

Recent insights into the role of Toll-like receptors in viral infection

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Summary

Toll-like receptors have a central role in innate immunity as they detect conserved pathogen associated molecular patterns (PAMPs) on a range of microbes including viruses, leading to innate immune activation and orchestration of the adaptive immune response. To date a large number of viruses have been shown to trigger innate immunity via TLRs suggesting that these receptors are likely to be important in the outcome to viral infection. This suggestion is supported by the observation that many viruses have evolved mechanisms not only to evade the innate immune system but also to subvert it for the benefit of the virus. 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.

Introduction

Innate immunity is made possible by a network of germ-line encoded pattern-recognition receptors (PRRs), which detect PAMPs on invading microbes and trigger immunological responses. PRRs include the Nod-like receptors (NLRs), RIG-like receptors (RLRs), Toll-like receptors (TLRs) and the recently identified cytosolic DNA receptors [1-4].

TLRs are type 1 membrane spanning receptors that consist of an extracellular leucine rich repeat (LRR) domain, a transmembrane spanning domain and a cytoplasmic Toll-interleukin 1 receptor-resistance domain (TIR) domain. TLRs can be broadly divided into those that are located at the cell surface and those that are located to the intracellular endosomal compartment. TLR4 and 5 are expressed on the cell surface and detect lipopolysaccharide and bacterial flagellin respectively, while TLR1/2 and TLR2/6 exist as heterodimers on the cell surface and detect bacterial triacylated and diacylated lipoprotein respectively. In contrast TLR3, 7, 8 and 9 are expressed on endosomes and detect microbial nucleic acids [5]. In order for signalling from these TLRs to occur endosomal acidification and maturation are required leading to the production of proinflammatory cytokines and type I interferons (IFNs) [6].

Signalling downstream of the TLRs is made possible by the existence of cytosolic TIR domain containing adaptor proteins MyD88 (myeloid differentiation factor 88), MAL (MyD88 adaptor like, also known as TIRAP), TRIF (TIR-domain-containing adaptor protein inducing IFN β , also known as TICAM-1) and TRAM (TRIF related adaptor molecule, also known as TICAM2) [7].

The fifth member of the group SARM (sterile α -and armadillo-motif containing protein) has been reported to be an inhibitor of TRIF [8]. Engagement of TLRs with these TIR adaptors results in the activation of cytosolic signalling complexes containing TRAF and IRAK proteins,

ultimately leading to the activation of NF κ B (Nuclear Factor κ B) and the IRF (Interferon Regulatory Factor) family of transcription factors. This triggers the production of pro-inflammatory cytokines and type I IFNs [7]. NF κ B is necessary for IL6 and TNF production, IFN β requires both NF κ B and IRF3, while IRF7 is required for IFN α production [9]. Recent studies on TLR signalling mechanisms have revealed that TLR-induced IRF3 & IRF7 activation is initiated only from the endosome, whereas other signals such as MAP kinase and NF κ B activation can be triggered from the plasma membrane or endosome [10].

Viral inhibitors of TLR signalling components

Early evidence indicating that TLRs might be important in sensing viruses came from observations that some viruses encoded proteins to target TLR signalling. For example studies of vaccinia virus (VACV) revealed 2 proteins that targets the TLR system for inhibition. A46 was shown to associate with the TIR adaptors to downregulate TLR signalling, while A52 was shown to target IRAK2 to inhibit TLR mediated NF κ B activation [11, 12]. Another protein of vaccinia, K7 was shown to target DDX3, required for IFN β induction [13]. Hepatitis C virus (HCV) is another virus that encodes proteins to inhibit TLR mediated signalling, in that its protease NS3/4A cleaves TRIF, while NS5A inhibits MyD88 [14, 15]. These and other TLR viral inhibitors contribute to virulence [16]. Figure 1 depicts the signalling events following TLR stimulation and the viral evasion strategies, that continue to be identified, which target these signalling proteins.

