication of viruses and mortality than control animals[60]. TLR4 is a sensor for glycoprotein of Ebola virus and induces the pro-inflammatory cytokines and suppression of cytokine signaling 1 in monocytes and macrophages that result in immuno-pathogenesis[61]. TLR4 activation causes the activation of NF-κB. This NF-κB is important inducer of HIV-1 and plays an important role in pathogenesis via regulating expression of NF-κB and proinflammatory cytokines in peripheral blood mononuclear cells and monocyte-derived macrophages. Thus modulation of TLR4 tends to depict the mechanism to promote HIV replication and progression of AIDS. TLR4 plays antiviral role against HBV in kidney and induces immune injury. These immune inflammatory reactions inhibit viral replication[62]. If transgenic mice are injected with TLR4 then replication of virus is inhibited in liver via IFN-alpha/beta dependent manner. TLR4 is upregulated on monocytes of patients having chronic HBV and induces regulatory T cell responses and immune tolerance[63]. After HCV infection, TLR4 shows three to seven fold upregulation. NS5A protein of HCV causes induction of TLR4 by upregulating its promoter in hepatocytes, thereby activating B cells by increasing production of interferon beta and IL-6[64]. Human T-lymphotropic virus-1 has p30 protein that interferes with TLR4 signaling. This protein results in downregulation of expression of TLR4 on surface and reduces the release of proinflammatory cytokines. In adult T cell leukemic patients, anti-inflammatory cytokines are released in macrophages after TLR4 activation resulting in low adaptive immunity[65]. TLR4-TRIF pathway has a vital role in immunity against H5N1 influenza A virus, however, TLR3 signaling is also involved[66]. TLR4 recognizes the glycoprotein of vesicular stomatitis virus. Macrophages activation of PI3 kinase pathway via TLR4 leads to type I interferon expression and antiviral immunity[67]. In myeloid DCs, TRIF/TRAM pathway is activated which leads to type I interferon response and ultimately antiviral immunity.

6. TLR7 and TLR8

TLR7 and TLR8 are phylogenetically and functionally related forms of TLRs[10,68]. TLR7 and TLR8 are expressed mainly in the endosomal compartments of specific immune cells. TLR7 is largely expressed by plasmacytoid dendritic cells (pDCs)[11,69-71]. TLR8, in contrast, is chiefly expressed by myeloid DCs and monocytes[72,73]. These TLRs are specialized for the recognition of ssRNAs from a number of viruses. TLR7 specifically recognizes ssRNA containing guanosine- and uridine-rich sequence motifs. A specialized sensing mechanism for a number of short interfering RNAs has also been associated with TLR7[12]. TLR7 is known to be involved in immune responses against dengue virus, influenza A virus, Sendai virus, vesicular stomatitis virus and HIV. TLR8, to date, has been shown to be mainly associated with HIV. But the potential of involvement in immune response against a number of ssRNA viruses is still to be discovered[74-77]. New insights have shown the potential role of TLR7 in sex-dependent patterns of HIV pathogenesis. The polymorphisms in TLR7, such as Gln11Leu, have been associated with accelerated disease progression in HIV-infected patients and high viral load[78]. Knockout studies in mice have shown that exact role of TLR7 and TLR8, in a viral infection might depend upon factors such as passage history, virus dose and route of administration[79]. TLR7

is localized in endoplasmic reticulum of unstimulated cells. Upon activation, TLR7 traffics to the endosomal compartment where it can be stimulated by binding of specific ligands. These ligand bound complexes of TLR7 are retained which leads to potent type I interferon response by pDCs[80]. TLR7-expressing pDCs have also been shown to be associated with the class switching of B cells, induction of type-I T helper responses and cross-priming in both human and mice[81].

