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REVIEW

Herpesviral infection and Toll-like receptor 2

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ABSTRACT

In the last decade, substantial progress has been made in understanding the molecular mechanisms involved in the initial host responses to viral infections. Herpesviral infections can provoke an inflammatory cytokine response, however, the innate pathogen-sensing mechanisms that transduce the signal for this response are poorly understood. In recent years, it has become increasingly evident that the Toll-like receptors (TLRs), which are germline-encoded pattern recognition receptors (PRRs), function as potent sensors for infection. TLRs can induce the activation of the innate immunity by recruiting specific intracellular adaptor proteins to initiate signaling pathways, which then culminating in activation of the nuclear factor kappa B (NF-κB) and interferon-regulatory factors (IRFs) that control the transcription of genes encoding type I interferon (IFN I) and other inflammatory cytokines. Furthermore, activation of innate immunity is critical for mounting adaptive immune responses. In parallel, common mechanisms used by viruses to counteract TLR-mediated responses or to actively subvert these pathways that block recognition and signaling through TLRs for their own benefit are emerging. Recent findings have demonstrated that TLR2 plays a crucial role in initiating the inflammatory process, and surprisingly that the response TLR2 triggers might be overzealous in its attempt to counter the attack by the virus. In this review, we summarize and discuss the recent advances about the specific role of TLR2 in triggering inflammatory responses in herpesvirus infection and the consequences of the alarms raised in the host that they are assigned to protect.

KEYWORDS herpesviruses, innate immune, Toll-like receptor (TLR), TLR2

INTRODUCTION

Herpesviruses (including herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV), etc.) infections of humans and other animals early in life are very common and prevalent in the population. These viruses are ubiquitous viruses and are characterized by a complex DNA genome, conserved virion structure and replication mechanisms. The majority of humans has had a primary infection with one or more herpesviruses and harbour these viruses in a latent state for the rest of their lives (Pellett PE, 2007). Therefore, herpesviruses are fascinating models for scientific research as they establish lifelong persistent infections in different tissues in immunocompetent and immunocompromised hosts. Human herpesviruses are of particularly medical importance because they are associated with severe diseases and cancers in immunocompromised hosts (Thorley-Lawson et al., 2008). Although the majority of infections with HSV and CMV are clinically mild or even asymptomatic, primary infection in the fetal and perinatal periods can be neurologically devastating or fatal (Crumpacker CS, 2005; L, 2005). The precise reasons for the increased severity of disease early in life are not clear, and may involve many aspects of immune defense. Thus, it is necessary to look at the innate immune response as it plays a key role in controlling the early replication of the virus, and can be instrumental in initiating an appropriate adaptive immune response.

THE INNATE IMMUNE RESPONSE

The immune system has evolved over time to recognize a

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wide variety of molecules expressed by microorganisms in order to exert a successful defense against infection. The innate immune response, mediated primarily by phagocytic cells and antigen-presenting cells, such as granulocytes, macrophages, and dendritic cells (DCs), is an early line of host defense during infection, which plays a crucial role in the immediate and rapid protection of a host from invading microbial pathogens. It is now known that viruses, similar to bacteria and fungi, are initially recognized by a class of host immune sensor molecules that are referred to as germline-encoded PRRs, via their encoded various proteins containing evolutionarily conserved pathogen-associated molecular patterns (PAMPs) that are broadly shared by pathogens and essential for their survival, hence make ideal targets for detection by the innate immune system (Janeway, 1989; Medzhitov, 2001). Several different classes of PRRs have been identified, each detecting diverse microbes or microbial components (Gordon, 2002).

The TLRs are the major and most well-characterized family of phylogenetically conserved PRRs from *Drosophila* to humans. These TLRs constitute a family of receptors that detect an increasingly broad range of pathogens that triggers a great deal of cellular responses, including NF-κB activation, secretion of inflammatory cytokines and chemokines, expression of immune costimulatory molecules, adhesion molecules, and induction of antiviral responses (Akira and Takeda, 2004; Ku et al., 2005). Overall, these responses help promote and shape the critical immunological processes that facilitate pathogen control and clearance. While the majority of works on TLRs have focused on detection of bacteria and fungi, it is becoming increasingly apparent that herpesviruses are also subject to innate sensing by multiple TLRs.

TLRs

TLRs, functioned as broad classes of microbial pattern recognition molecules (Akira et al., 2001; Zhang et al., 2004), are a group of type I transmembrane glycoproteins expressed predominantly on phagocytic cells, despite many other cell and tissue types also express TLRs (Hornung et al., 2002; Zarember and Godowski, 2002; Takeda and Akira, 2003). Although TLR expression patterns and levels in most cell and tissue types are still being defined, it appears that the majority of cells in the body express at least a subset of TLRs. Common features of TLRs are the varying numbers of N-terminal leucine-rich repeats (LRRs, 21-25) extracellular or luminal domain which are hypothesized to mediate the recognition of their respective PAMPs, followed by a conserved cysteine-rich region which is thought to contribute to receptor structure and function, the single transmembrane domain that determines the cellular localization, and the intracellular conserved signaling motif called the Toll-interleukin-1 (IL-1) receptor (TIR) domain, which is required for initiating intracellular signaling pathways and altered gene expression (Sabroe et al., 2003).

On activation of the receptor with its appropriate ligand, a specific set of TIR domain-containing adaptor molecules are recruited to the receptor and activate a downstream family of kinases containing IL-1 receptor-associated kinases (IRAKs) and tumor necrosis factor receptor-associated factors (TRAFs) (especially the IRAK4 and TRAF6), which then amplify the signal through NF-κB and IRFs (Akira and Takeda, 2004) that leads to the secretion of inflammatory cytokines, IFN I, chemokines, antimicrobial products and up-regulation of a subset of TLRs (Kawai and Akira, 2010). Specifically, these adaptor molecules include myeloid differentiation primary-response protein 88 (MyD88, utilized by all TLRs except for TLR3), TIR-domain-containing adaptor protein (TIRAP, also known as MyD88-adaptor-like protein (MAL), for TLR2 and TLR4), TIR domain-containing adaptor-inducing interferon-β (TRIF, also known as TIR-domain-containing adaptor molecule 1 (TICAM-1)), TRIF-related adaptor molecule (TRAM, also known as TIR-domain-containing adaptor molecule 2 (TICAM-2), for TLR3 and TLR4), and/or sterile and HEAT/armadillo (ARM) motif protein (SARM) (McGettrick and O'Neill, 2004). For more details of different TLR ligands and downstream factors, please refer the related reviews (Morrison, 2004; Chang et al., 2006; Herbst-Kralovetz and Pyles, 2006; Gay and Gangloff, 2007; Kawai and Akira, 2010, 2011).

PAMPs recognized by TLRs include lipids, lipoproteins, proteins, nucleic acids and/or combinations derived from a wide range of microbes such as bacteria, mycobacteria, parasites, fungi and viruses (Akira et al., 2006). And, the recognition of PAMPs by TLRs can occur in various cellular compartments, including the plasma membrane, endosomes, lysosomes and endolysosomes (Akira et al., 2006). Currently, 10 TLRs have been identified in humans (TLR1-10) and 12 have been identified in mice (TLR1-9, 11-13). TLRs are largely divided into two subfamilies depending on their cellular localization and respective PAMP ligands. One subfamily is composed of TLR1, TLR2, TLR4, TLR5, TLR6, TLR10, TLR11, TLR12 and TLR13, which are expressed on cell surfaces and recognize mainly microbial membrane components such as lipids, lipoproteins and proteins. In contrast, the other subfamily is composed of TLR3, TLR7, TLR8 and TLR9, which are expressed exclusively in intracellular compartments, such as the endoplasmic reticulum, endosomes, lysosomes and endolysosomes, where they specifically recognize microbial nucleic acids species (Medzhitov et al., 1997; Rock et al., 1998; Takeuchi et al., 1999; Chuang and Ulevitch, 2000, 2001; Du et al., 2000; Akira et al., 2006).

