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Recent advances on viral manipulation of NF- κ B signaling pathway

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Abstract

NF- κ B transcription factors regulate the expression of hundreds of genes primarily involved in immune responses. Signaling events leading to NF- κ B activation constitute a major antiviral immune pathway. To replicate and persist within their hosts, viruses have evolved diverse strategies to evade and exploit cellular NF- κ B immune signaling cascades for their benefit. We summarize recent studies concerning viral manipulation of the NF- κ B signaling pathway downstream of pattern recognition receptors. Signal transduction mediated by pattern recognition receptors is a research frontier for both infectious disease and innate immunology.

1. Introduction

NF- κ B proteins constitute a family of transcription factors (TFs) sharing Rel homology domain that regulate the transcription of genes downstream of promoters containing κ B sequences [1–3]. The NF- κ B-responsive genes encode a large array of effectors in the host antiviral defense system. Upon viral infection, cells initiate signaling events that activate NF- κ B TFs, either via pattern recognition receptors (PRRs) or in response to entry processes per se. Thus, cellular NF- κ B activation imposes selective pressure on viruses. Not surprisingly, numerous viruses modulate key components of the NF- κ B pathway to evade antiviral responses or, even to promote viral infection. The classic examples of NF- κ B activation by viral oncogenic proteins, such as Kaposi's sarcoma-associated herpesvirus (KSHV) vFLIP [4], Epstein-Barr virus (EBV) LMP1 [5] and human T cell leukemia virus (HTLV) Tax proteins [6], have been extensively reviewed elsewhere [7]. In this review, we will summarize recent studies concerning NF- κ B manipulation in the context of PRRs that represent a research frontier in infectious disease. To provide a view integrating viral modulation with host regulation of NF- κ B activation, we review viral factors according to the targeted component of the NF- κ B signaling pathway.

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2. NF- κ B activation by PRRs

In resting cells, NF- κ B TFs are kept as an inactive dimer by inhibitors of NF- κ B (I κ Bs) in the cytoplasm [8]. Upon viral infection, PRRs, such as Toll-like receptors (TLRs), RIG-like receptors (RLRs) and DNA sensors, recognize pathogen-associated molecular pattern (PAMPs). When bound to cognate PAMPs, TLR3 can engage TRIF, while the other TLRs signal through myeloid differentiation primary response gene 88 (MyD88) [9]. RIG-I-like receptors, e.g., retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5 (MDA-5), signal via mitochondria antiviral-signaling protein (MAVS) [10–13]. These adaptor molecules, in turn, recruit TNF receptor-associated factor (TRAF) and transforming growth factor β -activated kinase 1 (TAK1) [14]. TRAF, e.g., TRAF6, catalyzes the synthesis of K63-linked polyubiquitin chains that recruit and activate the IKK kinase complex, comprising IKK α , IKK β and IKK γ (also known as NEMO) [15]. Activated IKK phosphorylates I κ Bs, e.g., I κ B α , which primes I κ Bs for ubiquitination by SCF $^{\beta}$ -TrCP complex and subsequent degradation by the 26S proteasome [16,17]. Destruction of I κ Bs unleashes the NF- κ B dimer (primarily consisting of p65 [also known as RelA] and p50) which then translocates into the nucleus to up-regulate the expression of hundreds of genes including an array of inflammatory cytokines that constitute a robust antiviral innate immune response (Figure 1). Recent studies have implicated several DNA sensors, including cyclic GMP-AMP synthase (cGAS) [18], interferon- γ inducible protein 16 (IFI16) [19], DDX41 [20] and DAI [21], in sensing viral DNA and activating downstream NF- κ B and interferon regulatory factor (IRF) signaling via the STING adaptor.

As intracellular obligate “parasites”, viruses have evolved diverse strategies to modulate NF- κ B signaling. For example, human KSHV and EBV, two oncogenic gamma herpesviruses, activate NF- κ B during latency to exploit its pro-survival roles. This contributes to oncogenic transformation [22]. Human immune-deficient virus 1 (HIV-1) and herpes simplex virus 1 (HSV-1) usurp NF- κ B to activate viral gene expression [23,24]. Murine gamma herpesvirus 68 (γ HV68), a model herpesvirus for human KSHV and EBV, activates RIG-I and IKK β to promote viral lytic gene expression, while dampening host inflammatory immune gene expression [25–28]. Other viruses inhibit NF- κ B activation primarily to evade host inflammatory and immune responses (Table 1). These viruses deploy diverse factors that target nearly every key step of NF- κ B signaling pathway, from PRRs to NF- κ B-dependent transcription [29].

