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Viral Appropriation of Apoptotic and NF-κB Signaling Pathways

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Abstract Viruses utilize a variety of strategies to evade the host immune response and replicate in the cells they infect. The comparatively large genomes of the Orthopoxviruses and gammaherpesviruses encode several immunomodulatory proteins that are homologous to component of the innate immune system of host cells, which are reviewed here. However, the viral mechanisms used to survive host responses are quite distinct between these two virus families. Poxviruses undergo continuous lytic replication in the host cytoplasm while expressing many genes that inhibit innate immune responses. In contrast, herpesviruses persist in a latent state during much of their lifecycle while expressing only a limited number of relatively non-immunogenic viral proteins, thereby avoiding the adaptive immune response. Poxviruses suppress, whereas latent gammaherpesviruses activate, signaling by NF-κB, yet both viruses target similar host signaling pathways to suppress the apoptotic response. Here, modulation of apoptotic and NF-κB signal transduction pathways are examined as examples of common pathways appropriated in contrasting ways by herpesviruses and poxviruses. J. Cell. Biochem. 91: 1099–1108, 2004. © 2004 Wiley-Liss, Inc.

Key words: NF-κB; Epstein–Barr virus; vaccinia virus; poxvirus; bcl-2; apoptosis; caspase; inhibitor; innate immunity

Poxviruses replicate in the host cytoplasm and promptly undergo lytic replication that leads to cell death and viral dissemination. The large number of poxvirus genes that downmodulate host innate immune defenses (Table I) appear to be essential to the virus lifecycle in vivo [Moss, 2001]. In contrast, during much of the herpesvirus lifecycle, herpesviruses exist in a latent state expressing only a limited number of relatively non-immunogenic viral genes [Kieff and Rickinson, 2001] (Fig. 1). Herpesviruses encode fewer proteins that are known to downmodulate innate host defenses.

Both the gammaherpesviruses and orthopoxviruses modulate signaling to NF-κB and by signaling by apoptotic pathways. This may occur because several cellular receptors signal via both apoptotic cascades and the NF-κB pathway. For example, engagement of tumor necrosis factor receptors (TNFRs) aggregates procaspase 8 resulting in its cleavage to active caspase 8 and initiation of the caspase cascade that ultimately leads to apoptosis, or programmed cell death [Cohen, 1997]. Simultaneously, TNFR engagement leads to activation of the TNF receptor associated factors (TRAFs) permitting signaling to NF-κB [Karin and Ben-Neriah, 2000]. Signaling to NF-κB is predominantly associated with expression of cellular survival genes [Karin and Ben-Neriah, 2000]. Thus, pathways that mediate signaling to NF-κB and survival are linked at the level of TNF receptors to pathways that mediate apoptosis. Furthermore, bcl-2 family members have been

Abbreviations used: TNFRs, tumor necrosis factor receptors; TRAFs, TNF receptor associated factors; EBV, Epstein–Barr virus; LCLs, lymphocytoblastoid cells lines; LMP, membrane protein; KSHV, Kaposi's Sarcoma Herpes Virus; v-FLIP, viral FLICE inhibitory protein; BH4, Bcl-2 homology 4; IFN, interferon; TIR, toll/IL-1R; TLR, toll-like receptor; IKK, I-κB kinase; vv, vaccinia virus; LT, lymphotoxin.

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TABLE I. Poxviral Inhibitors of Innate Immune Signaling

Viral inhibitor	IFN	IL-1	LT/TNF	Fas	TLR3	TLR1, 2, 4, 6
Soluble receptor	+	+	+/−	—	—	—
PKR inhibitor	+(E3L)	—	—	—	+(E3L, K3L)	—
TIR inhibitor	—	+(A46R,A52R)	—	—	+(A52R)	+(A52R)
Caspase inhibitor	—	+(crmA)	+(crmA) MC159L (MCV)	+(crmA) MC159L (MCV)	MC159L (MCV)	—
Bcl-2 like apoptosis inhibitor	—	—	(M11L)	(M11L)	—	—

shown to mediate cell survival [Yin et al., 1994], and bcl-2 can stimulate signaling to NF-κB [Regula et al., 2002]. Following engagement of the appropriate lymphotoxin (LT) receptor, caspases proteolytically process the p100 Rel protein into p52 which translocates into the nucleus and drives transcription of certain NF-κB responsive genes [Dejardin et al., 2002].