TLR2

The human TLR2, located on chromosome 4q32, contains at least 89 single nucleotide polymorphisms (SNPs), including 17 that modify bases in the coding sequence of exon III

(Texereau et al., 2005). Nine SNPs are nonsynonymous and two (R677W and R753Q) were characterized to impair TLR2 function (Lorenz et al., 2000; Ogus et al., 2004; von Aulock et al., 2004). TLR2 was originally identified as the LPS receptor (Kirschning et al., 1998; Yang et al., 1998), but the TLR2 activation by LPS was subsequently attributed to the bacterial lipoprotein contamination of the LPS preparations (Lee et al., 2002; Hellman et al., 2003). Moreover, TLR2 can recognize a wide repertoire of microbial products, these ligands include lipoteichoic acid from Gram-positive bacteria (Schwandner et al., 1999; Takeda et al., 2003), lipoproteins from both Gram-negative bacteria and Gram-positive bacteria, mycobacteria, and spirochetes (Aliprantis et al., 1999), lipoarabinimannan from mycobacteria (Means et al., 1999), zymosan from fungi and envelope proteins from viruses (Underhill et al., 1999). Accordingly, there is considerable interest in investigating the potential association between functional impairment in this receptor and various human infections (Schwartz and Cook, 2005; Texereau et al., 2005).

To date, it is shown that TLR2 plays a key role in the microbial antigen activation of NF-κB (Zhang and Ghosh, 2001). Signaling through TLR2/TIR/MyD88/Mal activates NF-κB, promoting production of proinflammatory cytokines IL-1 , IL-6, IL-8, IL-12, and monocyte chemotactic peptide 1 (MCP-1) (Akira et al., 2001; Horng et al., 2002). In fact, TLR2 forms a heterodimer with its coreceptors TLR1 or TLR6 or perhaps other PRRs for detection of various microbial components, and in some cases, neither TLR1 nor TLR6 is required for molecular recognition (Buwitt-Beckmann et al., 2005). Furthermore, TLR2 may also signal as a homodimer to recognize different species of ligands.

CD14, a glycosylphosphatidyl inositol-anchored cell surface protein that has multiple leucine-rich regions, appears to function as a PRR for many different microbial antigens such as LPS, peptidoglycan and mannuronic acid polymers, etc. (Wright et al., 1990; Espevik et al., 1993; Haziot et al., 1996; Weidemann et al., 1997; Dziarski et al., 1998; Ingalls et al., 1999). CD14 also involves in TLR2-dependent initiation of immune response (Schroder et al., 2003), however, depending on the nature of the ligand, the presence of which is not absolutely required by all TLR2 complexes for signaling activity (Jiang et al., 2005). A recent study by Barbalat et al (2009) showed that TLR2 on inflammatory monocytes could recognize nonnucleic acid components and induce production of IFN I by internalization of TLR2 to the Golgi apparatus. In addition, other investigation has been shown that infection with viruses such as influenza A virus can induce an upregulation in TLR2 expression by human neutrophils (Lee et al., 2006). This strategy may be beneficial to the virus in order to induce upregulation of viral entry receptors but may also serve the host in favoring a rapid respond to viral invaders (Bieback et al., 2002; Zhu et al., 2007). Thus far, a large body of works demonstrate that TLR2 has been implicated in the recognition of structural components of several viruses (Bieback et al., 2002; Duesberg et al., 2002; Dolganiuc et al., 2004; Cooper et al., 2005), as well as members of the herpesvirus family, including HSV, VZV, murine gammaherpesvirus-68 (MHV-68), CMV, and EBV (Compton et al., 2003; Kurt-Jones et al., 2004; Wang et al., 2005a; Boehme et al., 2006; Sato et al., 2006; Szomolanyi-Tsuda et al., 2006; Gaudreault et al., 2007; Ariza et al., 2009; Michaud et al., 2010).

HSV and TLR2

HSV is a viral pathogen of the *Herpesviridae* family with two closely related subtypes HSV-1 and HSV-2 that commonly undergoes a lytic replication cycle at mucosal surfaces and spreads into sensory neurons where it establishes a latent infection for the lifetime of the individual (Roizman B, 2007). During an acute HSV infection, macrophages play important roles in both the innate response as well as in activating and shaping the adaptive immune response, at least partly through the production of chemokines, IFNs, and proinflammatory mediators such as RANTES, IL-6, and IL-12 (Heise and Virgin, 1995; Paludan, 2001; Vollstedt et al., 2001; Melchjorsen et al., 2002). Several studies have implicated TLRs as important players during a number of HSV infections, depending upon the cell type.

In the paradigmatic view of innate host responses, an acute inflammatory cell influx in infected tissue aids in virus clearance. Therefore, TLR signaling-deficient mice should have higher viral titers because they cannot mount an inflammatory response. Analysis of the TLR system in the pathogenesis of HSV infection further supports the notion that TLR interactions catalyze virus-induced inflammation. A number of recent studies have demonstrated that TLRs expressed by peritoneal macrophages and microglial cells, which are key immune cells that survey the brain parenchyma, are pivotal in identifying and generating the first line of innate immune responses against viral pathogens in the central nervous system (Kurt-Jones et al., 2004, 2005; Olson and Miller, 2004; Aravalli et al., 2005; Town et al., 2006). Although specific viral ligands for TLR2 have not been identified (Sato et al., 2006), which may have major roles in virus entry and initiate innate immune recognition at this early stage of the viral life cycle, recent studies suggest that TLR2 signaling may be involved in innate responses to HSV.

In vitro, most studies show that HSV-induced expression of proinflammatory cytokines and chemokines, such as TNF-α, IL-1, IL-6, IL-12, CCL7, CCL8, CCL9, CXCL1, CXCL2, CXCL4 and CXCL5, as well as the focal tissue damage (if left untreated, can result in prolonged brain inflammation and compromised brain function or death) (Aravalli et al., 2005; Marques et al., 2006, 2008a, 2008b; Armien et al., 2010), is diminished when TLR2 is not functional (Aravalli et al., 2005). In a recent report, Boivin and colleagues (Boivin et al., 2002) described the enhanced expression of TLR2 in the hindbrain

of mice infected with HSV-2. Moreover, in a separate study utilizing TLR2 knockout (TLR2$^{-/-}$) mice of related background, Kurt-Jones and colleagues showed that TLR2 mediated the NF-κB activation and induction release of inflammatory cytokines such as IL-6, IL-8, MCP-1, and other inflammatory cytokines, as well as an increase in encephalitic severity following intravenous inoculation with the lethal doses of HSV-1 KOS strain, resulting in the death of the mice from encephalitis (Kurt-Jones et al., 2004). However, in mice lacking functional TLR2, authors detected a blunted cytokine response or inflammatory infiltrates in the HSV-infected brain, but the mice did not have higher virus titers than wild-type (WT) counterparts, suggesting that TLR2 plays an important role in herpes encephalitis. Interestingly, similar observations have recently been made in a murine model of HSV eye infection (Sarangi et al., 2007). Moreover, a recent report also support a role for TLR2 signaling in HSV infection, which identified an association between naturally occurring polymorphisms in the human gene for TLR2 and the severity and recurrent infections of HSV-2 genital lesions and increased viral shedding in humans (Bochud et al., 2007), suggesting a role for TLR2 in the regulation of HSV-induced disease in the human population.

