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Review

Viruses and Toll-like receptors

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Abstract

Recently a number of viruses, including a poxvirus, herpesvirus, retrovirus and two paramyxoviruses, have been shown to activate cells via Toll-like receptor family members. Here we postulate that although activation via Toll-like receptor molecules can lead to anti-viral innate immune responses, in some cases viruses may use these responses to ameliorate infection.

Keywords: Toll-like receptor; Retrovirus; Herpesvirus; Poxvirus; Paramyxovirus

1. Introduction

To successfully establish infection, viruses must interact with various host components. For instance, viruses use a variety of cell surface proteins to achieve entry. Some of these cellular proteins are receptors that transduce signals upon ligand or virus binding. Viruses that also infect cells involved in immune response, i.e. lymphocytes or antigen-presenting cells, can trigger anti-viral responses via interaction with cell surface molecules. Examples of this are naturally occurring strains of measles virus (MV) that use the signaling/lymphocyte activation molecule (SLAM) CD150 for entry into T lymphocytes and thereby induce their activation and the subsequent production of inflammatory or suppressive cytokines [1,2]. Similarly, MV vaccine strains use CD46 as entry receptors and cause cell activation [3]. The Epstein–Barr virus (EBV) receptor, CD21, is known to be a signaling molecule and inhibitors of protein phosphorylation abrogate infection [4]. Receptor binding also triggers signaling when HIV binds to the CCR5 chemokine receptor that it uses as a co-receptor for cell entry, causing cell activation and the production of chemo-attractants that may play a role in amplifying the pool of virus-infected cells [5,6]. Myxoma virus, a member of the poxvirus family, requires signaling through activation of the Rantes chemokine receptors CCR1, CXCR4 or CCR5 to achieve infection [7].

This use of signaling molecules for entry may occur because viruses require cellular activation or the production of specific gene products to facilitate early steps of infection, such as entry or replication. Alternatively, early signaling could lead to altered or abrogated immune responses to virus-infected cells. Viruses may also induce cell signaling via interaction with cell surface molecules independently of virus entry to achieve cell activation. Recently, several diverse viruses have been shown to interact with proteins in the Toll-like receptor (TLR) family. The TLR proteins serve in the recognition of pathogen-associated ligands and function to activate the innate immune system. We postulate here that although viral activation of cells through TLR proteins can result in an anti-viral response, for some viruses this interaction creates a cellular environment more beneficial for virus replication.

2. TLRs function in innate and adaptive immunity

TLRs belong to a family of proteins involved in the innate immune response to various pathogens (for recent reviews, see [8,9]). Although the *Drosophila toll* gene was originally shown to function in embryonic development, it was later discovered to play a critical role in innate immunity in the adult fly, where activation of the Toll pathway results in the production of anti-microbial peptides [10]. TLR family members expressed on the cell surface have a characteristic extracellular leucine-rich repeat region (LRR) and an intracellular Toll/interleukin-1 (IL-1) receptor (TIR) domain (Fig. 1). These functional domains are also evolutionarily conserved in plants and are found in the genes important for resistance (R genes) to tobacco mosaic and potato X virus, among others [11,12].

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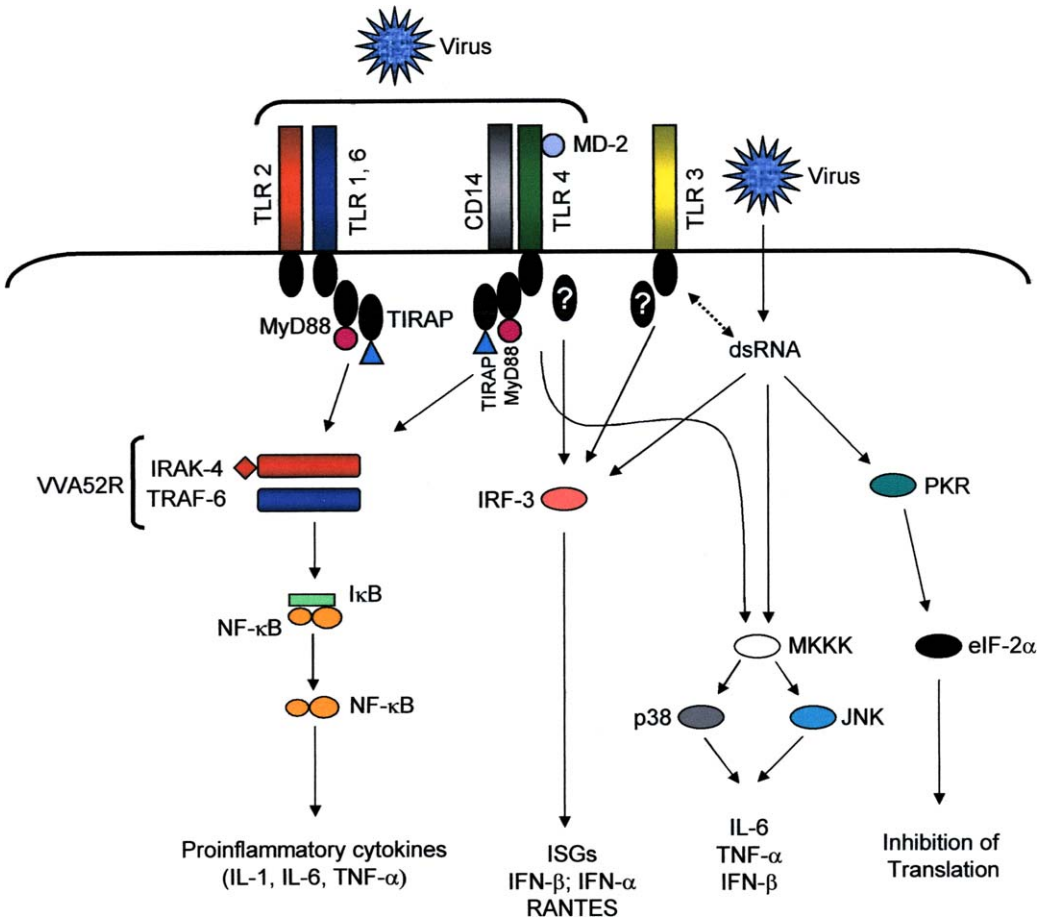

Fig. 1. Schematic representation of the intracellular signaling induced upon virus binding to Toll-like receptors or upon virus infection. Signaling results in the production of various proinflammatory cytokines, interferons and other cytopathic effects. Dashed line between dsRNA and TLR3 represents an unclear mechanism of interaction.

