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TLR3 immunity to infection in mice and humans

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Abstract

TLR3 is a receptor for dsRNA, which is generated during most viral infections. However, other cellular processes may also produce dsRNA and there are other receptors for dsRNA. The role of TLR3 in protective immunity to viruses has been investigated in mice and humans with genetically impaired TLR3 responses. TLR3-deficient mice responded to experimental challenge with 16 different viruses in various ways. They were susceptible to eight viruses, normally resistant to three other viruses, and their survival rates were higher than those of wild-type mice following infection with four other viruses. Conflicting results were obtained for the other virus tested. These data are difficult to understand in terms of a simple pattern based on virus structure or tissue tropism. Surprisingly, the known human patients with inborn errors of the TLR3 pathway have remained healthy or developed encephalitis in the course of natural primary infection with HSV-1. These patients display no clear susceptibility to other infections, including viral infections, such as other forms of viral encephalitis and other HSV-1 diseases in particular. This restricted susceptibility to viruses seems to result from impaired TLR3-dependent IFN-α/β production by central nervous system (CNS)-resident non-hematopoietic cells infected with HSV-1. These studies neatly illustrate the value of combining genetic studies of experimental infections in mice and natural infections in humans, to elucidate the biological function of host molecules in protective immunity.

Introduction

Like other vertebrate Toll-like receptors (TLRs), TLR3 consists of an extracellular leucine-rich repeat (LRR) motif, a transmembrane (TM) domain and an intracellular Toll and IL-1R (TIR) domain [1,2]. A comparison of the LRR and TIR domains of 366 vertebrate TLRs from 96 species, from fish to primates, revealed that the LRR domains of TLR3 and TLR7 are the most highly conserved [3]. Moreover, TLR3 is one of the four human TLRs (TLR3, TLR7, TLR8 and TLR9) that have evolved under the strongest purifying selection throughout human history [4]. These four TLRs are intracellular and are stimulated by nucleic acids [5,6]. TLR3 recognizes dsRNA, an intermediate generated during most viral infections [7]. It was thus thought that TLR3 acted as a sentinel against viruses. Mice

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genetically deficient for TLR3 have been engineered and experimentally challenged with various viruses. Surprisingly, TLR3-deficient mice were found to be susceptible to some viruses but normally resistant or even more resistant than the wild type to others [8-11]. Perhaps even more surprisingly, human patients with inborn errors of TLR3 immunity have been shown to suffer from life-threatening childhood herpes simplex virus 1 (HSV-1) encephalitis (HSE) while remaining normally resistant to other common viruses [12-16]. These observations are consistent with the concomitant discovery of other receptors for dsRNA in mice and humans, with less well documented roles in immunity to infection [11,17-19]. Moreover, dsRNA of non viral origin has been shown to trigger TLR3 signaling, raising the intriguing possibility that the continued investigation of TLR3-deficient mice or humans may reveal other phenotypes not necessarily related to viral infections. TLR3 has attracted considerable attention from investigators in fields as diverse as biochemistry, immunology and medicine. We review here current knowledge concerning TLR3, first focusing on TLR3 and its signaling pathway, then reviewing experimental infections in TLR3-deficient mice, and third describing TLR3-deficient humans.

TLR3 and its signaling pathway

The crystal structure of the human TLR3 ectodomain (ECD) revealed a large, horseshoe-shaped solenoid, potentially providing a large surface area for ligand recognition [20,21]. The TLR3-ECD was also thought to be essential for ligand binding-triggered multimerization [20-25]. More recent studies, performed on mouse or human TLR3-ECD, have demonstrated that TLR3-ECDs bind, as dimeric units, to dsRNA oligonucleotides of at least 45 bp in length, and that each TLR3-ECD binds dsRNA at two sites located at opposite ends of the TLR3 ‘horseshoe’. Intermolecular contacts between the C-terminal domains of two TLR3-ECDs coordinate and stabilize the TLR3 dimer [26-30]. In these models, the assembly of TLR3 as stable dimers on a single dsRNA ligand is necessary for TLR3 signaling [27,30]. The binding of dsRNA to the TLR3-ECD, the affinity of which increases with both buffer acidity and ligand size [23,27], is thus the first key step in TLR3 signaling. It remains unclear whether the natural dsRNA produced during viral infection can actually stimulate TLR3. Nevertheless, it has been shown that the length and structure of dsRNA agonists is important for TLR3 binding and activation [26-31]. Long dsRNAs are more potent inducers of TLR3 signaling than short dsRNAs [23,26-29,31]. Synthetic poly(I:C) is a nonspecific agonist of TLR3, which stimulates this receptor, leading to its robust activation [32]. Cell-endogenous mRNA, which is single-stranded but contains double-stranded regions [33] and is probably released from necrotic cells [34], can activate TLR3. Ultraviolet-damaged cell self noncoding snU1 (small nuclear U1) RNA, which contains stem-loop structures that could form dsRNA, also can activate TLR3 [35]. By contrast, total human cellular RNA cannot activate TLR3 [36], probably due to its high degree of posttranscriptional nucleoside modification.