Members of the TLR family that have been shown to be involved in responses to viral infection are TLR1, TLR2, TLR3, TLR4 TLR6, TLR7, TLR8 and TLR9 [9]. The contribution of each of

these receptors to viral infections is discussed below. In particular we will discuss the evidence emerging that suggests a role for human TLRs in the antiviral response.

TLR2

Immune responses against a number of DNA viruses such as, human cytomegalovirus (HCMV), Herpes simplex virus (HSV), Epstein-Barr virus (EBV), VACV and RNA viruses such as Lymphocytic choriomeningitis virus (LCMV), HCV and respiratory syncytial virus (RSV) are at least partially dependent on TLR2 [17-25] (See table 1 for virus nomenclature). These TLR2 dependent anti-viral responses occur across many different cell types. For example LCMV in glial cells of the CNS [18], EBV in monocytes [19] and HSV in microglial cells [20] all elicit TLR2 dependent responses. In human fibroblasts a TLR1/2 heterodimer recognises the envelope proteins of HCMV leading to the secretion of pro-inflammatory cytokines [21, 26]. In leukocytes a role for TLR2 in antiviral responses was demonstrated in a study of RSV [22]. It was shown that TLR2/6 heterodimers were important in cytokine responses to RSV and in controlling viral replication in vivo. In addition neutrophil migration and dendritic cell activation in the lung were dependent upon TLR2-RSV interactions [22].

A role for TLR2 in sensing VACV infection was first reported in a study using bone marrow-derived DCs, where the pro-inflammatory cytokine response was shown to be TLR2 dependent while the type I IFN response was shown to be TLR2 independent [23]. This was in agreement with a plethora of studies showing that MyD88 dependent TLR2 signalling was not involved in type I IFN production. Recently however this paradigm has been challenged as it has been reported that TLR2, in a specialised group of monocytes called Ly6C^{hi} inflammatory monocytes,

can induce type I IFNs following sensing VACV and MCMV virus particles [24, 27].

Interestingly, TLR2 internalisation was required for this process to occur while bacterial TLR2 agonists were unable to elicit this response. This situation is reminiscent to that of TLR4, where receptor internalization to endosomes is required for IRF3 activation and type I IFN production [28]. Thus the type I IFN-inducing TLRs appear to be those capable of endosomal localization, compared to those TLRs capable only of triggering pro-inflammatory cytokines, which are located at the cell surface.

There are however instances of viral infection where TLR2 may be subverted for the benefit of the virus. In a report of patients with chronic HCV infection it was demonstrated that the HCV core protein induced the expression of IL-10 and TNF α cytokines from monocytes. These cytokines caused both a reduction in IFN α release from pDCs and also triggered pDC apoptosis. Using blocking antibodies it was shown that the HCV core protein triggered release of these cytokines through TLR2 [29]. Thus HCV core protein engaging TLR2 accounts for HCV induced pDC loss and reduced IFN α , which may lead to viral persistence and also in part explains why IFN α is useful therapeutically in HCV infection [29] (Table 2).

TLR3

In myeloid dendritic cells TLR3 is expressed in endosomes, like the other TLR receptors of nucleic acids, however in fibroblasts and epithelial cells, TLR3 is expressed on the cell surface [30]. Among the TLR receptors, TLR3 is the most abundantly expressed in the brain and is found in neurons [31], astrocytes [32] and microglia [33]. TLR3 was shown to be a receptor for dsRNA and as well as sensing some RNA viruses, can also sense DNA viruses that generate

dsRNA during their life cycle [34]. On the other hand in TLR3-deficient mice immunity to LCMV, VSV, MCMV and Reovirus was unaffected [30, 35].

In other examples of viral infection, TLR3 was shown to mediate detrimental immunity to the host. Mice deficient in TLR3 exhibited greater resistance to infection with a number of viruses including Punta Toro, VACV and influenza virus, observations thought to be due to TLR3 mediated overproduction of inflammatory mediators [36-38]. These are examples where TLR3 mediated immunity favours the virus and may be viewed as viral subversion [16].

