Functional analysis of the bovine herpesvirus-1 gene encoding bICP0, a promiscuous trans-activator, that stimulates productive infection and inhibits Interferon (IFN) signaling pathways.

by

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Functional analysis of the bovine herpesvirus-1 gene encoding bICP0, a promiscuous trans-activator, that stimulates productive infection and inhibits Interferon (IFN) signaling pathways.

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University of Nebraska, 2008

Advisor: Dr. Clinton Jones

Virus-host interactions determine the outcome of the infection. Resistance to and recovery from a viral infection depends upon the efficiency by which the immune system inhibits viral spread. Most viruses encode factors to suppress the innate immune response. Bovine Herpesvirus-1 (BHV-1) infects cattle and similar to other alphaherpesvirinae subfamily members, establishes latency in sensory neurons. During acute infection and establishment of latency, BHV-1 viral genes interact with various cellular factors and repress the immune responses. One of the immediate early proteins, bICP0 has been identified as a viral component that suppresses innate immune responses. bICP0 can transactivate all viral promoters, and as such stimulates productive infection. Consequently, I have hypothesized that bICP0 has two functions that are crucial for stimulating productive infection: blocking of interferon signaling and activation of viral transcription. This hypothesis is the basis of my dissertation research.

The zinc RING finger and transactivation domains within bICP0 are believed to contribute significantly to the functions of bICP0. I have demonstrated that the bICP0 C-terminus domains are required for inhibiting IFN-β promoter activation. The C-terminus domains and the zinc RING finger are required for degrading interferon

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regulatory factor 3 (IRF3), a transcription factor involved in Type I IFN expression. The studies also suggest that bICP0 binds to interferon regulatory factor 7 (IRF7) and thus prevents it from activating the IFN-β promoter. Collectively these studies indicate that bICP0 disarms the innate immune response by targeting the transcription factors IRF3 and IRF7. To understand the role of zinc RING finger in productive infection, I generated a bICP0 zinc RING finger mutant BHV-1 virus. The mutant virus was characterized in cultured bovine cells and calves. In cell culture, the mutant virus has delayed growth as compared to the wild type or rescued virus. Similarly in calves, the mutant virus did not replicate as efficiently as the rescued virus and the immune response was also reduced. I believe these studies provide valuable information with respect to understanding BHV-1 pathogenesis, and the role that the zinc RING finger plays in virus-host interactions.

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ABBREVIATIONS

BHV-1	Bovine herpesvirus-1
bICP0	Bovine infected cell protein
bp	base pair
CAT	Chloramphenicol acetyl transferase
CBP	CREB binding protein
DEX	Dexamethasone
Dpi	days post infection
E	early
EBV	Epstein Barr virus
g	glycoprotein
GFP	Green fluorescence protein
h	Hour
HDAC	Histone deacetylase
h.p.i	hours post infection
HSV-1	herpes simplex virus
IETu	immediate early transcription unit
IFN-β	Interferon-beta
IRF7	Interferon regulatory factor 7
IRF3	Interferon regulatory factor 3
LAT	latency associated transcript
LR	latency related
NLS	Nuclear localization signal
TBK1	TANK binding kinase 1
RING	really interesting gene
Wt	wild type
13g/51g	13/51 glycine

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CHAPTER I

Literature Review

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I. Introduction

Viruses belonging to the *alphaherpesvirinae* subfamily of the *Herpesviridae* family are well known for establishment of a persistent or latent infection in sensory neurons of the respective hosts. The *Herpesviridae* family members infect a wide range of vertebrate hosts, including eight human viruses, several types in horses, cattle, mice, pigs, chickens, turtles, lizards or fish. The neurotropic *alphaherpesvirinae* subfamily members have a short replication cycle and efficiently destroy infected cells. Herpes simplex virus type -1 and 2 (HSV-1 and 2) cause ocular, facial and genital lesions while Varicella zoster virus (VZV) causes chicken pox and shingles. *Betaherpesvirinae* and *Gammaherpesvirinae* members are lymphotropic, exhibit a restricted host range and have a long replication cycle. Human cytomegalovirus (HCMV), Human Herpesvirus (HHV-6 and 8), Epstein-Barr virus (EBV) and Kaposi's sarcoma herpesvirus (KSHV) are members of the above mentioned subfamilies.