TLR3 expression has been shown to be relatively broad in mice and humans. The principal TLR3-expressing cells include peripheral leukocytes, such as dendritic cells [37], $CD8^+$ T cells [38] and NK cells [39], retinal [40], corneal [41], intrahepatic biliary [42,43] and intestinal [44] epithelial cells, keratinocytes [45,46], lung and dermal fibroblasts [12,13,47], vessel endothelial cells [48], hepatocytes [49] and CNS-resident cells, including neurons, oligodendrocytes, astrocytes and microglia [50-54]. Many *in vitro* studies have shown that the TLR3 expressed by these cell types are functional, triggering the production of IFN-α/β, -λ, and other cytokines in response to poly(I:C) [12,38,40,52,53,55-59]. TLR3 is intracellular in most cell types, human lung fibroblasts being the only cells for which TLR3 expression on the cell surface has been reported [47]. In resting cells, TLR3 is located in the endoplasmic reticulum (ER) [5], along with UNC-93B, a transmembrane protein required for the trafficking of TLR3, TLR7, TLR8 and TLR9 from the ER to the endosomal

compartment [12,60-62]. Upon stimulation with dsRNA, TLR3 interacts with UNC-93B via the transmembrane domains of the two molecules. It then moves to the endosomes [61,62], where it is colocalized with and phosphorylated by tyrosine kinases c-Src [63,64], EGFR [64] and, perhaps, PI3K [65,66]. The dimerized, phosphorylated TLR3 is thus primed to trigger downstream signaling.

The TLR3 signaling pathway is mediated exclusively by the TRIF adapter, which is recruited to TLR3 by interaction between the TIR domains of the two molecules [67,68]. Various branches of the signaling pathway emanating from TLR3-TRIF lead to the activation of IRF3, NF-κB and AP1 [10,69,70], and to the induction of apoptosis through procaspase-8 activation [71-73]. The signaling molecules involved have been characterized in detail, and this pathway has been shown to culminate in the activation of IRF3 and NF-κB, which together induce the production of antiviral IFNs and other cytokines [74] (Figure 1, Table 1). IRF3 activation is mediated by two kinases, TBK1 and IKKε [75,76], which themselves associate with TRIF through a signalosome complex also containing other key molecules, such as NAP1 and TRAF3 [77-79]. The activation of NF-κB is thought to be essentially mediated by RIP1 [80], and probably also by TRAF6 in some cell types [81,82]. RIP1 and TRAF6 subsequently recruit TAB2 [83] and TAK1, which phosphorylates IKKα and IKKβ [84]. These two kinases and the IKK adaptor protein IKKγ (NEMO) form the IKK complex [85]. IKKβ phosphorylates the NF-κB inhibitor IκB, eventually leading to its degradation and the nuclear translocation of NF-κB [84].

Other molecules appear to be involved in TLR3-IRF3/NF-κB-IFN pathways (Figure 1, Table 1), either as ‘positive regulators’, such as BS69, TAPE, TRAF2, TANK, TRIL, SINTBAD, TRADD, DDX3X, CaMKII, RAFLIN, GAB1, TRIM21, TRIM56, HMGB, ELMOD2, PELLINO1, SCARA1 and TRIS, which is a splice variant of TRIF [86-106], or as ‘negative regulators’, such as SHP-2, RIP3, TRAF1, TRAF4, A20, SARM1, PIASy, SIKE, TRIP, TIPE2 and DUBA [65,80,107-116]. Some molecules, such as NEMO [117] and TANK [90,91], have been shown to link the TLR3-IRF3 and TLR3-NF-κB pathways. Our knowledge of TLR3 signaling pathways has been greatly increased by *in vitro* studies in mouse and human cells, and *in vivo* studies in animals for a few molecules (Table 1). Recently developed transcriptional, proteomic and computational methods may help to paint a more comprehensive picture of the TLR3 signaling network [14,118-120]. Many of the molecules involved in TLR3 signaling are common to other signaling pathways, even the most upstream molecule, TRIF, which can be recruited by TLR4 [68] and by another cytosolic dsRNA sensor consisting of three RNA helicases, DDX1, DDX21 and DHX36 [121]. The degree of redundancy of most of the individual components of the TLR3-responsive pathway in cellular responses to dsRNA has not been assessed in mice or humans, in whom fewer genetic deficiencies have been described.

TLR3-mediated immunity to experimental infection in mice

At least six strains of TLR3-deficient mice have been challenged with up to 16 viruses, via at least 10 different routes of infection (Table 2). Various phenotypes have been studied in these mice, including the disease phenotype, viral load, and mortality. However, caution is required when comparing these experiments, due to the diversity of the protocols used. In these experimental models, the infectious phenotypes of the TLR3-deficient mice varied greatly, from enhanced susceptibility to enhanced resistance, with respect to the corresponding wild-type (WT) mouse. In terms of mortality, TLR3-deficient mice have been shown to be susceptible to encephalomyocarditis virus (EMCV) [122,123], coxsackievirus group B3 (CVB3) [124,125], CVB4 [126] and poliovirus [127,128]. While EMCV-infected TLR3-deficient mice have a higher viral load in the heart and display lower induction of proinflammatory cytokines and chemokines in the heart than WT mice [122], these mice

display high viral load in the pancreas and develop diabetes due to impaired IFN-α/β responses upon infection with an EMCV strain with a tropism for pancreatic β cells [123]. CVB3-challenged TLR3-deficient mice have a high viral load in the heart and acute myocarditis [124,125], which was accompanied by impaired systemic and cardiac IFN-γ induction in one study [124]. Similarly, CVB4 infection results in a high viral load in the heart and liver, together with severe cardiac damage and low levels of pro-inflammatory cytokines in the serum in TLR3-deficient mice [126]. The cardiac damage and poor survival of CVB4-infected TLR3-deficient mice can be rescued by the adoptive transfer of macrophages from WT mice, suggesting that these cells are protective. In mice transgenic for the poliovirus receptor, which are permissive to human poliovirus infection, TLR3 plays a key role in the antiviral response to poliovirus infection, with higher viral loads, in various organs, in TLR3-deficient mice than in WT mice [127].