The relationship of TLR3 to West Nile virus (WNV) infection was also explored. Initially it was reported that TLR3 contributed to WNV lethality by promoting peripheral inflammation that led to the breakdown of the blood brain barrier resulting in an increased viral load in the brain. Therefore mice lacking TLR3 were more resistant to lethal WNV infection compared to wild type (WT) mice [39]. However a subsequent study reported the opposite results, where TLR3 was shown to have a protective role against WNV infection whereby the absence of TLR3 decreased mice survival in response to WNV and enhanced viral load in the brain [40]. Although the data concerning TLR3 and West Nile virus is controversial, other evidence in support of a positive role for TLR3 in protective immunity against WNV comes from the observations that the WNV protein NS1 inhibits TLR3 signalling and the envelope protein inhibits RIP1, a protein required for signalling downstream of TLR3 [41, 42].

Many studies that have reported TLR dependent antiviral responses have utilized knockout mice, and the data regarding human TLRs in response to viral infection is more limited. However a role for human TLR3 in providing protective immunity against Herpes Simplex encephalitis

(HSE) was reported in two children who harboured a heterozygous mutation in TLR3 [43]. This mutation (C to T) resulted in replacement of a proline with a serine at amino acid 554, in leucine rich repeat 20 of the extracellular domain. This mutation behaved in an autosomal dominant manner to specifically predispose to HSE, as immunity to other viruses was not impaired. This is the first report that conclusively showed a link between human TLR3 and anti-viral immunity, particularly in the CNS [43]. In addition RNA released from EBV infected cells is capable of activating human TLR3 in a clinically relevant setting since sera from patients chronically infected with EBV triggered TLR3 signalling in EBV-transformed lymphocytes and peripheral mononuclear cells [44] (Table 2).

TLR4

Initial evidence that TLR4 might be an antiviral PRR was demonstrated in a study showing that the RSV fusion F protein stimulated cytokine production via TLR4 [45]. Further studies of RSV infection in TLR4 knockout mice showed reduced NK cell function, impaired interleukin-12 expression, and impaired virus clearance compared to WT mice [46]. In terms of VACV infection, mice lacking TLR4 exhibited greater viral replication and mortality compared to WT mice following respiratory infection [47]. It is thought that this TLR4 mediated protective immunity against VACV is due to the detection of a yet to be identified viral ligand by TLR4 [47]. In macrophages it was shown that VSV activated the PI3 kinase pathway via TLR4 leading to type I IFN expression and thus conferring antiviral immunity [48]. Other reports by contrast have shown that TLR4 contributes to detrimental immunity in response to some viral infections.

The recognition of Ebola virus glycoprotein by human TLR4 leads to the production of proinflammatory cytokines and thought to mediate viral immunopathogenesis [49].

Like TLR3, TLR4 has now been implicated in human anti-viral response. Studies using human microglia demonstrated that TLR4 activation was shown to inhibit HIV replication in a pathway requiring IRF3 [50]. Related to this it was found that polymorphisms in TLR4 were shown to influence viral load in HIV infected individuals [51]. The importance of human TLR4 in RSV infection was highlighted in a study reporting that in infants the presence of the TLR4 mutations Asp299Gly or Thr399Ile were associated with increased risk of severe RSV bronchiolitis [52]. Finally in patients with chronic hepatitis B infection, TLR4 (and TLR2) is overexpressed on monocytes and modulates the activities of Tregs which may contribute to immunotolerance [53] (Table 2).

