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NFκB inhibitors
Strategies from poxviruses

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The orchestration of the inflammatory responses to both infection and tissue damage is arguably the key physiological function of NFκB, and thus interference with the activation of NFκB represents an exceptional strategy for a successful pathogen to exploit to counter multiple host innate defense processes through the targeting of a single host regulatory pathway. Because of their large genomes, which typically encode ~200 proteins, and their unusual independence from the host nuclear transcriptional machinery, poxviruses are especially well suited to manipulate the cytoplasmic activation of NFκB. Indeed, poxviruses are known to encode multiple proteins that regulate the activation of NFκB in a variety of different ways and these can be considered potential paradigms for the development of novel anti-inflammatory therapies and more effective vaccines. Given the renewed interest in the pathogenesis of orthopoxviruses like smallpox and monkeypox, we review the current understanding of how the various classes of poxviral immunomodulatory proteins target and manipulate the NFκB pathway.

Introduction

Incessant communications between host and pathogens during their co-evolution have not only shaped the vertebrate immune system, but also the countermeasures used by successful pathogens. Survival of all viruses is strongly dependent on their ability to evade or subvert the cellular innate immune antiviral responses. Indeed, viruses from many families actively inhibit a diverse array of pathways and molecular targets to elude immune detection and destruction (reviewed in refs. 1–5). Among these, poxviruses are especially adept at interfering with various immune processes mounted by the host (reviewed in refs. 6–10).

Poxviruses belong to the family *Poxviridae*, which consists of large double-stranded DNA viruses that replicate exclusively in the cytoplasm of infected cells. The poxviruses are divided into eight recognized genera. Members from one genus, the orthopoxviruses, include variola (VARV), the causative agent of human smallpox, cowpox (CPXV), monkeypox (MPXV), vaccinia (VACV) and others. Poxviruses are among the more complex viruses that possess the genomic capacity to encode an array of inhibitors of host defenses. Because of their large genomes, which typically encode ~200 proteins, and their unusual independence from the host nuclear transcriptional machinery,[11] poxviruses can interfere with the expression of host genes without adversely affecting the expression of viral genes.

One of the hallmark signaling factors activated by tissue damage or microbial pathogens is the nuclear factor κB (NFκB), a key mediator of inducible transcription in the innate immune system. NFκB fulfills a central role in the cellular stress response and in inflammation by controlling the expression of a network of inducers and effectors that define responses to pathogens and other classes of danger signals.[12] Indeed, inducible regulation of self-protective gene expression is a central element of normal physiology and is key to the ability of multicellular organisms to adapt to various triggers of external stresses, including microbiological. Upon infection, microbial pathogens are sensed by the host and activate NFκB transcription factors via triggering of various sensors, like the TLRs (Toll-like receptors), which are expressed on cells of the innate immune system, including macrophages, DCs (dendritic cells) and mucosal epithelial cells.[13] Poxviruses, however, are especially well suited to block the cytoplasmic activation and transcriptional activities of NFκB. The interference with the activation of NFκB represents an exceptional strategy that poxviruses can exploit to counter multiple immune processes through the targeting of a single regulatory process, and as a consequence many poxvirus proteins have evolved to downregulate NFκB activation through different mechanisms. It is likely that poxvirus infection stimulates multiple sensors that are capable of activating NFκB, but whether the activation of NFκB-controlled defense genes actually occurs depends upon the competing inhibitory strategies mounted by each individual poxvirus. However, the outcome of this tug-of-war is not universal. For example, Modified Vaccinia Ankara (MVA), a promising replication-defective vaccine vector, is unusual among the orthopoxviruses in activating NFκB transcription factors in cells of several types,[14-16] presumably since it lacks too many of the inhibitors of NFκB signaling encoded by other orthopoxviruses. This review aims to highlight our current understanding of the strategies that poxviruses adopt to achieve the goal of controlling and neutralizing the host immune defenses triggered by the virus-activated IKK/NFκB signaling module.