In some other infections, TLR3 deficiency was found not to result in lower survival, but to underlie impaired virus control or high disease scores [9,124,129,130] (Table 2). TLR3-deficient mice are hypersusceptible to HSV-2 infection of the CNS, with a significantly higher viral load and disease score than WT mice, probably due to impaired virus-induced IFN-β production and virus control in astrocytes [9]. It would be interesting to evaluate the vulnerability of TLR3-deficient mice to intranasal or intracerebral HSV-1 infection of the CNS. However, when TLR3-deficient mice were challenged with HSV-1 by skin infection, high viral loads were detected only at the site of inoculation [129]. TLR3-deficient mice are also susceptible to respiratory syncytial virus (RSV) infection, but to a lesser extent, this infection resulting in a significant increase in mucus production in the airways [130]. TRIF-deficient mice have been challenged with EMCV, CVB3 and poliovirus, and have been shown to have infectious phenotypes similar to those of TLR3-deficient mice. Thus, TRIF-dependent pathways other than those mediated by TLR3 do not appear to play a major role in immunity to these viruses [124,127,128]. TRIF-deficient $CD8a^+/CD11c^+$ splenic dendritic cells and macrophages produce low levels of IFN-α/β in response to poliovirus infection. By inference, these cells may be responsible for the low levels of IFN-α/β production documented in poliovirus-infected TLR3- and TRIF-deficient mice, thereby contributing to poliovirus susceptibility [128].

Intriguingly, the strain of mouse used has a considerable influence over disease outcome and susceptibility to certain viruses (Table 2). TLR3-deficient mice have been found to be susceptible to mouse cytomegalovirus (MCMV) in some experimental conditions [8,131], as shown by lower survival rates, higher viral load in the spleen and the impaired production *in vivo* of cytokines, including IFN-α/β [8]. However, these mice were found to be normally resistant to MCMV in other experimental conditions [131]. The different mouse strains studied, C57BL/6 in the first study and C57BL/6xB129 in the second, may be one of the factors modifying the infectious phenotype in these mice, although other major differences in experimental conditions (such as infection site, the viral dose used for inoculation etc.) may also have played a role in the reported differences. Likewise, different strains of TLR3-deficient mice have been shown to display different degrees of susceptibility to Theiler's murine encephalomyelitis virus (TMEV) [132]. A more virulent strain of TMEV causes fatal encephalitis in C57BL/6 TLR3-deficient mice, whereas a less virulent strain of TMEV leads to high viral load in the CNS accompanied by severe CNS demyelination in SJL, but not C57BL/6 TLR3-deficient mice. It has also been suggested that TLR3 signaling in these experimental models may be either protective or pathogenic for the development of TMEV-induced demyelinating disease, depending on whether TLR3 signaling is activated during or before viral infection [132].

Moreover, TLR3-deficient mice are normally resistant to VSV, LCMV and T3 reovirus [131] (Table 2). TLR3-deficient mice display levels of IFN-γ production by $CD8^+$ T and

$CD4^+$ T cells in response to VSV and LCMV similar to those in WT mice; they are also similar to WT mice in terms of T3 reovirus-induced injury in the CNS [131]. Remarkably, TLR3-deficient mice appear to be even more resistant to other infections than WT mice, in terms of mortality (Table 2). They display enhanced resistance to influenza virus [133], punta toro virus [134], vaccinia virus [135] and West Nile virus (WNV) [136] infections. Viral load does not appear to underlie disease susceptibility in these infectious models. In some studies, high viral loads have been detected in the periphery (WNV) or in a specific organ (influenza virus in the lung). Conversely, in other studies, low viral loads were detected in the periphery (PTV) or in specific organs (vaccinia virus in the respiratory tract and WNV in the brain). A weak inflammatory response in TLR3-deficient animals might contribute to the low disease severity in these mice. Overall, studies in mice have provided unique insight, through carefully controlled experimental viral infections. However, only one virus (TMEV) has been assessed in direct comparative studies using both different viral strains (low virulence *vs* high virulence) and different mouse strains (SJL *vs* C57BL/6). Moreover, some important human-tropic viruses have not been studied in mice (e.g. human hepatitis viruses, varicella zoster virus (VZV), mumps virus, parvovirus), including some viruses for which mice are not permissive (e.g. human hepatitis viruses and VZV).

TLR3-mediated immunity to natural infection in humans

The first indication that human TLR3 immunity might be important in host defense against viral infection in humans was provided by the discovery of UNC-93B-deficiency in children with HSE, in 2006 [12]. Childhood HSE is a life-threatening complication of primary infection with HSV-1, a common virus that is innocuous in most children [137,138]. HSV-1 is a neurotropic dsDNA virus that produces dsRNA during its replication [139,140]. Although rare, HSE is the most common form of sporadic viral encephalitis in Western countries. The pathogenesis of HSE had long remained unclear, until our recent demonstration that some cases of the disease may result from single-gene inborn errors of TLR3-mediated immunity [141]. Two unrelated children with HSE and autosomal recessive (AR) complete UNC-93B deficiency have been identified [12]. TLR3 and TLR7/8/9 signaling was found to be abolished in the patients' fibroblasts and peripheral blood mononuclear cells (PBMCs), respectively. It had been suggested that TLR7-, TLR8- and TLR9-mediated IFN-α, -β and -λ production was largely redundant in human antiviral immunity, as IRAK-4- and MyD88-deficient patients display impaired responses to TLR7, TLR8 and TLR9 but normal responses to TLR3, and suffer from pneumococcal disease but are resistant to viruses, including HSV-1 [142-146]. Impaired TLR3-triggered, UNC-93B-dependent IFN-α, -β, or -λ immunity may therefore underlie HSE in UNC-93B-deficient patients. Autosomal dominant (AD) and AR TLR3 deficiencies were subsequently identified in other patients with HSE, providing evidence that human TLR3 is involved in host defense against HSV-1 in the CNS during primary infection in childhood [13,118,147,148]. The production of IFN-β and -λ by fibroblasts in response to stimulation with poly(I:C), HSV-1 and vesicular stomatitis virus (VSV) is impaired in TLR3-deficient fibroblasts, as in UNC-93B-deficient fibroblasts. Impaired TLR3 signaling also leads to high levels of viral replication and cell death in fibroblasts from patients following infection with HSV-1 or VSV, providing a plausible hypothesis for the molecular pathogenesis of the disease in the CNS.