TLR7/8

TLR7 and TLR8 are phylogenetically and functionally related and have been identified as important sensors of ssRNA from the viral genomes of influenza, VSV and HIV [54-56] While it was once thought that TLR8 was non-functional in mice [57], it is now known that murine TLR8 can be activated under some circumstances [58]. The contribution of these receptors to immunity comes from both TLR7 expressing pDCs and TLR8-expressing myeloid DCs [59]. Both mouse and human TLR7-expressing pDCs are responsible for the production of high levels of type I IFNs important in the induction of type I T helper responses, class switching of B cells and the promotion of cross priming [59]. In addition B cells expressing TLR7 respond to ssRNA viruses by the activation of co-stimulatory molecules and cytokine production [56].

An important contribution of TLR7 to immunity against WNV was demonstrated using knockout mice. Both TLR7 and MyD88 knockout mice had increased viral replication and mortality compared to control mice. Upon closer examination it was found that these knockout mice exhibited reduced homing of macrophages, CD4+ and CD8+ T cells to the brain infected with WNV, which was shown to be dependent on IL-23 [60, 61]. Another study however cast doubt on the requirement for TLR7 in immunity against WNV. It was shown that susceptibility to the virus did not differ in WT or TLR7 knockout mice following intradermal challenge, in contrast to the study where intraperitoneal infection was used [60]. In WT mice there was reduced numbers of CD11c + Langerhans cells in the epidermis following intradermal infection, an effect not observed in TLR7 deficient mice [62]. The authors concluded that upon cutaneous infection, the TLR7 mediated immune response contributes to viral pathogenesis by promoting WNV dissemination from the skin to other organs to initiate systemic infection. It was proposed that this process might reduce the TLR7-mediated protective immunity during the systemic stage of infection [62]. The case of WNV and TLR7 illustrates that defining the precise role of TLRs in viral infection is challenging and depends on factors such as virus dose, passage history of the virus and route of administration [62].

New insights into the importance of human TLR7 in HIV pathogenesis have been demonstrated. It was recently shown that TLR7 may be a crucial factor in explaining why HIV-1 infected women have lower viral loads in early HIV infection but progress to AIDS more rapidly than men [63]. It was found that pDCs from women produced significantly more IFN α in response to HIV-1 encoded TLR7 ligands when compared to pDCs from men after adjusting for viral load. In addition there was also higher levels of CD8+ T cell activation in women chronically infected with HIV-1. It is thought that this increased level of HIV immune activation in women may lead

to heightened disease progression. This data suggest that inhibition of the TLR7 pathway in pDCs might represent a new approach to treating HIV-1 infections. Interestingly a polymorphism in TLR7, Gln11Leu, has been associated with higher viral loads and accelerated disease progression in HIV infected individuals [64] (Table 2).

TLR9

Immune activation induces the expression of TLR9 in a number of cells type such as neutrophils, monocytes and CD4 T cells. Non-immune cells such as epithelial cells also express TLR9. In contrast TLR9 is constitutively expressed in B cells and plasmacytoid DCs [65]. Since TLR9 can sense hypomethylated CpG motifs of microbial DNA, immune responses to a number of DNA viruses are mediated by TLR9. Antiviral responses dependent on TLR9 are largely due to the production of large amounts of type I IFNs from pDCs. These responses occur in response to infection with HSV-1 [66-68], MCMV [66] adenovirus [69] and poxviruses [70]. An antiviral role for TLR9 against murine gammaherpesvirus 68 infection was also identified [71]. Bone marrow derived DCs deficient in TLR9 produced less proinflammatory cytokines and type I IFNs in response to this virus, and in an infection model greater viral loads were observed in mice lacking TLR9 [71].

In terms of human TLR9 a number of reports suggest that this TLR is important in HIV infection. TLR9 is reduced in B cells of HIV infected individuals, leading to impaired B cell responses to TLR9 agonists [72]. Furthermore CpG oligonucleotides were found to enhance B cell responsiveness in HIV-infected individuals [73]. In addition it has been shown that gp120 of HIV suppresses the activation of human pDCs following TLR9 stimulation [74]. Finally

polymorphisms in TLR9 affect the clinical outcome to HIV-1 infection, leading to a more rapid disease progression [75]. Since HIV is an RNA virus it is not known if TLR9 senses HIV directly, or if immune responses to HIV mediated by TLR9 occurs by some secondary means.