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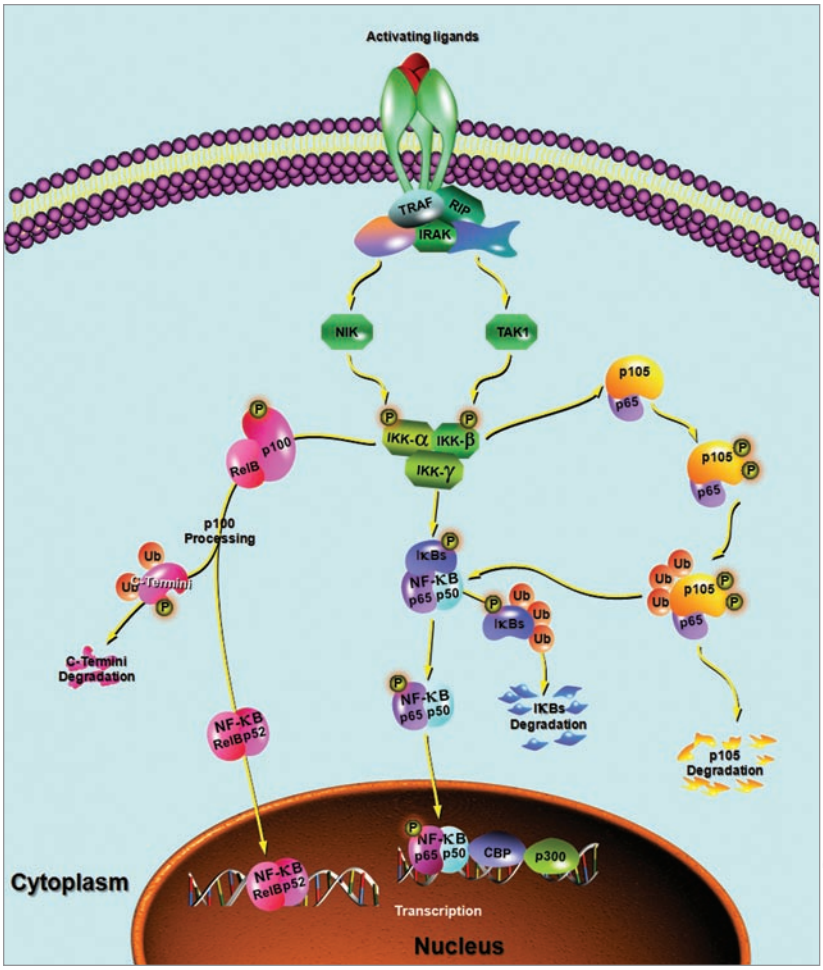

Figure 1. NFκB signaling pathways. Ligation-induced activation of various receptors for cytokines or PAMPs can induce the recruitment of receptor proximal adaptor proteins, where signaling to the IKK complex proceeds through TRAF/RIP complexes, generally in conjunction with TAK1, leading to canonical NFκB signaling, or through TRAFs and NIK leading to the noncanonical NFκB pathway. IKK activation induces IκB phosphorylation and degradation in the canonical pathway or p100 processing to p52 in the noncanonical pathway. In addition, active IKK may also induce the phosphorylation and degradation of p105, releasing associated NFκB subunits. Following their nuclear translocation, phosphorylated NFκB dimers bind to κB DNA elements and induce transcription of target genes. It is important to note that this simplified pathway is extremely general and that many inducers of the NFκB pathway will deviate from this outline to some extent.

NFκB Signaling

The NFκB complex consists of a family of dimeric transcription factors, which in mammals comprises RelA (p65), RelB, c-Rel, NFκB1 (p50) and NFκB2 (p52).[12] These structurally homologous proteins form various homodimers and heterodimers via their N-terminal Rel Homology Domains (RHDs). In unstimulated cells, NFκB dimers are sequestered in the cytoplasm via physical association of their RHDs with NFκB inhibitory proteins, called IκBs. NFκB1 and NFκB2 are first expressed as precursor proteins, p105 and p100 respectively, and have unique roles in the control of NFκB activity. Both p105 and p100 share structural homology with IκBs in their C-terminal portion, and thus act as IκB molecules when unprocessed, but provide the active subunits p50 and p52, respectively, upon processing.