TLRs have a ligand binding domain that binds the ssRNA ligands inside the endosomal compartments, a transmembrane domain that spans the membrane of the endosome, and TIR domain that faces the cytosol. Upon stimulation by a specific ssRNA ligand, TLR7 and TLR8 recruit an adaptor MyD88 to the cytoplasmic TIR domain. MyD88 in turn forms a complex with two IRAKs: IRAK-4 and IRAK-1. Upon activation, this complex recruits TRAF6. IRAK1/TRAF6 complex then dissociates from TLR. In addition to IκB kinase gamma (IKKγ), the TRAF6 also performs polyubiquitination of transforming growth factor beta and activated kinase 1. IKKγ subsequently associates with IKKα and IKKβ. A series of phosphorylation events then lead to IKK mediated degradation of IκB, which in the unphosphorylated state sequesters NF-κB in the cytosol. NF-κB now enters the nucleus to induce gene expression. TAK1 also triggers a mitogen activated protein kinase pathway leading to the formation of activator protein. Similar to NF-κB, activator protein enters into nucleus, and together both induce expression of proinflammatory genes[11,13].

Interferon regulatory factor 5 and 7 (IRF5 and IRF7) also interact with IRAKs and TRAF6 complex. This leads to IRAK1-dependent phosphorylation and both molecules are then transported to the nucleus. Animal model studies have shown that while IRF5 is primarily involved in regulating induction of pro-inflammatory cytokines, IRF7 is an important mediator in TLR7/TLR8-dependent type I interferon production[80,82-84].

Agonists of TLR7 and TLR8 can serve as potent therapeutic agents. These agents have shown promising results in several viral infections caused by a variety of viruses. TLR7/8 agonist, conjugated with HIV-1 gag protein, has shown to improve the quality and magnitude of T-cell response in non-primate animal models. TLR7 agonist, imiquimod has been effectively used against a number of viral infections caused by RNA viruses[85,86]. Synthetic agonists of TLR8, for example imidazoquinoline, exhibit immunostimulatory activity. In addition to their therapeutic potential, various conjugates of TLR7/8 agonists can be also used as adjuvants in vaccines[87]. It has been reported that the TLR7 agonist R848 can be used to enhance HBsAg-specific humoral and cellular immune responses. Furthermore, the R848 conjugated with CpG oligonucleotides can also be used as effective adjuvants for therapeutic and prophylactic HBV vaccine formulations.

7. TLR9

TLR9 is expressed in different cell types such as monocytes, neutrophils and CD^+ T cells where it is involved in immune activation. It is also expressed in non-immune cells. TLR9 is expressed on B cells and plasmacytoid DCs[14]. TLR9 mediates immune responses to viral DNA by identifying CpG motifs of microbial DNA. pDCs produce type 1 IFNs which in turn play key role in anti-viral responses. When infected with herpes simplex virus-1, murine cytomegalovirus (MCMV), adenovirus, poxvirus and murine gamma herpes virus 68, different TLR9 dependent responses occur. In case of murine gamma herpes virus infection, low levels of pro-inflammatory cytokines and type 1 IFNs are produced as DCs derived from bone marrow are TLR9 deficient cells. In human, TLR9 is important in HIV infection. There is impaired B cell response to TLR9 agonists because HIV infected individual's B cells have decreased amounts of TLR[88]. Activation of human pDCs and stimulation of TLR9 are inhibited by gp120 of HIV. As a result, there is a rapid disease progression due to TLR9 polymorphism[89]. It is not known that whether it recognizes HIV directly or by some secondary means. Expression of TLR9 is reduced in pDCs in patients with chronic hepatitis B infection[90]. In HCV dependent cirrhosis, TLR 9 is upregulated[91]. In RSV and measles infection, there is inhibition of production of type 1 IFN in pDCs[92].

TLR9 agonists can be used as vaccine adjuvant and for treatment of allergic and infectious diseases and cancer as mono- or combination therapies. TLR9 agonists are directly involved in activation as well as maturation of plasmacytoid DCs and tend to enhance differentiation of B cells into antibody-secreting plasma cells. TLR9 can enhance both cellular and humoral responses. Clinical trials have shown that when these agents are used as vaccine adjuvants, they increase the development of antitumor T cell responses and thus play role in antitumor activity. In different primates and rodents models, CpG oligodeoxynucleotide has been proved to be beneficial for asthma and other allergic diseases. The clinical contributions are enormous but it is still to be determined the safety and efficacy of the TLR9 agonist in human.