The relative importance of TLR2 signaling in HSV infection has also been called into question by the *in vitro* observation that a rare population of HSV, both in laboratory strains and in primary clinical isolates from humans of a variety of tissues and mucosal secretions, has the capacity to activate TLR2 (Sato et al., 2006). This study found that clinical and some laboratory HSV isolates generally exist as a collection of subspecies of viral clones, most of which do not activate TLR2. Using human cells expressing different TLRs, Kurt-Jones et al. then demonstrated that, rather than producing less IL-6 and IL-8 in response to HSV than adults do, peripheral blood mononuclear cells (PBMCs) from newborn humans produce more of these cytokines than adults do, indicating that TLR2 may play a role in the sepsis-like disease associated with human neonatal herpes infection.

While most children and adults undergoing their first HSV infection recover uneventfully (except in the case of encephalitis), neonates may die of fulminant disease characterized by multi-organ failure and accompanied by high levels of serum cytokines, suggesting that unlike the observation of dampened signaling through TLR3 in newborns relative to adults, there may be an enhanced response to signaling through TLR2, explaining the greater susceptibility of newborns to HSV disease (Kurt-Jones et al., 2005). Because this is an event that requires no protein synthesis, it occurs rapidly and does not require live virus, suggesting that structural components (except for the four essential viral glycoproteins gB, gD, and gHgL) of the virus are responsible for the alterations in cell physiology and intracellular signaling that occur during virus-cell contact and/or virus entry (Reske et al., 2008).

A role for TLR2 has been proposed in the outcome of HSV infection in human disease. In the case of HSV-1 (and HSV-2), the interaction between HSV-1 and TLR2 is important, since it affects the mortality of mice that die as a result of HSV encephalitis. Thus, these studies suggest the TLR2-mediated cytokine response to HSV-1 is detrimental to the host (Kurt-Jones et al., 2004). Experiments defining the effect of the elimination of this TLR2 specific response on the development of the acquired responses need to be addressed. It is anticipated that, as with other viruses, such as LCMV (Zhou et al., 2005), the elimination of these responses will result in a defective acquired immune response. It was recently shown that TLR2 and TLR9 act in a cooperative manner to activate conventional DCs (cDC)s during infection with HSV (Sato et al., 2006), and that TLR2 and TLR9 are sequentially engaged by HSV clones recognized by TLR2. A large fraction of this TLR2-dependent recognition of HSV by DCs requires TLR9. Furthermore, subspecies which do not stimulate TLR2 may still stimulate TLR9 (Sato et al., 2006). Together, these observations highlight the redundancy in innate detection and suggest the possibility of greater importance for TLR9 relative to TLR2 in detection of HSV. Importantly, control of murine infection in the brain may require synergistic activity of both TLR2 and TLR9 (Sorensen et al., 2008).

It is noteworthy that there is an additional report demonstrated that TLR2 induces apoptosis of HSV-infected microglial cells following immune mediator production, potentially implicating microglial cells in the regulation of the inflammatory response following HSV infection (Aravalli et al., 2005). However, the regulation of HSV-induced disease by TLR2 is not limited to models of HSV encephalitis, as TLR2$^{-/-}$ mice were also considerably resistant to ocular lesions associated with stromal keratitis caused by HSV-1 (Sarangi et al., 2007). Taken together, these data suggest that HSV infection detected by TLR2 resulted in an immoderate cytokine response that had lethal consequences for the host, although this is usually a protective mechanism. These studies support the concept advanced by several investigators that the inflammatory response may exacerbate pathology in HSV encephalitis (Sergerie et al., 2007; Fitch and van de Beek, 2008; Lundberg et al., 2008). Perhaps the ancient sentinels of the body have not received the benefits of evolutionary refinement from the 'double-edged sword' of TLR2 activation in response to virus infection. Thus, inhibition of TLR2 signaling might prove to be an effective approach in the treatment of superfluous neuroimmune responses seen during HSV-induced encephalitis and may provide important insights into the design of effective strategies to combat HSV infections.

VZV AND TLR2

Acute VZV infection is associated with an inflammatory re-

sponse characterized by fever and cytokine production (Arvin et al., 1986; Torigo et al., 2000). Recently, TLR2 has also been implicated in the response to VZV. Wang et al. demonstrated that VZV activates inflammatory cytokine responses via TLR2 (Wang et al., 2005a). Specifically, VZV induced the release of IL-6 in primary human monocytes, a natural host cell for VZV that expressing TLR2 (Compton et al., 2003; Koenig and Wolff, 2003), via TLR2-dependent activation of NF-κB, and small interfering RNA (siRNA) against TLR2 decreased the ability to secrete IL-6 response to VZV in human monocyte-derived macrophages (HMDM).

VZV has a narrow host range restricted to humans and selected cell types of primate and guinea pig origin, whereas mice and cells of murine origin are not susceptible to VZV infection (Weber, 2000). Therefore, unlike other herpesviruses such as HSV, which has a broader host range and activates both human and murine cells in a TLR2-dependent manner, VZV elicits species-specific cytokine response. VZV did not induce cytokines in murine embryonic fibroblasts or in a mouse cell line expressing TLR2 sequences, of either human or murine origin, although VZV did activate NF-κB in a human cell line expressing a murine TLR2. Accordingly, human coreceptors, e.g. CD14, including those participated in viral entry, may play a role in TLR2 activation and induction of inflammatory cytokines and thereby explain the species specificity of IL-6 induction by VZV. Together, these results suggest that, as with HSV, TLR2 may play a role in the early inflammatory response to VZV infection and contributes to the clinical presentation of symptomatic primary or reactivation disease with VZV (Kurt-Jones et al., 2004). This innate host immune response may be linked with specific cell-mediated immune responses that participate in severe complications of VZV infection, including thrombocytopenia, encephalitis, hepatitis, and pneumonitis. Such clinical findings are often associated with the production of inflammatory cytokines that may be linked with host TLR2 recognition of herpesviruses.

MHV-68 AND TLR2

MHV-68 is known to induce the secretion of a wide range of cytokines including IL-6, IL-10, IL-12, IFN I, and IFN-γ (Sarawar et al., 1996; Elsawa and Bost, 2004; Sarawar et al., 2004), and this induction is demonstrated to be involved in TLR2 until recently. Using transfected human embryonic kidney (HEK) 293 cells, MHV-68 leads to the activation of NF-κB reporter through TLR2. In addition, production of IL-6 and IFN-α upon MHV-68 challenge was lessen in murine embryonic fibroblasts derived from $TLR2^{-/-}$ and $MyD88^{-/-}$ mice as compared to their WT counterpart. In transgenic mice expressing a luciferase reporter gene under the control of the murine TLR2 promoter, MHV-68 stimulation activated TLR2 transcription, whereas TLR2 or MyD88 deficiency was associated with the decreased levels of IL-6 and IFN I production as well as increased viral burden during short-term challenges with MHV-68. Taken together, these results suggest that TLR2 contributes to the production of inflammatory cytokines and IFN I as well as to the control of viral burden during infection with MHV-68. Thus, the TLR2 pathway has a relevant role in the recognition of this virus and in the subsequent activation of the innate immune response (Michaud et al., 2010).

CMV AND TLR2

To better understand the immune responses triggered by herpesviruses, it is critical to identify not only which TLRs are activated during herpesviral infection but also the specific viral components that act as ligands/triggers for the different TLRs, leading to the stimulation of the host immune response. Viruses possess several structurally diverse PAMPs, including surface glycoproteins, which are a compelling target for the TLR system (Mogensen and Paludan, 2005). Although significantly less well studied, viral envelope glycoproteins that decorate the exterior of the virion are another broad class of molecules that are emerging as the subject of TLR2 detection (Mogensen and Paludan, 2005; Akira et al., 2006). One common theme among the viral glycoproteins implicated to date is that they play critical roles in the binding and/or entry of their respective viruses and permit the IFN and cytokine intruder alert systems to be activated by the host. Thus, viral pathogens can be detected at the earliest stages of infection simply via contact between glycoproteins and TLRs on the cell surface, rather than requiring viral gene expression and/or replication. The rapid recognition and response could provide a temporal advantage for the host immune response, which would be extremely beneficial for combating a viral infection.

