

Differential Regulation of NF- κ B Signaling during Human Cytomegalovirus Infection

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NF- κ B transcription factors are key regulators of immune and stress responses, apoptosis, and differentiation. Human cytomegalovirus (HCMV) activates or represses NF- κ B signaling at different times during infection. An initial increase in NF- κ B activity occurs within a few hours of infection. The virus appears to adapt to this change since initial viral gene expression is promoted by the elevated NF- κ B activity. Because NF- κ B upregulates innate immune responses and inflammation, it has also been suggested that HCMV needs to downregulate NF- κ B signaling. Recent studies have shown that HCMV has various mechanisms that inhibit NF- κ B signaling. HCMV reduces cell surface expression of tumor necrosis factor receptor 1 (TNFR1) and blocks the DNA binding activity of NF- κ B. Furthermore, some HCMV tegument proteins antagonize NF- κ B activation by targeting the key components of NF- κ B signaling at late stages of infection. In this review, we summarize the recent findings on the relationship between HCMV and NF- κ B signaling, focusing, in particular, on the viral mechanisms by which the NF- κ B signaling pathway is inhibited.

Key Words: Cytomegalovirus, NF- κ B, Immune response, Tegument protein

INTRODUCTION

Human cytomegalovirus (HCMV), a member of the β -herpesvirus subfamily, contains a large double-stranded DNA genome of approximately 235 kb. Infection of healthy individuals by HCMV is usually asymptomatic, but infection of immunocompromised individuals often causes severe or fatal disease. The viral particle is composed of an icosahedral capsid that encloses the genome. A structural feature unique to herpesviruses is the presence of a protein layer, called the tegument, between the capsid and the envelope. Upon initial fusion of the viral envelope with the host cell

plasma membrane, many of these tegument proteins are delivered into the cytoplasm, where they perform diverse functions that facilitate viral replication. The tegument functions include activation of viral gene transcription and antagonization of the host innate immunity (1). During productive infection, HCMV gene expression occurs in a three-step sequential fashion with immediate-early (IE), early, and late kinetics. IE1 and IE2 proteins play a pivotal role in regulating the expression of viral early and late genes as well as cellular genes (2). Viral early and late proteins mediate viral DNA replication and assembly and maturation of virion particles.

The NF- κ B transcription factors are critical regulators of

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a host cell's early response to viral infection (3). A variety of inflammatory events, including viral infection and exposure to inflammatory molecules, can induce NF- κ B's transcriptional activity, which subsequently drives expression of a number of different proinflammatory cytokines and chemokines. Upon binding of tumor necrosis factor alpha (TNF α) to its receptor, the canonical NF- κ B pathway is initiated. Activation of this pathway requires recruitment of adaptor molecules, such as TNF receptor type 1-associated death domain protein (TRADD), TNF receptor (TNFR)-associated factor 2 (TRAF2), Fas-associated death domain protein (FADD), and receptor-interacting protein kinase 1 (RIP1), which leads to an increase in polyubiquitination of NF- κ B essential modifier (NEMO) and subsequent assembly and activation of the I κ B kinase (IKK) complex. Activated IKK then phosphorylates I κ B α , resulting in its ubiquitin- and proteasome-dependent degradation. In the absence of I κ B α , the p65 and p50 NF- κ B subunits translocate into the nucleus to induce target gene expression. Non-canonical NF- κ B signaling occurs upon IKK activation by NF- κ B-inducing kinase (NIK) and induces nuclear translocation of the NF- κ B p52-RelB dimers. The nature of the transcriptional response induced by these different NF- κ B pathways varies depending on the subunit composition and posttranslational modifications of the specific NF- κ B dimers that are activated (3).