The subsequent discovery of AR and AD TRIF deficiencies in children with HSE completely validated the essential role of human TLR3 immunity in anti-HSV-1 defense in the CNS [149]. The TLR3 signaling pathway was found to be impaired in cells from patients with both AR and AD TRIF deficiencies, whereas the TRIF-dependent TLR4 pathway was affected only in patients with AR TRIF deficiency [68]. Interestingly, abnormally weak responses to stimulation of the DExD/H-box helicase pathway [121] have been observed in

both AR and AD TRIF-deficient fibroblasts. These results demonstrated the importance of TRIF for the TLR3-dependent production of antiviral IFNs in the CNS during primary infection with HSV-1. However, they also suggested that the TRIF-dependent TLR4 and, probably, the DExD/H-box helicase pathways are largely redundant in host defense in humans. Like AR and AD TRIF deficiencies, AD TRAF3 deficiency was found to be associated with a relatively broad cellular phenotype, but a narrow clinical phenotype, in another patient with isolated childhood HSE [150]. TRAF3 functions downstream from multiple TNF receptors as well as receptors inducing IFN-α, -β, and -λ production, including TLR3 [79,151-154]. AD TRAF3 deficiency has been identified in a young adult with a history of HSE in childhood. The mutant TRAF3 allele in this patient is a loss-of-expression, loss-of-function, dominant-negative mutant allele associated with impaired, but not abolished, TRAF3-dependent responses upon stimulation of both TNF receptors and receptors inducing IFN production, including TLR3 and cytosolic dsRNA sensors [150]. However, the clinical phenotype of this TRAF3-deficient patient was limited to HSE, presumably resulting from impairment of the TLR3-dependent induction of IFN in the CNS in the course of HSV-1 infection. The relatively broad cellular phenotype but narrow clinical phenotype (HSE) in patients with TRIF and TRAF3 deficiencies further implicated TLR3 in immunity to HSV-1 in the CNS.

The identification of AD TBK1 deficiency as a new genetic etiology of isolated childhood HSE was probably the greatest surprise to come out of human studies to date [14]. TBK1 is a kinase at the crossroads of multiple IFN-inducing signaling pathways, including those mediated by TLR3, cytosolic dsRNA sensors and dsDNA sensors [75,76,155]. Two unrelated children with HSE were found to carry different heterozygous mutations in the gene encoding TBK1. Both mutations are underlie a dominant phenotype, but presumably by different mechanisms: negative dominance or haplotype insufficiency. A defect in poly(I:C)-induced TLR3 responses could be detected only in fibroblasts from the patient carrying the dominant-negative *TBK1* mutation. Nevertheless, levels of viral replication and cell death due to two TLR3-dependent viruses (HSV-1 and VSV) were high in fibroblasts from both patients. However, the PBMCs and fibroblasts from both patients displayed normal IFN responses to the TLR3-independent agonists and viruses tested [14]. AD TBK1 deficiency therefore confers narrow, partial cellular phenotypes, probably accounting for the narrow clinical phenotype of these patients, which is limited to HSE. Overall, the five genetic etiologies of HSE, with mutations in *TLR3, UNC93B1, TRIF, TRAF3* and *TBK1*, have a similar cellular phenotype, consisting of impaired TLR3 signaling in fibroblasts, itself resulting in abnormally weak IFN-β and -λ production, enhanced viral replication and cell death in patients' cells, following infection with HSV-1 and VSV. The patients with these inborn errors of TLR3 immunity present no other viral diseases, or HSV-1 infections outside the CNS. Moreover, the clinical penetrance of these gene defects is incomplete. The viral phenotypes in the patients' fibroblasts were rescued by treatment with exogenous IFN-α, -β, and, to a lesser extent, IFN-λ. These findings, together with the previous observation of the development of HSE in combination with mycobacterial disease in patients with AR complete STAT1 deficiency and X-linked NEMO deficiency [156-159], strongly suggest that human TLR3- and IFN-mediated immunity is essential for defense against HSV-1 in the CNS during primary infection in childhood, but apparently otherwise largely redundant in host defense.