3. Inactivation of NF- κ B signaling cascades

3.1 TLRs, RLRs and their adaptors

PRRs detect PAMPs integral to pathogen infection, including pathogen structural components and replication intermediates. In viral infection, TLRs and RLRs often recognize viral nucleic acid. One common mechanism to disrupt innate immune signaling downstream of these receptors is an inactivating cleavage of the crucial adaptor molecules, namely MAVS for RLRs and TRIF or MyD88 for TLRs by virally encoded proteases. Following the discovery of MAVS cleavage by HCV NS3/4a protease [12,30], studies revealed that a number of RNA viruses deploy similar strategies to proteolytically destroy MAVS and TRIF molecules, which halts innate immune signaling at a very early step [31].

Recent work showed that hepatitis A virus, coxsackievirus B and enteroviruses encode cysteine proteases that target TRIF to disable TLR3 signaling [32–34]. A TLR2-mediated immune response is implicated in the pathogenesis of HSV-1 [35] and the ICP0 E3 ligase function targets MyD88 and Mal (TIRAP) for degradation to suppress the inflammatory response downstream of TLR2 [36]. Murine γ HV68 and human KSHV express multiple proteins interfering with TLR signaling, including ORF21, ORF31, ORF50 and K4.2 (KSHV-specific). The ORF50 (RTA) TF initiates ubiquitous gene expression for murine γ HV68 and human KSHV, and serves as an E3 ligase that downregulates TLR2 and TLR4 expression [37]. The mechanistic action of the other three proteins of KSHV and γ HV68 in evading TLR signaling remains unclear.

In resting cells, RIG-I is kept in a latent state by an intramolecular interaction that is secured by phosphorylation [38]. Thus, dephosphorylation is a prerequisite step for RIG-I activation by RNA [39]. Measles virus V protein blocks this action of the PP1 phosphatase to suppress RIG-I- and MDA-5-dependent innate immune responses [40,41]. Human metapneumovirus and respiratory syncytial virus express secreted glycosylated G proteins, in addition to their membrane-anchored longer isoform. The secreted G proteins bind to the CARD domain of RIG-I, but not that of MDA-5, blocking RIG-I dimerization with MAVS and nullifying RIG-I-mediated signal transduction [42,43].

3.2. IKK kinases

The core event of NF- κ B signaling cascade is the activation of the trimeric I κ B kinase (IKK) comprising catalytic IKK α or IKK β and the scaffold protein IKK γ . While IKK α and IKK β show some redundancy in their phosphorylation of downstream substrates, the scaffolding IKK γ is absolutely required for the recruitment and activation of IKKs [3]. Hepatitis A virus 3c protease cleaves IKK γ to block activation of the IKK kinase complex, whereas the NSP4 protein of porcine reproductive and respiratory syncytial virus binds to IKK γ and effectively sequesters it from IKK α and IKK β [44,45]. Additionally, IKK kinases are targeted by the polymerase of hepatitis B virus, the NS1 protein and the PB1-F2 polypeptide of influenza A virus [46–48]. These viral factors physically associate with IKK kinases and prevent their activation in response to viral infection, thereby terminating upstream signaling. Collectively, viruses target the assembly and phosphorylation of the IKK complex to prevent proper activation of IKK kinases.

