

TLR3 Sensing of Viral Infection

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Abstract: Viral infection is detected by the innate immune system which mounts a rapid semi-selective defence involving inflammation and production of type 1 interferons. Several sensors, both cell surface and intracellular, exist to detect different types of viral motifs. Double-stranded RNA viruses and dsRNA replication intermediates are detected by toll-like receptor 3 (TLR3) as well as by retinoid-inducible gene 1 (RIG-I) like receptors. Binding of dsRNA or its synthetic analogue poly I:C to TLR3 recruits the adaptor protein TRIF and stimulates distinct pathways leading to activation of interferon regulatory factor (IRF) and NF- κ B. Here, we review the signalling cascades initiated by TLR3 and the modulation of these pathways.

Keywords: TLR3, virus, dsRNA, signalling, type 1 interferons, NF- κ B.

TLR3: SENSING OF VIRAL INFECTION

Human cells are equipped with a range of pattern-recognition receptors (PRRs) which recognise microbial pathogen-associated molecular patterns (PAMPs), and mount innate immune responses following infection. Anti-viral sensors can be broadly classified into two groups; toll-like receptor family members and retinoid-inducible gene 1 (RIG-I) like receptors (RLRs). TLR3 is activated by double stranded (ds) RNA [1] whilst TLR7 and TLR8 recognise single stranded RNA [2, 3]. RLRs are located in the cytoplasm and include RIG-I and MDA5, both of which recognise dsRNA [4]. DNA dependent activator of IFN-regulatory factors (DAI), a cytosolic DNA sensor, also recognises B and Z formation DNA from a variety of microbial sources [5]. Recognition of a viral PAMP by its cognate PRR activates an intracellular signalling cascade culminating in the production of type 1 interferons (IFN α and IFN β) and pro-inflammatory cytokines. Type 1 interferon in turn activates interferon stimulated genes (ISGs) and production of anti-viral proteins, thus amplifying the anti-viral immune response.

TLR3, when triggered by dsRNA, can activate several signalling cascades including those leading to the activation and nuclear translocation of the transcription factors IRF3 and NF- κ B, upregulating expression of interferon- β and pro-inflammatory cytokines (Fig. 1). These pathways can be intercepted and modulated at several steps, causing the balance of signalling to shift between the anti-viral and pro-inflammatory response. This review will discuss the signalling pathways induced by TLR3 activation and how these pathways can be modulated to the benefit or detriment of viral disease.

TLR3 LIGANDS

The ligand for TLR3 was identified as dsRNA relatively recently in 2001 [1, 6]. Eight families of dsRNA viruses exist including the rotaviruses which are implicated in infant mortality [7]. A further source of dsRNA is derived from the replication of other RNA and DNA viruses which produce dsRNA as a by-product of replication [8]. TLR3 can also potentially be activated by endogenous host single stranded mRNA though the presence of secondary structures, such as hairpins, which have dsRNA regions [9]. However, a later study from this group reported that common mammalian modifications of RNA such as methylation reduced signalling through TLR3 compared to minimally modified viral nucleic acids [10]. RNA released from necrotic cells in rheumatoid arthritis synovial fluid activates TLR3 on synovial fibroblasts [11]. Furthermore co-culture of macrophages from TLR3 $-/-$ mice with necrotic neutrophils resulted in abrogated production of pro-inflammatory cytokines compared to wild type macrophages. Pre-treatment of the necrotic neutrophils with RNase attenuated cytokine production from WT macrophages [12]. Small interfering RNA (siRNA) is an increasingly popular method for silencing gene expression but can also result in IFN release via the TLR3 pathway, depending on cell type and duplex length [13]. Polyinosinic:polycytidylic acid (poly I:C) is a synthetic dsRNA often used in studies investigating TLR3 function and signalling. The unmodified nature of poly I:C is further evidence that TLR3 recognises the RNA duplex rather than any modifications or other structures of dsRNA [14].

STRUCTURE OF TLR3

The structure of TLR3 was simultaneously elucidated by two separate groups who both published their findings in 2005. The basic structure of TLR3 is similar to the rest of the TLR family, comprising an extracellular ligand binding domain, a transmembrane domain and a cytoplasmic Toll/Interleukin-1 receptor (TIR) domain. The extracellular