NFκB activation is tightly controlled by both typical and atypical pathways, which can regulate the proteolysis of the inhibitory IκB and IκB-related proteins.[12] In the typical (i.e., canonical) pathway, NFκB activation is classically mediated by proteasomal degradation of the prototypical IκB member, IκBα[12] (**Fig. 1**). Upon stimulation by various innate immune receptors that have sensed pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), signal transduction events rapidly lead to the activation of the IκB kinase (IKK) complex, composed of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit, NEMO (NFκB essential modulator). Activated IKK phosphorylates IκBα, predominantly via the action of IKKβ, triggering its polyubiquitination and proteasomal degradation and inducing the nuclear translocation of associated NFκB subunits.

Atypical NFκB pathways, involving either p105 or p100, also have important immune functions.[17] In the p100-mediated pathway (i.e., non-canonical NFκB pathway), upon agonist stimulation, activated IKKα phosphorylates p100, triggering its polyubiquitination and subsequent partial proteolysis by the proteasome to produce p52, which translocates into the nucleus predominantly in association with RelB.[17] Unlike the p100 pathway, the processing of p105 to produce the active p50 subunit by the proteasome is constitutive and is not regulated by agonist stimulation. However, p105 is phosphorylated by IKKβ after activation of the canonical pathway, targeting it for complete degradation by the proteasome, which relieves its repression of associated NFκB subunits.

Poxvirus Proteins that Modulate the Activation of NFκB Signaling

Secreted poxvirus ligand inhibitors. For many years, poxviruses have been known to encode multiple proteins that can interfere with NFκB function[9,16] either directly, by inhibiting the immediate signaling members of the NFκB family, or indirectly by targeting upstream events that trigger the activation of NFκB (**Fig. 2**). In one strategy, many poxviruses express soluble, secreted versions of innate immune receptors or ligand-binding proteins for numerous cytokines that can cause the activation of NFκB, such as TNF, lymphotoxin-α (LTα), interleukin 1β (IL-1β), IL-18 and CD153.[6,10] Expression of these soluble viral receptors or cytokine binding proteins can intercept cellular ligand-receptor interactions, thus blocking the signaling events that lead to the activation of NFκB. The T2 protein from the leporipoxviruses Shope fibroma virus and myxoma virus was the first virus-encoded TNF receptor (vTNFR) to be

characterized.[18,19] T2 is expressed as a secreted glycoprotein that binds and inhibits TNF[18] and was shown to be important for myxoma virus virulence in the infected rabbit host.[19] In addition, the related CPXV proteins cytokine response modifier B (CrmB), C, D and E were also shown to act as soluble vTNFRs to intercept TNF ligand-receptor interaction.[20-23] These related vTNFRs encoded by cowpox virus also target LTα, another prototypic member of the TNF superfamily,[24] and orthologs were also found in other orthopoxviruses, including VACV, ectromelia virus (ECTV), MPXV and VARV.[25] CrmB is the only gene of this vTNFR family present in the genomes of multiple orthopoxvirus species, including VARV, MPXV and CPXV.[26] In addition, CPXV was also shown to encode an additional member of the TNFR family, a soluble, secreted form of CD30, the receptor for CD153.[24] More recently, another class of secreted poxvirus-encoded TNF inhibitors was described for members of the *Yatapoxvirus* genus.[27-31] For example, the 2L protein of tanapox virus (TPV-2L) is a potent inhibitor of human TNF, but more closely resembles a secreted version of the MHC-I heavy chain, rather than any known TNF receptor species.[29] Thus, these poxviral proteins are referred to as viral TNF-binding proteins (vTNF-BPs) rather than vTNFRs. Notably, secreted TPV-2L was shown to inhibit TNF-mediated NFκB signaling as measured by IκBα degradation.[32]