Human TLR4 was the first mammalian TLR to be identified by mining the human EST database for sequences similar to *Drosophila toll* [13]. Subsequently, it was shown to be the lipopolysaccharide (LPS) receptor by positional cloning of the gene encoding LPS resistance in C3H/HeJ mice [14,15]. TLRs recognize pathogen-associated molecular patterns (PAMPs) produced by microbial agents. To date, at least 10 different mammalian TLRs have been identified that recognize various microbial PAMPs, including peptidoglycans from Gram-positive bacteria and other microorganisms, double-stranded RNA (dsRNA) and unmethylated bacterial GpC DNA (reviewed in [16]). It has been proposed that PAMPs, which make up structures such as bacterial cell walls, are critical for microbial survival, so that microorganisms that express such molecules cannot avoid the innate immune response through mutation [9].

TLR4 functionally assembles on the cell surface with a complex of proteins that includes CD14, MD-2 and LPS-binding protein, and mice lacking any one of these molecules through targeted mutagenesis have a defective LPS response [9]. Signaling through TLR2, the family member whose ligands include peptidoglycan, lipoprotein, and zymosan, also results in NF-κB activation and proinflammatory cytokine expression [9]. Interestingly, TLR2 functions through heterodimerization with TLR1 or TLR6 family members to effectively broaden the range of ligands that are recognized [17,18]. It is not currently known if TLR2 heterodimerizes with additional TLRs or if heterodimerization occurs between other TLR proteins.

TLRs use intracellular adaptor molecules that contain the TIR but not the extracellular domain for signaling. Several adaptor proteins have been discovered, including MyD88 [19], MyD88-like (MAL)/TIR domain-containing adapter protein (TIRAP) [20,21] and TIR domain-containing adapter inducing IFN-β (TRIF)/TIR-containing adaptor molecule (TICAM-1) [22,23] (Fig. 1). After TLR activation, several signaling pathways are activated, and through the use of these adaptor molecules, different responses are achieved (Fig. 1). For example, binding of LPS to TLR4 activates both MyD88-dependent and -independent pathways. The MyD88-dependent pathway signals through IRAK-4 and TRAF6, resulting in the degradation of IκB and the subsequent activation of NF-κB, as well as through JNK and p38 MAP kinase, leading to the expression of proinflammatory cytokines such as TNFα, IL-1, and IL-6 [9]. TIRAP/MAL, which functions together with MyD88, is involved in the TLR4 and TLR2/1 and TLR2/6 but not TLR3, 5, 7 and 9 signaling cascade [24,25]. In contrast, TRIF/TICAM-1 is

involved in TLR3- and TLR4-mediated activation of the interferon-β promoter and functions independently from TIRAP/MAL [22,23].

Type I interferon induction (IFN-α and -β) is also linked to signaling by certain TLRs through the activation of the transcription factor, interferon regulatory factor 3 (IRF3). Infection by some viruses typically results in IRF3 phosphorylation by an unknown mechanism, leading to the expression of interferon-stimulated genes (ISGs) and the production of IFN-α and -β, which play a major role in the cellular response to infection (for review, see [26]) (Fig. 1). IRF3 phosphorylation and gene activation are induced by TLR3 and TLR4 but not TLR2 or TLR9 ligands. The TLR4 ligand LPS stimulated MyD88-independent IFN-β gene expression in macrophages [27] and dendritic cells (DCs) [28]. Moreover, pre-treatment of cells with poly I:C, a TLR3 ligand or lipid A, the moiety of LPS involved in TLR4 recognition, inhibited the replication of the murine herpesvirus MHVγ68 in macrophages [29]. Since replication of MHVγ68 and other viruses often generates dsRNA, this ability to induce TLR3 may play an important role in the anti-viral response. It is not clear, however, that the TLR4 pathway would be activated during an MHVγ68 infection, although at least one β-herpesvirus, human cytomegalovirus (CMV) has been shown to activate cells via interaction with TLR2 (see below). Interestingly, IFNs themselves increase TLR gene expression, which could result in further amplification of this effect [30].

Recent evidence also links TLR-mediated innate immunity to adaptive immune responses (for reviews, see [31,32]). In addition to inducing expression of proinflammatory cytokines, TLR-mediated activation leads to DC maturation and to increased expression of molecules important for the adaptive immune response, such as IL-10, IL-12 and those required for antigen presentation (major histocompatibility class II and co-stimulatory molecules CD80 and CD86) [33]. DCs are crucial to the initiation of the adaptive immune response because they drive differentiation of CD4+ T cells into a Th1 or Th2 effector phenotype. Activation of the MyD88-dependent pathway appears to influence the development of Th1 responses. MyD88-deficient mice exhibited normal Th2 responses, but defective Th1 responses [33] and stimulation of PBMCs with TLR2 ligands resulted in T cell proliferation and the production of IFN-γ, a type 1 cytokine [34]. However, there is also evidence that TLR signaling influences Th2 responses. A functional TLR4 molecule is required for optimal development of a Th2 response to inhaled antigen [35]. The outcome of the response to inhaled antigen is dependent on the level of TLR ligand, since stimulation with low levels of LPS resulted in a Th2 response and high levels in a Th1 response [36]; whether this is due to differential activation of the MyD88-dependent and -independent pathways remains to be determined. The *Yersinia pestis* LcRV protein has also been shown to induce IL-10, a type 2 cytokine that suppresses Th1 responses, in mice lacking a functional TLR4 gene [37]. This response was ablated in TLR2 –/– mice, indicating that it is the result of LcRV interaction with this family member [38]. Thus, the particular pathway activated by TLR ligands plays a role in the adaptive immune response.