BHV-1, a member of *alphaherpesvirinae* subfamily, causes Infectious bovine rhinotracheitis (IBR). IBR is a respiratory disease of feeder cattle that causes significant loss to the cattle industry every year. The causative agent was initially identified as Bovine Herpes Virus type–1 (BHV-1/BoHV-1) in 1928 by Reisinger and Reimann (Muylkens et al., 2007e). The common definition of this agent is given as "A species of VARICELLOVIRUS that causes INFECTIOUS BOVINE RHINOTRACHEITIS and other associated syndromes in cattle". Cattle trading between countries lead to rapid spread of IBR in Europe and North America (Edwards et al., 1990). The clinical signs of the disease vary depending on the type of animal tissue infected by the virus. These symptoms are categorized as (a) respiratory tract infections, (b) eye infections, (c) abortions, (d) genital infections, (e) brain infections or (f) generalized infections of newborn calves. The respiratory tract infection is localized to the upper respiratory tract,

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nose or throat for example. Ocular infections and respiratory infections typically occur in the same animal. Abortion occurs when the uterus of the susceptible animal is invaded by the circulating virus in the blood stream, it then infects the fetal blood system and the fetus (Chow et al., 1964). Genital infections often lead to a disease referred to as "Infectious Pustular Vulvovaginitis" (IPV). IBR brain infections, although rare, can lead to seizures, mental depression, coma and eventually death. The ability of BHV-1 to cause immunosuppression can initiate severe IBR by promoting secondary opportunistic bacterial infections. This disorder is frequently referred to as bovine respiratory disorder or "Shipping fever".

BHV-1 is among all the other eight herpesviruses that are isolated naturally from cattle, for example BHV-2, BHV-4, or BHV- 5 also infect cattle. Based on antigenic and genomic analyses, BHV-1 is classified into three subtypes: BHV-1.1, BHV-1.2a and BHV-1.2b. Differences between BHV-1.1 and BHV-1.2 are restricted to distinct genomic regions that are characterized by loss or gain of restriction sites (Engels et al., 1986). BHV-1.1 is referred as the classical virus causing IBR. BHV-1.2a is prevalent in Brazil whereas BHV-1.2b is less pathogenic and is predominantly found in Australia and Europe. Comparative analysis of both subtypes BHV-1.1 and BHV-1.2a demonstrated that both subtypes were able to induce clinically undistinguishable respiratory disease in calves, either subsequent to a primary infection or following reactivation (Spilki et al., 2004). BHV-1.2b is associated with respiratory disease and IPV, but does not usually cause abortion (Van Oirschot, 1995;D'Arce et al., 2002).

BHV-1 is not the sole infectious agent for "Shipping Fever" (Tikoo et al., 1995) because it immunosuppresses the infected animal leading to secondary infections. *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus* are bacterial strains typically associated with secondary bacterial pneumonia. The symptoms include

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high fever, anorexia, coughing, excessive salivation, nasal discharge and conjunctivitis. Acute infection is followed by latent infection during which there are no visible clinical signs. Virus reactivates from latency during stress or immunosuppression (Jones, 2003c). Antimicrobial therapy prevents the secondary pneumonia but eradication of the virus is a challenge due to establishment of latency. Modified live or inactivated vaccines are used for repeated vaccination or immunization but do not prevent BHV-1 infection and establishment of latency (Muylkens et al., 2007). Many of the modified live vaccines can be pathogenic in young calves, and cause immunosuppression.

BHV-1 virion morphology is similar to other members of the *Herpesviridae* family. The virion can be divided into four parts: (i) envelope, (ii) tegument, (iii) nucleocapsid and, (iv) core. Virions are spherical or pleomorphic in nature varying from 150-200 nm in diameter. The capsid is surrounded by an electron-dense zone of tegument and a lipid bilayer envelope (Valicek and Smid, 1976). The nucleocapsid has icosahedral symmetry (T=16) and consists of 162 capsomeres (Armstrong et al., 1961;Babiuk et al., 1996). The BHV-1 genome is a linear, 135.3 kb long double stranded DNA molecule (Muylkens et al., 2007). The genome arrangement is a typical Class D Herpesvirus, with the genome divided into two segments: a unique long (U_L) sequence and a unique short (U_S) sequence. U_L is approximately 102 kb long whereas U_S is approximately 11 kb, and is flanked on both sides by inverted internal (IR) and terminal repeats (TR). The U_L and U_S regions can flip-flop relative to each other, which gives rise to four isomeric forms during DNA replication (Schynts et al., 2003). The genome has a high G + C content of 72% (Plummer et al., 1969). The BHV-1 genome was completely sequenced by using DNA fragments from Cooper, p8-2, 34 and Jura strains (Meyer et al., 1997). The Genbank Accession number is AJ004801. The nomenclature for BHV-1 genes is similar to HSV-1 genes (Manservigi and Cassai,

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1991). BHV-1 contains 73 open reading frames (ORFs) that are homologous to genes found in other *alphaherpesviruses*, hence they are referred to the related genes of HSV-1 (Muylkens et al., 2007b).