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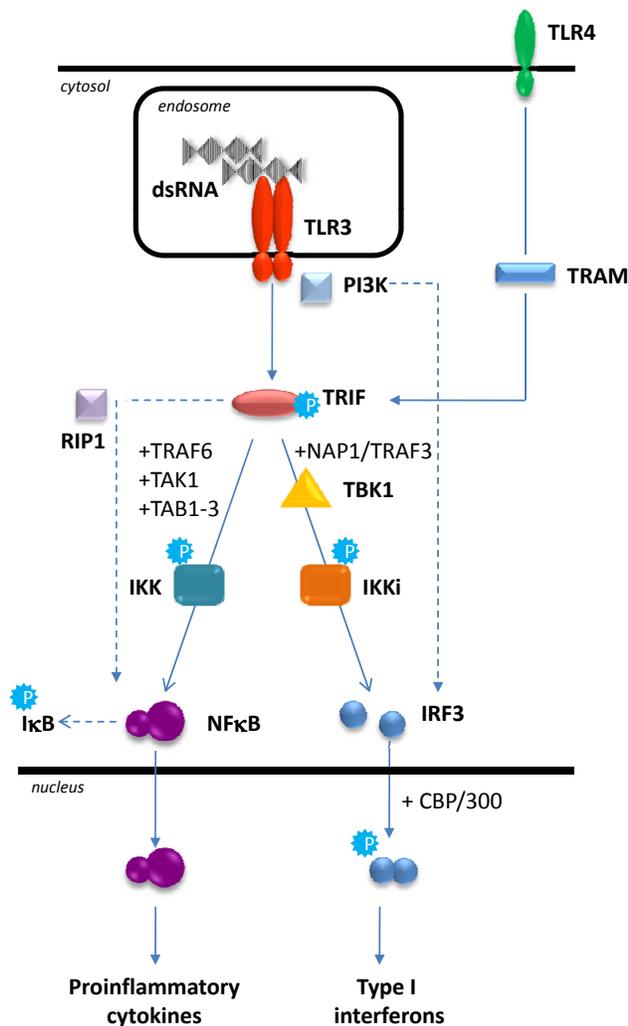


Fig. (1). TLR3 signalling pathways. dsRNA binds to TLR3 in endosomes activating TRIF. TLR4 can also activate TRIF via the adaptor protein TRAM. *Type I IFN pathway:* TBK1 interacts with TRIF via NAP1 and TRAF3 and the signal is transduced to IKKi leading to phosphorylation of IRF3 (and IRF7, not shown). PI3K, which is recruited to phosphotyrosine residues on TLR3, is also required for complete IRF activation. IRFs dimerise then translocate to the nucleus where they bind to DNA and transactivate type 1 IFN genes expression in conjunction with the co-activator CBP/p300. *Proinflammatory cytokine pathway:* TLR3 activates NFκB by a TRAF-6 mediated interaction with TRIF involving the TAK1/TAB1/TAB2/TAB3 complex which activates TAK1 causing it to phosphorylate and activate IKK. This phosphorylates IκB and targets it for degradation unmasking nuclear localisation sequences on NFκB. Signalling from TRIF via RIP-1 is required for full activation of NFκB and transcriptional induction of target genes.

domain of TLR3 has 23 leucine rich repeats (LRRs) arranged in a horseshoe solenoid structure. The concave surface of the horseshoe is heavily glycosylated with many negatively charged residues and is therefore not a candidate location for nucleic acid binding [15, 16]. Glycosylation is however required for full function of TLR3 signalling [17]. Amino acids in LRR20 on the convex surface of TLR3 were later found to be essential for ligand binding [18]. Binding of dsRNA to the ectodomain was found to be dependent on

acidic pH [19]. It has been proposed that upon dsRNA binding to TLR3, a second TLR3 molecule recognises the opposite strand of the dsRNA duplex [18], implicating formation of a TLR3 homodimer in dsRNA signalling. Leonard and colleagues later demonstrated that dsRNA binding induces TLR3 multimerisation and that the number of participant monomers increases with the length of the dsRNA duplex [20].

DISTRIBUTION AND EXPRESSION OF TLR3

TLR3 is expressed in a wide variety of cells including epithelial cells [21], fibroblasts [6], microglia [22], astrocytes [23], mast cells [24, 25], eosinophils [26], endothelial cells [27] and dendritic cells [28]. In contrast TLR3 expression is minimal in T cells [29, 30] and entirely absent in neutrophils which use alternative sensors to detect dsRNA [31, 32]. TLR3 expression is barely detectable [28] to absent [33] in monocytes but expression was markedly increased following cytokine-induced maturation of monocytes to immature dendritic cells (iDC). Muzio and colleagues detected TLR3 in mature DCs but not iDC [33]. In contrast, other studies found that expression of TLR3 is lost during progression of DCs to the mature form [28, 34]. TLR3 induced DC maturation is believed to bridge the innate and adaptive immune systems and readers are directed to the review by Salio and colleagues for more information [35]. TLR3 mRNA was not detected in T-lymphocytes, B cells or NK cells [33]. Sha *et al.* reported TLR3 to be the most abundant TLR expressed in Beas-2b airway epithelial cells. In agreement with this, poly I:C was found to be the most effective TLR agonist at upregulating gene expression in micro-arrays, with 7-fold upregulation of IL-8 and IL-6 expression observed [36].