3.3. Degradation of I κ Bs

I κ Bs keep NF- κ B TFs in an inactive state that is readily activated upon acute stimulation by viral infection or TNF- α treatment. The degradation of I κ Bs is governed by serine phosphorylation within the N-terminal domain, subsequent ubiquitination by the β -TrCP E3 ligase and ultimately proteolytic cleavage by the proteasome [49,50]. Poxviruses express a family of multiple proteins containing ankyrin-repeat sequences, the signature structural element of I κ Bs and some of these viral proteins interfere with the degradation of I κ Bs [51]. For example, the vaccinia virus A49 protein harbors a sequence mimicking I κ B α to block the action of the β -TrCP E3 ligase responsible for recognizing and ubiquitinating phosphorylated I κ Bs, thus stabilizing I κ Bs and inhibiting NF- κ B activation [52]. The non-structural protein 1 (NSP1) of human rotavirus instead blocks the degradation of I κ Bs by

inducing the degradation of β -TrCP [53]. In essence, viral proteins display structural mimicry of I κ Bs and β -TrCP to stabilize I κ Bs and avoid NF- κ B activation.

3.4 NF- κ B TFs

Degradation of I κ B subunits unleashes the p50–p65 dimer that translocates into the nucleus and, in collaboration with other TFs and activators, up-regulates anti-viral gene expression. Recent studies have identified viral proteins that inhibit NF- κ B activation by targeting its nuclear translocation and transcriptional activation.

HSV-1 expresses a serine/threonine kinase, US3, which potently phosphorylates p65 at serine 75 [54]. Mutations that abolished the kinase activity of US3 also impaired its ability to block NF- κ B activation. With kinase-active US3, hyperphosphorylated p65 was retained in the cytoplasm, even when cells were stimulated with TNF- α , a cytokine that induces acute NF- κ B activation. It is not clear whether the US3 homologues of other herpesviruses possess a similar function. Additionally, the ICP0 E3 ligase of HSV-1 antagonizes NF- κ B activation via both p50 and p65. While ICP0 degraded p50 via the RING finger domain that interacts with the Rel homology domain of p50, ICP0 bound p65 and blocked its nuclear translocation [55]. A similar strategy is deployed by the mouse poxvirus [56], ectromelia virus, in blocking p65 nuclear translocation. In cells expressing the ectromelia virus EMV150 protein, one member of the EMV ankyrin-repeat-containing family, I κ B degradation was observed while p65 resided in the cytoplasm of cells stimulated with TNF- α . Mutational analysis showed the BTB/Kelch domain of EMV150 was sufficient to interact with and retain p65 in the cytoplasm, implicating the BTB/Kelch domains which are shared by various cellular proteins in regulating p65 nuclear translocation [57]. These studies emphasized that the nuclear translocation of NF- κ B subunits is a crucial step in mounting a cellular anti-viral response.

The transcriptional activity of NF- κ B is modulated by post-translational modifications and transcription co-activators/co-repressors. For example, the phosphorylation of p65 at ser529 is necessary for its interaction with and subsequent acetylation by CBP/p300. Further enhanced by ser536 phosphorylation, acetylation of p65 at lys310 increases its transcription activity [58]. The p65-CBP/p300 interaction is targeted by vaccinia virus and HSV-1. These viruses encode ORF002 and VP16 that compete with CBP/p300 for binding to p65 [59,60]. P65 acetylation and transcription are then severely attenuated, thereby suppressing inflammatory cytokine expression. UXT is a transcriptional co-activator that enhances NF- κ B-mediated gene expression. The EBV BGLF4 kinase phosphorylates UXT and dampens the transcription activity of NF- κ B [61].

4. Activation of the NF- κ B signaling pathway by viral proteins

Some viruses activate the NF- κ B signaling pathway to promote viral infection and/or cell proliferation [62]. These viruses have evolved distinct mechanisms to target key signaling components of the NF- κ B pathway, such as PRRs and IKKs. We highlight viral factors of NF- κ B activation in relation to their cellular targets (Table 2).

4.1 PRRs

A few viral proteins directly engage TLR2 and RIG-I to elicit NF- κ B activation. Recent studies have shown that glycoproteins gH/gL of HSV directly interacts with TLR2 and is sufficient to activate NF- κ B [63]. Similarly, HSV gB associates with TLR2 and purified gB activates NF- κ B in cells expressing TLR2 [64]. We have identified viral glutamine amidotransferases (vGATs) of γ HV68 and KSHV that activate NF- κ B via RIG-I. vGATs physically associate with and activate RIG-I in the absence of any viral RNA [28]. Though lacking intrinsic enzyme activity, vGATs recruit their cellular homologue, phosphoribosylformylglycinamide synthase (PFAS), to deaminate RIG-I, thereby activating RIG-I independent of RNA. When exogenously expressed, deaminated RIG-I is competent to trigger NF- κ B and IRF activation without viral RNA.