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Ia. Productive Infection

Based upon their expression during productive infection, BHV-1 genes are classified temporally into three classes: (i) Immediate early (IE), (ii) Early (E) or (iii) Late (L) genes. The IE phase genes are expressed first and do not require newly synthesized proteins. The E gene expression begins as early as 2 hours post infection (h.p.i) and requires protein synthesis. During the early phase, the viral proteins involved in DNA replication are synthesized. The late genes encode structural proteins and generally are expressed after DNA replication. Assembly of virions also occurs during the late phase of productive infection (Fraefel et al., 1993). Components of virions (αTIF) entering the host cell trigger the IE phase of the next cycle of viral infection. The IE1 promoter is stimulated by the αTIF protein that is encoded by the late UL48 gene (Misra et al., 1994b). IE RNAs are expressed from two transcriptional units, IEtu1 and IEtu2. IEtu1 encodes two proteins, infected cell protein 0 (bICP0), and infected cell protein 4 (bICP4) whereas IEtu2 encodes a protein bICP22, which is similar to the HSV-1 IE protein ICP22 (Jones, 2003c). IE proteins activate E gene expression, and viral DNA replication ensues. Expression of certain IE proteins like bICP0 and bICP4 is essential for late gene transcription. The other immediate early proteins are bICP4-another regulatory factor in viral replication, circ- myristylated virion component and bICP22. bICP22 trans-represses viral promoters and is transcriptionally regulated by bICP0. The tegument protein VP22 (BVP22) enhances biotherapeutic potential in tumor gene therapy by altering the cell cycle, making cells more sensitive to therapeutic genes (Qiu et al., 2005). bICP27, an early protein in contrast to HSV-1 ICP27 which is an immediate early protein, is involved in regulation of structural genes, the glycoprotein gC (Hamel and Simard, 2003).

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Early genes that encode nonstructural proteins are located in a region of 10.5 kb (map position 0.332 to 0.410 of BHV-1 genome) that contains three open reading frames (ORFs) homologous to herpes simplex virus DNA polymerase catalytic subunit (DNApol, UL30), major DNA-binding protein (MDBP, UL29) and ICP18.5 assembly protein (ICP18.5, UL28) (Meyer *et al.*, 1997).

BHV-1 encodes at least 33 structural proteins. Among these, eight are known glycoproteins- gB, gC, gD, gE, gH, gI, gK and gL. gC is the major glycoprotein found on surface of infected cells and virion envelope. gC is one the major target for the innate and cellular immune response (Gupta et al., 2001). BHV-1 encodes at least 10 glycoproteins that are similar to those expressed by HSV-1 (Schwyzer and Ackermann, 1996). Productive infection of the permissive cells is initiated by low affinity binding of viral structural proteins gB/gC to heparin sulfate sugar moieties (Okazaki et al., 1994;Li et al., 1995), which facilitates stable binding of gD to cellular receptors. Next, gD, gB and a heterodimer of gH and gL aid in fusion of the virion envelope with the plasma membrane (Meyer et al., 2001). Fusion with the host cell membrane leads to release of the nucleocapsid into the cytoplasm, from where the virions are transported to nuclear pores by associating with microtubules. During transport to the nucleus, the virion sheds its tegument proteins that are the first viral proteins exposed to the intracellular environment. The tegument proteins, bTIF (VP16) and VP8 are crucial for initiation of transcription of IE genes. The UL41 gene encoded viral host shut off (vhs) protein causes shut off of host cell protein synthesis. VP16 or α-TIF (transducing factor of alpha genes) and cellular proteins transactivate IE promoter by binding to a TAATGAGCT motif leading to expression of bICP0, bICP4 and bICP22 which in turn initiate the cascade of early and late viral gene expression (Wirth et al., 1992e;Misra et al., 1994a;Saydam et al., 2004).