TLR3 expression is upregulated by poly I:C [36-38] or viral infection e.g. RSV [39, 40] and *H. influenza* [21, 41]. TLR3 expression in human monocytes and monocyte-derived macrophages is inducible by treatment with IFNβ. The human promoter for TLR3 contains interferon response elements (IREs) and a STAT element; the cognate transcription factors are suggested to be IRF1 and STAT1 [34]. Indeed dominant-negative expression of STAT1 can abolish poly I:C induced TLR3 expression in murine cells [42]. LPS also upregulates TLR3 expression via the autocrine action of LPS-induced IFNβ secretion. LPS does not however upregulate TLR3 expression in human cells [34].

INTRACELLULAR DISTRIBUTION OF TLR3

TLR3 is mostly thought of as an intracellular receptor, resident on the plasma membranes of endosomal vesicles. Indeed in various subsets of dendritic cells and macrophages TLR3 is exclusively intracellular [43-45]. Other cell types however can display a proportion or all of their TLR3 on the cell membrane. Cell surface TLR3 has been demonstrated in fibroblasts [6], astrocytes [23] and epithelial cells [40, 46, 47]. A neutralising monoclonal antibody for TLR3 prevents dsRNA signalling in fibroblasts where TLR3 is on the cell surface [6] but not in dendritic cells where TLR3 is intracellular [45]. Studies on the localisation of TLR3 in epithelial cells have been conflicting. Several studies have concluded that TLR3 is located intracellularly in airway [21, 41] and endometrial epithelial cells [48]. As well as

upregulating TLR3 expression, viral infection of epithelial cells appears to induce localisation of TLR3 to the cell surface [40, 46] where it serves to sensitise or “prime” the cells to better recognise and respond to subsequent viral challenge [40]. A short 26 amino acid “linker” domain between the cytoplasmic TIR domain and the transmembrane portion of TLR3 is necessary for intracellular vesicular localisation of TLR3. Truncated versions of TLR3 lacking this region localise to the cell surface [49]. UNC93B is a membrane protein normally resident in the endoplasmic reticulum (ER), which is reported to be involved in trafficking of TLR7 and 9 from the ER to the endolysosome. Although this wasn’t demonstrated for TLR3 [50], Brinkmann and colleagues demonstrated that UNC93B interacts with TLR3 *via* its transmembrane domain and that point mutation of UNC93b in mice abrogates TLR3, 7 and 9 signalling, leaving the mice extremely susceptible to viral infection [51].

TLR3 and TLR7 localise in the same intracellular compartments often found adjacent to phagosomes containing apoptotic bodies, suggesting that TLR3 and 7 can be triggered by nucleic acids from apoptotic cells [52].

TLR DEFICIENCY: FUNCTIONAL ROLE FOR TLR3

The exact role that TLR3 plays during viral infection remains controversial with conflicting reports implicating TLR3 in both disease pathogenesis and anti-viral innate immunity.

TLR3 deficiency can be detrimental to the outcome of viral infection, demonstrating the importance of TLR3 in the anti-viral host defence. When infected with RSV, TLR3 *-/-* mice and WT mice were shown to have the same amount of viral growth. TLR3 *-/-* mice however, had an elevated pathological response to infection including increased mucus production in the airways [53]. TLR3 deficient mice also exhibit increased mortality and viral titre in the heart compared to WT following encephalomyocarditis virus infection with an associated decrease in the pro-inflammatory cytokine profile [54]. Infection with herpes simplex encephalitis virus (HSV) in several children was reported to be associated with point mutations in TLR3 suggesting a non-redundant role for TLR3 in defence of the central nervous system against HSV infection [55]. Deficiency of UNC93b, a TLR3 associated protein discussed in the previous section, was also associated with HSV infection in children [56]. The importance of TLR3 signalling was also demonstrated by knockout of its adaptor protein Toll/IL-1R domain-containing adaptor inducing IFN- β (TRIF). Following infection with mouse cytomegalovirus, mice homozygous for mutations in TRIF did not produce type 1 IFN and exhibited higher splenic viral titres. Additionally LPS and poly I:C challenge of TRIF *-/-* macrophages failed to induce phosphorylation or dimerisation of IRF3 [57, 58]. A further study did not observe any changes in mortality between TLR3 *-/-* mice and wild type mice infected with mouse cytomegalovirus (MCMV) infection despite higher splenic viral titres in TLR3 *-/-* mice [59]. Upon infection with coxsackievirus B4 TLR3 *-/-* mice displayed higher morbidity & mortality [60,61] as well as increased (100-1000 fold) viral titres and significantly reduced cytokines at day 4. Transfer of WT

macrophages but not DCs to TLR3 *-/-* mice significantly extended survival post infection, suggesting that the antiviral role of TLR3 in this infection was cell specific [60]. TRIF deficient mice also had higher viral titres [61].