There is evidence that infection of fibroblasts with human CMV (HCMV) strongly triggers the production of inflammatory cytokines and stimulates transcription of the IFN pathway. In contrast to paramyxoviruses and retroviruses, CMV is extremely structurally complex, displaying at least 12 envelope glycoproteins. Inactive virus particles or even glycoprotein B, the HCMV entry mediator, largely mimic the response to intact virus (Simmen et al., 2001), suggesting that interaction of CMV envelope proteins with a cell surface sensing mechanism transduces the signal for antiviral activation. Like HSV, using HEK293 cells stably transfected with TLR2 expression vector, TLR2 in patients has been demonstrated to play a role in the early stage of CMV infection. And, HCMV virions are capable of stimulating an inflammatory cytokine secretion from human and mouse macrophages through TLR2-dependent activation of NF-κB (Compton et al., 2003; Boehme et al., 2006). Furthermore, similar to most TLR2-mediated responses, signaling of TLR2 in response to CMV is strongly enhanced by the presence of CD14. The importance of TLR2 in the early control of CMV infection

mediated by natural killer (NK) cells has also been shown for mice. Mice deficiency of TLR2 shows elevated murine CMV (MCMV) replication in the spleen and liver 4 days post-infection as compared to WT C57BL/6 mice, and this difference in viral titers was abrogated by *in vivo* depletion of NK cells between the two groups using anti-NK1.1 antibodies, suggesting that the effect of TLR2 on MCMV clearance on day 4 was NK cell mediated. Therefore, the defect in early antiviral control was associated with reduction of NK cell population in the spleen and liver 4 days post-infection and may be related to NK cell recruitment, proliferation, or sensitivity to apoptosis (Szomolanyi-Tsuda et al., 2006). Although the structural component(s) important for the recognition of HSV, VZV, and MHV-68 have not been identified, interaction with TLR2 for NF-κB activation and inflammatory cytokine secretion, likely in heterodimeric form with TLR1, involves two CMV entry-mediating envelope glycoproteins gB and gH in permissive cells (Boehme et al., 2006), suggesting that multiple glycoproteins from a single virus can display recognition motifs.

Activation of TLRs by envelope glycoprotein also suggests that the processes of virus entry and innate immune activation are coordinated. The viral envelope is studded with numerous copies of each glycoprotein, and each copy is able to interact with one or more cellular receptors. Multiple interactions between viral envelope glycoproteins and different types of cellular receptors may induce the formation of an organized structure reminiscent of the immunological synapse (Kinashi, 2005). This type of receptor clustering would allow the cell to synchronize innate immune activation with the process of viral entry. Cellular integrins have been identified as receptors for HCMV gB and gH (Feire et al., 2004; Wang et al., 2005b) and have also been linked to TLRs (Ogawa et al., 2002; Triantafilou and Triantafilou, 2002). It is possible that HCMV binding to integrins could facilitate interaction with TLR2/TLR1 heterodimers. Furthermore, receptor clustering may provide a mechanism by with integrin and TLR signaling can be coordinated. As NF-κB activation is also a downstream consequence of integrin usage, it is possible that TLR2 and integrins both contribute to the activation of NF-κB upon HCMV infection (Guo and Giancotti, 2004). However, it remains to be determined whether NF-κB activation by TLR2 and integrins is coordinated or coincidental.

Clinically, a R753Q SNP in TLR2 in liver transplant patients is associated with a significantly higher degree of CMV replication, and homozygosity for this polymorphism confers increased risk of CMV disease (Kijpittayarit et al., 2007), whereas Boehme et al. provides the biologic explanation for this clinical observation by demonstrating that variant cells with TLR2 R753Q SNP failed to recognize CMV gB (Boehme et al., 2006). This immunologic impairment could serve as a biologic mechanism underlying the association between TLR2 R753Q polymorphism and CMV disease in humans. Moreover, this impaired innate viral recognition may impede development of a more robust antiviral immune mechanism thereby translating to a higher incidence of clinical disease. It is unclear whether R753Q SNP results in an impaired ligand-receptor attachment or impaired engagement of downstream adapter molecules. The location of the R753Q SNP within a group of highly conserved amino acids at the C-terminal of TLR2 suggests that R753Q SNP may impede the engagement of MyD88 adapter protein; however, this remains to be demonstrated. Currently, what is known is that, *in vitro*, R753Q SNP in TLR2 is associated with impaired NF-κB activation during exposure to CMV and bacterial lipoproteins. Therefore, these findings expand on the previous observations (Kijpittayarit et al., 2007) and those of others (Compton et al., 2003) that TLR2 is an important component in the immunopathogenesis of CMV in humans. Treatment of cultured human ectocervical tissue with the TLR2 agonist lipoteichoic acid leads to inhibition of CMV replication in an IFN-β-dependent manner, but similar treatment in foreskin fibroblasts, which do not express TLR2, did not demonstrate inhibition (Harwani et al., 2007).

EBV AND TLR2

EBV infection of primary B cells causes B cell activation and proliferation. Previous studies demonstrated that EBV-encoded dUTPase stimulates the expression of proinflammatory cytokines in human PBMCs and HMDM and that the increased cytokine expression was dependent on NF-κB activation (Glaser et al., 2006; Waldman et al., 2008). Recent reports have indicated that TLR2 is also shown to play an important role in immune responses directed against EBV infection. Infectious and UV-inactivated EBV virions are demonstrated to lead to the activation of NF-κB through TLR2 signaling pathway (Gaudreault et al., 2007). However, NF-κB activation was not enhanced by the presence of CD14. The effect of EBV was abolished by pretreating HEK293-TLR2 cells with blocking anti-TLR2 antibodies or by preincubating viral particles with neutralizing anti-EBV antibodies. In addition, siRNA designed against TLR2 significantly reduce the chemokine response to EBV (Gaudreault et al., 2007). Recently, further investigations show that the purified EBV-encoded dUTPase could potentially modulate the innate immune response in EBV-permissive cells through the TLR2 and the adaptor molecule MyD88 signal transduction pathway but not CD14, leading to the activation of NF-κB and the production of various proinflammatory cytokines in a dose-dependent manner, including IL-6, and the key anti-inflammatory cytokine IL-10. Furthermore, activation of NF-κB was abrogated by blocking anti-TLR2, anti-EBV-encoded dUTPase antibodies and the overexpression of a dominant negative form of MyD88 in HEK293 cells expressing TLR2. In addition, administration of HMDM with the anti-EBV-encoded dUTPase antibody or the anti-TLR2 antibody blocked the production of IL-6 triggered by the

EBV-encoded dUTPase.

Lately, the IL-6 and IL-10 have received much attention because of their important roles in both innate and adaptive immune responses. Given the ability of EBV to establish persistent infections in the host, it might be expected that the virus has evolved to develop mechanism(s) to regulate the expression and function of IL-6, IL-10 and TNF-α as part of the virus strategy to evade immune surveillance. Therefore, understanding how EBV-encoded dUTPase is important in determining immune response outcomes during EBV infection. Taken together, these results indicate that TLR2 may be an important PRR in the immune response directed against EBV infection and the nonstructural protein encoded by EBV, dUTPase, which is a PAMP and that it has immunomodulatory functions, could be a potential target for the development of novel therapeutic agents against infections caused by EBV, although additional studies are necessary to define the signaling pathways activated by the EBV-encoded dUTPase and to determine its role in modulating immune responses to EBV infection.