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BHV-1 genome circularizes following entry into the nucleus (Fraefel, Wirth et al., 1993b). The alphahervesviruses genomes, for example, HSV-1 genome replicate by Theta (θ) replication using the circular genome as the template (Thiry et al., 2006). The replication begins at the origin of replication (*ori*), BHV-1 is suggested to have two *ori* within the inverted repeats of Us (Chew et al., 2005). Replication of the circular genome by the θ and rolling circle modes of replication results in a concatemer that consists of newly synthesized viral genomes fused together in a head-to-tail arrangement (Jacob et al., 1979). Leaky late gene expression is initiated during the replication process. After replication, true late genes are expressed (Muylkens et al., 2007). Structural proteins are assembled together like a scaffold to form an intermediate particle within the nucleus. On maturation, the inner scaffold is displaced with the viral genome generating mature virions. Although many models have been proposed for the egress of the mature capsids, the widely accepted model is proposed to occur in three-steps (Mettenleiter, 2006). During the release from the nucleus, the budding capsid acquires inner nuclear membrane as a primary envelope, which is then fused with the outer nuclear membrane and then the particle enters the cytoplasm. This de-envelopement requires viral U_S3 kinase activity (Reynolds et al., 2002). In the last step, the virions acquire the tegument and the secondary envelope by budding from the *trans*-golgi compartment (Mettenleiter and Minson, 2006). Other models propose transport through the endoplasmic reticulum and dilation of nuclear pores creating direct contact between virions and cytoplasm (Leuzinger et al., 2005) followed by budding of naked capsids from the cell membrane.

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Ib. Latency

Following productive infection, alpha-herpesviruses establish lifelong latent or persistent infection in sensory neurons of the trigeminal ganglia (TG) (Jiang et al., 1998). BHV-1 establishes latency primarily in ganglionic neurons because BHV-1 DNA can readily be detected in TG of latently infected cattle following ocular infection (Ackermann et al., 1982). Also, BHV-1 DNA can be detected within the germinal centers of pharyngeal tonsils (Winkler et al., 2000a). Establishment of latent infection occurs soon after initial replication in the mucosal epithelium of the peripheral nervous system. Viral replication occurs within ganglia for approximately one week (Knotts et al., 1974) after infection. The latency-reactivation cycle occurs in a stepwise manner with establishment occurring first, followed by maintenance and reactivation from latency. During establishment, viral gene expression is repressed except for the latency-related (LR) gene. LR gene product represses viral gene expression and is the only transcript that is abundantly expressed in the TG during maintenance of latency(Rock et al., 1987). bICP22 may also aid in establishment of latent infection by repressing certain BHV-1 promoters (Koppel et al., 1997). Reactivation from latency is triggered by stress or immunosuppression. In animal models, treatment with dexamethasone (DEX) can induce reactivation (Tanaka et al., 1994). Transcripts from ICP4, ICP0, ICP27 and TK were detected 24 h after DEX treatment (Minagawa et al., 1994). BHV-1 from latently infected rabbits or cattle can be reactivated by DEX (Rock et al., 1992). Reactivation can also be induced by administration of glucocorticoids or corticosteroids (DEX) (Hofmann et al., 1986). Lytic or productive infection of permissive cells or calves leads to apoptosis. During the onset of latency, lytic viral gene expression is inhibited and there is an induction of an anti-apoptotic state mediated by the abundantly expressed LR transcript (Lovato et al., 2003;Henderson et al., 2004).

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bICP0

bICP0 is encoded by the IE transcription unit 1 (IEtu1). It is expressed constitutively during productive infection because it contains IE and E promoters (Fraefel et al., 1994). Originally identified as a transactivator protein and referred as p135, bICP0 encodes a 676 aa long open reading frame that is present in the spliced IER2.9 and IER2.6 RNAs. The bICP0 protein contains a cysteine-rich zinc RING finger domain at the N-terminus, which is similar to the HSV-1 immediate early protein ICP0. The C3H4 zinc RING finger domain is the only conserved region between ICP0 and bICP0 (Figure 1.2c). Similar to bICP0, other members of the family encode functional homologs of ICP0, for example, p61 of varicella-zoster virus, or early protein 0 (EICP0) of equine herpes virus. These proteins transactivate all classes of viral genes and contain C3H4 zinc RING finger (Bowles et al., 1997). bICP0 is antisense to the latency-related (LR) transcript and the sequence is conserved within different strains of BHV-1 (K22 and Jura) (Wirth et al., 1992d). bICP0 is a 97 kDa nuclear protein in the infected cells (Fraefel et al., 1994).