3. Viral interactions with Toll-like receptors result in different pathogenic effects

Several different viruses that infect mammals have now been shown to interact with TLRs, including the paramyxoviruses respiratory syncytial virus (RSV) and MV, the poxvirus vaccinia virus (VV), the murine retroviruses mouse mammary tumor virus (MMTV), and a herpesvirus (CMV) (Table 1). The outcome of TLR activation can differ between viruses—from an anti-viral response to the creation of a cellular environment that enhances viral replication and spread. Because of the potent cellular activation that results from TLR signaling, some viruses may utilize these proteins to a replication advantage even in the presence of an anti-viral immune response.

3.1. Respiratory syncytial virus

RSV, a paramyxovirus that infects the upper respiratory tract and causes an acute infection in children, was the first virus shown to activate cells via TLRs [39]. Incubation of human and mouse peripheral blood mononuclear cells (PBMCs) with the viral attachment F protein elicited IL-6, IL-8 and TNF-α secretion at levels comparable to that seen after LPS stimulation. F protein-induced cytokine expression was dependent on both CD14 and TLR4, since mice deficient in either protein failed to produce IL-6. In contrast, influenza virus induced cytokine production in both wild-type and TLR4-null mice, suggesting that this virus stimulates a TLR4-independent response [40]. A direct physical interaction between F protein and TLR4 or CD14 has yet to be demonstrated, however.

RSV has also been shown to activate NF-κB activity in vivo in the murine lung at two different phases of virus infection. At very early stages of infection, TLR4-dependent

Table 1
Viral proteins that interact with TLR proteins or TLR signaling proteins

Virus	Viral protein	TLR protein	Reference
Respiratory syncytial virus	F	TLR4	[39]
Vaccinia virus	A52R, A46R	IRAK4, TRAF6	[44,46]
Mouse mammary tumor virus	Env	TLR4, TLR2	[55]
Measles virus	H	TLR2	[62]
Cytomegalovirus	gB (?)	TLR2 + TLR1	[66]

NF-κB activation occurs in alveolar macrophages; this step is independent of viral replication and appears to be an initial response to the input virus. In contrast, NF-κB activation in lung epithelial cells as well as alveolar macrophages and additional immune cells at later stages of infection (24 h postinfection) was shown to be TLR4 independent and to require viral replication [41].

F protein-mediated signaling through TLR4 is thought to stimulate an anti-viral innate immune response, since C57BL/10ScCr mice that have a genomic deletion encompassing the TLR4 gene showed impaired RSV clearance [39]. Indeed, these mice also showed decreased IL-12 expression, deficient natural killer (NK) cell function and reduced NK and CD14+ cell pulmonary trafficking in response to virus [40]. However, the interpretation of these results has recently been complicated by the finding that C57BL/10ScCr mice also have a point mutation in their IL-12 receptor gene that renders their cells unresponsive to this cytokine and as a result, they do not produce IFN-γ [42]. Other strains of mice that lack functional TLR4 molecules will have to be tested for their ability to clear RSV.

These data indicate that RSV F protein is a potent activator of TLR4 signaling that has the potential to lead to virus clearance. It is surprising that a virus capable of a high mutation frequency such as RSV has maintained a domain on the F protein that serves to elicit such a strong immune response. Another possibility is that RSV utilizes this immune response in infection. Activation of cells through a TLR pathway may lead to the production of some cellular factors that enhance intracellular replication. Alternatively, utilization of the innate immune response may skew the Th1/Th2 balance of the immune response to result in a non-productive adaptive immune response. It also remains to be determined whether the immune response to RSV in its natural host, humans, differs from that in mice.

3.2. Vaccinia virus

At least one of the poxviruses, VV, has developed a unique strategy to inhibit anti-viral responses mediated by both TLRs and the IL-1 receptor (IL-1R). Poxviruses like VV have large genomes with many genes dedicated to subverting the immune response [43]. Using a sequence homology search, Bowie and colleagues [44] recently identified two VV proteins (A46R and A52R) that contain TIR domains. However, both A46R and A52R lack a highly conserved BB loop found in the cytoplasmic tails of TIR domains [45] that contains the Pro712 to His mutation in the TLR4 gene from C3H/HeJ mice and renders it unable to signal. This region of the TIR is also essential for MyD88 binding [45], suggesting that the VV proteins inhibit TIR-based signaling by interacting with other downstream effector molecules.

Both A46R and A52R blocked IL-1 signaling, although A52R was more potent [44]. In addition, A52R inhibited signaling through TLR1/2, TLR6/2, TLR3, TLR4, TLR5, IL-18 and TLR3, indicating that it acted at some common step in the pathway [46]. Indeed, A52R co-immunoprecipitated with both IRAK2 and tumor necrosis factor receptor-associated factor 6 (TRAF6), two downstream components involved in TIR signaling (Fig. 1). It is not yet known how A46R works to block IL-1R signaling or whether it targets TLR family members.

The presence of viral proteins that block the innate immune response suggests that VV must elicit an anti-viral immune response through the IL-1R, IL-18R or TLRs. Previous work has shown that type I IFNs are required to control VV infection [47]. Thus, blocking TLR-mediated activation of IRF3 in response to virus infection may prevent IFN production. That these proteins block the innate immune response to virus is also supported by the observation that VV infection does not induce DC differentiation, but instead blocks their maturation by poly (I:C) [48]. There is also functional evidence that the A52R gene has a role in blocking innate immune responses, since VV mutants lacking this gene were moderately attenuated in a murine intranasal model of infection [46]. It is interesting to note that the A52R and A46R viral proteins block separate cell-signaling pathways by targeting protein domains that have overlapping function. Thus, unlike many viral proteins that function as decoy receptors for specific cytokines or chemokines, these poxvirus proteins have the potential to have a broader effect on the host immune response.