Counter-intuitively, TLR3 deficiency can also positively affect the outcome of viral infection, particularly by dampening the pro-inflammatory response. TLR3 *-/-* mice exhibited less viral load, decreased morbidity and mortality compared to wild type mice in response to infection with the DNA vaccinia virus [62]. TLR3 deletion also appears to protect against infection with Punta Toro virus (PTV). TLR3 *-/-* mice infected with PTV display similar viral loads but less inflammatory mediators (IL-6 in particular), increased survival, and reduced liver disease compared to WT [63]. TLR3 deficiency also appears to protect against influenza A, with TLR3 *-/-* mice exhibiting increased viral load upon infection with influenza A compared to WT; they also had less inflammation, increased survival, and less CD8+ T lymphocytes in the bronchoalveolar lavage fluid [64]. The severity of rabies virus, which exclusively infects neuronal cells is also reduced when TLR3 is deficient, with increased survival of TLR3 *-/-* mice following infection. Following rabies infection, TLR3 localises with viral proteins to form perinuclear protein aggregates known as Negri bodies which contribute towards the pathogenesis of the disease [65].

The consequences of TLR3 deficiency in mice infected with West Nile virus (WNV), a mosquito-borne flavivirus, are controversial. Peripheral infection with WNV breaks down the blood brain barrier, leading to brain infection and lethal encephalitis. TLR3 *-/-* mice display less viral load in the brain, decreased cytokine load in the periphery and better survival compared to wild type mice [66]. Indeed the deleterious contribution of TLR3 to the pathogenesis of WNV infection was further demonstrated by delineation of the molecular events behind the observation that elderly people (average age 72.3 ± 8.8 years) have a more severe disease progression than younger patients (age 20 - 36 years). WNV infection of macrophages from young people reduces expression of TLR3 *via* a STAT1 dependent mechanism. TLR3 expression in macrophages from older people was elevated and the subsequent elevation of inflammatory cytokines is thought to mediate permeability of the blood brain barrier and progression of encephalitis [67]. Conversely, another group demonstrated that mouse mortality following WNV infection is increased in the absence of TLR3. The authors suggest that the culture method of the virus as well as the delivery route explain the difference in results [68].

Another function of TLR3 is to act as an endogenous sensor for host dsRNA released from necrotic cells. In mouse models of sepsis, peritonitis and gut injury, TLR3 deficiency protected against sustained deleterious inflammation, an effect mediated by the failure of TLR3 *-/-* macrophages to recognise dsRNA from necrotic neutrophils [12]. TLR3 is also required for normal inflammation of skin keratinocytes following exposure to necrotic material [69].

TLR3 SIGNALLING: TRIF

TLRs transduce signalling *via* the intracellular toll/IL-1 receptor (TIR) domain by recruitment of adaptor proteins such as MyD88, TRAM, TIRAP or TRIF. TIR-domain

containing adaptor inducing interferon- β (TRIF) (also called TICAM-1) is the only adaptor protein available for use by TLR3 due to the presence of alanine in position 795 in the protruding BB loop of the TIR domain rather than the proline conserved amongst other TLRs [70]. TRIF comprises a TIR domain flanked by proline rich C and N terminal domains. In resting cells TRIF does not co-localise with TLR3 and is found diffusely throughout the cytosol of the cell. dsRNA binding to TLR3 transiently recruits TRIF to localise with TLR3 at the membrane before dissociating to form cytosolic speckle structure to which NAK-associated protein 1 (NAP1) and receptor interacting protein (RIP)-1 also localised [71].

TRIF interacts with TLR3 *via* their respective TIR domains. TRIF can also transduce intracellular signals leading to type I interferon production by TLR4. Following TLR3 or TLR4 activation, TRIF deficient mice don't produce IFN and fail to activate IRF3 [72]. Interestingly TRIF expression is upregulated by an NF- κ B dependent pathway [73].

TLR3 Signalling to Type 1 Interferon

dsRNA induces the production of type 1 interferon by signalling along a TRIF/TBK1/IRF3 pathway, culminating in the phosphorylation and nuclear localisation of IRFs. TANK-binding kinase 1 (TBK1) binds to TRIF with preference for the phosphorylated form of TRIF [74] and knockdown of TBK1 by siRNA can block IRF3 activation [75]. This interaction is thought to be mediated by NAK associated protein 1 (NAP1) [76]. TRAF3 has also been proposed as a link molecule bridging the interaction between TRIF and TBK1 [77, 78]. I κ B kinase ϵ (IKK ϵ , also known as IKK inducible [IKK i]) and TBK1 have been identified as the kinases responsible for phosphorylation of IRF3 and IRF7 [79]. Additional phosphorylation of IRF3/7 by phosphoinositide 3-kinase (PI3K), which is recruited to phosphotyrosine residues on TLR3, is also required for complete IRF activation. Although IRF3 can translocate to the nucleus in the absence of PI3K activity, full phosphorylation of IRF3 and full activity as a transcription factor requires PI3K [80].