Like HSV-1 ICP0, bICP0 also colocalizes with nuclear bodies called "proto-oncogene promyelocytic leukemia protein-containing nuclear domains" (ND10 or PODS) (Inman et al., 2001) .The activity of bICP0 is zinc-dependent and is inhibited in the presence of thionein, a chelator for zinc (Fraefel et al., 1994). The functional domains of bICP0 were identified by using transposon (Zhang et al., 2005). It contains three major functional domains: two from aa 78-256 and one at aa 457, which are essential for transactivating viral promoters. bICP0 has a nuclear localization signal (NLS) at its C-terminus spanning from aa 607-676. The sequence for the NLS is KRRR (622-625), which resembles other monopartite NLS signals. The NLS core sequence of HSV-1 ICP0 is PRLRR (Paterson and Everett, 1988). A bICP0 mutant lacking the NLS

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is not targeted to nucleus in transiently transfected cells, and has reduced transactivation potential (Inman et al., 2001; Zhang et al., 2005). Another C-terminus deletion mutant Δ*NcoI* demonstrated that nuclear localization was essential for the activation of a minimal thymidine kinase promoter (Zhang et al., 2005). The domain from aa 280-330 is the acidic domain, which is rich in asparatic, glutamic acid, serine or threonine residues. This domain is not present in HSV-1 ICP0. Although the subcellular localization of transposon mutants within the acidic domain was different than the wild type bICP0, there was no obvious effect either on protein stability or transactivation of the TK promoter. This region might be required for activating certain promoters or in certain cell types (Zhang et al., 2005). The zinc RING finger domain is crucial for viral transcription and productive infection. Expression of full-length bICP0 protein in the absence of other viral genes can be toxic to transiently transfected mouse neuroblastoma cells (neuro-2A) (Inman et al., 2001b). bICP0 is predicted to be a ubiquitin (Ub) E3 ligase because of the presence of the zinc RING finger domain. The zinc RING finger domain plays an important role in protein degradation pathways.

In general, ubiquitination is a marker for proteasome-mediated protein degradation. Ubiquitination also regulates DNA repair (Ulrich and Jentsch, 2000), lysosomal targeting (Dunn and Hicke, 2001), nuclear export (Lohrum et al., 2001), gene transcription (Salghetti et al., 2001) and trafficking of membrane proteins. Ubiquitination of a target protein is achieved by sequential action of three proteins: Ub activating enzyme (E1), Ub conjugating enzyme (E2) and Ub ligase (E3). Identification of E3s was difficult as they constitute a group of proteins with diverse size and domain structure. Several E3 proteins in yeast, plants, viruses and mammals have been identified and characterized (Barry and Fruh, 2006). E3 ligases determine the specificity for the substrate and covalently link the Ub chain to the lysine (Lys) residue of the substrate

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protein. If the target protein is linked to the Ub chain through the Lys48 residue, it is targeted for proteasomal degradation. If linked through a Lys63 residue, it participates in other cellular functions (Elsasser and Finley, 2005). E3 Ubiquitin ligases primarily regulate polyubiquitination of the target protein for degradation. In some cases, ubiquitation and degradation of a target protein (IκB subunit) can lead to its activation (NFκB) (Karin and Ben Neriah, 2000).

E3s are categorized into **HECT-E3** (*Homologous to E6-associated protein C-terminus*) and **RING-E3** (*Really interesting new gene*) ligases. Cysteines (Cys) within HECT E3 form thiol-ester bond with the Ub chain and are directly involved in catalysis of ubiquitination. A RING finger is typically represented as a consensus sequence of zinc binding Cys and His arranged in the following manner: **CysX2CysX(9-39)CysX(1-3)HisX(2-3)Cys/HisX2CysX(4-48)CysX2Cys** (Figure. 1.2b). The eight conserved cysteines and histidines coordinate two Zn^{2+} ions in a "cross-braced" fashion (Lovering et al., 1993;Freemont, 1993). Based on the residue (Cys/ His) occupying the fifth coordination site, RING fingers are further divided into RING-H2 and RING-HC. Unlike HECT-E3, RING-E3 facilitates target protein ubiquitination by bringing substrate and E2 in close proximity (Ardley and Robinson, 2005). The discovery of various plant and yeast N-end rule E3's containing this domain lead to linking of RING finger domain with ubiquitination (Potuschak et al., 1998). For example, yeast N-end rule E3, ubr1 depends on its RING finger for ubiquitinating activity, Rbx1 of SCF E3 complex and cdc53/culin-1 is essential for ubiquitoination *in vitro* (Joazeiro and Weissman, 2000). MDM2 uses its C-terminus RING finger for p53 degradation (Honda and Yasuda, 2000;Fang et al., 2000). Unlike HECT E3's, RING finger E3s are believed to mediated direct transfer of Ub from E2s to the target protein. The RING finger

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containing proteins are regulated by phosphorylation. For example, phosphorylation of MDM2 inhibits p53 binding (Fuchs et al., 1998).