3.3. Mouse mammary tumor virus

Retroviruses require target cell activation and division for efficient entry of reverse-transcribed proviral DNA into the nucleus and integration into the chromosomes. Since the pre-integration complex generated in the cytoplasm is not transported through the nuclear pore, cell activation and cycling, which results in the breakdown of the nuclear membrane during mitosis, is thought to allow the viral genome access to the host chromosomes for integration [49,50].

At least one retrovirus, MMTV, may accomplish this cell activation via TLRs. MMTV is a milk-borne virus that initially infects lymphoid targets in the Peyer's patches of the small intestine (reviewed in [51]). It had been known for many years that the C3H/HeJ mice exhibit significantly reduced MMTV-induced tumor formation compared to the highly related C3H/HeN or C3H/OuJ mice [52]. The TLR4 molecule in C3H/HeJ mice has a point mutation in the cytoplasmic domain that both abrogates its ability to signal and causes it to function as a co-dominant negative receptor [53]. The genetic link between resistance to MMTV-induced tumorigenesis and a mutant TLR4 allele led to the hypothesis that MMTV might utilize this receptor to activate its initial cellular targets and thereby achieve infection. This hypothesis was supported by previous work showing that MMTV binding to B lymphocytes resulted in their activation [54].

In vivo injection of MMTV into C3H/HeN mice resulted in increased numbers of activated B cells in draining lymph nodes 18–20 h postinjection of virus [54,55]. In contrast, MMTV did not activate B cells in C3H/HeJ mice or in congenic BALB/cJ mice containing the defective C3H/HeJ

LPS allele, indicating TLR4 dependence [55]. B cell activation occurred in the absence of viral gene expression, indicating that the MMTV particles themselves were capable of activating cells and most likely occurs through direct binding of the viral envelope protein to TLR4, since co-transfection/co-immunoprecipitation assays showed that the MMTV envelope protein binds to both TLR2 and TLR4 [55]. This ability to activate cells without entry explains the large increase in MMTV-activated B cells that do not become infected [54].

MMTV activation in vivo was accompanied by a significant increase in NF-κB activity in lymphocytes from C3H/HeN but not C3H/HeJ mice [55]. Recently we have also found that MMTV binding activates TLR4-dependent cytokine (IL-6, IL-12 and TNF-α) production by DCs (Burzyn et al., manuscript in preparation). Interestingly, MMTV binding to DCs but not B cells up-regulates expression of the entry receptor, transferrin receptor 1 (Burzyn et al., manuscript in preparation). Thus, DCs may be the initial targets of MMTV infection, and the ability of this virus to activate the TLR4 signaling pathway may represent a mechanism to increase the expression of its own receptor, similarly to MV (see next section).

MMTV can utilize additional TLRs to activate cells. MMTV activates B and DCs from C57BL/10ScN mice that have a genomic deletion of the *Tlr4* gene and thus have no TLR4 receptor protein instead of the dominant negative molecule produced in C3H/HeJ mice (Rassa et al., in preparation; Burzyn et al., in preparation). However, MMTV failed to activate B cells or DCs from doubly null animals that were generated by crossing C57BL/10ScN with TLR2 knockout mice. These data indicate that MMTV also utilizes the TLR2 pathway. Indeed, the MMTV Env co-immunoprecipitates with TLR2, indicating a potential physical interaction between these two proteins (Rassa et al., in preparation). Thus, it appears that dominant negative TLR4 protein in C3H/HeJ cells suppresses signaling, either through direct heterodimerization, as has been reported for TLR1 and TLR6 [17] or through interaction with one of the TLR adaptor proteins.

There are several consequences of MMTV-mediated cell activation. LPS treatment of infected cells has been shown to increase MMTV viral gene transcription in lymphocytes [56]. Additionally, we and others have observed reduced lymphocyte infection at early times in C3H/HeJ mice, perhaps due to the reduced levels of B cell activation in the initial rounds of infection ([57]; Rassa and Ross, unpublished). However, this activation is also likely to lead to cytokine and chemokine production that could result in anti-viral innate and adaptive immune responses. Recently, Golovkina and colleagues [57] observed that MMTV-mediated activation of the TLR4 pathway might also subvert the anti-viral adaptive immune response. In C3H/HeN mice, MMTV-mediated activation of B cells led to production of IL-10, a cytokine known to repress induction of Th1-type cellular immune responses. In contrast, IL-10 induction did not occur in C3H/HeJ mice, suggesting that mice of this strain could produce a stronger Th1 response against MMTV. When wild-type MMTV virus was passaged through C3H/HeJ mice by breast-feeding, a novel recombinant form of the virus arose after several generations [58]. This recombinant acquired some of the MMTV structural genes from an endogenous MMTV present in the C3H mouse genome. Since similar recombinants were not generated in mice with a wild-type TLR4 gene, these data indicate that MMTV induces a cell-mediated anti-viral response in C3H/HeJ mice, resulting in the selection of viruses that acquire genes from an endogenous MMTV and thus escape the response because their proteins are not recognized as foreign. In contrast, the cell-mediated anti-viral response in C3H/HeN mice might be reduced, perhaps due to the induction of IL-10 via TLR4 signaling, and such recombinants do not arise.

Other retroviruses may also interact with TLRs. Ardavin and colleagues [54] previously showed that Moloney murine leukemia virus (M-MLV) could activate B cells. We also showed that there is a physical interaction between M-MLV Env protein and TLR4 and that the MMTV and MoMulv Env compete with each other for binding to this receptor [55]. This suggests that the two Env molecules share a common binding domain on the TLR4 protein. Given the common requirement for cellular activation by retroviruses, interaction with TLR proteins may be a shared phenotype of several retroviruses.