Childhood HSE is not accompanied by the dissemination of HSV-1 disease via the bloodstream or epithelium or by other severe viral infections. Consistent with this clinical feature, most types of TLR3-expressing cells from AD or AR TLR3-deficient patients display a normal IFN response to poly(I:C) or HSV-1, including leukocytes in particular. This suggests that TLR3-independent or residual TLR3-dependent signaling in such cell types may contribute to antiviral immunity [13,118]. The human TLR3-IFN-α, -β and -λ

circuit therefore seems to confer selective protective immunity to HSV-1 in the CNS. The data for fibroblasts *in vitro* first provided a plausible mechanism for the pathogenesis of HSE *in vivo* in the CNS [12,13], where TLR3 is indeed expressed and can stimulate IFN production, particularly in neurons, astrocytes, oligodendrocytes and microglial cells [52,53,160,161]. These CNS cells have all been shown to be permissive for HSV-1 infection *in vitro* [52,162-164]. The hypothesis that HSE pathogenesis in patients with inborn errors of TLR3 deficiencies involves CNS-specific, non hematopoietic resident cells was recently tested, by deriving induced pluripotent stem cells (iPSCs) from patients with TLR3 pathway deficiencies or from healthy controls [165]. UNC-93B- and TLR3-deficient neurons and oligodendrocytes were much more susceptible to HSV-1 infection than control cells, due to the lack of TLR3-dependent production of IFN-β and IFN-λ. By contrast to the high degree of vulnerability of astrocytes to HSV-2 infection in TLR3-deficient mice [9], *in vitro* differentiated UNC-93B-deficient astrocytes or neural stem cells (NSCs) were no more susceptible to infection than control cells. However, human astrocytes and NSCs may play a role in protective anti-HSV-1 immunity in the CNS *in vivo*. These findings provided a cellular basis for the pathogenesis of childhood HSE in human patients with inborn errors of TLR3 immunity, involving impaired CNS-intrinsic TLR3-dependent IFN-α/β and IFN-λ immunity to HSV-1[165] (Figure 2).

Concluding remarks and perspectives

Consistent with its high degree of conservation across vertebrate species [3] and its distinctive signature of purifying selection in humans [4,15], TLR3 is the only TLR to date that has been shown to play a non redundant role in host defense in both mice and humans. TLR3 is essential for host defense in mice, at least under some experimental conditions and against some viruses, and under natural conditions in humans, at least in some individuals, for protection of the CNS against HSV-1 during primary infection. Human studies have taught us that human TLR3 immunity is essential for protective intrinsic immunity to HSV-1 primary infection in the CNS, but otherwise largely redundant in host defense. Most human TLR3 pathway deficiencies seem to confer a relatively narrow predisposition to viruses, limited to HSE in the patients identified to date [16]. Exceptionally, one recently reported TLR3-deficient patient developed CVB3 myocarditis as an adult, which is consistent with the vulnerability of TLR3-deficient mice to related viruses [124,126]. There may be other, as yet unknown viral phenotypes to be discovered in TLR3-deficient patients. It is still too soon to draw definitive conclusions regarding the range of clinical phenotypes associated with TLR3 pathway deficiencies, given the limited number of patients identified and studied to date. It will be interesting to search for mutations in TLR3-IFN pathway genes in patients with HSE and other viral diseases, particularly those affecting other TLR3-expressing organs. Such studies should provide us with a broader vision of TLR3 protective immunity in natural conditions of infection.

Regardless of the specific role of human TLR3 in host defense against viruses, which will be defined more precisely as more patients are discovered, the redundancy of TLR3 is understandable, as it is not the only receptor that recognizes dsRNA. Several known cytosolic dsRNA sensors, including protein kinase R [166], 2'-5'-oligoadenylate synthetases [167], and the more recently identified RNA helicases RIG-I (retinoic acid-inducible gene I) and MDA5 (melanoma differentiation-associated gene 5), also recognize dsRNA and have been shown to confer distinct antiviral activity in studies carried out *in vitro* and *in vivo* in mice [17-19,168,169]. Other IFN-inducing pathways, including those mediated by the cytosolic dsDNA sensors DAI and IFI16 [170-172], may also contribute to host antiviral immunity. It is still largely unknown whether the multiple antiviral pathways are redundant with each other in host immunity to infections. TLR3 differs from the IFN-inducing cytosolic dsRNA or dsDNA sensors in terms of its ligand specificity, its unique cellular

distribution and downstream signaling pathway, and these differences may govern a unique mechanism in host defense. Intriguingly, the clinical penetrance of human TLR3 pathway deficiencies was found to be incomplete even for HSE, as some HSV-1-infected genetically affected relatives have not developed HSE, perhaps due to diverse environmental, pathogen-related (such as viral infection load and, less likely, virus strains), or host factors (age at infection, modifying genes, etc). This, in turn, paradoxically implies that although TLR3 function is life-saving in at least some individuals, it may be largely redundant in host defense in most individuals, and that HSE may be an uncommon event even among TLR3-deficient patients. This makes determination of the precise clinical penetrance of TLR3 pathway deficiencies for HSE all the more important.

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Highlights

1. Upon stimulation by dsRNA, TLR3 dimerizes and recruits TRIF, and the subsequent activation of IRF3 and NF-κB leads to induction of target genes.
2. TLR3-deficient mice and their wild-type (WT) littermates have been experimentally infected with 16 different viruses.
3. Consequences of TLR3 deficiency in mice range from enhanced susceptibility to normal or even greater resistance to certain viruses than WT mice.
4. Some human patients with inborn errors of the TLR3 pathway develop herpes simplex virus 1 encephalitis during primary infection.
5. Human patients with inborn errors of the TLR3 pathway are apparently normally resistant to most other infections, including viral infections.