Other examples of modulation of human TLR9 by viral infection include patients with chronic hepatitis B infection, where TLR9 expression is reduced in pDCs [76] and HCV dependent cirrhosis, where TLR9 was found to be upregulated, suggesting that TLR9 might be important during this infection [77]. Further, in RSV and measles infection TLR9 (and TLR7) mediated type I IFN production is inhibited in human pDCs [78].

Although it has been reported that TLR9 can mediate detrimental immunity to bacterial infection [79] and CpG DNA leads to HIV-LTR trans-activation and HIV replication in HIV-1 transgenic murine splenic cells [80], there are as yet no reports indicating that TLR9 promotes detrimental immunity to viral infection, as has been reported for TLR3, or that TLR9 is manipulated by the virus as a means of systemic dissemination. In contrast it has been reported that TLR7 and TLR8 on human neutrophils might contribute to the pathogenesis of influenza infection [81] (Table 2).

Co-operation of TLRs in viral recognition

In natural viral infections multiple PRRs are likely to be engaged in responding to a particular virus and this appears to be the case for TLRs. The importance of such co-operation between different TLRs in providing anti-viral immunity is highlighted in a study by Sorensen et al [17]. Using knockout mice they showed that TLR2 and TLR9 were both required for immunity to HSV-2. Systemic infection induced cytokine response was significantly affected in the double knockouts while only partially affected in either single knockout. Interestingly viral loads in the

brain were increased in the TLR2/9 double knockouts compared to the single knockouts or control mice. Similar findings were also reported in HSV-infected dendritic cells [82]. Another example of such co-operation occurs in MCMV infection, where it was shown that both TLR7 and TLR9 mediate the pDC response to virus and this is first study showing that TLR7 can respond to a DNA virus [83]. In other examples of viral infection it is clear that TLRs co-operate with other PRRs to provide anti-viral immunity. The modified vaccinia virus Ankara (MVA) strain is currently being developed as a vaccine vector against HIV/AIDS and innate immune sensing of this virus is mediated by TLR2/6, MDA5 and the NALP3 inflammasome [84].

Therapeutic use of TLR agonists in antiviral immunity

A number of studies have shown that a variety of TLR agonists may have a positive effect in antiviral immunity. For example a bacterial ligand of TLR2/6, FSL-1, was shown to induce significant resistance to experimental HSV-2 infection [85]. Ampligen, an analogue of the synthetic TLR3 agonist poly(IC), has been explored for the treatment of HIV [86]. Preclinical studies have shown that another derivative of poly(I:C), poly ICLC, confers protective immunity to a range of viruses including influenza, RSV and SARS [87]. PIKA, another TLR3 agonist, has been proposed as a possible treatment in an influenza pandemic due to inhibition of influenza replication and potency as an adjuvant [88]. Agonists for TLR3 and TLR9 were shown to induce potent antiviral responses to HSV-2 [89]. Interestingly another group showed that this effect was dependent on the release of Type III interferons, which like type I IFNs display anti-viral activity [90]. Currently imiquimod is used for the topical treatment of genital warts caused by human papilloma virus and for some skin cancer conditions such as basal cell carcinoma and actinic keratosis. In addition the TLR7/8 ligand resiquimod (R848) was also examined in clinical studies of HSV and HCV, unfortunately with mixed results [91].

Recently it has been shown that agonists for TLR7/8 may affect viral latency, since they and viruses acting via TLR7/8 have been shown to reactivate latent Kaposi's sarcoma-associated herpesvirus (KSHV), which sounds a note of caution in the therapeutic use of such agonists [92]. Finally stimulation of TLR9 with CpG oligonucleotides provided protection against influenza [87] and TLR9 agonists have also been proposed to be useful in the treatment of HIV [93].