4.2 Kinases

IKK α/β , IKK ϵ and TAK1 are targeted by viral proteins to stimulate NF- κ B activation. KSHV GPCR (vGPCR) requires both IKK ϵ and TAK1 to activate NF- κ B [65,66]. vGPCR interacts with and activates both IKK ϵ and TAK1, and loss or depletion of IKK ϵ and TAK1 impairs vGPCR-induced NF- κ B activation [65,66]. While TAK1 phosphorylation and lysine 63-linked polyubiquitination are induced by vGPCR [65], IKK ϵ also promotes p65 phosphorylation and so promotes tumor formation [66]. The link between TAK1 and IKK ϵ in vGPCR-induced NF- κ B activation remains undefined. The cytoplasmic domain of HIV-1 glycoprotein gp41 (gp41CD) induces NF- κ B activation via TAK1 [67]. gp41CD physically interacts with TAK1 and TAK1 activity is essential for gp41CD-induced NF- κ B activation. Additionally, HIV-1 Vpr targets both IKK α/β and TAK1 to activate NF- κ B [68,69]. Vpr interacts with and activates IKK α/β through enhancing the phosphorylation of IKK α/β , and Vpr also directly promotes the phosphorylation of p65 to activate NF- κ B [68]. Both IKK α/β and TAK1 are required for Vpr-induced NF- κ B activation. In activating TAK1, Vpr interacts with TAK1 and enhances the TAB3-TAK1 interaction, thereby increasing TAK1 polyubiquitination, phosphorylation and consequent activation.

4.3 Other signaling components

HIV-1 Tat interacts with the p65 subunit of NF- κ B, thus enhancing the DNA-binding ability of p65 and the transcription of HIV-1 LTR by p65 [70]. This is consistent with the corollary that HIV utilizes activated NF- κ B to promote viral gene expression. Tat also associates with I κ B α and prevents its binding to the NF- κ B complex. Hepatitis B virus X protein (HBx) physically associates with the cleaved p22 form of cFLIP (p22-FLIP) and NEMO to form a ternary complex [71], which is required for HBx-induced NF- κ B activation. This result is consistent with that p22-FLIP associates with and activates IKK α/β to instigate NF- κ B activation. Human papillomavirus E6 activates NF- κ B through targeting NFX1-91 for degradation [72]. NFX1-91 binds to the promoter of the NF- κ B inhibitor p105 and up-regulates its expression.

5. Hijacking NF- κ B signaling events by virus

A recurring theme is that virus hijacks the NF- κ B pathway to promote infection by using NF- κ B TFs to enhance viral gene expression. Examples include HIV [73], HSV-1 [24] and

bovine foamy virus [74]. This has been extensively reviewed previously [62]. Our recent studies demonstrate that murine γ HV68 exploits the activation of MAVS and IKK β to promote viral lytic replication [25]. Specifically, γ HV68 activates IKK β in a MAVS-dependent manner and activated IKK β phosphorylates viral replication transactivator (RTA), an essential TF for gamma herpesvirus replication, thereby elevating the expression of viral lytic genes. On the other hand, γ HV68 directs the IKK β kinase to phosphorylate p65, the critical subunit for the transcriptionally active NF- κ B dimer, and induces p65 degradation in conjunction with the RTA E3 ligase [26,27]. As such, MAVS and IKK β are necessary to abrogate antiviral cytokine production and loss of MAVS results in the secretion of increased cytokines (Figure 2). We have discovered a family of viral pseudo enzymes that activate RIG-I via deamidation, a molecular action distinct from RNA-induced RIG-I activation [28]. Our study illustrates an intriguing mechanism whereby innate immune activation is intercepted by a viral TF/E3 ligase to enable the expression of viral lytic genes while disabling the production of host antiviral cytokines.