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III. Innate Immune responses:

Virus infection triggers the host immune system and, in general viruses encode genes that help the virus to evade immune detection. Following entry into the host cell, the viral genome is transcribed, translated and followed by assembly and release of new virions. The host cell responds by recognizing the entry of virus, and initiates actions to eliminate the virus quickly and efficiently. The elimination process occurs in a stepwise manner that requires signals from the infected cell as well as the immune system. The initial response to viral infection is provided by the innate immune system, which is followed by the adaptive immune responses. The innate immune response is the first line of defense against invading pathogens. Antiviral cytokines like interferons (IFNs) are crucial for innate immunity, and also stimulate development of adaptive immunity to pathogens (Le Bon and Tough, 2002). Secretion of IFN-α/β is initiated when viral components are recognized by the host cell. Viral components associated with events like entry, genome replication, transcription, translation or assembly of virions is detected by host pattern-recognition receptors (PRRs). These viral components are referred as pathogen-associated molecular patterns (PAMPs) (Janeway, Jr. and Medzhitov, 2002). In general, PAMPs can be genomic DNA, double-stranded RNA (dsRNA), single-stranded RNA generated during viral replication, lipoproteins or viral envelope proteins. Recognition of the PAMPs activates various signaling pathways that lead to stimulation of immune cells and production of cytokines (Akira, 2006). The PRRs are of two kinds: membrane-bound or cytoplasmic.

Toll-like receptors (TLRs) are the key membrane-bound PRRs that recognize conserved molecular motifs found in bacteria, fungi, protozoa, or viruses (Takeda et al., 2003). The TLR family has 13 members with each recognizing a different PAMP and initiating a unique signaling pathway (Akira, 2006). TLR-1, 2, 4, 5 and 6 detect

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microbial peptidoglycans, lipopolysaccharides or lipoproteins. TLR-3, 7, 8 and 9 recognize distinct pathogen specific nucleic acids like dsRNA, ssRNA and CpG DNA respectively. TLR-7/8 recognize guanosine or uridine rich ssRNA from HIV, VSV, and influenza viruses (Heil et al., 2004). TLR-3, 7, 8 and 9 are expressed on endosomes whereas TLR-2 and 4 are expressed on the cell surface.

TLRs mediate signaling through the interaction of their Toll/IL-1 R (TIR) domain with intracellular adaptors. These adaptors include MyD88 (myeloid differentiation factor 88), TRIF (TIR domain-containing adaptor-inducing IFN-β), TRAM (TRIF related adaptor molecule) or TIRAP/MAL (TIR domain-containing adapter protein). Interaction of TIR domains with any of these adaptors stimulates a specific downstream signaling pathway. Except for TLR3, which triggers a TRIF-dependent pathway, all other TLRs mediate signaling through a MyD88-dependent pathway (Takeda and Akira, 2005). These signaling pathways culminate in the activation of transcription factors: NF-κB, interferon regulatory factors (IRFs) and ATF/c-Jun for example. These transcription factors cooperate to activate IFN-α/β promoters (Seth et al., 2006b). MyD88 recruits IL-1R-associated kinases (IRAKs) to form a complex (TLR-MyD88-IRAK4/1) along with TRAF6. IRAK-1 and TRAF6 dissociate from the complex and interact with another complex of TAK1 (Transforming growth factor-beta-activated kinase-1), TAB1 and TAB2 (TAK1–binding protein). This interaction leads to activation of canonical IκB kinases (IKKs). The IKK kinases cause phosphorylation and subsequent degradation of IκB which releases NF-κB transporting it into nucleus (Akira, 2006). TAK1 also activates c-Jun terminal kinases and p38 which in turn activates ATF-c-Jun (Akira S, 2004). The TRIF-mediated signaling also leads to activation of IRF3 and NF-κB. TRIF interacts with TRAF-associated NF-κB activator (TANK) binding kinase 1 (TBK1) and the noncanonical kinase IKKi/IKKε that

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