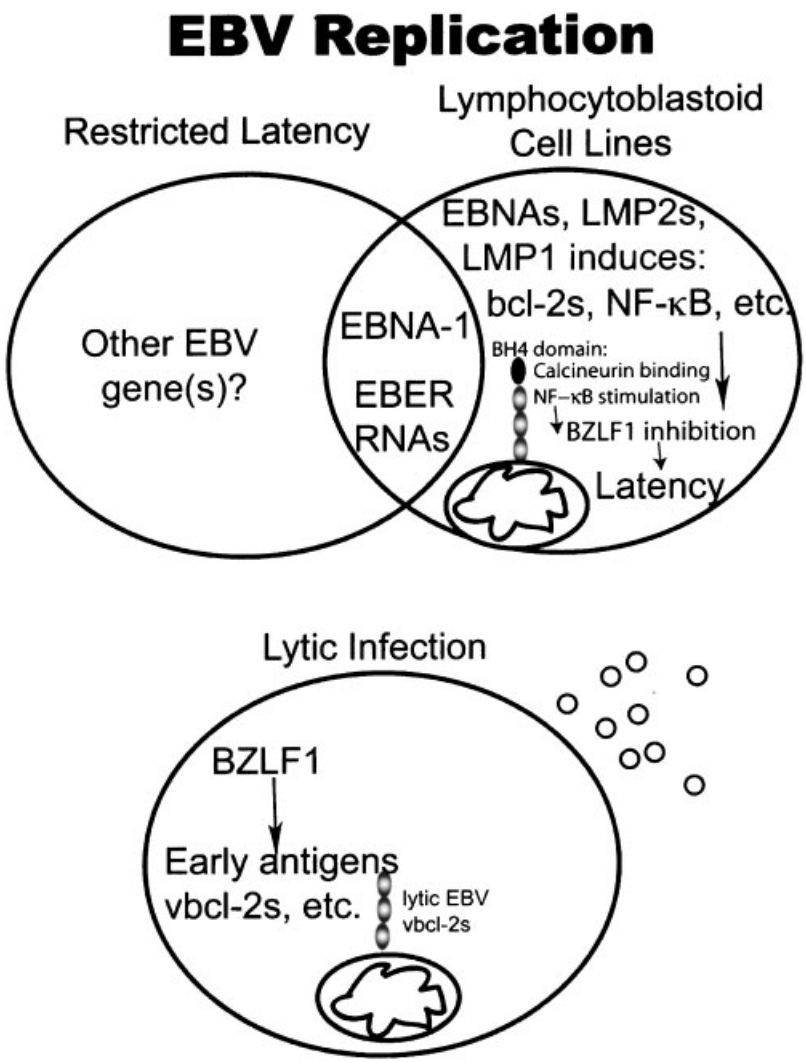

Fig. 1. EBV latency as an immune escape mechanism. Lytic replication. Once expressed, the EBV viral transactivator, BZLF1, drives gammaherpesvirus early genes and eventually may lead to the production of over 80 viral proteins, viral DNA synthesis, and virion release. Only a minority of cells undergo lytic replication at a given time. Latency. Signaling to NF-κB induced by latently expressed EBV LMP-1 proteins, and perhaps by LMP-1 induced cellular bcl-2 proteins, may suppress activation of the BZLF1 lytic transactivaton. This mechanism is consistent with the observation that NF-κB is induced by LMP-1, and is one factor responsible for the maintenance of latency [Prince et al., 2003]. The mechanisms suppressing lytic replication in the majority of cells that are latently EBV infected (center of diagram) is unclear.

Thus, the apoptotic and NF-κB signaling pathways, which are critical mediators of innate immune responses, are linked at the level of both bcl-2 and caspases.

Given that signaling to NF-κB and apoptosis utilize related pathways that also modulate viral infections, it is unsurprising that Orthopoxviruses and gammaherpesviruses encode proteins to appropriate these antiviral pathways for the benefit of the virus (Tables I and II). The modulation of signal transduction pathways utilized by these distinct classes of DNA viruses provides insight into common mechanisms used by many viruses. These common mechanisms may provide insight into rational anti-viral therapies that target viral signal transduction.

GAMMAHERPESVIRUS LATENCY AS AN IMMUNE ESCAPE MECHANISM

The Epstein–Barr virus (EBV) exists in two states: (1) lytic viral replication, when many EBV genes are expressed to permit EBV DNA to replicate independently of cellular DNA, and (2) latent replication, when EBV episomes replicate in tandem with chromosomal DNA. The switch from latent to lytic replication determines whether the fate of the latent EBV virus is tied to that of the cell, versus being dependent upon infection of another susceptible cell by EBV virions. Latent infection permits the virus to largely escape immune surveillance, whereas lytic replication makes EBV susceptible to a highly effective adaptive immune response [Rooney et al., 1997]. BZLF1 is the EBV protein that regulates the switch to lytic replication is BZLF1 in EBV. BZLF1 is an immediate early gene of EBV, which is the principal transactivator of lytic EBV gene expression [Grogan et al., 1987]. Once expressed, the viral transactivators drive gammaherpesvirus early genes and eventually may lead to the production of viral particles and viral DNA synthesis (Fig. 1). Gammaherpesvirus genes expressed during latency serve to make EBV virtually undetectable by the immune system and permit EBV episomes to replicate in tandem with the cellular chromosomes.

EBV persists in most host cells via expression of the protein, EBNA-1, which functions independently of other known EBV proteins to tether the EBV episome to chromosomal DNA without pathological consequences [Kieff and Rickinson, 2001]. EBNA-1 is poorly immunogenic due to a series of Gly-Ala repeats that inhibit its proteosomal processing, thereby diminishing presentation of EBNA1 peptides by host cell Class I MHC [Levitskaya et al., 1997]. Burkitt's lymphoma is a disease of *c-myc* proto-oncogene overexpression often requiring EBV co-expression, where EBNA 1 is also the only EBV protein known to be expressed [Ruf et al., 1999]. During an in vitro EBV infection, EBV establishes a latent growth-transforming infection of B cells, allowing them to proliferate in culture as lymphocytoblastoid cells lines (LCLs) [Kieff and Rickinson, 2001]. EBV lymphoproliferative disease of the immunocompromised host phenotypically resembles LCLs. Out of over 80 proteins encoded by the EBV genome, LCLs only express three latent membrane proteins (LMP 1, 2A, and 2B), eight EBV nuclear antigens (EBNAs), and the 'EBER RNAs' [Kieff and Rickinson, 2001]. The EBERs are also expressed in both forms of EBV latency and mediate resistance to PKR-mediated apoptosis [Komano et al., 1999]. Members of the gammaherpesvirus family are characterized by their ability to establish latent infection, while expressing few viral proteins. Because immune recognition of latently infected cells is impaired [Rooney et al., 1997], viral signaling mechanisms that favor latency tend to promote

TABLE II. Viral Inhibitors of Apoptosis Conditionally Modulate NF-κB Signaling

Virus/lifecycle stage	Anti-apoptotic gene	Modulated NF-κB signaling
Gammaherpesvirus (lytic)	KSHVorf16	Unknown
	BALF1	Unknown
	BHRF1	None
	KSHV K7	None
Gammaherpesvirus (latent)	LMP1	Increased TRAFs→NF-κB
	LMP1-induced cellular bcl-2s	Increased NF-κB via IKK-β
	VFLIP	Increased NF-κB via binds IKK-γ
Orthopoxviruses	CrmA	Decreased via inhibited IL-1 & IL-18
	M11L and unidentified vv ORF	Unknown
	E3L (anti-PKR)	Decreased NF-κB signaling

viral survival. In this regard, it is interesting to note that NF-κB inhibits the lytic replication of gammaherpesviruses by interacting with EBV BZLF1 and Kaposi's Sarcoma Herpes Virus (KSHV) Rta to inhibit transactivation of early gammaherpesvirus genes [Brown et al., 2003]. This raises the question of which gammaherpesvirus genes regulate latent gammaherpesvirus replication.