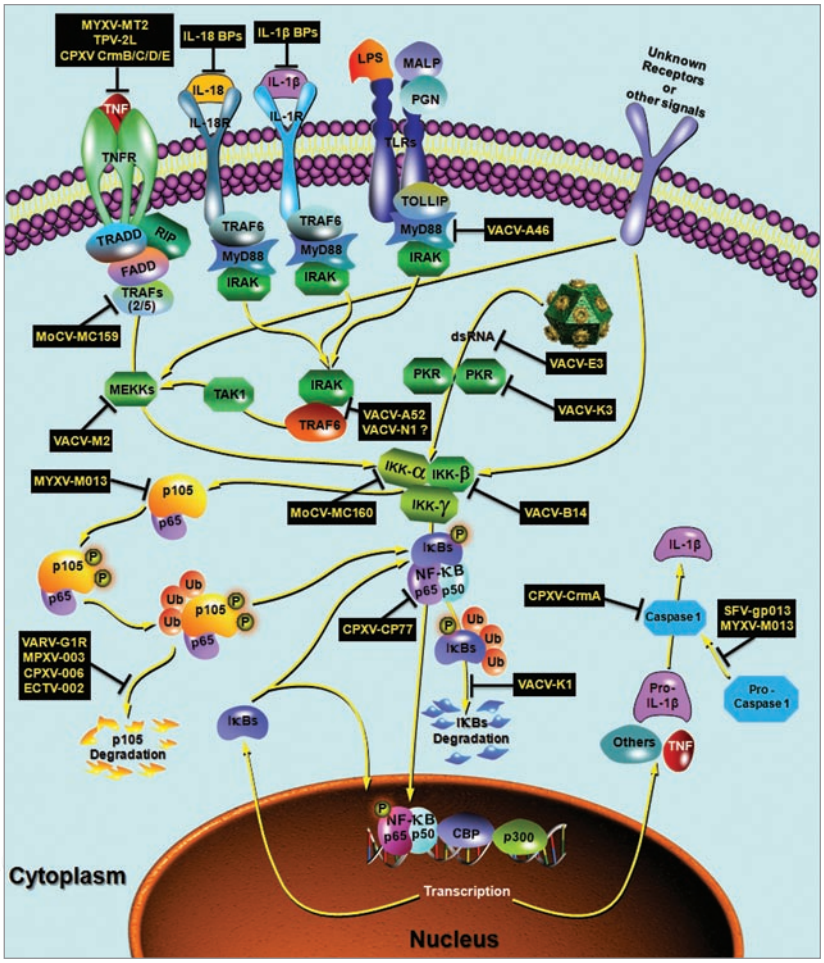

Figure 2. Poxvirus inhibitors of NFκB signaling pathways. A diagrammatic representation of the NFκB signaling pathways induced by various ligands, with focus on those known to be inhibited by poxviral proteins. The major points at which poxviral proteins have been reported to interfere with the NFκB pathway are indicated using black arrows and are described in the text.

Other cytokine binding proteins that interfere with the activation of NFκB have also been reported from various poxviruses. VACV and CPXV were shown to encode an abundant, secretory glycoprotein that functions as a soluble IL-1 receptor, which in contrast with cellular counterparts, binds only IL-1β and not IL-1α or the natural competitor IL-1 receptor antagonist.[33,34] Similarly, soluble IL-18 binding proteins (IL-18BP) were shown to be secreted from the molluscum contagiosum virus (MoCV) (gene MC54L),[35,36] as well as VACV, ECTV and CPXV.[35,37] Notably, the ECTV protein was found to block NFκB activation in response to IL-18.[35] In addition, related inhibitory proteins are encoded by other poxviruses, including VARV,[38] Yaba-like disease virus (YLDV),[30] MPXV[39] and swinepox virus (SPXV).[27] Moreover, deletion of the IL-18BP (gene C12L) from VACV strain western Reserve (WR) caused virus attenuation.[40] Furthermore, many poxviruses encode other classes of secreted proteins such as vaccinia growth factor (VGF), a protein similar to epidermal growth factor (EGF), which is capable of inducing the activation of NFκB of cells that are close to virus-infected cells in a paracrine like fashion.[41-45] VGF was shown to utilize and induce tyrosine phosphorylation of EGF receptors in A431 cells[46,47] and is important to the virulence of vaccinia virus.[48]