IRFs dimerise *via* a domain in the C-terminal region called the IRF association domain (IAD). Phosphorylation of IRF3 by the IKK ϵ /TBK1 complex and by PI3K occurs in a cluster of serine/threonine residues in the IAD domain and removes the autoinhibitory barrier to dimerisation, allowing full activation of IRF. IRF dimers then translocate to the nucleus where they bind to DNA *via* the N-terminal DNA binding domain with the help of the co-activators CBP/p300 [81].

TLR3 SIGNALLING PATHWAYS NF- κ B

TLR3 activates NF- κ B by two distinct pathways; a TRAF6 mediated pathway and a RIP-1 mediated pathway.

Following activation of TRIF, TRAF6 associates with N-terminal motifs in TRIF. Prevention of this association reduced NF- κ B activity but not IRF3 activity, providing evidence of divergence of TRIF signalling [74, 82]. Recruitment of TRAF6 to TRIF and the subsequent TRAF6 oligomerisation activates its E3 ubiquitin ligase activity, resulting in self-ubiquitination of TRAF6 which facilitates the recruitment of a TAK1/TAB1/TAB2/TAB3 complex

[83,84]. Transforming growth factor-beta-activated kinase 1 (TAK1) becomes activated by autophosphorylation of amino acids in its activation loop which allows TAK1 to phosphorylate and activate the IKK complex. Active IKK phosphorylates inhibitor of κ B (I κ B) which is then ubiquitinated and degraded, releasing NF- κ B dimers to translocate into the nucleus and initiate transcription of target genes.

TLR3 can also activate NF- κ B *via* the RIP homotypic interaction motif (RHIM) domain in the C-terminal region of TRIF which can bind both RIP1 and RIP3. RIP1 activity is required for full activation of NF- κ B [85-87] but RIP3 was actually found to downregulate signalling by competing with RIP1 for binding to TRIF [87]. The importance of Peli1, an E3 ubiquitin ligase, has recently been shown in TRIF mediated signalling with the observation that Peli1 deficiency abrogates TRIF-induced production of pro-inflammatory mediators, an effect mediated by the ubiquitination of RIP1 [88].

There is a growing evidence that TLR3 stimulation can activate the mitogen activated protein kinase cascade (MAPK) *via* the intermediate TAK1. IL-1 stimulated activation of TAK1 resulted in downstream activation of MKK6 and the c-Jun N-terminal kinase (JNK) pathway [84, 89, 90].

TLR3 AND APOPTOSIS SIGNALLING

Another unique feature of TRIF is that it is the only TLR adaptor protein to be strongly involved in apoptotic signalling [91], indeed Han *et al.* showed that HEK293T cells undergo significantly more apoptosis when TRIF was overexpressed [92]. The RIP homotypic interaction motif (RHIM) domain in the C-terminal region is necessary for this signalling and it has also been shown that mutations in the TIR domain also block apoptosis [91]. TRIF binding of RIP1 and RIP3 *via* the RHIM domain induces downstream signalling through Fas-Associated protein with Death Domain (FADD) and caspase 8 [91, 92]. Interestingly TRIF can be cleaved by caspases resulting in decreased NF- κ B and IRF activation [93]. The RHIM domain is also necessary for TRIF-induced maturation of the cytokine IL-1 β which has been demonstrated to occur *via* a TLR3/4 – TRIF – caspase-8 dependent mechanism [94]. Weber and colleagues also demonstrated the necessity of caspase-8 in poly I:C induced TRIF dependent apoptosis in HaCaT keratinocytes [95]. Given the pro-apoptotic nature of TRIF signalling, it has been suggested that TLR3 agonists may be useful therapeutic agents in cancer to induce apoptosis [96].

TRIF AND TLR4 SIGNALLING

TLR4 mediated production of type 1 interferon is dependent on the presence of TRIF but a direct interaction between TRIF and TLR4 has not been found. Rather TLR4 utilises another adaptor protein TRAM (also called TICAM-2) to link with the TRIF dependent pathway [70]. A recent paper showed that LPS stimulation of TLR4 recruited TRIF to the plasma membrane *via* interaction with TRAM. The entire complex then translocated to the endosome to complete signalling [97]. This is further supported by the report from Kagan and colleagues who propose a two step sequence of events following TLR4 stimulation. First the

signal is transduced through the MyD88 pathway, after which TLR4 is endocytosed and proceeds to signal through the TRAM-TRIF pathway in the endosome [98].

MODULATION OF TLR3 SIGNALLING PATHWAYS

Inhibition of TLR3 signalling has been reported to occur via a number of mechanisms including targeting of TRIF, downstream mediators and other as yet unidentified factors (Table 1).