SEVERAL LATENT GAMMAHERPESVIRUS GENES THAT ARE INVOLVED IN SIGNALING TO NF-κB MAY HELP MAINTAIN LATENCY

The KSHV viral FLICE inhibitory protein (v-FLIP) signals to NF-κB by associating with IKK-γ, which is the regulatory subunit of the IKK complex [Field et al., 2003]. Furthermore, the LMP-1 of EBV signals to NF-κB [Mosialos et al., 1995]. Signaling to NF-κB by both the latently expressed KSHV vFLIP and EBV LMP-1 proteins, would hypothetically serve to suppress activation of the BZLF1 lytic transactivator [Brown et al., 2003]. This hypothetical mechanism is consistent with the observation that NF-κB is induced by LMP-1 [Mosialos et al., 1995] and is one factor responsible for the maintenance of latency [Prince et al., 2003]. These findings are consistent with the hypothesis that signaling to NF-κB favors the survival of gammaherpesviruses [Brown et al., 2003]. In support of this hypothesis, KSHV-transformed cells undergo cell death when treated with proteasome inhibitors to block signaling to NF-κB [Keller et al., 2000].

Cellular bcl-2 proteins are induced by LMP-1, and in addition to promoting cellular survival, bcl-2 proteins would be predicted to promote latency via several mechanisms. Signaling by Rel family members such as NFAT and NF-κB, is modulated via the N-terminal Bcl-2 homology 4 (BH4) domain. Human bcl-2 was found to mediate increased signaling to NF-κB in human cells; whereas a BH4 domain-deleted mutant bcl-2 does not signal to NF-κB [Regula et al., 2002]. Thus, during the dormant phase of the viral lifecycle, the BH4 domain of cellular bcl-2 proteins might favor maintenance of latent infection, viral persistence and, incidentally, immortalization. EBV encodes the viral bcl-2 homolog, BHRF1, which is expressed during lytic infection, inhibits apoptosis, but lacks a BH4 domain [Henderson et al., 1993]. Recently, EBV was found to encode another vbcl-2 that suppresses apoptosis [Marshall et al., 1999]; however an N-terminally truncated construct did not [Bellows et al., 2002]. N-terminal truncations of anti-apoptotic human and viral bcl-2 family members have been reported to activate their proapoptotic functions [Bellows et al., 2000]. One hypothesis for the absence of a BH4 domain in most vbcl-2s is that N-terminally truncated bcl-2s would escape caspase cleavage and conversion to proapoptotic molecules [Bellows et al., 2000]. An alternative hypothesis is that N-terminal truncation prevents signaling to NF-κB by certain viral bcl-2 family members, just as N-terminal modifications do in cellular bcl-2 family members [Regula et al., 2002]. The lack of a BH4 domain encoded by most vbcl-2s does not hinder prosurvival effects during lytic replication [Henderson et al., 1993; Sarid et al., 1997; Marshall et al., 1999], but hypothetically may prevent signaling to NF-κB associated with full-length bcl-2s [Regula et al., 2002] that would inhibit lytic replication [Brown et al., 2003].

Sequestration of calcineurin by bcl-2 family members that possess a BH4 domain inhibits NFAT from translocating to the nucleus and activating NFAT responsive genes. The promoter for the EBV transactivator, BZLF1, which controls the switch from latent to lytic infection, is suppressed by a pharmacologic inhibitor of calcineurin [Liu et al., 1997]. This suggests another role for bcl-2 in the lytic switch of EBV. In EBV-infected cells only expressing EBNA-1 protein, cellular bcl-2 that is reported to be induced by EBV EBERs [Komano et al., 1999] might suppress lytic reactivation by BZLF1; however, others have not reproduced the finding of increased bcl-2 due to EBERs in BL cells [Ruf et al., 2000]. Thus, it is still unclear how EBV-infected Burkitt's lymphoma cells downmodulate lytic infection. Nevertheless, there are many potential mechanisms whereby bcl-2 may suppress BZLF1 transactivator levels.

ANTIAPOPTOTIC MECHANISMS ALSO FUNCTION AS SIGNALING PATHWAYS FAVORING VIRAL SURVIVAL

Apoptosis is a cell suicide program characterized by DNA digestion that allows for the elimination of dangerous cells, such as cells that have acquired genetic damage or viral nucleic acids. Many DNA viruses appear to possess viral bcl-2 homologs that inhibit apoptosis [Henderson

et al., 1993; Sarid et al., 1997; Marshall et al., 1999]. There are several other viral mechanisms for abrogating the apoptotic pathway that destroys virus-infected cells, such as the EBV genes *EBNA5* and *BZLF1*, which interfere with p53-induced apoptosis (reviewed by Davis and Rouse, 1997). Thus, several viral mechanisms for suppressing apoptosis are similar to anti-apoptotic pathways overexpressed in cancer cells.

EBV differs from KSHV, herpesvirus saimirii, and murine herpesvirus 68 in that EBV does not possess a specific caspase inhibitor (Table II). Caspases effect apoptosis through a programmed series of proteolytic events that lead to digestion of cellular components [Cohen, 1997]. Viral caspase inhibitors are believed to neutralize immune responses of the host that activate the caspase pathway of apoptotic cell death. At least three different types of viral proteins inhibit the caspase pathway of apoptosis: (1) the vFLIP inhibitory protein of the gamma-2-herpesviruses [Thome et al., 1997], (2) the murine herpesvirus 68 M1 homolog of the serpins [Virgin et al., 1997] which resembles crmA, a caspase inhibitor encoded by the cowpox virus genome [Ray et al., 1992], and (3) the baculovirus p35 inhibitor of apoptosis protein [Hershberger et al., 1994]. BHRF1, EBV's known bcl-2 homolog, functions to inhibit Fas in a crmA-like manner; however, BHRF1, like bcl-2, does not function to inhibit Fas-mediated apoptosis in B cells [Foghsgaard and Jaattela, 1997]. Thus, various mechanisms may inhibit caspase activity in viral infection.