Response to viruses is by secretion of IL-12 and type 1 interferon by natural interferon producing cells. Infection prevention and control (IPC) of some of the viruses *in vitro* is recognized by TLR9. Ly49H receptor of MCMV is expressed by NK cells which are involved in viral clearance through cytokine secretion mediated by TLR. Depletion of IPC leads to a dramatic reduction in IFN-α, which lead other cell types to release IL-12, as a result normal NK cell and IFN-gamma responses to MCMV. It is concluded that antiviral cytokine responses by DC, IPC and other cell types, are mediated by TLR9/MyD88 pathway and their coordination is necessary for NK function clearance of MCMV.

Small pox is caused by poxviruses. Virus use multiple process to inactivate DC and ultimately suppression of immune response has developed multiple strategies to suppress immune responses. It is hypothesized that as these are large DNA viruses, their recognition is through DCs involving endosomal DNA recognition receptor TLR9. According to a study, TLR9 recognizes the causative agent of mousepox, ectromelia virus (ECTV) by DC recognition. Mice lacking TLR9 are more susceptible to ECTV infection indicating the importance of TLR9. In contrast, DCs are activated by vaccinia virus Ankara (MVA) which are modified by strongly attenuated poxvirus through both TLR9-independent and dependent pathways. It is therefore tested whether to protect ECTV lethally infected mice. There is a need for stimulation of immune responses by MVA. In fact, mice are protected from death when at the same time MVA given as a lethal dose of ECTV. Importantly, MVA if administered for 2 days rescued TLR9 deficient mice. Therefore, these data purpose an important role of TLR9 in the defense against poxviruses[93].

Some of the infections are caused by human gamma herpes viruses like Kaposi's sarcoma associated herpes virus and Epstein Barr virus. For the study of gamma herpes virus pathogenesis, murine gamma herpes virus 68 serves as a model. Unmethylated CpG DNA motifs which are present in viral and bacterial DNA are recognized by TLR9. DCs from wild type mice are compared with Flt3L cultured DCs which secreted very low levels of IFN-alpha, IL-6 and IL-12 when stimulated by gamma herpes virus. After infection, there is higher viral load which is seen in both latent and lytic infection.

This suggests involvement of TLR9 during gamma herpes virus infection[94].

VACV encodes two proteins that target the TLR system for inhibition. A46R was shown to associate with the TIR adaptors to downregulate TLR signaling, and it inhibits all the four adaptor proteins, MyD88, MAL, TRAM, and TRIF, while A52R was shown to target IRAK2 to inhibit TLR mediated NF-κB activation[95,96]. Another protein of VACV, K7 was shown to target DDX3, a component of IKKε/TBK1 complex, required for IFNβ induction[97]. HCV is another virus that encodes protein to inhibit TLR mediated signaling in that its protease NS3/4A cleaves TRIF, while NS5A inhibits MyD88[98]. Thus these and other viral inhibitors contribute to virulence. Another newly discovered proteins which inhibit TLR3 are 14-3-3ε and 14-3-3σ which play important role in signaling of different TLRs. Both these proteins impair the normal function of TLR2, TLR3, TLR4, TLR7/8 and TLR9 ligand-induced IL-6, TNFα and NF-κB and IFN-β production and their reporter gene activity. 14-3-3ε and 14-3-3σ also bind to both TRAF3 and TRAF6. So through modulation of TLR signaling pathway, these two proteins play major regulatory role in balancing the host inflammatory response to viral infections. It is shown that 14-3-3ε and 14-3-3σ inhibit TLR2, -3, -4, -7/8 and TLR9 mediated signaling. In contrary, 14-3-3ε and 14-3-3σ enhance TLR2, -4, -7/8 and TLR9 mediated RANTES production and inhibit TLR3 mediated RANTES production. Researches until now have shown that following LPS stimulation of cell, PKCε is recruited to TLR4, which is followed by phosphorylation of PKCε and subsequent association with 14-3-3β in a MyD88 dependent manner[99]. This binding of 143-3β to PKCε has been shown to lock PKCε in an open conformation thus regulating its lipid binding activity[100]. Thus regarding the role of viral infection, it is proposed that initial sensing of virus by the TLR leads to suppression of 14-3-3ε and 14-3-3σ expression thereby permitting the induction of TLR-driven early proinflammatory cytokines and type 1 IFN production towards the elimination of infection[101].

Conflict of interest statement

We declare that we have no conflict of interest.

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