HESPERVIRAL EVASION OF TLR2 SIGNALLING PATHWAY

Viruses, which are obligate parasites that depend on host cells for survival, are the class of the most abundant, diverse and rapidly evolving pathogens that the host immune system is challenged by and they therefore represent a serious threat to human health. Given the fact that mounting an inflammatory response through PRRs is a prerequisite for containment and eradication of invading pathogens, it is not surprising that most pathogens, in turn, have evolved to encode the most diverse and sophisticated strategies to evade and counteract the host immune responses (Haller et al., 2006; Roy and Mocarski, 2007; Weber and Haller, 2007; Bowie and Unterholzner, 2008; Randall and Goodbourn, 2008; Unterholzner and Bowie, 2008; Tsuchida et al., 2009). Virulence strategies used by pathogens are diverse, and involve various mechanisms such as inhibition of cytokines and chemokines, blocking apoptosis, and interfering with cellular pathways such as TLR signaling. Successful evasion of host immune responses by microbial pathogens may result in severe infection and disease. For the virus to be successful, these evasive strategies have to be balanced with the pathology induced and the possibilities of transmission to new susceptible individuals. Due to the ability of viruses to exploit the cellular machinery during their replication cycle, an intricate virus-host relationship has developed throughout evolution, and this allows many types of viruses to interfere profoundly with host signaling (Hiscott et al., 2006). Overall, viruses can interfere in multiple ways with NF-κB and IRF pathways to inhibit induction of proinflammatory molecules and IFN.

It has recently been demonstrated that mammalian TLR signaling can be regulated by viral gene products. The herpesviruses have co-evolved with their vertebrate hosts for more than one hundred million years to establish lifelong infections (Soderberg-Naucler and Nelson, 1999; McGeoch et al., 2000). This co-evolution has created viruses which are well adapted to the human host and environment. Thus, herpesviruses are capable of coping with the human immune defence in a balanced manner generally without serious threads to the life of the host. Infection with a foreign herpesvirus, normally hosted by another species, does not always hold this balance, and the pathology is unpredictable. This is seen when humans are infected with the simian B virus, which often shows serious clinical outcome (Whitley RJ, 2001). Accordingly, all herpesviruses employ a multitude of strategies to modulate the host immune response, facilitating this persistence in the face of a robust innate and adaptive immune response.

One of the example of herpesviral interference with TLR-induced signaling to be described is that the HSV-1 immediate-early protein ICP0 can diminish the TLR2-driven proinflammatory cytokine production in respond to HSV-1 infection. When expressed alone, ICP0 is sufficient to obstruct TLR2-mediated responses to both viral and nonviral ligands at or downstream of the MyD88 adaptor and upstream of p65. Moreover, ICP0 can also decrease the levels of MyD88 and Mal by its E3 ligase function and the cellular proteasomal activity, suggesting that ICP0 facilitates the degradation of TLR adaptor molecules and restriction of the inflammatory response, thus hampering the antiviral host response to infection, much as it inhibits the interferon response by sequestration and degradation of IRF3 (van Lint et al., 2010). Further examination of the role that TLRs play at both the cellular and organismal levels may provide further clues toward understanding the complex relationship between herpesviruses and their hosts.

CONCLUSIONS AND PERSPECTIVES

As described in this review, how advances made in the recent research we presented have increased our understanding of how host innate immunity senses and responds to herpesviruses through TLR2 and how herpesviruses encode sophisticated and successful strategies for evading and subverting their detection. For some aspects of TLR2-mediated signalling there is now unprecedented molecular detail, provided in part by ‘seeing’ the host immune response from the perspective of the virus. To date, the gB and gH of HCMV and dUTPase of EBV have been found to be subject to innate immune surveillance by TLR2, however, much work remains to be performed. Specifically, it will be particularly interesting to determine the TLR2 ligands of other herpesviruses and works for determining the TLR2 agonist of HSV-1 are undergoing in our lab.

Dissection of the molecular basis of herpesvirally dis-

played PAMPs is also fundamental to understanding the molecular basis of TLR2 recognition of herpesviruses. However, herpesviruses may adopt many different strategies that inhibit TLR2 signalling, to manipulate the cytokine environment and benefit herpesviruses, to prevent the activation of transcription factors. A key challenge in future studies will be to harness the information from herpesviral evasion studies for the benefit of human health, as some of the identified viral proteins themselves, or derivatives of them, may have therapeutic uses in suppressing a range of inappropriate TLR2 signalling, as viruses have been 'examining' the host immune machinery for millions of years, so their proteins are optimized to specifically and maximally inhibit their targets, which is analogous to a naturally occurring drug-development programme.

In the instances of TLR2 activation in concert with herpesvirus entry, we need to further investigate how entry events such as binding and fusion are coordinated with innate sensing. Do herpesviral entry receptors and their associated interactions with envelope glycoproteins contribute to or augment TLR2 activation? How does TLR2 activation modulate and promote host immunity, and what is the relationship of pathogenicity, virulence, and TLR2 responses to herpesviruses? How do TLR2 responses influence the processes of persistence and latency after primary infection? The answers to these questions will shed light on the delicate balance between pathogen and host and may lead to improve the design of more efficacious viral vaccines.

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ABBREVIATIONS

ARM, armadillo; CMV, cytomegalovirus; DCs, dendritic cells; EBV, Epstein-Barr virus; HCMV, human CMV; HEK, human embryonic kidney; HMDM, human monocyte-derived macrophages; HSV, herpes simplex virus; IFN I, type I interferon; IL-1, interleukin-1; IRAKs, IL-1 receptor-associated kinases; IRFs, interferon-regulatory factors; LRRs, N-terminal leucine-rich repeats; MAL, MyD88-adaptor-like protein; MCMV, murine CMV; MCP-1, monocyte chemotactic peptide 1; MHV-68, murine gammaherpesvirus-68; MyD88, myeloid differentiation primary-response protein 88; NF-κB, nuclear factor kappa B; PAMPs, pathogen-associated molecular patterns; PBMCs, peripheral blood mononuclear cells; PRRs, pattern recognition receptors; SARM, sterile and HEAT/ARM motif protein; siRNA, small interfering RNA; SNPs, single nucleotide polymorphisms; TICAM-1, TIR-domain-containing adaptor molecule 1; TICAM-2, TIR-domain-containing adaptor molecule 2; TIR, Toll-IL-1 receptor domain; TIRAP, TIR-domain-containing adaptor protein; TLRs, Toll-like receptors; TRAFs, tumor necrosis factor receptor-associated factors; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor-inducing interferon-β; VZV, varicella-zoster virus; WT, wild-type

REFERENCES

Akira, S., and Takeda, K. (2004). Toll-like receptor signalling. Nat Rev Immunol 4, 499-511.

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

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. Cell 124, 783–801.

Aliprantis, A.O., Yang, R.B., Mark, M.R., Suggett, S., Devaux, B., Radolf, J.D., Klimpel, G.R., Godowski, P., and Zychlinsky, A. (1999). Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. Science 285, 736–739.

Aravalli, R.N., Hu, S., Rowen, T.N., Palmquist, J.M., and Lokensgard, J.R. (2005). Cutting edge: TLR2-mediated proinflammatory cytokine and chemokine production by microglial cells in response to herpes simplex virus. J Immunol 175, 4189–4193.

Ariza, M.E., Glaser, R., Kaumaya, P.T., Jones, C., and Williams, M.V. (2009). The EBV-encoded dUTPase activates NF-kappa B through the TLR2 and MyD88-dependent signaling pathway. J Immunol 182, 851–859.

Armien, A.G., Hu, S., Little, M.R., Robinson, N., Lokensgard, J.R., Low, W.C., and Cheeran, M.C. (2010). Chronic cortical and subcortical pathology with associated neurological deficits ensuing experimental herpes encephalitis. Brain Pathol 20, 738–750.

Arvin, A.M., Koropchak, C.M., Williams, B.R., Grumet, F.C., and Foung, S.K. (1986). Early immune response in healthy and immunocompromised subjects with primary varicella-zoster virus infection. J Infect Dis 154, 422–429.

Barbalat, R., Lau, L., Locksley, R.M., and Barton, G.M. (2009). Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. Nat Immunol 10, 1200–1207.