Certain lytic EBV genes also mediate survival in lytically infected cells by modulating signaling. The lytic EBV gene *BcRF1*, which is a vIL-10 homolog, inhibits IFN-γ, IL-17, and IL-8 receptor synthesis by the host and downmodulates MHC class I [Kieff and Rickinson, 2001]. Poxviruses also encode homologs of IL-10 [Moss, 2001]. EBV also expresses an inhibitor of protein kinase R (PKR), a cellular activator of the antiviral innate immune response [Poppers et al., 2003]. Other modulators of the innate immune response by EBV during lytic replication so far appear to be restricted to BARF1, which inhibits effects of colony stimulating factor-1 on monocyte production of IFN-α [Kieff and Rickinson, 2001]. Lack of identified innate immune modulators may reflect the technical constraints of restricted latency of EBV that limits induction of lytic replication and generation of recombinants or may indicate that the establishment of the initial EBV infection is not rate-limiting for replication. Whereas comparatively little is known of the effects of EBV on innate immunity, poxviruses encode numerous genes to regulate innate immunity.

POXVIRUS INHIBITION OF SIGNALING TO NF-κB

Poxviruses are lytic DNA viruses that replicate in the host cytoplasm and encode several modulators of the innate immune response. Unlike the case with gammaherpesviruses, the presence of a robust animal model of disease has facilitated identification of poxvirus virulence factors. Consistent with their lytic lifecycle, and host-to-host spread where viral dissemination is rapid compared to EBV, many of the genes involved in poxvirus virulence target the innate immune response by neutralizing cytokines, interferons, and chemokines [Moss, 2001] or their signaling pathways [Bowie et al., 2000].

Interleukin 1 (IL-1) is an inflammatory cytokine thought to be responsible for a host of inflammatory responses [CA Dinarello, 1991]. Upon engagement of the IL-1 receptor (IL-1R), a Toll/IL-1R (TIR) adapter protein, in this case MyD88 [Akira et al., 2000], is clustered by its association with the intracellular TIR domain of the IL-1R. The MyD88 TIR domain is common to the IL-1R, IL-18R, and many members of Toll-like receptor (TLR) family, and thereby mediates a common signaling cascade ultimately leading to activation of NF-κB. Similarly, following ligand-dependent engagement of TLRs, signal transduction is thought to occur via homotypic interactions of TLRs and TIR adapters physically associated with the cytoplasmic TIR domain [Akira et al., 2000] (Fig. 2). Among the ligands recognized by the TLRs are viral nucleic acids and glycoproteins, which trigger the production of cytokines, chemokines, and type I interferons via induction of the transcription factors, NF-κB and IRF3 [Rassa and Ross, 2003]. Relevant TLR signaling is mediated in part by the TIR adapter TRIF, which mediates TLR3 and some TLR4 signaling [Yamamoto et al., 2002; Oshiumi et al., 2003]. TRIF is essential to most antiviral innate immune responses [Hoebe et al., 2003]. TRIF signaling induces the transcription factor, IRF3, that together with NF-κB drives transcription

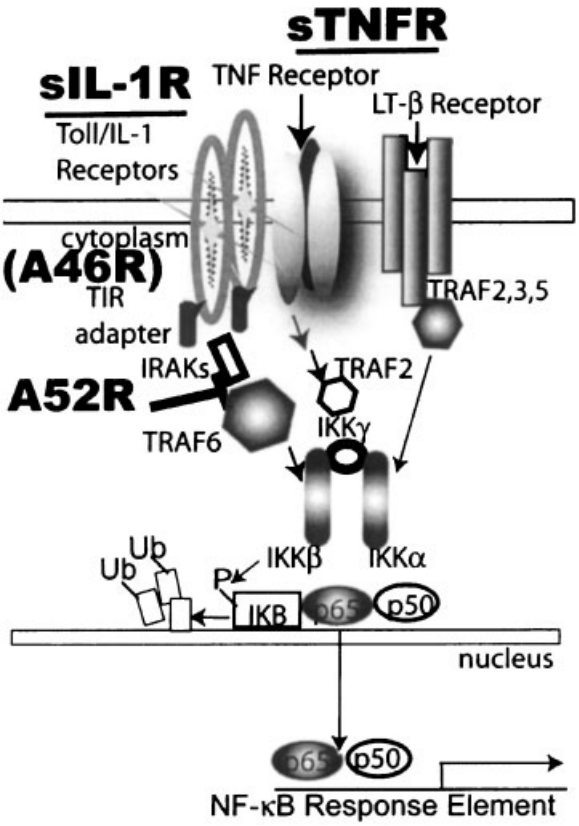

Fig. 2. Pathways inhibited by vaccinia virus inhibitors of NF-κB signaling. Multiple components of signaling to NF-κB are inhibited by vaccinia virus, which encodes soluble receptors and viral inhibitors of signaling to NF-κB that is common to the Toll/IL-1, TNF, and lymphotoxin (LT) pathways that regulate viral replication.

of the antiviral IFN-α/β stimulated response element regulated genes [Fitzgerald et al., 2003].

Recruitment of TIR adapter proteins leads to the recruitment, activation, and phosphorylation of IRAK family members, which subsequently bind to and activate TRAF6 [Janssens and Beyaert, 2003]. Activated TRAF6 is thought to auto-ubiquitinate, and then bind and activate a TAK1 complex. TAK1 then phosphorylates and activates the I-κB kinase (IKK) complex. The IKK complex is composed of two structurally similar kinases, IKK-α and IKK-β, as well as the non-catalytic regulatory subunit IKK-γ (also called NEMO). The activated IKK complex phosphorylates I-kBα leading to its polyubiquitination and subsequent degradation by the proteasome. Degradation of I-kBα permits nuclear translocation of NF-κB dimers, ultimately driving transcription of NF-κB responsive genes (reviewed in Karin and Ben-Neriah, 2000).