Inhibition of TRIF signalling

Targeted inhibition of TRIF blocks all downstream signalling including activation of NF- κ B and IRF3. Hepatitis C viral protease NS3/4a cleaves TRIF and the RLH adaptor interferon-beta promoter stimulator (IPS)-1, thereby specifically targeting and evading TLR3- and TLR4- as well as RLH-signalling [99]. In addition to inhibition of TLR3 by caspase cleavage [93], RIP3 has been shown to downregulate signalling by competing with RIP1 for binding to TRIF [87]. TRIF mediated activation of NF- κ B and IRF is also repressed by PIASy, a protein inhibitor of activated STAT (PIAS) [100] but the exact mechanism is as yet unclear. Sterile alpha and TIR motif –containing protein (SARM), another TIR domain interacting adaptor molecule, inhibits TLR3 and TLR4 signalling downstream of TRIF and was demonstrated to interact with TRIF by both co-immunoprecipitation and yeast two hybrid experiments [101].

TRIF signalling is also regulated by A20, a TNF α inducible protein with deubiquitinase activity which inhibits NF- κ B activation in response to several stimuli. Wang and colleagues demonstrated that A20 associates with TRIF and inhibits activation of NF- κ B, the IFN β promoter and ISRE following TLR3 and TRIF activation with poly I:C and Sendai virus. A20 did not inhibit activation of NF- κ B, ISRE or IFN β promoter when signalling was induced by components downstream of TRIF such as TBK1 and IKK proteins [102]. In contrast, a later study showed that A20 associates with TBK1 and the IRF3 kinases IKKi/IKK ϵ , reduces phosphorylation and therefore activation of IRF3 [103].

Modulation of Signalling Downstream of TRIF

A recent paper by An *et al.* demonstrated that Src-homology 2 -domain-containing tyrosine phosphatase (SHP)-1 inhibits production of pro-inflammatory cytokines whilst concurrently augmenting production of type 1 interferons by binding and inactivating Interleukin-1 receptor-associated kinase (IRAK)1 [104]. The signalling cascade leading to the production of type 1 interferon can also be interrupted by the protein tyrosine phosphatase SHP-2 which is ubiquitously expressed and is present in the cytoplasm of all cells. Mutations in SHP-2 lead to Noonan syndrome which is characterised by short stature, facial dysmorphism and increased risk of heart defects and leukemia [105]. TLR3-induced signalling culminates in serine/threonine phosphorylation of IRF which is required for its dimerisation and nuclear translocation. Despite this, tyrosine phosphatases were found to block dsRNA induced signalling [106]. SHP-2 knockdown in macrophages allows greater IFN β production in response to LPS and poly I:C and SHP-2 over-expression can inhibit activation of IRF3. Further

investigation revealed that SHP-2 actually interacts with TBK1 and blocks its kinase activity, thus attenuating signalling to IRF3 [107]. Another molecule, the inositol 5' phosphatase (SHIP-1), also interacts with TBK1, negatively regulating production of IFN β following TLR3 stimulation [108].

Interestingly SHP-2 appears to be necessary for complete activation of NF- κ B [109-111]. SHP-2 deficient cells display impaired phosphorylation of I κ B and NF- κ B activation following IL-1/TNF stimulation but restoration of SHP-2 into cells can restore NF- κ B activation and IL-6 production. SHP-2 can be co-immunoprecipitated in a complex with IKK and was therefore deemed to be functionally necessary for efficient phosphorylation of I κ B and subsequent activation of NF- κ B [109]. The involvement of SHP-2 in the IRF and NF- κ B pathways appears to be modulated by signal regulatory protein (SIRP α), a transmembrane protein which acts as a substrate and adaptor protein for SHP-1 and SHP-2. The intracellular cytoplasmic domain has two ITIM motifs which become tyrosine phosphorylated upon activation. Phosphorylated SIRP α then recruits SHP-1 and SHP-2 which dephosphorylate members of the signalling cascade, negatively modulating signalling [112]. SIRP α knockdown increases LPS-induced association of SHP-2 with IKK, resulting in prolonged activation of NF- κ B [111]. Overexpression of SIRP α can suppress TNF α induced SHP-2 mediated stimulation of NF- κ B [110]. TRADD, another component of the TLR3 pathway can affect viral defence. TRADD -/- mice displayed a survival advantage following LPS or poly I:C challenge compared to WT. Poly I:C and LPS induced activation of the TRIF dependent pathway was impaired in TRADD-/- mice by measurement of cytokine secretion. These authors also demonstrated a cell specific role for TRADD with fibroblasts from TRADD -/- mice having impaired NF- κ B and MAPK signalling; this was not observed in bone marrow macrophages [113].