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

Bochud, P.Y., Magaret, A.S., Koelle, D.M., Aderem, A., and Wald, A. (2007). Polymorphisms in TLR2 are associated with increased viral shedding and lesional rate in patients with genital herpes simplex virus Type 2 infection. J Infect Dis 196, 505–509.

Boehme, K.W., Guerrero, M., and Compton, T. (2006). Human cytomegalovirus envelope glycoproteins B and H are necessary for TLR2 activation in permissive cells. J Immunol 177, 7094–7102.

Boivin, G., Coulombe, Z., and Rivest, S. (2002). Intranasal herpes

simplex virus type 2 inoculation causes a profound thymidine kinase dependent cerebral inflammatory response in the mouse hindbrain. Eur J Neurosci 16, 29–43.

Bowie, A.G., and Unterholzner, L. (2008). Viral evasion and subversion of pattern-recognition receptor signalling. Nat Rev Immunol 8, 911–922.

Buwitt-Beckmann, U., Heine, H., Wiesmuller, K.H., Jung, G., Brock, R., Akira, S., and Ulmer, A.J. (2005). Toll-like receptor 6-independent signaling by diacylated lipopeptides. Eur J Immunol 35, 282–289.

Chang, J.H., McCluskey, P.J., and Wakefield, D. (2006). Toll-like receptors in ocular immunity and the immunopathogenesis of inflammatory eye disease. Br J Ophthalmol 90, 103–108.

Chuang, T., and Ulevitch, R.J. (2001). Identification of hTLR10: a novel human Toll-like receptor preferentially expressed in immune cells. Biochim Biophys Acta 1518, 157–161.

Chuang, T.H., and Ulevitch, R.J. (2000). Cloning and characterization of a sub-family of human toll-like receptors: hTLR7, hTLR8 and hTLR9. Eur Cytokine Netw 11, 372–378.

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

Cooper, A., Tal, G., Lider, O., and Shaul, Y. (2005). Cytokine induction by the hepatitis B virus capsid in macrophages is facilitated by membrane heparan sulfate and involves TLR2. J Immunol 175, 3165–3176.

Crumpacker CS, W.S. (2005). Cytomegalovirus. In Principles and Practice of Infectious Diseases, B.J. Mandell GL, Dolin R, ed. (New York, Churchill Livingstone), pp. 1786–1801.

Dolganiuc, A., Oak, S., Kodys, K., Golenbock, D.T., Finberg, R.W., Kurt-Jones, E., and Szabo, G. (2004). Hepatitis C core and nonstructural 3 proteins trigger toll-like receptor 2-mediated pathways and inflammatory activation. Gastroenterology 127, 1513–1524.

Du, X., Poltorak, A., Wei, Y., and Beutler, B. (2000). Three novel mammalian toll-like receptors: gene structure, expression, and evolution. Eur Cytokine Netw 11, 362–371.

Duesberg, U., von dem Bussche, A., Kirschning, C., Miyake, K., Sauerbruch, T., and Spengler, U. (2002). Cell activation by synthetic lipopeptides of the hepatitis C virus (HCV)–core protein is mediated by toll like receptors (TLRs) 2 and 4. Immunol Lett 84, 89–95.

Dziarski, R., Tapping, R.I., and Tobias, P.S. (1998). Binding of bacterial peptidoglycan to CD14. J Biol Chem 273, 8680–8690.

Elsawa, S.F., and Bost, K.L. (2004). Murine gamma-herpesvirus-68-induced IL-12 contributes to the control of latent viral burden, but also contributes to viral-mediated leukocytosis. J Immunol 172, 516–524.

Espevik, T., Otterlei, M., Skjak-Braek, G., Ryan, L., Wright, S.D., and Sundan, A. (1993). The involvement of CD14 in stimulation of cytokine production by uronic acid polymers. Eur J Immunol 23, 255–261.

Feire, A.L., Koss, H., and Compton, T. (2004). Cellular integrins function as entry receptors for human cytomegalovirus via a highly conserved disintegrin-like domain. Proc Natl Acad Sci U S A 101, 15470–15475.

Fitch, M.T., and van de Beek, D. (2008). Drug Insight: steroids in CNS infectious diseases–new indications for an old therapy. Nat Clin Pract Neurol 4, 97–104.

Gaudreault, E., Fiola, S., Olivier, M., and Gosselin, J. (2007). Epstein-Barr virus induces MCP-1 secretion by human monocytes via TLR2. J Virol 81, 8016–8024.

Gay, N.J., and Gangloff, M. (2007). Structure and function of Toll receptors and their ligands. Annu Rev Biochem 76, 141–165.

Glaser, R., Litsky, M.L., Padgett, D.A., Baiocchi, R.A., Yang, E.V., Chen, M., Yeh, P.E., Green-Church, K.B., Caligiuri, M.A., and Williams, M.V. (2006). EBV-encoded dUTPase induces immune dysregulation: Implications for the pathophysiology of EBV-associated disease. Virology 346, 205–218.

Gordon, S. (2002). Pattern recognition receptors: doubling up for the innate immune response. Cell 111, 927–930.

Guo, W., and Giancotti, F.G. (2004). Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 5, 816–826.

Haller, O., Kochs, G., and Weber, F. (2006). The interferon response circuit: induction and suppression by pathogenic viruses. Virology 344, 119–130.

Harwani, S.C., Lurain, N.S., Zariffard, M.R., and Spear, G.T. (2007). Differential inhibition of human cytomegalovirus (HCMV) by toll-like receptor ligands mediated by interferon-beta in human foreskin fibroblasts and cervical tissue. Virol J 4, 133.

Haziot, A., Ferrero, E., Kontgen, F., Hijiya, N., Yamamoto, S., Silver, J., Stewart, C.L., and Goyert, S.M. (1996). Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14-deficient mice. Immunity 4, 407–414.

Heise, M.T., and Virgin, H.W.t. (1995). The T-cell-independent role of gamma interferon and tumor necrosis factor alpha in macrophage activation during murine cytomegalovirus and herpes simplex virus infections. J Virol 69, 904–909.

Hellman, J., Tehan, M.M., and Warren, H.S. (2003). Murein lipoprotein, peptidoglycan-associated lipoprotein, and outer membrane protein A are present in purified rough and smooth lipopolysaccharides. J Infect Dis 188, 286–289.

Herbst-Kralovetz, M., and Pyles, R. (2006). Toll-like receptors, innate immunity and HSV pathogenesis. Herpes 13, 37–41.

Hiscott, J., Nguyen, T.L., Arguello, M., Nakhaei, P., and Paz, S. (2006). Manipulation of the nuclear factor-kappaB pathway and the innate immune response by viruses. Oncogene 25, 6844–6867.

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

Hornung, V., Rothenfusser, S., Britsch, S., Krug, A., Jahrsdorfer, B., Giese, T., Endres, S., and Hartmann, G. (2002). Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 168, 4531–4537.

Ingalls, R.R., Heine, H., Lien, E., Yoshimura, A., and Golenbock, D. (1999). Lipopolysaccharide recognition, CD14, and lipopolysaccharide receptors. Infect Dis Clin North Am 13, 341–353, vii.

Janeway, C.A., Jr. (1989). Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 54 Pt 1, 1–13.

Jiang, Z., Georgel, P., Du, X., Shamel, L., Sovath, S., Mudd, S., Huber, M., Kalis, C., Keck, S., Galanos, C., *et al.* (2005). CD14 is required for MyD88-independent LPS signaling. Nat Immunol 6, 565–570.

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11, 373–384.

Kawai, T., and Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34, 637–650.

Kijpittayarit, S., Eid, A.J., Brown, R.A., Paya, C.V., and Razonable, R.R. (2007). Relationship between Toll-like receptor 2 polymorphism and cytomegalovirus disease after liver transplantation. Clin Infect Dis 44, 1315–1320.