3.4. Measles virus

MV is another virus that may use TLR activation to create a cellular environment more permissive to infection. MV has profound effects on the host's immune system; both stimulating a strong anti-viral immune response and a general immunosuppression that predisposes infected individuals to secondary infections. Previous work had demonstrated that MV interacted with DCs and monocytes [59] and that it was capable of activating NF-κB at early stages of virus infection [60]. Moreover, wild-type but not vaccine strains were shown to have a more profound effect on DCs and monocytes [61]. Wild-type MV uses SLAM/CD150 as its entry receptor, while vaccine strains use CD46 [1–3]. Interestingly CD150 is not expressed on unstimulated DCs or monocytes.

Recent work by Schneider-Schaulies and colleagues [62] has provided an explanation as to how wild-type MV can infect DC or monocytes. They demonstrated that the MV hemagglutinin protein (H) activates cells through either human or murine TLR2. This activation was independent of viral gene expression and resulted in IL-6 and IL-1β production. Interestingly, only wild-type and not vaccine MV strains activated cells via TLR2. Thus, a single amino acid change at position 481 in H (Asn→Tyr) is responsible for both the tropism change for the entry receptor (SLAM/CD150 for wild type, CD46 for vaccine strains) and the ability of MV to interact with TLR2. It remains to be seen if this particular amino acid is part of a binding site that makes contact with TLR2 or if the mutation found in the

vaccine strains results in an altered H structure that precludes interaction.

Activation through TLR2 not only resulted in the expression of inflammatory cytokines, but also in increased expression of the wild-type MV entry receptor CD150. This represents an excellent example of a virus that uses cellular activation to up-regulate expression of its own receptor, thereby allowing infection of target cells. These data also suggest that viral activation of TLR2 could result in increased infection and viral spread. Indeed, expression of CD150 and MV infection were amplified in DCs stimulated with either TLR2 or TLR4 ligands [63].

3.5. Cytomegalovirus

CMV is a member of the β-herpesvirus family and is widely disseminated human pathogen that causes significant morbidity and mortality in immunocompromised individuals. Previous work showed that binding of CMV particles to cells led to the induction of inflammatory cytokines and interferon-stimulated genes that were characteristic of an innate immune response [64]. Moreover, this induction of gene expression could be achieved with purified CMV gB envelope protein [65], suggesting that gB binding to a membrane receptor led to innate immune activation. Recent work by Compton and colleagues indicates that CMV virion-mediated activation of cells involves both TLR2 and CD14. In cells lacking either of these molecules, CMV was unable to activate the NF-κB pathway or trigger inflammatory cytokine production (IL-6 and -8), in contrast to cells expressing both molecules [66]. This activation did not require productive infection, since both ultraviolet-inactivated and defective particles induced cytokine production. It appears that at least some, if not all of this activation is mediated by the interaction of gB with a heterodimer of TLR2 and TLR1 but not TLR6 (Guerrero, and Compton, personal communication).

Many of the pathogenic affects associated with CMV infection are facilitated or directly mediated by inflammatory cytokines, which could be a by-product of this innate immune pathway activation. Additionally, CMV induction of the inflammatory response may play a role in virus replication and dissemination, through up-regulation of NF-κB viral, gene transcription and the recruitment of CMV targets such as neutrophils and monocytes [66].

3.6. Viral ligands for TLRs; PAMPS or site-specific binding?

To date, all of the viral ligands for TLRs are proteins. One unanswered question is how a single receptor such as TLR4 or TLR2 can bind so many divergent ligands. Direct ligand binding to mammalian TLR has only been demonstrated in a few cases, and the interaction domains have yet to be mapped. However, in plants it has been demonstrated that recombination events between the LRRs of related R genes result in the reversal of ligand specificity, indicating that this domain is responsible for binding the virulence factor expressed by the pathogen [67]. Structural studies of mammalian ribonuclease inhibitor, which is an LRR protein, show a very large contact area with the ligand RNase A. This large area of interaction may explain the ability of ribonuclease inhibitor to inhibit the activity of various RNAses with limited protein sequence identity [68]. Since the extracellular portion of all TLR molecules contains LRR domains, there may also be a large region on the TLR proteins capable of interacting with viral envelope proteins.

Another possibility is that TLRs recognize particular patterns on viral particles, as is thought to occur for the PAMPs. For example, virtually all viral attachment proteins assemble as trimers on the surface of virions [69]. Alternatively, since all the viral proteins are highly glycosylated, the TLR proteins may function as lectins and bind to the carbohydrate additions to the viral protein. However, at least in the case of MMTV, the ability of the Env to bind to TLR4 was not sensitive to tunicamycin treatment or endoglycosidase H, indicating that N-linked carbohydrates were not involved in the interaction [55]. Interestingly, treatment of cells with brefeldin A could disrupt the co-immunoprecipitation, indicating that Env and TLR4 either interact through O-linked carbohydrates or at a post-Golgi domain when expressed in the same cell (Rassa and Ross, unpublished).

4. Conclusions

Viral interactions with the TLR proteins and manipulations of the innate immunity system likely represent a fundamental aspect of viral pathogenesis. We are in the very early stages of understanding both basic TLR biology as well as the interactions of viruses with these proteins. Based on the divergent collection of viruses that have been shown to interact with TLR proteins, we can only expect the list to grow over the next few years.

Viruses have a high rate of mutation and thus would be expected to rapidly evolve to avoid innate immune responses. It remains to be determined whether many viruses retain the ability to activate cells via TLRs as part of their replication pathway, or as is thought for bacterial PAMPs, the domains that interact with the TLRs are fundamental to other aspects of infection, and thus escape mutants are not viable. It will be important to identify other viruses that have developed new strategies to exploit the potent activation of the immune system for their own replication as well as to determine the mechanisms they have developed to avert the potential antiviral effects.