Vaccinia virus is a DNA poxvirus used to vaccinate against smallpox and several of vaccinia's viral proteins have been demonstrated to modulate the intracellular signalling pathway of TLR3. The vaccinia proteins A46R and A52R inhibit NE and IL-1 induced NF- κ B activation and IL-8 secretion in airway epithelial cells [114]. A46R also inhibits NF- κ B activation following stimulation with agonists of TLRs 1, 2, 4, 5, 6, 7 and 9 possibly by virtue of its association with the adaptor protein MyD88. Interaction between A46R and TRIF was also demonstrated by co-immunoprecipitation and GST-pulldown. Overexpression of A46R blocked IRF3 activation following poly I:C stimulation. A52R was found to inhibit poly I:C induced NF- κ B activation but not IRF3 activation. This is mediated by interaction with TRAF6 and IRAK-2 [115]. Suppressor of IKK ϵ (SIKE) inhibits the IKK ϵ /TBK1 complex under resting condition but dissociates from TBK1 following viral challenge allowing antiviral signalling to proceed [116].

Uncharacterised Inhibition of TLR3 Signalling

Other mechanisms of TLR3 inhibition include modulation by viral proteins as well as endogenous factors. Macrophages from CD14 -/- mice show impaired responses to poly I:C suggesting a role for CD14 in TLR3 signalling. Lee *et al.* found that TLR3 and CD14 can co-localise in

Table 1. Inhibitors and Modulators of TLR3 Signalling

Name	Mode of inhibition	Ref.
HCV NS3/4a protease	Cleavage of TRIF	[99]
Caspases	Cleavage of TRIF	[93]
RIP3	Competes with RIP1 for binding to TRIF	[87]
PIASy	Exact mechanism unknown	[100]
SARM	Inhibits TRIF	[101]
A20	Associates with (i) TRIF or (ii) TBK1 and IKKs	[102, 103]
SHIP-1	Interacts with TBK1	[108]
SHP1	Inactivates IRAK-1 and promotes type I IFN production	[104]
SHP2	Inhibits TBK1 and blocks type I IFN production	[107]
VVA46R	Binds to TRIF and inhibits signalling	[115]
VVA52R	Inhibits TRAF6 and IRAK2-mediated NF- κ B activation	[115]

lysosomes [44]. Guggul is a plant steroid used in traditional medicines for the treatment of inflammatory diseases including arthritis and cardiovascular disease. Guggul inhibits TRIF-induced NF- κ B and IRF3 activation [117]. Suppressor of cytokine signalling (SOCS)1 is a target gene of poly I:C stimulation. Expression of SOCS1 can inhibit poly I:C induced STAT1 phosphorylation activity. This may represent an autocrine regulatory mechanism to control TLR3 signalling [42]. West Nile virus non structural protein 1 (NS1) inhibited activation and nuclear translocation of both NF- κ B and IRF3 in response to poly I:C stimulation of HeLa cells [118].

ALTERNATIVE SENSORS FOR dsRNA

The fact that poly I:C can still induce interferon responses in the absence of TLR3 or TRIF [72] suggests that there are alternative mechanisms that sense intracellular dsRNA. The RIG-I-like receptors (RLRs) RIG-I and MDA5 recognise specific viruses. RIG-I was demonstrated to enhance poly I:C induced interferon production [119, 120], suggesting that it may have the ability to sense dsRNA. Further work has since elucidated that RIG-I recognises several ssRNA viruses such as influenza and Japanese encephalitis virus [4]. Another RLR related to RIG-I called MDA5 recognises picornaviruses [4] and poly I:C [121].

RIG-I and MDA5 have several domains including two caspase recruitment domains (CARD) at the N-terminus, a middle DExD/H RNA helicase domain and a C-terminal repressor domain. RLRs bind dsRNA *via* their helicase domain and stimulate downstream signalling through the CARD domain.

RIG-I is thought to discriminate between endogenous host RNA and microbial RNA by specifically recognising the uncapped 5' triphosphorylation characteristic of certain viruses but not host RNA [122-124], but as discussed in more detail in a recent review RIG-I can be activated by a range of RNA ligands with and without a 5' triphosphate group [125]. RLR signalling is mediated by the adaptor protein IFN β promoter stimulator 1 (IPS-1) (also known as mitochondrial antiviral signalling [MAVS]) which binds to activated RLR through interaction of the IPS-1 CARD

domain with the RLR CARD domain. IPS-1 recruits TRAF3 [77, 78] which in turn recruits TBK1 and IKKi. From this point the signalling cascade is similar to that of TRIF mediated signalling. TBK1/IKKi phosphorylate IRF3/7 which dimerises, translocates to the nucleus and stimulates production of interferon related genes [75]. Viral evasion of RIG-I signalling can be modulated by the influenza A non-structural protein 1 (NS1) [126]. RIG-I can also be degraded by viral proteinases during picornavirus infection, attenuating the antiviral defence [127]. Interestingly, Manuse and Parks reported that TLR3 activation was associated with increased expression of RIG-I suggesting the possibility of cross-talk between the two modes of dsRNA sensing [128]. Overexpression of RIG-I has been implicated in the pathogenesis of lupus nephritis with the finding that knockdown of RIG-I prevents activation of IRF7 which is involved in downstream inflammatory responses [129].