When *in vivo* function of NF- κ B signaling events is considered, γ HV68 infection in mice contributes substantially to our understanding in host immune defense and viral manipulation. Although blockade of NF- κ B activation by the I κ B α super-suppressor did not impact γ HV68 lytic replication [75], γ HV68 exploits signaling events upstream of NF- κ B to facilitate lytic gene transcription and productive infection [25]. On the other hand, inhibition of NF- κ B activation via host factors or engineered recombinant γ HV68 demonstrated crucial roles of NF- κ B activation in viral latent infection [75,76]. Interestingly, activation of NF- κ B appeared to originate from CpG DNA-triggered TLR9 signaling. Despite that loss of TLR9 expression increased γ HV68 reactivation from latently-infected B cells, the frequency of latently-infected B cells was comparable in wild-type and *Tlr9*^{-/-} mice [77]. MyD88-deficiency or expression of the I κ B α super-suppressor, but not MAVS-deficiency, reduced the frequency of γ HV68 latently-infected splenocytes [25,75,76], supporting the corollary that TLR-dependent NF- κ B activation facilitates the establishment of latent infection. In conclusion, signaling events upstream of NF- κ B are important for γ HV68 lytic replication, whereas NF- κ B activation per se is critical for γ HV68 latent infection.

6. Concluding remarks

We anticipate that the list of viral factors modulating cellular NF- κ B activation will rapidly expand with research into innate immune signaling. Studies on viral modulation of NF- κ B-dependent gene expression in the nucleus, such as epigenetic modifications, will likely uncover fundamental principles governing the specificity and coordination of NF- κ B-mediated transcription. PRRs constitute another research frontier in virus-host interaction and viral manipulation of PRRs should elucidate significant mechanisms of host defense and viral evasion. Virus-host interactions shaped by millions of years of co-evolution are sure to surprise us with elegant examples of viral manipulation of both host NF- κ B activation and related signaling pathways.

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Highlight

1. NF- κ B transcription factors regulate the expression of hundreds of genes in immune response.
2. NF- κ B activation by PRRs constitutes a research frontier in infection and immunity.
3. Viruses have evolved distinct and intricate strategies to modulate NF- κ B signaling.
4. Viral modulation offers mechanistic view into key regulation governing NF- κ B activation.

Figure 1. Signaling of NF-κB activation downstream of pattern recognition receptors

Pattern recognition receptors, including Toll-like receptors, RIG-I-like receptors and DNA receptors, interact with their cognate adaptors and activate the canonical IKKαβγ complex. The TRAF molecules and TAB-TAK1 complex link distinct adaptors to the IKKαβ kinase. IKKα or IKKβ phosphorylates inhibitors of NF-κB that is subsequently degraded by the proteasome, releasing the p65-p50 dimer to translocate into the nucleus and up-regulate the expression of a large set of inflammatory cytokines. Dashed lines indicate that the signaling events are not well defined.

Figure 2. Modulators of NF-κB encoded by human KSHV and murine γHV68

In response to viral infection, pattern recognition receptors activate the NF-κB pathway, while viruses target key signaling components to modulate NF-κB activation. Highlighted components are diagrammed in conjunction with viral modulators of human KSHV and murine γHV68.