Kinashi, T. (2005). Intracellular signalling controlling integrin activation in lymphocytes. Nat Rev Immunol 5, 546–559.

Kirschning, C.J., Wesche, H., Merrill Ayres, T., and Rothe, M. (1998). Human toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. J Exp Med 188, 2091–2097.

Koenig, A., and Wolff, M.H. (2003). Infectibility of separated peripheral blood mononuclear cell subpopulations by varicella-zoster virus (VZV). J Med Virol 70 Suppl 1, S59–63.

Ku, C.L., Yang, K., Bustamante, J., Puel, A., von Bernuth, H., Santos, O.F., Lawrence, T., Chang, H.H., Al-Mousa, H., Picard, C., et al. (2005). Inherited disorders of human Toll-like receptor signaling: immunological implications. Immunol Rev 203, 10–20.

Kurt-Jones, E.A., Belko, J., Yu, C., Newburger, P.E., Wang, J., Chan, M., Knipe, D.M., and Finberg, R.W. (2005). The role of toll-like receptors in herpes simplex infection in neonates. J Infect Dis 191, 746–748.

Kurt-Jones, E.A., Chan, M., Zhou, S., Wang, J., Reed, G., Bronson, R., Arnold, M.M., Knipe, D.M., and Finberg, R.W. (2004). Herpes simplex virus 1 interaction with Toll-like receptor 2 contributes to lethal encephalitis. Proc Natl Acad Sci U S A 101, 1315–1320.

L, C. (2005). Herpes Simplex Virus. In Principles and Practice of Infectious Diseases, B.J. Mandell GL, Dolin R, ed. (New York, Churchill Livingstone), pp. 1762–1780.

Lee, H.K., Lee, J., and Tobias, P.S. (2002). Two lipoproteins extracted from Escherichia coli K-12 LCD25 lipopolysaccharide are the major components responsible for Toll-like receptor 2-mediated signaling. J Immunol 168, 4012–4017.

Lee, R.M., White, M.R., and Hartshorn, K.L. (2006). Influenza a viruses upregulate neutrophil toll-like receptor 2 expression and function. Scand J Immunol 63, 81–89.

Lorenz, E., Mira, J.P., Cornish, K.L., Arbour, N.C., and Schwartz, D.A. (2000). A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. Infect Immun 68, 6398–6401.

Lundberg, P., Ramakrishna, C., Brown, J., Tyszka, J.M., Hamamura, M., Hinton, D.R., Kovats, S., Nalcioglu, O., Weinberg, K., Openshaw, H., et al. (2008). The immune response to herpes simplex virus type 1 infection in susceptible mice is a major cause of central nervous system pathology resulting in fatal encephalitis. J Virol 82, 7078–7088.

Marques, C.P., Cheeran, M.C., Palmquist, J.M., Hu, S., and Lokensgard, J.R. (2008a). Microglia are the major cellular source of inducible nitric oxide synthase during experimental herpes encephalitis. J Neurovirol 14, 229–238.

Marques, C.P., Cheeran, M.C., Palmquist, J.M., Hu, S., Urban, S.L., and Lokensgard, J.R. (2008b). Prolonged microglial cell activation and lymphocyte infiltration following experimental herpes encephalitis. J Immunol 181, 6417–6426.

Marques, C.P., Hu, S., Sheng, W., and Lokensgard, J.R. (2006). Microglial cells initiate vigorous yet non-protective immune responses during HSV-1 brain infection. Virus Res 121, 1–10.

McGeoch, D.J., Dolan, A., and Ralph, A.C. (2000). Toward a comprehensive phylogeny for mammalian and avian herpesviruses. J Virol 74, 10401–10406.

McGettrick, A.F., and O'Neill, L.A. (2004). The expanding family of MyD88-like adaptors in Toll-like receptor signal transduction. Mol Immunol 41, 577–582.

Means, T.K., Lien, E., Yoshimura, A., Wang, S., Golenbock, D.T., and Fenton, M.J. (1999). The CD14 ligands lipoarabinomannan and lipopolysaccharide differ in their requirement for Toll-like receptors. J Immunol 163, 6748–6755.

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

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

Melchjorsen, J., Pedersen, F.S., Mogensen, S.C., and Paludan, S.R. (2002). Herpes simplex virus selectively induces expression of the CC chemokine RANTES/CCL5 in macrophages through a mechanism dependent on PKR and ICP0. J Virol 76, 2780–2788.

Michaud, F., Coulombe, F., Gaudreault, E., Kriz, J., and Gosselin, J. (2010). Involvement of TLR2 in recognition of acute gammaherpesvirus-68 infection. PLoS One 5, e13742.

Mogensen, T.H., and Paludan, S.R. (2005). Reading the viral signature by Toll-like receptors and other pattern recognition receptors. J Mol Med (Berl) 83, 180–192.

Morrison, L.A. (2004). The Toll of herpes simplex virus infection. Trends Microbiol 12, 353–356.

Ogawa, T., Asai, Y., Hashimoto, M., and Uchida, H. (2002). Bacterial fimbriae activate human peripheral blood monocytes utilizing TLR2, CD14 and CD11a/CD18 as cellular receptors. Eur J Immunol 32, 2543–2550.

Ogus, A.C., Yoldas, B., Ozdemir, T., Uguz, A., Olcen, S., Keser, I., Coskun, M., Cilli, A., and Yegin, O. (2004). The Arg753GLn polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. Eur Respir J 23, 219–223.

Olson, J.K., and Miller, S.D. (2004). Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol 173, 3916–3924.

Paludan, S.R. (2001). Requirements for the induction of interleukin-6 by herpes simplex virus-infected leukocytes. J Virol 75, 8008–8015.

Pellett PE, R.B. (2007). The family Herpesviridae: a brief introduction. In Fields virology, H.P. Knipe DM ed. (Philadelphia, Lippincott Williams and Wilkins), pp. 2479–2500.

Randall, R.E., and Goodbourn, S. (2008). Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol 89, 1–47.

Reske, A., Pollara, G., Krummenacher, C., Katz, D.R., and Chain, B.M. (2008). Glycoprotein-dependent and TLR2-independent

innate immune recognition of herpes simplex virus-1 by dendritic cells. J Immunol 180, 7525–7536.

Rock, F.L., Hardiman, G., Timans, J.C., Kastelein, R.A., and Bazan, J.F. (1998). A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci U S A 95, 588–593.

Roizman B, K.D., Whitley RJ. (2007). Herpes simplex viruses. In Fields Virology, H.P. Knipe DM, ed. (Philadelphia, Lippincott Williams and Wilkins), pp. 2501–2602.

Roy, C.R., and Mocarski, E.S. (2007). Pathogen subversion of cell-intrinsic innate immunity. Nat Immunol 8, 1179–1187.

Sabroe, I., Read, R.C., Whyte, M.K., Dockrell, D.H., Vogel, S.N., and Dower, S.K. (2003). Toll-like receptors in health and disease: complex questions remain. J Immunol 171, 1630–1635.

Sarangi, P.P., Kim, B., Kurt-Jones, E., and Rouse, B.T. (2007). Innate recognition network driving herpes simplex virus-induced corneal immunopathology: role of the toll pathway in early inflammatory events in stromal keratitis. J Virol 81, 11128–11138.

Sarawar, S.R., Cardin, R.D., Brooks, J.W., Mehrpooya, M., Tripp, R.A., and Doherty, P.C. (1996). Cytokine production in the immune response to murine gammaherpesvirus 68. J Virol 70, 3264–3268.

Sarawar, S.R., Lee, B.J., and Giannoni, F. (2004). Cytokines and costimulatory molecules in the immune response to murine gammaherpesvirus-68. Viral Immunol 17, 3–11.

Sato, A., Linehan, M.M., and Iwasaki, A. (2006). Dual recognition of herpes simplex viruses by TLR2 and TLR9 in dendritic cells. Proc Natl Acad Sci U S A 103, 17343–17348.