THE POXVIRUSES EXPRESS PROTEINS THAT DOWNMODULATE THE HOST IMMUNE RESPONSE: SOLUBLE RECEPTORS AND INHIBITORS OF INTRACELLULAR SIGNALING PATHWAYS

For example, the variola and vaccinia Orthopoxviruses encode secreted decoy receptors for cytokines such as IL-1 [Moss, 2001]. The importance of IL-1 to the anti-poxviral immune response is suggested by the attenuated, nonpyrogenic phenotype of vaccinia virus strains expressing soluble IL-1 receptors that bind IL-1 beta. Experiments that engineered an effective sIL-1R from the defective sIL-1R encoded by vaccinia virus Copenhagen resulted in a less pyrogenic, less virulent virus [Alcami and Smith, 1996].

In contrast to the case with soluble viral IL-1R, the number and properties of virally encoded TNFRs vary among the Orthopoxviruses. TNFRs such as crmB, C, D, and E are soluble or membrane bound TNFRs. Some TNFR reading frames such crmC encoded by the vvUSSR A53R open reading frame are functional, whereas crmC is not functional in vvCopenhagen [Reading et al., 2002]. The finding of soluble CD30R solely from ectromelia virus suggests unique host selective pressures [Panus et al., 2002]. Differences in sTNFR expression among Orthopoxviruses have been attributed to episodic TNF-driven evolutionary selection [Johnston and McFadden, 2003]. Soluble IL-18R is encoded by the Orthopoxviruses and prevents binding of IL-18 to its receptors, thereby preventing signaling to NF-κB and production of IFN-γ [Gracie et al., 2003]. Finally, receptors for interferons are present in many poxviruses and all of the Orthopoxviruses [Alcami et al., 2000]. Thus, Orthopoxviruses encode soluble IL1Rs, IL-18Rs and, sometimes, TNFRs all of which can downmodulate innate immune responses.

Many vaccinia virus proteins are involved in inhibition of innate immune signaling pathways. For example, the E3L and K3L vaccinia virus proteins each inhibit the host PKR protein that is responsible for initiating the host cell response to dsRNA [Der and Lau, 1995]. Furthermore, vaccinia virus encodes two proteins, A46R and A52R, which have been shown to

inhibit Toll/IL-1 receptor signaling [Bowie et al., 2000]. A52R was shown to block TLR-1, -2, -4, and -6 signaling, presumably via the physical association of A52R with TRAF6 and IRAK2 [Harte et al., 2003]. While the existence of A52R predicts a role for the TLRs in responding to vaccinia virus, the exact TLRs required to regulate poxvirus infection are unknown. Recent studies of polymorphisms in the TRIF TIR adapter suggest that viral signaling via TLR3 and TLR4 is critical to antiviral innate immune responses against vaccinia virus [Hoebe et al., 2003]. Furthermore, the crmA serpin prevents signaling to NF-κB by inhibiting the cleavage of proIL-1 and proIL-18. Thus, poxviruses tend to possess mechanisms that suppress signaling to IRF3 and NF-κB, whereas gammaherpesviruses possess mechanisms that stimulate signaling to NF-κB during latency.

Signaling to NF-κB is generally thought to confer resistance to apoptosis [Denk et al., 2000]. However, in contrast to the gammaherpesviruses during latency, antiapoptotic mechanisms in poxviruses are not known to signal to NF-κB (Table II). Poxviruses possess an antiapoptotic homolog of the caspase inhibitor crmA, a caspase inhibitor encoded by the cowpox virus genome [Ray et al., 1992] that also inhibits signaling to NF-κB by inhibiting caspase processing of pro-IL-1β and pro-IL-18. Like the vFLIP of Gammaherpesviruses, the MC159L vFLIP inhibits apoptosis [Field et al., 2003]; however, unlike the KSHV vFLIP, MC159L does not signal to NF-κB [Gil et al., 2001]. The myxoma M11L protein prevents apoptosis by a direct interaction with the mitochondria [Everett et al., 2002], similarly to gammaherpesvirus vbcl-2s. It is not known whether M11L influences signaling to NF-κB. However, unlike cellular bcl-2, BHRF1 does not signal to NF-κB [Foghsgaard and Jaattela, 1997]. Thus, various mechanisms may inhibit apoptosis in lytic gammaherpesvirus infection and in poxvirus infection without signaling to NF-κB that might trigger the production of inflammatory cytokines.

INNATE IMMUNE SIGNALING PATHWAYS ARE OFTEN REDUNDANTLY TARGETED BY POXVIRUS PROTEINS AT DISTINCT SITES IN THE SAME SIGNALING PATHWAY

For example, vaccinia virus encodes decoy receptors for, IL-1β [Alcami and Smith, 1996]. Further downstream in the IL-1β signaling pathway, A46R inhibits IL-1β signaling [Bowie et al., 2000] (Fig. 2). A52R inhibits IL-1β signaling by interacting with TRAF6 and IRAK2 [Harte et al., 2003]. Finally, the crmA gene inhibits caspase 1 mediated processing of proIL-1 to IL-1. Thus, four separate vaccinia virus proteins are known to modulate host IL-1 responses—many via targeting individual components of the NF-κB signaling pathway (Table I). Similarly, the soluble IFN-α/β R of vaccinia virus interdicts the interferon response at the receptor level [Alcami et al., 2000]. A52R inhibits the dsRNA-specific TLR3 [Harte et al., 2003], although it remains unclear whether PKR is truly downstream of TLR3 since TLR3−/− cells still partially responded to dsRNA [Alexopoulou et al., 2001]. Finally, E3L inhibits signaling to IRF3 via PKR [Xiang et al., 2002], as does K3L [Langland and Jacobs, 2002]. Thus, it generally appears that innate immune signaling pathways are redundantly targeted by poxvirus immunomodulatory genes.