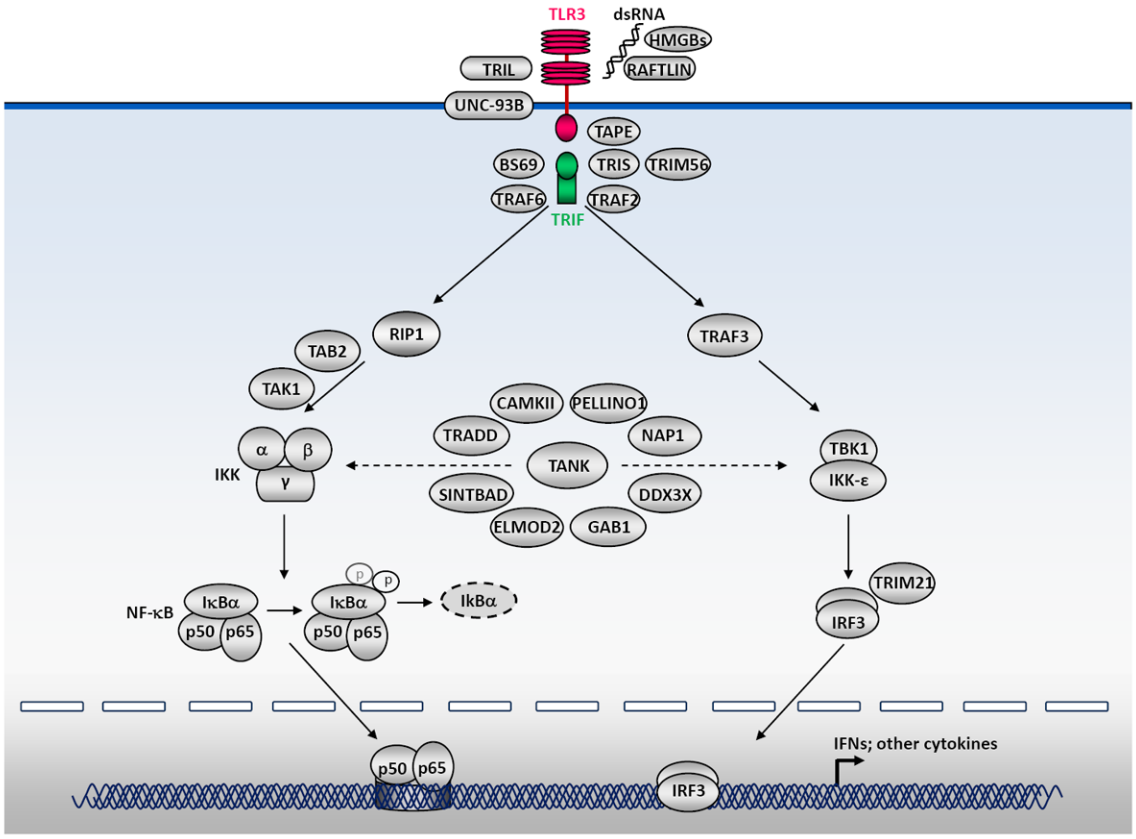

Figure 1. TLR3 signaling pathways leading to the activation of NF-κB and IRF3

The TLR3 signaling pathway is mediated exclusively by the TRIF adapter. TLR3-TRIF interaction and the downstream signaling it induces lead to the activation of IRF3 and NF-κB. The phosphorylation of IRF3 is mediated by two kinases, TBK1 and IKKε, which themselves associate with TRIF through a signalosome complex also containing other key molecules, including TRAF3. The activation of NF-κB is thought to be essentially mediated by RIP1, and probably also by TRAF6 in some cell types. RIP1 and TRAF6 subsequently recruit TAB2 and TAK1, which phosphorylates IKKα and IKKβ. These two kinases and the IKK adaptor protein IKKγ (NEMO) form the IKK complex. IKKβ, in turn, phosphorylates the NF-κB inhibitor IκB, eventually leading to its degradation and the nuclear translocation of NF-κB. Other molecules that have been experimentally implicated in the TLR3-IRF3/NF-κB-IFN pathways are also indicated, including BS69, TAPE, TRAF2, TANK, TRIL, SINTBAD, TRADD, DDX3X, CaMKII, RAFLIN, GAB1, TRIM21, TRIM56, HMGB, ELMOD2, PELLINO1 and TRIS. The activation and nucleus translocation of IRF3 and NF-κB lead to the induction of antiviral IFNs and other cytokines.

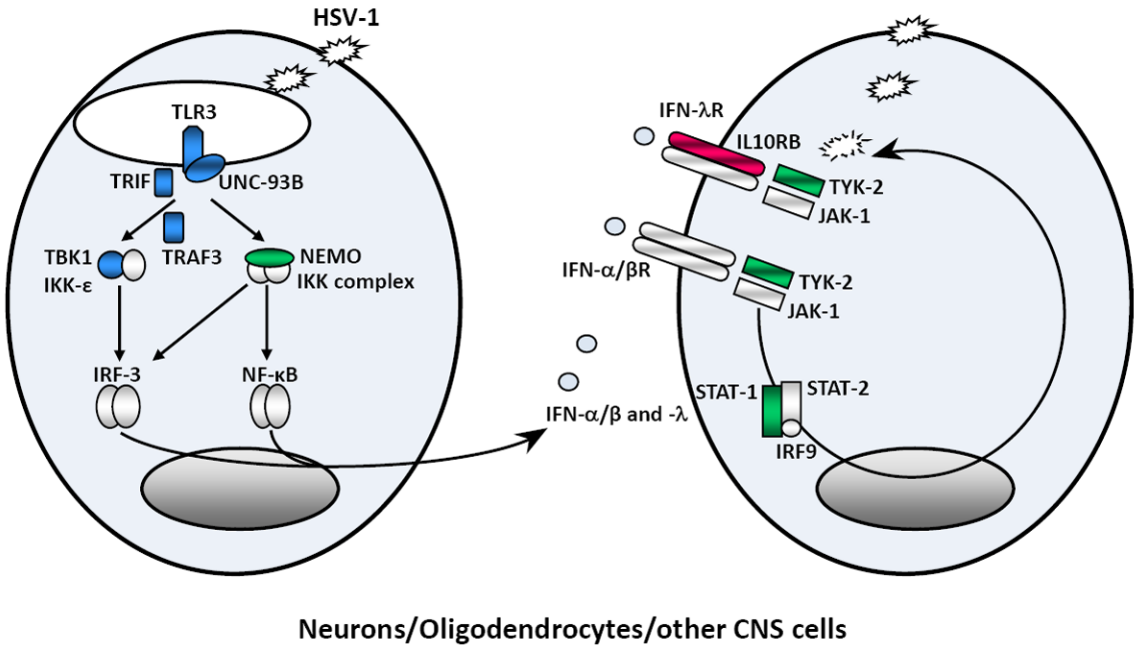

Figure 2. Inborn errors of TLR3-mediated and IFN-mediated immunity in childhood herpes simplex virus 1 encephalitis