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References

[1] E.C. Hsu, C. Iorio, F. Sarangi, A.A. Khine, C.D. Richardson, CD150(SLAM) is a receptor for a lymphotropic strain of measles virus and may account for the immunosuppressive properties of this virus, Virology 279 (2001) 9–21.

[2] H. Tatsuo, N. Ono, K. Tanaka, Y. Yanagi, SLAM (CD150) is a cellular receptor for measles virus, Nature 406 (2000) 893–897.

[3] R.E. Dorig, A. Marcil, A. Chopra, C.D. Richardson, The human CD46 molecule is a receptor for measles virus (Edmonston strain), Cell 75 (1993) 295–305.

[4] N. Sugano, W. Chen, M.L. Roberts, N.R. Cooper, Epstein–Barr virus binding to CD21 activates the initial viral promoter via NF-κB induction, J. Exp. Med. 186 (1997) 731–737.

[5] Q.H. Liu, D.A. Williams, C. McManus, F. Baribaud, R.W. Doms, D. Schols, E. De Clercq, M.I. Kotlikoff, R.G. Collman, B.D. Freedman, HIV-1 gp120 and chemokines activate ion channels in primary macrophages through CCR5 and CXCR4 stimulation, Proc. Natl. Acad. Sci. USA 97 (2000) 4832–4837.

[6] D. Weissman, R.L. Rabin, J. Arthos, A. Rubbert, M. Dybul, R. Swofford, S. Venkatesan, J.M. Farber, A.S. Fauci, Macrophage-tropic HIVand SIV envelope proteins induce a signal through the CCR5 chemokine receptor, Nature 389 (1997) 981–985.

[7] A.S. Lalani, J. Masters, W. Wang, J. Barrett, R. Rannu, H. Everett, C.W. Arendt, G. McFadden, Use of chemokine receptors by poxviruses, Science 286 (1999) 1968–1971.

[8] A. Aderem, R.J. Ulevitch, Toll-like receptors in the induction of the innate immune response, Nature 406 (2000) 782–787.

[9] R. Medzhitov, Toll-like receptors and innate immunity, Nat. Rev. Immunol. 1 (2001) 135–145.

[10] B. Lemaitre, E. Nicolas, L. Michaut, J.-M. Reichhart, J.A. Hoffmann, The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults, Cell 86 (1996) 973–983.

[11] S.P. Dinesh-Kumar, W.-H. Tham, B.J. Baker, Structure-function analysis of the tobacco mosaic virus resistance gene N, Proc. Natl. Acad. Sci. USA 97 (2000) 14789–14794.

[12] A. Bendahmane, K. Kanyuka, D.C. Baulcombe, The Rx gene from potato controls separate virus resistance and cell death responses, Plant Cell 11 (1999) 781–792.

[13] R. Medzhitov, P. Preston-Hurlburt, C.A. Janeway Jr., A human homologue of the Drosophila Toll protein signals activation of adaptive immunity, Nature 388 (1997) 394–397.

[14] A. Poltorak, X. He, I. Smirnova, M.-Y. Liu, C. van Huffel, X. Ku, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciarid-Castagnoli, B. Layton, B. Beutler, Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *tlr4* gene, Science 282 (1998) 2085–2088.

[15] S.T. Qureshi, L. Lariviere, G. Leveque, S. Clermont, K.J. Moore, P. Gros, D. Malo, Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (*Tlr4*), J. Exp. Med. 189 (1999) 615–625.

[16] S. Akira, H. Hemmi, Recognition of pathogen-associated molecular patterns by TLR family, Immunol. Lett. 85 (2003) 85–95.

[17] A. Ozinsky, D.M. Underhill, J.D. Fontenot, A.M. Hajjai, K.D. Smith, C.B. Wilson, L. Schroeder, A. Aderem, The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors, Proc. Natl. Acad. Sci. USA 97 (2000) 13766–13771.

[18] A.M. Hajjar, D.S. O'Mahony, A. Ozinsky, D.M. Underhill, A. Aderem, S.J. Klebanoff, C.B. Wilson, Functional interactions between Toll-like receptor (TLR) 2 and TLR1 or TLR6 in reponse to phenol-soluble modulin, J. Immunol. 166 (2001) 15–19.

[19] T. Kawai, O. Adachi, T. Ogawa, K. Takeda, S. Akira, Unresponsiveness of MyD88-deficient mice to endotoxin, Immunity 11 (1999) 115–122.

[20] T. Horng, G.M. Barton, R. Medzhitov, TIRAP: an adaptor molecule in the Toll signalling pathway, Nat. Immunol. 2 (2001) 835–841.

[21] K.A. Fitzgerald, E.M. Palsson-McDermott, A.G. Bowie, C.A. Jefferies, A.S. Mansell, G. Brady, E. Brint, A. Dunne, P. Gray, M.T. Harte, D. Mc Murray, D.E. Smith, J.E. Sims, T.A. Bird, L.A. O'Neill, Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction, Nature 413 (2001) 78–83.

[22] M. Yamamoto, S. Sato, K. Mori, K. Hoshino, O. Takeuchi, K. Takeda, S. Akira, A novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-β promoter in the Toll-like receptor signaling, J. Immunol. 169 (2002) 6668–6672.

[23] H. Oshiumi, M. Matsumoto, K. Funami, T. Akazawa, T. Seya, TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-β induction, Nat. Immunol. 4 (2003) 161–167.

[24] T. Horng, G.M. Barton, R.A. Flavell, R. Medzhitov, The adaptor molecule TIRAP provides signaling specificity for Toll-like receptors, Nature 420 (2002) 329–333.

[25] M. Yamamoto, S. Sato, H. Hemmi, H. Sanjo, S. Uematsu, T. Kaisho, K. Hoshino, O. Takeuchi, M. Kobayashi, T. Fujita, K. Takeda, S. Akira, Essential role for TIRAP in activation of the signaling cascade shared by TLR2 and TLR4, Nature 420 (2002) 324–329.