Upregulation of NF- κ B signaling by HCMV

(1) NF- κ B activation during virus entry and the early phase of infection

Several reports have shown that at early times after infection, HCMV activates the key components of both the interleukin-1 β (IL-1 β) and TNF α signaling pathways, including NF- κ B (4~8) and various mitogen-activated protein kinases (MAPKs) (9, 10). This activation occurs in two distinct phases. The initial activation is mediated by virus attachment and entry. It has been demonstrated that HCMV envelope glycoprotein B (gB) and glycoprotein H (gH) bind to cellular Toll-like receptor 2 (TLR2). This interaction induces recruitment of adaptor proteins including Toll-interleukin 1 receptor (TIR) domain-containing adaptor

protein (TIRAP), also termed myeloid differentiation primary response 88 (MyD88) adaptor-like (MAL), and MyD88. Downstream recruitment of TRAF6, a ubiquitin E3 ligase, leads to the activation of NF- κ B by the IKK complex, resulting in the secretion of proinflammatory cytokines, such as TNF α and IL-1 β (7).

The second phase of activation is mediated by viral IE1 protein. IE1 upregulates NF- κ B through the induction of the cellular transcription factor Sp1, which activates the NF- κ B p65 and p105/p50 promoters (8, 11). IE1 also selectively induces the formation of the nuclear RelB-p50 NF- κ B complex through Jun kinase and c-Jun/Fra-2 AP-1 in vascular smooth muscle cells (12, 13). HCMV appears to have adapted to these activation events, since NF- κ B activity is considered beneficial for initial viral gene expression. Activation of IL-1 β and TNF α signaling, which increases the secretion of chemokines, is thought to facilitate virus dissemination by recruiting HCMV susceptible cells (14~18).

(2) Activation mechanisms observed in clinical strains

The HCMV clinical isolates have a long 15 kb unique region termed UL/b'. Two of the UL/b' region gene products, UL144 and UL138, upregulate the TNF α -mediated NF- κ B signaling (19, 20). UL138, which is expressed during latent infection, modulates the cell surface expression of TNFR1, suggesting that TNF signaling may affect HCMV latency (20, 21). UL144, which is expressed early in lytic infection, is a transmembrane glycoprotein with a short intracellular cytoplasmic tail. UL144 upregulates chemokine (C-C motif) ligand 22 (CCL22) via the activation of NF- κ B in a TRAF6-dependent manner. CCL22 is a chemoattractant for T helper 2 (Th2) and regulatory T (Treg) cells. Therefore, upregulation of NF- κ B signaling by UL144 may be a viral strategy to reduce Th1-mediated immune responses (19).

HCMV functions that inhibit NF- κ B signaling

There is increasing evidence that HCMV can also inhibit NF- κ B signaling during lytic infection. This negative regulation may be necessary to suppress excessive immune responses that are detrimental to viral infection. Although a viral function in downregulating NF- κ B signaling at the early stage of infection has been found, it is believed that the

viral late functions are critical to suppress NF- κ B signaling.

(1) IE2 (UL122)

HCMV IE2 acts as a strong transactivator of viral and cellular genes, as a repressor of its own major IE (MIE) promoter, and as a regulator of cell cycle progression. IE2 is mainly localized in the nucleus and interacts with numerous cellular proteins (1). IE2 inhibits the expression of interferon beta (IFN- β) and proinflammatory cytokines. The efficient induction of IFN- β during HCMV infection requires the activation of both interferon regulatory factor 3 (IRF3) and NF- κ B pathways. Although IE2 does not affect IRF3 activation, it inhibits virus-induced binding of NF- κ B to the IFN- β promoter, resulting in the attenuation of gene expression that is dependent on IFN- β and NF- κ B (22).

(2) pp65 (UL83)

The phosphoprotein pp65 is encoded by UL83 and is the most abundant tegument protein. DNA microarray data have shown that many cellular RNAs are elevated to a greater extent in response to UL83-deficient virus than to wild-type virus, indicating that pp65 is required to suppress the induction of numerous cellular RNAs early in infection (23, 24). These RNAs include those expressed from the IFN-responsive genes and the proinflammatory cytokine genes. The mechanism by which pp65 inhibits the production of IFNs is controversial. One study demonstrated that pp65 inhibits IRF1 and NF- κ B activity (23), while another study suggested that pp65 inhibits IRF3 activation (24). It is likely that tegument pp65 rapidly moves to the nucleus after it is delivered to infected cells and suppresses excessive antiviral cellular gene expression at very early times after infection. However, the mechanism by which pp65 inhibits the activation of NF- κ B needs to be further investigated.