Schroder, N.W., Morath, S., Alexander, C., Hamann, L., Hartung, T., Zahringer, U., Gobel, U.B., Weber, J.R., and Schumann, R.R. (2003). Lipoteichoic acid (LTA) of Streptococcus pneumoniae and Staphylococcus aureus activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. J Biol Chem 278, 15587–15594.

Schwandner, R., Dziarski, R., Wesche, H., Rothe, M., and Kirschning, C.J. (1999). Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J Biol Chem 274, 17406–17409.

Schwartz, D.A., and Cook, D.N. (2005). Polymorphisms of the Toll-like receptors and human disease. Clin Infect Dis 41 Suppl 7, S403–407.

Sergerie, Y., Boivin, G., Gosselin, D., and Rivest, S. (2007). Delayed but not early glucocorticoid treatment protects the host during experimental herpes simplex virus encephalitis in mice. J Infect Dis 195, 817–825.

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

Soderberg-Naucler, C., and Nelson, J.Y. (1999). Human cytomegalovirus latency and reactivation - a delicate balance between the virus and its host's immune system. Intervirology 42, 314–321.

Sorensen, L.N., Reinert, L.S., Malmgaard, L., Bartholdy, C., Thomsen, A.R., and Paludan, S.R. (2008). TLR2 and TLR9 synergistically control herpes simplex virus infection in the brain. J Immunol 181, 8604–8612.

Szomolanyi-Tsuda, E., Liang, X., Welsh, R.M., Kurt-Jones, E.A., and Finberg, R.W. (2006). Role for TLR2 in NK cell-mediated control of murine cytomegalovirus in vivo. J Virol 80, 4286–4291.

Takeda, K., and Akira, S. (2003). Toll receptors and pathogen resistance. Cell Microbiol 5, 143–153.

Takeda, K., Kaisho, T., and Akira, S. (2003). Toll-like receptors. Annu Rev Immunol 21, 335–376.

Takeuchi, O., Kawai, T., Sanjo, H., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Takeda, K., and Akira, S. (1999). TLR6: A novel member of an expanding toll-like receptor family. Gene 231, 59–65.

Texereau, J., Chiche, J.D., Taylor, W., Choukroun, G., Comba, B., and Mira, J.P. (2005). The importance of Toll-like receptor 2 polymorphisms in severe infections. Clin Infect Dis 41 Suppl 7, S408–415.

Thorley-Lawson, D.A., Duca, K.A., and Shapiro, M. (2008). Epstein-Barr virus: a paradigm for persistent infection – for real and in virtual reality. Trends Immunol 29, 195–201.

Torigo, S., Ihara, T., and Kamiya, H. (2000). IL-12, IFN-gamma, and TNF-alpha released from mononuclear cells inhibit the spread of varicella-zoster virus at an early stage of varicella. Microbiol Immunol 44, 1027–1031.

Town, T., Jeng, D., Alexopoulou, L., Tan, J., and Flavell, R.A. (2006). Microglia recognize double-stranded RNA via TLR3. J Immunol 176, 3804–3812.

Triantafilou, M., and Triantafilou, K. (2002). Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. Trends Immunol 23, 301–304.

Tsuchida, T., Kawai, T., and Akira, S. (2009). Inhibition of IRF3-dependent antiviral responses by cellular and viral proteins. Cell Res 19, 3–4.

Underhill, D.M., Ozinsky, A., Hajjar, A.M., Stevens, A., Wilson, C.B., Bassetti, M., and Aderem, A. (1999). The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature 401, 811–815.

Unterholzner, L., and Bowie, A.G. (2008). The interplay between viruses and innate immune signaling: recent insights and therapeutic opportunities. Biochem Pharmacol 75, 589–602.

van Lint, A.L., Murawski, M.R., Goodbody, R.E., Severa, M., Fitzgerald, K.A., Finberg, R.W., Knipe, D.M., and Kurt-Jones, E.A. (2010). Herpes simplex virus immediate-early ICP0 protein inhibits Toll-like receptor 2-dependent inflammatory responses and NF-kappaB signaling. J Virol 84, 10802–10811.

Vollstedt, S., Franchini, M., Alber, G., Ackermann, M., and Suter, M. (2001). Interleukin-12- and gamma interferon-dependent innate immunity are essential and sufficient for long-term survival of passively immunized mice infected with herpes simplex virus type 1. J Virol 75, 9596–9600.

von Aulock, S., Schroder, N.W., Traub, S., Gueinzius, K., Lorenz, E., Hartung, T., Schumann, R.R., and Hermann, C. (2004). Heterozygous toll-like receptor 2 polymorphism does not affect lipoteichoic acid-induced chemokine and inflammatory responses. Infect Immun 72, 1828–1831.

Waldman, W.J., Williams, M.V., Jr., Lemeshow, S., Binkley, P., Guttridge, D., Kiecolt-Glaser, J.K., Knight, D.A., Ladner, K.J., and Glaser, R. (2008). Epstein-Barr virus-encoded dUTPase

enhances proinflammatory cytokine production by macrophages in contact with endothelial cells: evidence for depression-induced atherosclerotic risk. Brain Behav Immun 22, 215–223.

Wang, J.P., Kurt-Jones, E.A., Shin, O.S., Manchak, M.D., Levin, M.J., and Finberg, R.W. (2005a). Varicella-zoster virus activates inflammatory cytokines in human monocytes and macrophages via Toll-like receptor 2. J Virol 79, 12658–12666.

Wang, X., Huang, D.Y., Huong, S.M., and Huang, E.S. (2005b). Integrin alphavbeta3 is a coreceptor for human cytomegalovirus. Nat Med 11, 515–521.

Weber, F., and Haller, O. (2007). Viral suppression of the interferon system. Biochimie 89, 836–842.

Weber, O. (2000). Novel mouse models for the investigation of experimental drugs with activity against human varicella-zoster virus. Antivir Chem Chemother 11, 283–290.

Weidemann, B., Schletter, J., Dziarski, R., Kusumoto, S., Stelter, F., Rietschel, E.T., Flad, H.D., and Ulmer, A.J. (1997). Specific binding of soluble peptidoglycan and muramyldipeptide to CD14 on human monocytes. Infect Immun 65, 858–864.

Whitley RJ, H.J. (2001). Cercopithecine herpesvirus (B virus). In Fields Virology, H.P. Knipe DM, ed. (Philadelphia, Lippincott Williams and Wilkins), pp. 2835–2848.

Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J., and Mathison, J.C. (1990). CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 249, 1431–1433.

Yang, R.B., Mark, M.R., Gray, A., Huang, A., Xie, M.H., Zhang, M., Goddard, A., Wood, W.I., Gurney, A.L., and Godowski, P.J. (1998). Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. Nature 395, 284–288.

Zarember, K.A., and Godowski, P.J. (2002). Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J Immunol 168, 554–561.

Zhang, D., Zhang, G., Hayden, M.S., Greenblatt, M.B., Bussey, C., Flavell, R.A., and Ghosh, S. (2004). A toll-like receptor that prevents infection by uropathogenic bacteria. Science 303, 1522–1526.

Zhang, G., and Ghosh, S. (2001). Toll-like receptor-mediated NF-kappaB activation: a phylogenetically conserved paradigm in innate immunity. J Clin Invest 107, 13–19.

Zhou, S., Kurt-Jones, E.A., Mandell, L., Cerny, A., Chan, M., Golenbock, D.T., and Finberg, R.W. (2005). MyD88 is critical for the development of innate and adaptive immunity during acute lymphocytic choriomeningitis virus infection. Eur J Immunol 35, 822–830.

Zhu, J., Martinez, J., Huang, X., and Yang, Y. (2007). Innate immunity against vaccinia virus is mediated by TLR2 and requires TLR-independent production of IFN-beta. Blood 109, 619–625.