[26] M.J. Servant, N. Grandvaux, J. Hiscott, Multiple signaling pathways leading to the activation of interferon regulatory factor 3, Biochem. Pharm. 64 (2002) 985–992.

[27] T. Kawai, O. Takeuchi, T. Fujita, J.-I. Inoue, P.F. Muhlradt, S. Sato, K. Hoshino, S. Akira, Lipopolysaccharide stimulates the Myd88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes, J. Immunol. 167 (2001) 5887–5894.

[28] K. Hoshino, T. Kaisho, T. Iwabe, O. Takeuchi, A. Shizuo, Differential involvement of IFN-β in Toll-like receptor-stimulated dendritic cell activation, Int. Immunol. 14 (2002) 1225–1231.

[29] S.E. Doyle, S.A. Vaidya, R. O'Connell, H. Dadgostar, P.W. Dempsey, T.-T. Wu, G. Rao, R. Sun, M.E. Haberland, R.L. Modlin, G. Cheng, IRF3 mediates a TLR3/TLR4-specific anti-viral gene program, Immunity 17 (2002) 251–263.

[30] M. Miettinen, T. Sareneva, I. Julkunen, S. Matikainen, IFNs activate toll-like receptor gene expression in viral infections, Genes Immun. 2 (2001) 349–355.

[31] S. Akira, K. Takeda, T. Kaisho, Toll-like receptors: critical proteins linking innate and acquired immunity, Nat. Immunol. 2 (2001) 675–680.

[32] G.M. Barton, R. Medzhitov, Control of adaptive immune responses by Toll-like receptors, Curr. Opinion Immunol. 14 (2002) 380–383.

[33] M. Schnare, G.M. Barton, A.C. Holt, K. Takeda, S. Akira, R. Medzhitov, Toll-like receptors control activation of adaptive immune responses, Nat. Immunol. 2 (2001) 947–950.

[34] P.A. Sieling, W. Chung, B.T. Duong, P.J. Godowski, R.L. Modlin, Toll-like receptor 2 ligands as adjuvants for human Th1 responses, J. Immunol. 170 (2003) 194–200.

[35] K. Dabbagh, M.E. Dahl, P. Stepick-Biek, D.B. Lewis, Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells, J. Immunol. 168 (2002) 4524–4530.

[36] S.C. Eisenbarth, D.A. Piggott, J.W. Huleatt, I. Visintin, C.A. Herrick, K. Bottomly, Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen, J. Exp. Med. 196 (2002) 1645–1651.

[37] A. Sing, A. Roggenkamp, A.M. Geiger, J. Heesemann, *Yersinia enterocolitica* evasion of the host innate immune response by V antigen-induced IL-10 production of macrophages is abrogated in IL-10-deficient mice, J. Immunol. 169 (2002) 1315–1321.

[38] A. Sing, D. Rost, N. Tvardovskaia, A. Roggenkamp, A. Wiedemann, C.J. Kirschning, M. Aefelbacher, J. Heesemann, *Yersinia* V-antigen exploits toll-like receptor 2 and CD14 for interleukin 10-mediated immunosuppression, J. Exp. Med. 196 (2002) 1017–1024.

[39] E.A. Kurt-Jones, L. Popova, L. Kwinn, L.M. Haynes, L.P. Jones, R.A. Tripp, E.E. Walsh, M.W. Freeman, D.T. Golenbock, L.J. Anderson, R.W. Finberg, Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus, Nat. Immunol. 1 (2000) 398–401.

[40] L.M. Haynes, D.D. Moore, E.A. Kurt-Jones, R.W. Finberg, L.J. Anderson, R.A. Tripp, Involvement of Toll-like receptor 4 in innate immunity to respiratory syncytial virus, J. Virol. 75 (2001) 10730–10737.

[41] H.A. Haeberle, R. Takizawa, A. Casola, A.R. Brasier, H.J. Dieterich, N. Van Rooijen, Z. Gatalica, R.P. Garofalo, Respiratory syncytial virus-induced activation of nuclear factor-κB in the lung involves alveolar macrophages and toll-like receptor 4-dependent pathways, J. Inf. Dis. 186 (2002) 1199–1206.

[42] A. Poltorak, T. Merlin, P.J. Nielsen, O. Sandra, I. Smirnova, I. Schupp, T. Boehm, C. Galanos, M.A. Freudenberg, A point mutation in the IL-12Rβ2 gene underlies the IL-12 unresponsiveness of LPS-defective C57BL/10ScCr mice, J. Immunol. 167 (2001) 2106–2111.

[43] B.T. Seet, J.B. Johnston, C.R. Brunetti, J.W. Barrett, H. Everett, C. Cameron, J. Sypula, S.H. Nazarian, A. Lucas, G. Grant McFadden, Poxviruses and immune evasion, Annu. Rev. Immunol. 21 (2003) 377–423.

[44] A. Bowie, E. Kiss-Toth, J.A. Symons, G.L. Smith, S.K. Dower, L.A. O'Neill, A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling, Proc. Natl. Acad. Sci. USA 97 (2000) 10162–10167.

[45] Y. Xu, X. Tao, B. Shen, T. Horng, R. Medzhitov, J.L. Manley, L. Tong, Structural basis for signal transduction by the Toll/interleukin-1 receptor domains, Nature 408 (2000) 111–115.

[46] M.T. Harte, I.R. Haga, G. Maloney, P. Gray, P.C. Reading, N.W. Bartlett, G.L. Smith, A. Bowie, L. O'Neill, The poxvirus protein A52R targets Toll-like receptor signaling complexes to suppress host defence, J. Exp. Med. 197 (2003) 343–351.

[47] M.F. van den Broek, U. Muller, S. Huang, R.M. Zinkernagel, M. Aguet, Immune defence in mice lacking type I and/or type II interferon receptors, Immunol. Rev. 148 (1995) 5–18.