Schematic representation of the production of and response to IFN-α/β, and IFN-λ in anti-HSV-1 immunity. HSV-1 produces viral dsRNA during its replication. TLR3 is a transmembrane receptor of dsRNA in the endoplasmic reticulum and endosome. The recognition of dsRNA by TLR3 induces activation of the IRF-3 and NF-kB pathways via TRIF, leading to the production of IFN-α/β and/or IFN-λ. TLR3, UNC-93B, TRIF, TRAF3, TBK1 and NEMO deficiencies are associated with impaired IFN-α/β and/or IFN-λ production, particularly during HSV-1 infection. The binding of IFN-α/β or IFN-λ to its receptor induces the phosphorylation of JAK1 and TYK-2, activating the signal transduction proteins STAT-1, STAT-2 and IRF9. This complex is translocated, as a heterotrimer, to the nucleus, where it acts as a transcriptional activator, binding to specific DNA response elements in the promoter region of IFN-inducible genes. STAT-1 and TYK2 deficiencies are associated with impaired IFN-α, -β and -λ responses. Proteins for which genetic mutations have been identified and associated with susceptibility to isolated HSE are shown in blue. Proteins for which genetic mutations have been identified and associated with susceptibility to mycobacterial, bacterial and viral diseases, including HSE, are shown in green. Proteins for which genetic mutations have been identified but not associated with susceptibility to viral diseases are shown in red.

Table 1

List of molecules involved in the TLR3 pathway, with experimental evidence

No.	Gene	Protein	Positive (+) or negative (-) regulator	*In vitro* study: cell type	*In vivo* model?	Refs
1	TLR3	TLR3	+	HEK293; human dermal fibroblasts; iPSC-derived CNS cells	mouse; human	8, 9, 12, 32, 118
2	UNC93B1	UNC93B	+	HEK293; mouse macrophages; human dermal fibroblasts; iPSC-derived CNS cells	mouse, human	13, 60, 61
3	TICAM1	TRIF	+	HEK293; human dermal fibroblasts	human	67, 68, 149
		TRIS	+	HEK293	none	104
4	TRAF3	TRAF3	+	Human dermal fibroblasts, HEK293; RAW cells	human	79, 150
5	TBK1	TBK1	+	HEK293; mouse embryonic fibroblasts; human dermal fibroblasts	human	14, 75, 76
6	NEMO	NEMO	+	mouse embryonic fibroblasts; human dermal fibroblasts	human	117, 159
7	IRF3	IRF3	+	mouse macrophages	none	74
8	CHUK	IKBKA/IKKa	+	HEK293	none	85
9	IKBKB	IKKb	+	HEK293	none	85
10	IKBKE	IKKe	+	HEK293	none	75
11	NFKB1	NFKB	+	HEK293; human monocyte-derived dendritic cells	none	85
12	NFKB2	NFKB	+	HEK293; human monocyte-derived dendritic cells	none	85
13	NFKBIA	IkBa	+	HEK293	mouse; human	85
14	RELA	NFKB	+	HEK293; human esophageal epithelial cells	none	85
15	AZI2	NAP1	+	HEK293	none	77
16	TBKBP1	SINTBAD	+	HEK293	none	92
17	CAMK2A	CAMKII	+	murine macrophages; HEK293	none	96
18	CC2D1A	TAPE	+	HEK293	none	88
19	DDX3X	DDX3X	+	HEK293	none	95
20	ELMOD2	ELMOD2	+	A549 human adenocarcinoma cell line; human primary macrophages	none	101
21	GAB1	GAB1	+	mouse macrophages, mouse embryonic fibroblasts	none	98
22	HMGB1	HMGB	+	mouse embryonic fibroblasts	none	99
23	HMGB2	HMGB	+	mouse embryonic fibroblasts	none	99
24	HMGB3	HMGB	+	mouse embryonic fibroblasts	none	99
25	RIPK1	RIP1	+	HEK293; mouse embryonic fibroblasts	none	80