There are certain apparent exceptions to the general observation that innate signaling pathways are redundantly targeted by vaccinia (see Table I). First, except for the TLR3 pathway discussed above, Toll signaling is only inhibited by A52R [Harte et al., 2003]. Some obstacles to immunomodulation via vaccinia virus-encoded soluble Toll receptors may be the number of Toll receptors and the possible antagonism of viral components by a hypothetical virally encoded TLR homolog. Second, although certain poxviruses encode decoy TNF-α receptors, many are non-functional, only rare decoy receptors neutralize LT-α, and no other poxviral LT antagonist has been identified to date [Reading et al., 2002]. One group reported that several Orthopoxviruses except for the Ankara strain blocked TNF-mediated signaling to NF-κB, and they suggested that several Orthopoxviruses encode a novel inhibitor of TNF-mediated signaling to NF-κB [Oie and Pickup, 2001].

The apparent absence of redundant poxvirus inhibitors of TNF-α, LTs, and TLRs-1, -2, -4, and -6 may be because these pathways are: (a) irrelevant to viral replication, (b) inhibited by as yet uncharacterized vaccinia virus proteins [Johnston and McFadden, 2003], or (c) targeted more distally in the signaling pathway, as with TLRs and A52R, [Harte et al., 2003]. Further, in vivo virulence factors such as vaccinia virus TNFRs may be lost as a result of repeated viral

passage in vitro [Reading et al., 2002]. The minimal inhibition of TNF-α and LT is striking because TNF-α is an antiviral cytokine. Furthermore, the LTs: LT-α, LIGHT, and LT α1β2 (LT-β), stimulate antiviral responses [Berger et al., 1999; Benedict and Ware, 2001]. Based upon (1) the number of uncharacterized poxviral genes [Moss, 2001], (2) the presence of five putative A52R homologs in the swinepox virus genome [Afonso et al., 2002], and (3) the redundancy in many poxvirus inhibitory pathways, poxviruses may encode uncharacterized proteins functioning in concert with the 'orphan' poxvirus signaling inhibitors represented by A52R in the Toll pathway. Thus, we hypothesize that other poxvirus proteins will be identified that inhibit signaling by TNF-α, Toll ligands, and LTs.

Viral modulation of signaling via the IKK complex is a critical step in signaling to NF-κB and is a target of many other viruses [Tait et al., 2000; Xiao et al., 2000; Ye et al., 2000; Spitkovsky et al., 2002], but IKK complex signaling to NF-κB is not known to be targeted by poxviruses (Fig. 2). Examples of viral targeting of the IKK complex include the adenovirus 14.7 kDa protein that mediates resistance to TNF-induced apoptosis by associating with the IKK-γ subunit of the IKK complex [Ye et al., 2000]. As previously outlined, the gammaherpesvirus KSHV encoded v-FLIP modulates the IKK-γ component of the IKK complex [Field et al., 2003]. The HTLV-1 tax protein associates with the IKK-γ subunit of the IKK-complex and induces signaling to NF-κB [Xiao et al., 2000]. Similarly, the human papillomavirus E7 protein associates with the IKK-complex, but inhibits signaling to NF-κB [Spitkovsky et al., 2002]. Degradation of I-κBα following its phosphorylation is a step inhibited by the African Swine Fever Virus that encodes A238L, a non-degradable homolog of I-κBα that thereby inhibits signaling to NF-κB [Tait et al., 2000]. Thus, several viruses encode proteins that target the IKK complex. Because of these findings, we hypothesize that poxviruses may encode unidentified inhibitors of signaling to NF-κB via the IKK complex.

CONCLUSION: PROSPECTS FOR THERAPY

Anti-IFN and anti-apoptotic proteins are encoded by Orthopoxviruses and Gammaherpesviruses, which utilize common and distinct mechanisms to subvert normal cellular signaling. These viral mechanisms appear vulnerable to therapeutic intervention. Progressive advances in understanding viral pathogenesis initially led to anti-viral therapies based upon knowledge of viral macromolecular synthesis. Recent advances in understanding viral modulation of cellular signal transduction pathways promise to ameliorate viral pathogenesis by blocking viral signaling.

Many viruses, including those reviewed here, encode genes to suppress IFN. Direct replacement of IFN-α or IFN-β is an effective therapy for chronic viral hepatitis [Lawrence, 2000], but is toxic. Imidazoquinolones used in the treatment of papillomaviruses were recently found to function by engaging TLR7 to stimulate IFN-α production, expanding the prospect of targeted IFN-α therapy [Stanley, 2002]. Arginine butyrate had been used to induce signaling pathways that induce lytic EBV replication in EBV infected and malignantly transformed cells in vivo, making these cells vulnerable to antiviral agents [Mentzer et al., 1998]. NF-κB is now known to promote EBV and KSHV latency [Brown et al., 2003], which is a disease state linked to several malignancies [Kieff and Rickinson, 2001]. Thus, it is logical to pursue therapies that inhibit signaling to NF-κB in patients with gammaherpesvirus-associated malignancies.

REFERENCES

Afonso CL, Tulman ER, Lu Z, Zsak L, Osorio FA, Balinsky C, Kutish GF, Rock DL. 2002. The genome of swinepox virus. J Virol 76:783–790.

Akira S, Hoshino K, Kaisho T. 2000. The role of Toll-like receptors and MyD88 in innate immune responses. J Endotoxin Res 6:383–387.

Alcami A, Smith G. 1996. A mechanism for the inhibition of fever by a virus. Proc Natl Acad Sci USA 96(7):3706–3711. 93:11029–11034.

Alcami A, Symons J, Smith G. 2000. The vaccinia virus soluble alpha/beta interferon (IFN) receptor binds to the cell surface and protects cells from the antiviral effects of IFN. 74:11230–11239.

Alexopoulou L, Holt A, Medzhitov R, Flavell R. 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413:732–738.

Bellows D, Chau B, Lee P, Lazebnik Y, Burns W, Hardwick J. 2000. Antiapoptotic herpesvirus Bcl-2 homologs escape caspase-mediated conversion to proapoptotic proteins. J Virol 74:5024–5031.