[48] J. Engelmayer, M. Larsson, M. Subklewe, A. Chahroudi, W.I. Cox, R.M. Steinman, N. Bhardwaj, Vaccinia virus inhibits the maturation of human dendritic cells: a novel mechanism of immune evasion, J. Immunol. 163 (1999) 6762–6768.

[49] J. Harel, E. Rassart, P. Jolicoeur, Cell cycle dependence of synthesis of unintegrated viral DNA in mouse cells newly infected with murine leukemia virus, Virology 110 (1981) 202–207.

[50] T. Roe, T.C. Reynolds, G. Yu, P.O. Brown, Integration of murine leukemia virus DNA depends on mitosis, EMBO J. 12 (1993) 2099–2108.

[51] S.R. Ross, Using genetics to probe host-virus interactions: the mouse mammary tumor virus model, Microb. Infect. 2 (2000) 1215–1223.

[52] H.C. Outzen, D. Morrow, L.D. Shultz, Attenuation of exogenous murine mammary tumor virus virulence in the C3H/HeJ mouse substrain bearing the LPS mutation, J. Natl. Canc. Inst. 75 (1985) 917–923.

[53] S.N. Vogel, D. Johnson, P. Perera, A. Medvedev, L. Lariviere, S.T. Qureshi, D. Malo, Functional characterization of the effect of the C3H/HeJ defect in mice that lack an *LPS*^{n} gene: in vivo evidence for a dominant negative mutation, J. Immunol. 162 (1999) 5666–5670.

[54] C. Ardavin, F. Luthi, M. Andersson, L. Scarpellino, P. Martin, H. Diggelmann, H. Acha-Orbea, Retrovirus-induced target cell activation in the early phases of infection: the mouse mammary tumor virus model, J. Virol. 71 (1997) 7295–7299.

[55] J.C. Rassa, J.L. Meyers, Y. Zhang, R. Kudaravalli, S.R. Ross, Murine retroviruses activate B cells via interaction with Toll-like receptor 4, Proc. Natl. Acad. Sci. USA 99 (2002) 2281–2286.

[56] L.B. King, R.B. Corley, Lipopolysaccharide and dexamethasone induce mouse mammary tumor proviral gene expression and differentiation in B lymphocytes through distinct regulatory pathways, Mol. Cell Biol. 10 (1990) 4211–4220.

[57] B.A. Jude, Y. Pobezinskaya, J. Bishop, S. Parke, R.M. Medzhitov, A.V. Chervonsky, T.V. Golovkina, Subversion of the innate immune system by a retrovirus, Nat. Immunol. 4 (2003) 537–538.

[58] L.M. Hook, Y. Agafonova, S.R. Ross, S.J. Turner, T.V. Golovkina, Genetics of mouse mammary tumor virus-induced mammary tumors: linkage of tumor induction to the *gag* gene, J. Virol. 74 (2000) 8876–8883.

[59] I.M. Klagge, S. Schneider-Schaulies, Virus interactions with dendritic cells, J. Gen. Virol. 80 (1999) 823–833.

[60] E. Helin, R. Vainionpaa, T. Hyypia, I. Julkunen, S. Matikainen, Measles virus activates NF-kappaB and STAT transcription factors and production of IFN-alpha/beta and IL-6 in the human lung epithelial cell line A549, Virology 290 (2001) 1–10.

[61] J.J. Schnorr, S. Xanthakos, P. Keikavoussi, E. Kaempgen, V. ter Meulen, S. Schneider-Schaulies, Induction of maturation of human blood dendritic cell precursors by measles virus is associated with immunosuppression, Proc. Natl. Acad. Sci. USA 94 (1997) 5326–5331.

[62] K. Bieback, E. Lien, I.M. Klagge, E. Avota, J. Schneider-Schaulies, W.P. Duprex, H. Wagner, C.J. Kirschning, V. ter Meulen, S. Schneider-Schaulies, Hemagglutinin protein of wild-type measles virus activates toll-like receptor 2 signaling, J. Virol. 76 (2002) 8729–8736.

[63] N. Murabayashi, M. Kurita-Taniguchi, M. Ayata, M. Matsumoto, H. Ogura, T. Seya, Susceptibility of human dendritic cells (DCs) to measles virus (MV) depends on their activation stages in conjunction with the level of CD150: role of Toll stimumlators in DC maturation and MV amplification, Microb. Infect. 4 (2002) 785–794.

[64] H. Zhu, J.P. Cong, T. Shenk, Use of differential display analysis to assess the effect of human cytomegalovirus infection on the accumulation of cellular RNAs: induction of interferon-responsive RNAs, Proc. Natl. Acad. Sci. USA 94 (1997) 13985–13990.

[65] K.A. Simmen, J. Singh, B.G. Luukkonen, M. Lopper, A. Bittner, N.E. Miller, M.R. Jackson, T. Compton, K. Fruh, Global modulation of cellular transcription by human cytomegalovirus is initiated by viral glycoprotein B, Proc. Natl. Acad. Sci. USA 98 (2001) 7140–7145.

[66] T. Compton, E.A. Kurt-Jones, K.W. Boehme, J. Belko, E. Latz, D.T. Golenbock, R.W. Finberg, Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2, J. Virol. 77 (2003) 4588–4596.

[67] B.F. Holt, D.A. Hubert, J.L. Dangl, Resistance gene signaling in plants—complex similarities to animal innate immunity, Curr. Opinion Immun. 15 (2003) 20–25.

[68] B. Kobe, J. Deisenhofer, Mechanism of ribonuclease inhibition by ribonuclease inhibitor protein based on the crystal structure of its complex with ribonuclease A, J. Mol. Biol. 264 (1996) 1028–1043.

[69] J.J. Skehel, D.C. Wiley, Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin, Annu. Rev. Biochem. 69 (2000) 531–569.