Intracellular poxvirus inhibitors of NFκB. Another strategy adopted by poxviruses is to express intracellular factors to regulate signaling pathways leading to NFκB activation in virus-infected cells. For example, some poxviruses encode caspase inhibitors, such as CrmA, which can inhibit caspase-1-mediated processing of pro-IL-1β and pro-IL-18 to the mature, active forms,[49] leading essentially to the inhibition of NFκB activation. Similarly, other caspase-1 inhibitors have been reported from VACV,[50] MYXV[10] and Shope fibroma virus (SFV).[51] In addition, as the activation of NFκB is an integral part of Toll-like receptor (TLR) signaling pathways, which recognize PAMPs such as viral glycoproteins and nucleic acids,[52,53] it is not surprising that many poxviruses have developed multiple mechanisms to modulate these pathways.[54] For example, two VACV encoded proteins, A46 and A52, that share amino acid sequence similarity with the Toll/IL-1 receptor (TIR) cytoplasmic domain, a motif that defines the IL-1/TLR superfamily of receptors, were reported to be capable of antagonizing signal transduction through IL-1 and TLRs.[55,56] A46 inhibits TLR-induced signaling by associating with TIR domain containing adaptor molecules, including myeloid differentiation factor 88 (MyD88), Mal (MyD88 adaptor-like),

TRIF, and TRAM and interferes with the activation of NFκB and MAP kinases.[56,57] On the other hand, A52 associates with IRAK2 and TRAF6, disrupts signaling complexes containing these proteins and blocks NFκB activation signaled through multiple TLRs, including TLR3.[56,58,59] Both A46 and A52 were also shown to inhibit signaling from TLR2, TLR4 and TLR9 in primary microglial cells.[55]

Another example of a TIR signaling inhibitor is the N1 protein from VACV, an intracellular homodimeric virulence factor,[60] that has been reported to inhibit NFκB activation by a variety of stimuli including IL-1β, TNF, and agonists for TLR2, TLR3 and TLR4.[61] Moreover, N1 protein was recently shown to inhibit signaling from TLR2, TLR4 and TLR9 in primary microglial cells.[55] N1 was also reported to inhibit receptor-, adapter-, TRAF- and IKK-α and IKK-β-dependent signaling to NFκB via its ability to associate with several components of the multisubunit IκB kinase complex.[61] Interestingly, a recent study by Graham et al. (2008) demonstrated the ability of N1 to inhibit IL-1-induced NFκB activation, however, failed to observe any N1 mediated inhibition of TNF-induced NFκB activation.[59] In addition, another report by Cooray et al. (2007) indicated that N1 does not inhibit IL-1-induced NFκB-dependent gene expression in VACV-infected cells, presumably due to the presence of other VACV NFκB signaling inhibitors. Moreover, a recent study showed that N1 did not co-purify or co-precipitate with the IKK complex, unlike another VACV protein, B14.[62] However, given that N1 inhibits TRAF6-induced NFκB activation but does not seem to interact with the IKK complex,[62] in contrast to previous suggestions,[61] it is likely that N1 acts at the level of TRAF6 or on downstream proteins in the pathway that precede the IKK complex.[59] In addition, the crystal structure of N1 reveals it is a Bcl-2-like protein[63,64] and N1 was shown to inhibit staurosporine-induced apoptosis in transfected and infected cells.[64] N1 was also shown to bind BH3 peptides from pro-apoptotic Bcl-2 family proteins Bid, Bim and Bak in vitro[63] and to co-precipitate Bid, Bad and Bax from VACV-infected cells.[64]