(3) UL26

UL26 is an early viral protein (25) that initially localizes to the nucleus. As the infection progresses, UL26 becomes cytoplasmic and eventually localizes to virion assembly sites (26). The mechanism by which UL26 contributes to HCMV replication is largely unknown. It has recently been reported that UL26 is able to antagonize NF- κ B activation. Infection with UL26-deficient virus fails to block the canonical NF- κ B activation induced by TNF α , and the expression

of UL26 in the absence of other viral proteins is sufficient to block TNF α -induced NF- κ B activation. Therefore, UL26 appears to attenuate IKK phosphorylation and subsequent I κ B α degradation (27).

(4) Other viral functions

It has been shown that HCMV infection prevents external signaling to the cell by disrupting the function of TNFR1. HCMV infection of monocytic cell lines and U373 cells results in a reduction of cell surface expression of TNFR1. This reduction may result from relocalization of TNFR1 from the cell surface and inhibition of TNF α -induced Jun kinase activation. It is likely that the viral functions responsible for TNFR1 relocalization are associated with viral IE or early gene expression. However, infection with a recombinant HCMV, which contained a deletion of the viral genes involved in downregulation of major histocompatibility complex (MHC) class I (US2 to US11), still resulted in relocalization of TNFR1 (28).

Recent studies have suggested that the viral gene products expressed at late phases of infection are involved in the inhibition of the IL-1 β - and TNF α -induced NF- κ B signaling (29, 30). These inhibitory effects correspond to impaired IL-1 β - and TNF α -induced I κ B α phosphorylation and NF- κ B activation, and decreased expression of multiple inflammatory chemokines. HCMV infection has a different effect on the IL-1 β signaling pathway than on the TNF α signaling pathway, with a greater effect on I κ B α phosphorylation and NF- κ B activation observed for the IL-1 β signaling pathway, suggesting that HCMV targets the cellular components upstream of the IKK complex, the convergence point of two pathways (29). Since HCMV suppression of the IL-1 β and TNF α signaling pathways occurs at late stages of infection, the ability of HCMV to regulate these pathways may represent a critical viral adaptation for persistence infection within the host.

CLOSING REMARKS

The NF- κ B signaling pathway is critical for innate immunity and defense against viruses. Recent studies have shown that HCMV upregulates or downregulates NF- κ B

Table 1. Summary of the HCMV functions that regulate NF- κ B signaling

	Viral gene products	Cellular target	Mechanism	References
<i>Upregulation</i>	gB (UL55), gH (UL75)	TLR2	Induces recruitment of adaptor molecules	7
	IE1 (UL123)	Sp1	Activates the p65 and p105/p50 promoter	8, 11
		Jun kinase, AP-1	Induces the RelB-p50 complex	12, 13
	UL138	TNFR1	Increase TNFR1 surface expression	20, 21
	UL144	TRAF6	Increases CCL22 expression	19
<i>Downregulation</i>	IE2 (UL122)	NF- κ B	Reduces DNA binding of NF- κ B to the IFN promoter	22
	pp65 (UL83)	IRFs, NF- κ B	Reduces IFN- β production	23, 24
	UL26	Unknown	Inhibits IKK phosphorylation	27
	Unknown (early protein)	TNFR1	Reduces TNFR1 surface expression	28
	Unknown (late protein)	Unknown	Inhibits TNF α - and IL-1 β -mediated NF- κ B activation	29, 30

signaling at different times of infection (Table 1). Initial activation of NF- κ B signaling is observed during the early phase of infection. Interestingly, the virus appears to have adapted to this change since initial viral gene expression is promoted by the elevated NF- κ B activity. The upregulation of NF- κ B signaling may also help virus dissemination. However, because NF- κ B activation leads to antiviral immune responses and inflammation, it is not surprising that HCMV has also developed strategies to downregulate NF- κ B signaling. Indeed, evidence is accumulating that HCMV inhibits NF- κ B signaling at late stages of infection. The viral gene products responsible for this late inhibition of NF- κ B signaling have yet to be discovered. Certainly, further information on the interplay between virus and NF- κ B signaling will be necessary to understand the pathogenesis of HCMV.

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