Unanswered questions and future perspectives

Huge strides have been made in understanding the immune response mediated by TLRs following infection with different viruses. However not all of the signalling events are fully defined and further details of the proteins required and how they are regulated are likely to be revealed. It is also clear that the outcome of a virus –TLR interaction is complex and depends on the particular TLR and the virus in question as well as the host species. For example the TLR4 dependent immune response to VACV in mice is beneficial while the TLR3 contribution is detrimental [37, 47]. In contrast TLR3 in human keratinocytes provides protective immunity against VACV infection [94]. Also it is now appreciated that immune responses for at least some viral infections requires the contribution of many PRRs. How all these signals are integrated in natural infection is unknown. Regarding TLR signalling important differences in mice and humans have been observed, therefore therapeutic manipulation of TLRs will require a more complete understanding of the human system [16]. Since TLRs can mediate harmful immune responses following viral infection, further studies should reveal drug targets of TLR signalling pathways that may be modulated in order to improve outcome to different viral infections. It is possible that some of these drugs might be based on known viral inhibitors of TLR signalling.

Conversely given the many promising TLR agonists that have been reported to have antiviral activities there is reason to be optimistic that such strategies may be employed either as treatments for viral infection or possibly as vaccine adjuvants.

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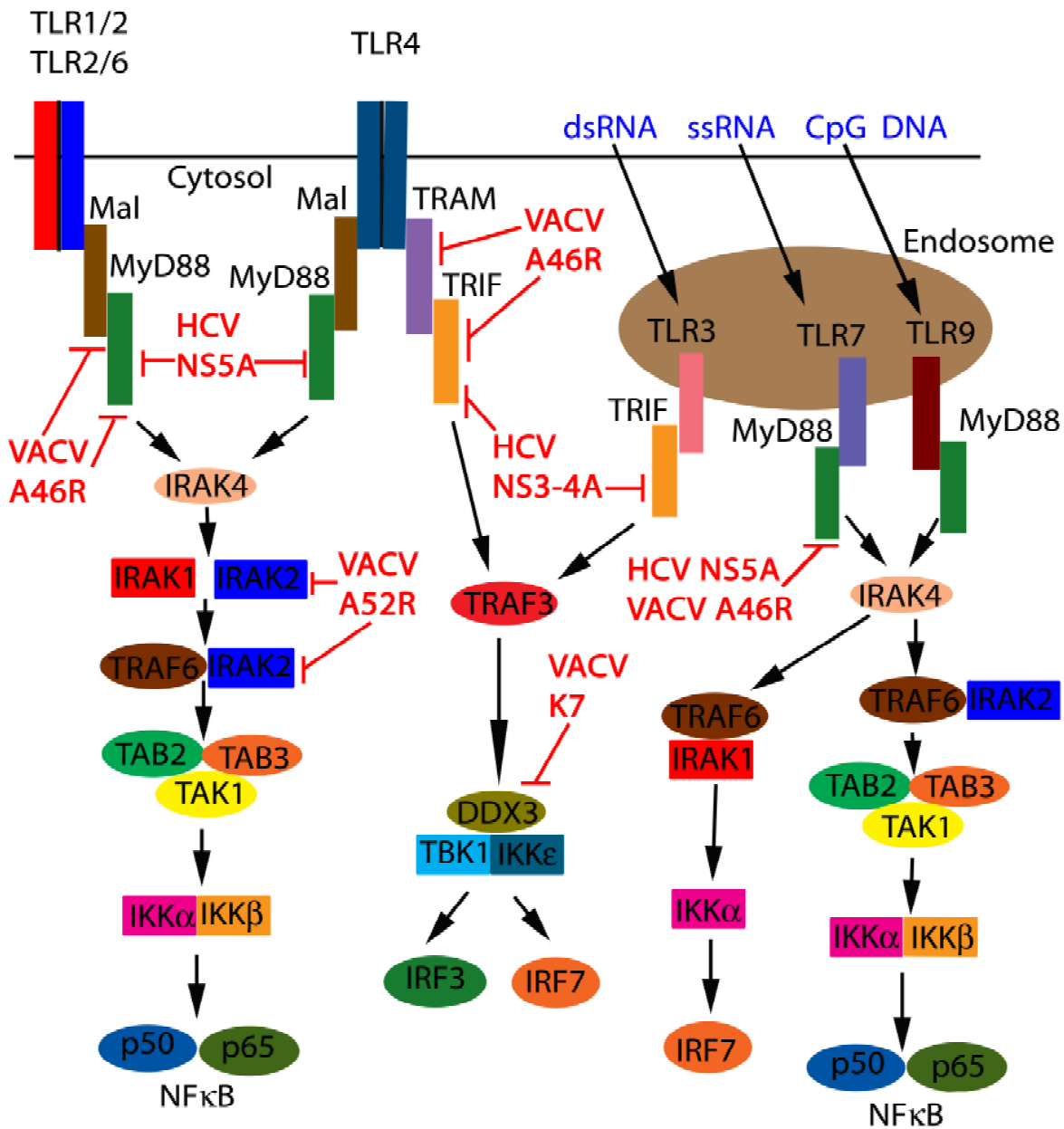