Given the importance of controlling NFκB activation during viral infection, many poxviruses encode several proteins that act on different cellular targets with the goal of tightly controlling all aspects of the NFκB pathway. For example, VACV gene B14R encodes a 17-kDa cytosolic protein (B14) that contributes to VACV virulence[65] and inhibits the IKK complex.[62] The interaction of B14 with the IKK complex depends on the presence of IKKβ, and B14 binding to the IKK complex prevents phosphorylation of the IKKβ activation loop. Consequently, B14 inhibits the phosphorylation and subsequent ubiquitin-mediated degradation of IkBα, the inhibitor of NFκB.[62] Given the ability of B14 to inhibit signaling from the IKK complex via its interaction with IKKβ: the nexus where IL-1 receptor, TNF receptor and TLR signaling pathways to NFκB converge, only B14, but not A52 or N1, is able to block signaling and activation of NFκB downstream of a variety of stimuli including TNF, IL-1, poly (I:C) and phorbol myristate acetate.[62] Despite sharing no significant sequence similarity with other viral or cellular Bcl-2-like proteins, B14, similar to A52, adopts a Bcl-2-like fold.[59] However, unlike cellular and viral Bcl-2-like proteins such as N1, both A52 and B14 lack a surface groove for binding BH3 peptides from pro-apoptotic Bcl-2-like proteins, a step necessary for antagonizing their function, and therefore they do not modulate apoptosis.[59] Another example is the VACV M2 protein, whose corresponding gene is absent from the MVA genome and was shown to interfere with step(s) that would otherwise enable ERK2 phosphorylation and the consequent activation of an NFκB response.[66] In addition, recombinant M2-expressing MVA virus restored the "wild-type" NFκB-inhibitory phenotype of VACV, as indicated by decreased NFκB migration to infected cell nuclei and interference in transcription.[66] M2 possesses motifs characteristic of ER-localized proteins and was shown to co-localize with cellular ER proteins.[67] Notably, elimination of the N-terminal leader sequence or the putative ER retention and retrieval motifs from the M2 protein compromised both its ER location and its ability to inhibit virus-induced NFκB activation, signifying the importance of ER location to the M2 ability to inhibit NFκB activation.[67]

Furthermore, MoCV encodes two proteins, MC159 and MC160, containing death effector domains (DEDs), which belong to a family of cellular and viral FLIP (vFLIP)-like proteins.[68-70] Although proteins MC159 and MC160 likely share a similar structure, only MC159 has been shown to inhibit apoptosis induced in response to death receptor FADD.[68-70] MC159 was also shown to inhibit NFκB activation induced by the overexpression of TNF-RI molecules[71] and by PKR.[72] Further analysis revealed that MC159 prevents TNF-induced IκBβ degradation and consequently the persistent NFκB transcriptional activation and NFκB-mediated expression of cellular genes that are initiated later in response to TNF.[73] However, MC159 did not prevent IκBα degradation, correlating with its inability to inhibit transient NFκB transcriptional activation, or inhibit the upregulation of transcription of early host genes.[73] In addition, the previously reported MC159-RIP interaction[71] was shown not to be important for MC159 inhibitory functions.[73,74] In contrast, MC159-TRAF2 interaction[71,73] was shown to be critical for the inhibitory function of MC159 and was proposed to be responsible for preventing MEKK2–IKK complex formation to prevent IκBβ degradation.[73] As for MC160, expression of the protein was shown to significantly reduce TNF-mediated NFκB activation as well as activation by receptor-interacting proteins such as TRAF2, NIK or MyD88, via its ability to reduce IKK kinase activity and IKK subunit phosphorylation.[75] Furthermore, IKKα-IKKβ interactions were not detected in MC160-expressing cells, under conditions demonstrated to induce IKK complex formation, however, interactions between the MC160 protein and the major IKK subunits were undetectable.[75] Interestingly, MC160 was shown to induce IKKα degradation by competitively interacting, through its C-terminal region, with the cellular heat shock protein 90 (HSP90), necessary for IKKα stabilization.[76] In addition, the death effector domain (DED)-containing N-terminal region of MC160 was also shown to associate with procaspase-8 and inhibit procaspase-8-mediated NFκB activation, indicating that the MC160 protein utilizes at least two distinct mechanisms for impeding NFκB activation.[76]