Although RLR detection of dsRNA is utilised in all cells (except plasmacytoid dendritic cells), it is the only sensor for dsRNA in neutrophils which lack expression of TLR3 [31]. Challenge of human neutrophils with intracellular poly I:C results in increased expression of type 1 interferons and proinflammatory cytokines though antiviral signalling mediated by both RIG-I and MDA. Both are strongly expressed in the neutrophil [32].

RNA activated protein kinase (PKR) is a serine/threonine kinase which has two binding domains for dsRNA. Upon activation, PKR homodimerises and undergoes autophosphorylation, and in turn phosphorylates a protein called eukaryotic initiation factor 2 (eIF2 α) which is involved in translation of eukaryote mRNA. eIF2 α activity is impaired by phosphorylation thereby inhibiting protein synthesis [130].

CONCLUSIONS

TLR3 is the only TLR that recognises dsRNA and is also unique through its exclusive use of TRIF, rather than other adaptor proteins such as MyD88. Although generally thought to initiate host anti-viral defences, TLR3 deficiency studies have paradoxically implicated TLR3 in the pathogenesis of several viral diseases such as that caused by West Nile virus.

The TLR3 activated pathways can be modulated at many points, sometimes bending signalling in a pro-inflammatory direction at the expense of the anti-viral response. Further study of signalling induced by dsRNA may provide novel therapeutic targets for the treatment of viral induced pro-inflammatory disease.

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ABBREVIATIONS

ER	=	Endoplasmic reticulum
CARD	=	Caspase recruitment domains
DAI	=	DNA-dependent activator of IFN-regulatory factors
DC	=	Dendritic cell
dsRNA	=	Double stranded RNA
eIF2 α	=	Eukaryotic initiation factor 2
FADD	=	Fas-Associated protein with Death Domain
IAD	=	IRF association domain
iDC	=	Immature dendritic cell
IFN	=	Interferon
IKK	=	I κ B kinase
IL-6	=	Interleukin 6
IL-8	=	Interleukin 8
IPS-1	=	IFN β promoter stimulator
IRAK	=	Interleukin-1 receptor associated kinase
IRE	=	Interferon response element
IRF	=	Interferon regulatory factor
ISG	=	Interferon stimulated gene
ISRE	=	Interferon stimulated response element
ITIM	=	Immunoreceptor tyrosine-based inhibitory motif
LPS	=	Lipopolysaccharide
LRR	=	Leucine rich repeat
MAPK	=	Mitogen activated protein kinase
MDA5	=	Melanoma differentiation-associated gene-5
MEF	=	Mouse embryonic fibroblast
NAK	=	NF κ B-activating kinase
NAP1	=	NAK associated protein 1
NF- κ B	=	Nuclear Factor-KappaB
NS1	=	West Nile virus (WNV) nonstructural protein NS1

PAMP	=	Pathogen associated molecular pattern
PI3K	=	Phosphoinositide 3-kinase
PIASy	=	Protein inhibitor of activated STAT
PKR	=	Protein kinase R
poly I:C	=	Polyinosinic:polycytidylic acid
PRR	=	Pattern recognition receptor
PTV	=	Punta toro virus
RHIM	=	RIP homology interaction motif
RIG-I	=	Retinoid inducible gene
RIP-1	=	Receptor interacting protein-1
RLR	=	RIG-I like receptor
RSV	=	Respiratory syncytial virus
SARM	=	Sterile alpha and TIR motif –containing protein
SHP-1	=	Src-homology 2 -domain-containing tyrosine phosphatase
SIKE	=	Suppressor of IKK ϵ
siRNA	=	silencing RNA
SIRP α	=	Signal regulatory protein
SOCS1	=	Suppressors of cytokine signalling
STAT1	=	Signal transducer and activator of transcription
TAK1	=	Transforming growth factor β -activated kinase 1
TANK	=	TRAF family member-associated NFKB
TBK1	=	TANK-binding kinase 1
TICAM	=	TIR-containing adapter molecule
TIR	=	Toll/Interleukin-1 receptor
TLR	=	Toll like receptor
TRADD	=	TNFR associated death domain
TRAF	=	TNF Receptor Associated Factor
TRAM	=	TLR related adaptor protein
TRIF	=	TIR-domain-containing adapter-inducing interferon- β
WNV	=	West Nile virus
WT	=	Wild type

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