Figure 1. TLR signalling and inhibition by viral proteins

Upon ligation of the TLRs with their respective ligands, TIR domain containing adaptors are recruited to the receptors. MyD88 is the prototypical member of the group being utilised by all TLRs except TLR3, which utilises TRIF only. TLR2 requires MAL along with MyD88 and

TLR4 requires all four adapters including TRAM, as a bridging adapter to TRIF. Adapter recruitment leads to the activation of IRAK4 and the phosphorylation of IRAK1. IRAK1 and IRAK2 signal to TRAF6, which leads to the activation of the TAK1 containing complex. This stimulates the kinase activity of the IKK complex and triggers the activation of NF κ B, allowing its movement into the nucleus and the expression of pro-inflammatory cytokines. The pathway to IRF3 and IRF7 activation following TRIF stimulation from either TLR3 or TLR4 requires TRAF3 and the IKK ϵ /TBK1 complex resulting in type I IFN production. TLR7 and TLR9 activate the IRAK1/TRAF6 complex that stimulates IKK α phosphorylation and activation of IRF7 allowing the production of type I IFNs. A46R from the vaccinia virus associates with and inhibits all four adaptor proteins, MyD88, MAL, TRAM and TRIF. In contrast A52R from VACV binds to and inhibits IRAK2, while K7 inhibits DDX3, a component of the IKK ϵ /TBK1 complex. The HCV protease NS3-4A cleaves and inactivates TRIF, while NS5A also from HCV sequesters MyD88.

Abbreviation	Virus	Family	Genome structure
HCMV	Human cytomegalovirus	Herpesviridae	dsDNA
HSV	Herpes Simplex virus	Herpesviridae	dsDNA
EBV	Epstein-Barr virus	Herpesviridae	dsDNA
VACV	Vaccinia virus	Poxviridae	dsDNA
LCMV	Lymphocytic choriomeningitis virus	Arenaviridae	ssRNA(-)
HCV	Hepatitis C virus	Flaviviridae	ssRNA(+)
RSV	Respiratory syncytial virus	Paramyxoviridae	ssRNA(-)
PTV	Punta Toro virus	Bunyaviridae	ssRNA (-)
IAV	Infuenza A virus	Orthomyxoviridae	ssRNA (-)
WNV	West Nile Virus	Flaviviridae	ssRNA(+)
EBOV	Ebola virus	Filoviridae	ssRNA (-)
HIV	Human Immunodeficiency virus	Retroviridae	ssRNA(RT)
VSV	Vesicular stomatitis virus	Rhabdoviridae	ssRNA(-)
MCMV	Murine cytomegalovirus	Herpesviridae	dsDNA
ADV	Adenovirus	Adenoviridae	dsDNA
MHV-68	murine gammaherpesvirus 68	Herpesviridae	dsDNA
HBV	Hepatitis B virus	Hepadnaviridae	dsDNA-RT
KSHV	Kaposi's sarcoma-associated herpesvirus	Herpesviridae	dsDNA