A central enzyme involved in anti-viral activity is the dsRNA dependent protein kinase, PKR, a serine/threonine kinase that is activated upon dsRNA binding and subsequent autophosphorylation. In addition to its role as a translational controlling factor, PKR is a key transcriptional regulator exerting anti-viral and anti-tumoral activities (reviewed in refs. 77 and 78). PKR affects diverse transcriptional factors such as interferon regulatory factor 1, STATs, p53, activating transcription factor 3, and NFκB.[77] Although PKR is known to trigger a cascade of events involving IKK phosphorylation of IκBα and NFκB nuclear translocation, the nature of the PKR effect is unclear.[78] However, it seems likely that the catalytic activity of PKR is necessary to signal dsRNA-dependent activation of IKK and the subsequent NFκB activation.[79,80] The extent and strength of the anti-viral action of PKR are clearly understood by the findings that unrelated viral proteins of animal viruses have evolved to inhibit PKR action by using diverse strategies, such as the VACV E3 and K3 proteins.[81,82] K3 is thought to act as a competitive inhibitor of PKR through its homology to eIF-2α, whereas E3, a dsRNA-binding protein, was shown to interfere with the binding of the kinase to dsRNA.[83] Moreover, E3 has been previously shown to inhibit both p38 and NFκB,[84] as well as cytokine expression.[85] Recently, a requirement for PKR in the activation of p38 signal transduction and NFκB nuclear translocation has been described, highlighting the function of E3 in suppressing these events to limit cytokine expression.[86]

Ankyrin repeat NFκB inhibitors encoded by poxviruses. Ankyrin repeat (ANK) proteins form the largest family of poxvirus proteins and are encoded by almost all chordopoxviruses.[87] These proteins are composed largely of multiple copies of the ANK motif that in many eukaryotic proteins mediates protein-protein interactions.[88] Several studies have already reported the ability of some of these ANK-repeat proteins to inhibit NFκB activation. For example, VACV K1, an ANK-repeat containing protein, has been shown to inhibit NFκB activation in rabbit kidney RK-13 cells by preventing IκBα degradation[89] and was recently shown to inhibit signaling from TLR2, TLR4 and TLR9 in primary microglial cells.[55] However, K1 inhibition of host NFκB activation doesn't seem to be related to the host range function of K1 since this inhibition was observed both in permissive and nonpermissive cells.[89] Given that K1 contains ANK-repeats like those present in IκB family members, it was initially predicted to displace IκBα and subsequently bind to and inhibit NFκB. This scenario would be similar to that adopted by the A238L protein of the African swine fever virus (ASFV), which shares 40% homology with IκBα[90] and binds to free NFκB/p65 to prevent its nuclear translocation.[91,92] However, since IκBα was shown to remain intact in the presence of K1, in contrast to the case in presence of the A238L of the ASFV,[91,93] it was instead suggested that K1 may inhibit IκBα degradation by interfering either directly with IKK or indirectly with kinases that act upstream of IKK to prevent IκBα phosphorylation.[89] Another ANK-containing protein from MYXV, M150, has been shown to co-localize with NFκB following TNF treatment of cells.[94] In vivo experiments using an M150 knockout virus showed that M150 is a critical virulence factor, with its deletion generating an increase in the inflammatory process, consistent with the interference of M150 with the NFκB-induced pro-inflammatory pathway.[94]