No.	Gene	Protein	Positive (+) or negative (-) regulator	*In vitro* study: cell type	*In vivo* model?	Refs
26	TRADD	TRADD	+	HEK293; mouse embryonic fibroblasts	mouse	93, 94
27	TRAF2	TRAF2	+	HEK293	none	87
28	TRAF6	TRAF6	+	HEK293	none	82, 87
29	ZMYND11	BS69	+	HEK293; HeLa	none	86
30	TAB2	TAB2	+	HEK293	none	83
31	NR2C2	TAK1	+	mouse embryonic fibroblasts; HEK293	none	83, 84
32	PELI1	PELI1	+	mouse embryonic fibroblasts	mouse	106
33	MSR1	SCARA1	+	Human monocytoid THP-1 cells; human foreskin fibroblasts	none	103
34	TRIM56	TRIM56	+	HeLa, HEK293, Huh7.5	none	102
35	EGFR	EGFR	+	HEK293; HT1080 cells; mouse embryonic fibroblasts	none	64
36	SRC	Src	+	HEK293; human monocyte-derived dendritic cells; HeLa	none	63
37	PIK3CA	PI3K	+	HEK293	none	65, 66
38	TRIL	TRIL	+	HEK293; U373 (human glioblastoma astrocytoma)	none	89
39	RFTN1	RAFTLIN		HEK293, HeLa	none	97
40	TANK	TANK		HEK293	mouse	90, 91
41	TRIM21	TRIM21/Ro52		HEK293	none	100, 105
42	OTUD5	DUBA	-	HEK293	none	116
43	PTPN11	SHP-2	-	mouse macrophages, RAW264.7, mouse embryonic fibroblasts	none	107
44	LRRFIP1	TRIP	-	HEK293; mouse macrophages	none	115
45	PIAS4	PIASγ	-	HEK293	none	113
46	TNFAIP3	A20	-	HEK293	mouse	110, 111
47	SARM1	SARM1	-	HEK293	none	112
48	SIKE1	SIKE	-	HEK293	none	114
49	TNFAIP8L2	TIPE2	-	mouse dendritic cells	mouse	65
50	TRAF1	TRAF1	-	HEK293	none	108
51	TRAF4	TRAF4	-	HEK293	none	109

Table 2

Viral infections of mice with TLR3 deficiency

Virus	Mouse strain	Inoculation route	Phenotype			Refs
			Survival rate	Viral load	Other	
EMCV (myocarditic variant)	C57BL/6	i.p.	↓	↑ in the heart and liver	↓proinflammatory cytokine and chemokine expression in the heart	122
EMCV (variant of pancreas β cells tropism)	C57BL/6	i.p.	↓	↑ in the pancreas	↑blood glucose; ↓ IFN serum levels at early time points after EMCV infection	123
MCMV	C57BL/6	i.p.	↓	↑in the spleen	↓IFN-α/β levels in the serum	8
	C57BL/6XB129	i.p.	NT	NT	No difference in CD8 T or CD4 T response	131
CVB3	C57BL/6 (>=8X)	i.p.	↓	↑ in the heart and serum	↓IFN-γ and proinflammatory cytokine levels in the heart and serum	124
	C57BL/6XB129	i.p.	↓	↑ in the heart	↑ inflammation in the heart	125
CVB4	NOD	i.p.	↓	↑ in the heart and liver	Greater cardiac damage; ↓TNF-α in the serum; phenotypes rescued by WT NOD macrophage adoption	126
HSV-1	C57BL/6	Skin flank scarification	NT	↑at infection site	↓HSV-1-specific CD8 T-cell response	129
HSV-2	C57BL/6J	Intravaginal s.c.	NT	↑ in the cerebellum and medulla spinalis	↑ CNS disease score	9
Poliovirus	C57BL/6- PVRTg21 (X7-10)	i.v.	↓	↑ in the liver, spleen and kidney	↓IFN-α levels in the serum, spleen and spinal cord	127
RSV	C57BL/6 (10X)	Intratracheal	NT	No difference	↑Th2-cytokine production, mucus production and gob5 expression in the airways	130
TMEV (BeAn)	SJL (6X)	Hemisphere injection	NT	↑ in the brain and spinal cord	Severe demyelination and inflammation in the CNS ↓late CD8 T and CD4 T responses in the CNS	132
	C57BL/6	Hemisphere injection	NT	↑ in the brain and spinal cord	No obvious demyelinating disease	132

Virus	Mouse strain	Inoculation route	Phenotype			Refs
			Survival rate	Viral load	Other	
TMEV (GDVII)	C57BL/6	Hemisphere injection	↓	↑ in the brain and spinal cord	Fatal encephalitis	132
VSV	C57BL/6xB129	i.v.	NT	NT	No difference in CD8 T or CD4 T response	131
LCMV	C57BL/6xB129	i.p. and foot pad	NT	NT	No difference in CD8 T or CD4 T response	131
T3 reovirus	C57BL/6xB129	i.cb. or i.c.	NT	NT	No difference in CNS injury	131
Vaccinia virus	C57BL/6	i.n.	↑	↓in the respiratory tract	↓inflammatory cytokines in serum and bronchoalveolar lavage fluid ↓lung inflammation	135
Influenza virus	C57BL/6 (>=8X)	i.n.	↑	↑in the lung	↓inflammatory cytokines in bronchoalveolar air space	133
PTV	C57BL/6	s.c.	↑	Slightly ↓in the serum	↓inflammatory cytokines in serum and liver	134
WNV	C57BL/6 (10X)	i.p. and i.cbvt.	↑	↑in the periphery ↓in the brain	↓inflammatory cytokines and IFN in serum and brain ↓neuropathology in the brain	136

Notes:

Note 1.

EMCV: encephalomyocarditis virus	i.p. intraperitoneal
MCMV: mouse cytomegalovirus	i.v. intravenous
CVB3: Coxsackievirus B3	i.cb. intracerebral
CVB4: Coxsackievirus B4	i.c., intracranial
RSV: Respiratory syncytial virus	i.n., intranasal
TMEV: Theiler's murine encephalomyelitis virus	s.c. subcutaneous
VSV: vesicular stomatitis virus	i.cbvt. intracerebroventricular
PTV: Punta toro virus	
LCMV: lymphocytic choriomeningitis virus	
WNV: West Nile virus	

Note 2.
↑ increased; ↓ decreased; NT not tested.

Note 3.
When available, the number of backcross generations of the TLR3-deficient mice is indicated in brackets after the mouse strain e.g. (10X).