Bellows D, Howell M, Pearson C, Hazlewood S, Hardwick J. 2002. Epstein–Barr virus BALF1 is a BCL-2-like antagonist of the herpesvirus antiapoptotic BCL-2 proteins. J Virol 76:2469–2479.

Benedict C, Ware C. 2001. Virus targeting of the tumor necrosis factor superfamily. Virology 289(1):1–5.

Berger D, Naniche D, Crowley M, Koni P, Flavell R, Oldstone M. 1999. Lymphotoxin-beta-deficient mice show defective antiviral immunity. Virology 260:136–147.

Bowie A, Kiss-Toth E, Symons J, Smith G, Dower S, LA ON. 2000. A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. Proc Natl Acad Sci USA 97:10162–10167.

Brown H, Song M, Deng H, Wu T, Cheng G, Sun R. 2003. NF-kappaB inhibits gammaherpesvirus lytic replication. J Virol 77:8532–8540.

CA Dinarello C. 1991. Interleukin-1 and interleukin-1 antagonism. Blood 77:1627–1652.

Cohen G. 1997. Caspases: The executioners of apoptosis. Biochem J 326:1–16.

Davis I, Rouse B. 1997. Caveat lector: A skeptical look at viral immune evasion. Front Biosci 2:596–605.

Dejardin E, Droin N, Delhase M, Haas E, Cao Y, Makris C, Li Z, Karin M, Ware C, Green D. 2002. The lymphotoxin-beta receptor induces different patterns of gene expression via two NF-kappaB pathways. Immunity 17:525–535.

Denk A, Wirth T, Baumann B. 2000. NF-kappaB transcription factors: Critical regulators of hematopoiesis and neuronal survival. Cytokine Growth Factor Rev 11:303–320.

Der S, Lau A. 1995. Involvement of the double-stranded-RNA-dependent kinase PKR in interferon expression and interferon-mediated antiviral activity. Proc Natl Acad Sci USA 92:8841–8845.

Everett H, Barry M, Sun X, Lee S, Frantz C, Berthiaume L, McFadden G, Bleackley R. 2002. The myxoma poxvirus protein, M11L, prevents apoptosis by direct interaction with the mitochondrial permeability transition pore. J Exp Med 196:1127–1139.

Field N, Low W, Daniels M, Howell S, Daviet L, Boshoff C, Collins M. 2003. KSHV vFLIP binds to IKK-gamma to activate IKK. J Cell Sci 116:3721–3728.

Fitzgerald K, Rowe D, Barnes B, Caffrey D, Visintin A, Latz E, Monks B, Pitha P, Golenbock D. 2003. LPS/TLR4 signaling to IRF-3/7 and NF-kB involves the Toll adapters TRAM and TRIF. J Exp Med 198:1043–1055.

Foghsgaard L, Jaattela M. 1997. The ability of BHRF1 to inhibit apoptosis is dependent on stimulus and cell type. J Virol 71:7509–7517.

Gil J, Rullas J, Alcami J, Esteban M. 2001. MC159L protein from the poxvirus molluscum contagiosum virus inhibits NF-kappaB activation and apoptosis induced by PKR. J Gen Virol 82:3027–3034.

Gracie J, Robertson S, McInnes I. 2003. Interleukin-18. J Leukoc Biol 73:213–224.

Grogan E, Jenson H, Countryman J, Heston L, Gradoville L, Miller G. 1987. Transfection of a rearranged viral DNA fragment, WZhet, stably converts latent Epstein–Barr viral infection to productive infection in lymphoid cells. Proc Natl Acad Sci USA 84:1332–1336.

Harte M, Haga I, Maloney G, Gray P, Reading P, Bartlett N, Smith G, Bowie A, O'Neill L. 2003. The poxvirus protein A52R targets Toll-like receptor signaling complexes to suppress host defense. J Exp Med 97:343–351.

Henderson S, Huen D, Rowe M, Dawson C, Johnson G, Rickinson A. 1993. Epstein–Barr virus-coded BHRF1 protein, a viral homologue of Bcl-2, protects human B cells from programmed cell death. Proc Natil Acad Sci 90:8479–8483.

Hershberger P, LaCount D, Friesen P. 1994. The apoptotic suppressor p35 is required early during baculovirus replication and is targeted to the cytosol of infected cells. J Virol 68:3467–3477.

Hoebe K, Du X, Georgel P, Janssen E, Tabeta K, Kim S, Goode J, Lin P, Mann N, Mudd S, Crozat K, Sovath S, Han J, Beutler B. 2003. Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. Nature 424:743–748.

Janssens S, Beyaert R. 2003. Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. Mol Cell 11:293–302.

Johnston J, McFadden G. 2003. Poxvirus immunomodulatory strategies: Current perspectives. J Virol 77:6093–7100.

Karin M, Ben-Neriah Y. 2000. Phosphorylation meets ubiquitination: The control of NF-[kappa]B activity. Annu Rev Immunol 18:621–663.

Keller S, Schattner E, Cesarman E. 2000. Inhibition of NF-kappaB induces apoptosis of KSHV-infected primary effusion lymphoma cells. Blood 96:2537–2542.

Kieff E, Rickinson A. 2001. Epstein–Barr virus and its replication. Philadelphia: Lippincott Williams and Wilkins.

Komano J, Maruo S, K K, T O, Takada K. 1999. Oncogenic role of Epstein–Barr virus-encoded RNAs in Burkitt's lymphoma cell line Akata. J Virol 73:9827–9831.

Langland J, Jacobs B. 2002. The role of the PKR-inhibitory genes, *E3L* and *K3L*, in determining vaccinia virus host range. Virology 299(1):133–141.

Lawrence S. 2000. Advances in the treatment of hepatitis C. Adv Intern Med 45:65–105.

Levitskaya J, Sharipo A, Leonchiks A, Ciechanover A, Masucci M. 1997. Inhibition of ubiquitin/proteasome-dependent protein degradation by the Gly-Ala repeat domain of the Epstein–Barr virus nuclear antigen 1. Proc Natl Acad Sci USA 94:12616–12622.