Table 1 Virus Nomenclature; (+), positive strand RNA; (-), Negative stranded RNA; (RT), Reverse transcribed

TLR	Virus	Macromolecule detected	Model system used	Reference
TLR1 Human	HCMV	Envelope proteins B and H	HEK-293s	[26]
TLR2 Murine	HSV	Unknown	Knockout mice	[17]
	VACV	Unknown	Murine Ly6Chi monocytes.	[24]
	VACV	Unknown	Knockout mice	[23]
	LCMV	Unknown	Murine Glia	[18]
	RSV	Unknown	Knockout mice	[22]
TLR2 Human	HCV	Core protein, NS3	Human monocytes and murine macrophages	[25]
	EBV	Unknown	Human monocytes	[19]
	HCMV	Envelope proteins B and H	HEK-293s, human fibroblasts	[21, 26]
TLR3 Murine	VACV	Unknown	Knockout mice	[37]
	PTV	Unknown	Knockout mice	[36]

TLR3 Human	IAV	Unknown	Knockout mice	[38]
	WNV	Unknown	Knockout mice	[39, 40, 62]
	VACV	Unknown	Human Keratinocytes	[94]
	HSV	Unknown	Human Polymorphisms	[43]
	EBV	dsRNA	Human PBMCs and lymphocytes	[44]
TLR4 Murine	RSV	Fusion F protein	Knockout mice	[45]
TLR4 Human	VACV	Unknown	Knockout mice	[21][47]
	RSV	Unknown	Human polymorphisms	[52]
	VACV	Unknown	Human keratinocytes	[94]
	EBOV	Glycoprotein	THP1 cells	[49]
	HIV	Unknown	Primary human microglia	[50]
	HIV	Unknown	Human polymorphisms	[51]
	HBV	Unknown	Primary human monocytes	[53]
TLR6 Murine	RSV	Unknown	Knockout mice	[22]
TLR7 Murine	IAV	ssRNA	Knockout mice	[54]
TLR7 Human	HIV	ssRNA	Knockout mice	[55]
	VSV	ssRNA	Knockout mice	[56]
	WNV	Unknown	Knockout mice	[39, 40, 62]
	HIV	Unknown	Patient study	[63]
	HIV	Unknown	Human polymorphisms	[64]
	KSHV	Unknown	Latent infected	[92]

TLR7 Murine	IAV	ssRNA	Knockout mice	[54]
	HIV	ssRNA	Knockout mice	[55]
	VSV	ssRNA	Knockout mice	[56]
	WNV	Unknown	Knockout mice	[39, 40, 62]
TLR7 Human	HIV	Unknown	Patient study	[63]
	HIV	Unknown	Human polymorphisms	[64]
	KSHV	Unknown	Latent infected primary effusion lymphoma cell lines	[92]
TLR8 Human	HIV	ssRNA	HEK 293 cells	[55]
	KSHV	Unknown	Latent infected primary effusion lymphoma cell lines	[92]
TLR9 Murine	HSV-1	Unknown	Knockout mice	[67]
	MCMV	Unknown	Knockout mice	[66]
	ADV	Unknown	Knockout mice	[69]
	Poxviruses	Unknown	Knockout mice	[70]
	MHV-68	Unknown	Knockout mice	[71]
	HIV	CpG	Transgenic mice	[80]

Table 2 The interaction of viruses with mouse and human TLRs in different model systems.

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