Table 1

Viral inhibitors of the NF- κ B signaling pathway

Cellular target		Viral protein	Virus	Mechanism	Refs	
PRR Receptor and Adaptor	RIG-I	V protein	Measles virus	Blocks PP1 phosphatase activities	[41]	
		G protein	Human metapneumovirus virus	Binds to CARD domain of RIG-I and blocks its dimerization with MAVS	[42]	
			Human respiratory syncytial virus		[43]	
	MDA5	V protein	Measles virus	Blocks PP1 phosphatase activities	[40]	
	TLR2	RTA	Gamma-herpesvirus 68, Kaposi's sarcoma-associated virus (KSHV)	Down-regulates TLR2 expression	[37]	
	TLR4			Down-regulates TLR4 expression	[37]	
	TLR3	NS1	West Nile Virus (WNV)	Unknown	[78]	
	MAVS	NS3/4a	Hepatitis C Virus (HCV)	Cleaves MAVS into non-functional fragments	[30]	
		Protease 3C	Coxsackievirus B		[32]	
		Protease 3CD	Hepatitis A Virus (HAV)	Cleaves TRIF into non-functional fragments	[34]	
	Protease 3C	Enteroviruses		[33]		
IKK kinases		ICP0	Human Simplex Virus 1 (HSV-1)	Targets MyD88 for proteasomal degradation	[36]	
		TIRAP		Targets TIRAP for proteasomal degradation	[36]	
		TRAF6	Epstein-Barr Virus (EBV)	Interacts with and deubiquitinates TRAF6	[79]	
	IKK γ (NEMO)	Protease 3C	HAV	Cleaves NEMO into non-functional fragments	[44]	
		NSP4	Porcine reproductive and respiratory syncytia virus	Sequesters IKK γ from IKK α and IKK β	[45]	
	IKK α IKK β	PB1-F2	Influenza A virus	Interacts with and blocks IKK kinases activation	[47]	
		NS1			[46]	
		Polymerase	Hepatitis B Virus (HBV)		[48]	
	IkBs	IkBs	Ankyrin-repeat proteins	Poxvirus	Competes for SCF ubiquitin ligase complex	[51]
			A49	Poxvirus	Mimics IkBs to target β -TrCP	[52]
NF κ B		NSP1	Human Rotavirus	Targets β -TrCP for proteasomal degradation	[53]	
	RelA	US3	HSV-1	Hyperphosphorylates RelA to prevent its nuclear translocation	[54]	
		EMV150	Ectromelia	Interacts with RelA and blocks its nuclear translocation	[57]	
		ICP0	HSV-1		[55]	
		ORFV002	Poxvirus		[56]	

Cellular target	Viral protein	Virus	Mechanism	Refs
	ORF002	Vaccinia virus	Competes RelA for CBP/p300	[59]
	VP16	HSV-1		[60]
	ORF61	Varicella-zoster virus	Blocks RelA nuclear translocation, mechanism unknown	[80]
	ICP0	HSV-1	Targets p50 for proteasomal degradation	[55]
	BGLF4	EBV	Phosphorylates co-activator UXT to dampen NFκB activity	[61]

Table 1 additional: [78], [79], [80]

Table 2

Viral activators of the NF- κ B signaling pathway

Cellular target		Viral protein	Virus	Mechanism	Refs
Receptor	RIG-I	vGAT	Gamma-herpesvirus 68, Kaposi's sarcoma-associated virus (KSHV)	vGAT interacts with and activates RIG-I via deamidation	[28]
	TLR2	gB gH/gL	Herpes simplex virus-1 (HSV-1) HSV-1	gB interacts with and activates TLR2 in a MyD88/TRAF6 dependent manner gH/gL interacts with and activates TLR2	[64] [63]
Kinase	IKK α / β	Vpr	Human immunodeficiency virus type 1 (HIV-1)	Vpr interacts with and activates IKK α / β through enhancing their phosphorylation	[68]
	IKK ϵ	vGPCR	KSHV	vGPCR interacts with and activates IKK ϵ , which in turn promotes p65 phosphorylation	[66]
	TAK1	gp41CD	HIV-1	gp41CD interacts with TAK1 and TAK1 activity is required for gp41CD-induced NF- κ B activation	[67]
		vGPCR	KSHV	vGPCR interacts with and activates TAK1 through inducing its phosphorylation and ubiquitination	[65]
Others		Vpr	HIV-1	Vpr interacts with and activates TAK1 through inducing its phosphorylation	[69]
	I κ B α	Tat	HIV-1	Tat interacts with I κ B α and prevents its binding to the NF- κ B complex	[70]
	NEMO	HBx	Hepatitis B virus	HBx interacts with p22-FLIP and NEMO to form a ternary complex	[71]
	NFX1-91	E6	Human papillomavirus	E6 targets NFX1-91 for degradation	[72]
	RelA	Tat	HIV-1	Tat interacts with p65 and enhances the DAN-binding ability and transcriptional activity of p65	[70]
		Vpr	HIV-1	Vpr promotes the phosphorylation of p65	[68]
	Unknown	mu2	Reovirus	Unknown	[81]
		N	Porcine epidemic diarrhea virus	Unknown	[82]
		N	Porcine reproductive and respiratory syndrome virus (PRRSV)	Unknown	[83]
		Nsp2	PRRSV	Unknown	[84]

Table 2 additional: [81], [82], [83], [84]