Liu S, Liu P, Borras A, Chatila T, Speck S. 1997. Cyclosporin A-sensitive induction of the Epstein–Barr virus lytic switch is mediated via a novel pathway involving a MEF2 family member. EMBO J 16:143–153.

Marshall W, Yim C, Gustafson E, Graf T, Sage D, Hanify K, Williams L, Fingeroth J, Finberg R. 1999. Epstein–Barr virus encodes a novel homolog of the bcl-2 oncogene that inhibits apoptosis and associates with bax and bak. J Virol 73:5181–5185.

Mentzer S, Fingeroth J, Reilly J, Perrine S, Faller D. 1998. Arginine butyrate-induced susceptibility to ganciclovir in an Epstein–Barr-virus-associated lymphoma. Blood Cells Mol Dis 24:114–123.

Mosialos G, Birkenbach M, Yalamanchili R, VanArsdale T, Ware C, Kieff E. 1995. The Epstein–Barr virus transforming protein LMP1 engages signalling proteins for the tumor necrosis factor receptor family. Cell 80:389–399.

Moss B. 2001. Poxviridae: The viruses and their replication. Philadelphia: Lippincott Williams and Wilkins.

Oie K, Pickup D. 2001. Cowpox virus and other members of the orthopoxvirus genus interfere with the regulation of NF-kappaB activation. Virology 288:175–187.

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

Panus J, Smith C, Ray C, Smith T, Patel D, Pickup D. 2002. Cowpox virus encodes a fifth member of the tumor necrosis factor receptor family: A soluble, secreted CD30 homologue. Proc Natl Acad Sci USA 99:8348–8353.

Poppers J, Mulvey M, Perez C, Khoo D, Mohr I. 2003. Identification of a lytic-cycle Epstein–Barr virus gene product that can regulate PKR activation. J Virol 77:228–236.

Prince S, Keating S, Fielding C, Brennan P, Floettman E, Rowe M. 2003. Latent membrane protein 1 inhibits Epstein–Barr virus lytic cycle induction and progress via different mechanisms. J Virol 77:5000–5007.

Rassa J, Ross S. 2003. Viruses and Toll-like receptors. Microbes Infect 5:961–968.

Ray C, Black R, Kronheim S, Greenstreet T, Sleath P, Salvesen G, Pickup D. 1992. Viral inhibition of inflammation: Cowpox virus encodes an inhibitor of the interleukin-1B converting enzyme. Cell 69:597–604.

Reading P, Khanna A, Smith G. 2002. Vaccinia virus CrmE encodes a soluble and cell surface tumor necrosis factor receptor that contributes to virus virulence. Virology 292:285–298.

Regula K, Ens K, Kirshenbaum L. 2002. IKK beta is required for Bcl-2-mediated NF-kappa B activation in ventricular myocytes. J Biol Chem 277:38676–38682.

Rooney C, Smith C, Heslop H. 1997. Control of virus-induced lymphoproliferation: Epstein–Barr virus-induced lymphoproliferation and host immunity. Mol Med Today 3:24–30.

Ruf IK, Rhyne PW, Yang H, Borza CM, Hutt-Fletcher LM, Cleveland JL, Sample JT. 1999. Epstein–Barr virus regulates c-MYC, apoptosis, and tumorigenicity in Burkitt lymphoma. Mol Cell Biol 19:1651–1660.

Ruf I, Rhyne P, Yang C, Cleveland J, JT S. 2000. Epstein–Barr virus small RNAs potentiate tumorigenicity of Burkitt lymphoma cells independently of an effect on apoptosis. J Virol 74:10223–10228.

Sarid R, Sato T, Bohenzky R, Russo J, Chang Y. 1997. Kaposi's sarcoma-associated herpesvirus encodes a functional Bcl-2 homologue. Nat Med 3:293–298.

Spitkovsky D, Hehner S, Hofmann T, Moller A, Schmitz M. 2002. The human papillomavirus oncoprotein E7 attenuates NF-kappa B activation by targeting the Ikappa B kinase complex. J Biol Chem 277:25576–25582.

Stanley M. 2002. Imiquimod and the imidazoquinolones: Mechanism of action and therapeutic potential. Clin Exp Dermatol 27:571–577.

Tait S, Reid E, Greaves D, Wileman T, Powell P. 2000. Mechanism of inactivation of NF-kappa B by a viral homologue of I kappa b alpha. Signal-induced release of i kappa b alpha results in binding of the viral homologue to NF-kappa B. 275:34656–34664.

Thome M, Schneider P, Hofman K, Fickenscher H, Meini E, Neipel F, Mattman C, Burns K, Bodmer J-L, Schroter M, Scaffidi C, Krammer P, Peter M, Tschopp J. 1997. Viral FLICE-inhibitory proteins (FLIPS) prevent apoptosis induced by death receptors. Nature 386:517–521.

Virgin H, Latreille P, Wamsley P, Hallsworth K, Weck K, Dal Canto A, Speck S. 1997. Complete sequence and genomic analysis of murine gammaherpesvirus 68. J Virol 71:5894–5904.

Xiang Y, Condit R, Vijaysri S, Jacobs B, Williams B, Silverman R. 2002. Blockade of interferon induction and action by the E3L double-stranded RNA binding proteins of vaccinia virus. J Virol 76:5251–5259.

Xiao G, Harhaj E, Sun S. 2000. Domain-specific interaction with the I kappa B kinase (IKK)regulatory subunit IKK gamma is an essential step in tax-mediated activation of IKK. J Biol Chem 275:34060–34067.

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

Ye J, Xie X, Tarassishin L, Horwitz M. 2000. Regulation of the NF-kappaB activation pathway by isolated domains of FIP3/IKKgamma, a component of the IkappaB-alpha kinase complex. J Biol Chem 275:9882–9889.

Yin X-M, Oltvai Z, Korsmeyer S. 1994. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. Nature 369:321–323.