Recently, additional ANK-containing NFκB inhibitors have been reported from several orthopoxviruses including CPXV, MPXV, ECTV and VARV.[95-97] CP77, a CPXV protein with nine predicted ankyrin repeats, is expressed in the early phase after viral infection and is necessary for VACV growth in non-permissive cells.[98-102] A recent study reported that CP77 was able to block either IL-1 or TNF-induced NFκB activation at a step downstream of IKK kinase activation.[95] In addition, CP77 was shown to bind to the NFκB/p65, similar to A238L of the ASFV,[91,92] through the N-terminal six-ANK-repeat region and to Cullin-1 and Skp1 of the SCF complex through a C-terminal F-box-like domain, with both regions being required to block NFκB activation.[95] However, in contrast to CP77, A238L binds to free NFκB without the need for binding to SCF complex, so the mode of CP77 action does not seem to be the same as that of the A238L protein. Instead, it was suggested that when signal-induced phosphorylation and degradation of IκBα occurs in TNFα-treated cells, the N-terminal region of CP77 serves as a "surrogate" IκB-like domain, while the C-terminal region binds to the SCF complex, preventing NFκB from being released into cell nucleus.[95] Moreover, an ANK-containing protein from VARV, G1R, which is substantially divergent from any vaccinia counterparts but is highly conserved among several other pathogenic orthopoxvirus, including CPXV, MPXV and ECTV, was shown to interact with both NFκB1/p105 and Skp1 and to inhibit NFκB activation.[96] This was the first reported interaction between a virally-encoded protein and NFκB1/p105. Members of this family of ANK-repeat proteins were shown to block TNF-induced NFκB1/p105 degradation as well as NFκB activation in transfected cells.[96] Characterization of the targeted cowpox gene (CPXV-006) knockout virus that lacks the expression of this ANK-containing NFκB inhibitor was also recently reported, showing that, in contrast to wild type CPXV, infection with the CPXV-006-knockout virus rapidly induces high levels of a variety of NFκB-controlled pro-inflammatory cytokines from infected human myeloid (THP1) cells.[97] Furthermore, the CPXV-006-knockout virus was attenuated for disease pathogenesis in the murine intratrachael model, and induced a significantly elevated cellular inflammatory response at tissue sites of virus replication in the lung.[97] These data indicate that the CPXV ANK-repeat 006 protein interferes with the NFκB-induced pro-inflammatory pathway in vitro and in vivo.[96,97]

PYRIN domain NFκB inhibitors encoded by poxviruses. PYRIN domain (PYD) proteins have recently emerged as important signaling molecules involved in the development of innate immunity to intracellular pathogens through activation of inflammatory mediator pathways. Caspase-1-mediated processing of pro-IL-1β and IL-18 as well as activation of the transcription factor NFκB have been described as effectors of PYD-mediated signal transduction.[103-105] A MYXV-encoded M013 protein, with sequence similarity to the cellular PYD-only protein (cPOP1), which can block recruitment of ASC to activated PAN receptors and inhibit the PYD-mediated signal

transduction pathway,[106] was shown to directly associate with ASC and inhibit PYD-mediated activation of pro-caspase-1 and subsequent processing of pro-IL-1β and pro-IL-18.[107] Similarly, another PYD-containing protein from SFV, gp013, was also shown to directly associate with ASC and inhibit PYD-mediated activation of pro-Caspase-1 and subsequent processing of pro-IL-1β.[108] Additional PYD-only proteins were identified in the genomes of swinepox virus (SPXV), YLDV, and deerpox virus (DPXV). Interestingly, both MYXV and SFV proteins were claimed in one report to induce NFκB activation in transiently transfected cells,[108] in contrast to cPOP1.[106] However, these results are in a conflict with more recent results indicating that transfected MYXV-M013 inhibits TNF-induced nuclear translocation and transcriptional activities of NFκB, presumably through its ability to interact with NFκB1/p105.[109] Further studies on the mechanism of action of these viral PYD-containing modulators of NFκB signaling and inflammasomes are likely to shed new light on how the NFκB pathway is integrated with other pathogen-defense response pathways as well.

Concluding Remarks

Significant advances in our understanding of the sensing, recognition and response to viral pathogens have been made in the past decade; at pace with these discoveries has been the increase in our understanding of the mechanisms used by viruses to interfere with, and manipulate, the diverse host immune responses. For millions of years, viruses have been probing for weaknesses in host immune pathways and this exploration is yielding important clues about which pathways must be pro-actively compromised in order for virus infection to successfully perpetuate. The NFκB pathway is a prime target for viral evasion and many viruses have developed strategies to manipulate NFκB signaling through the use of multifunctional viral proteins that target either its activation or its direct signaling cascade members. By targeting NFκB-regulated gene expression, the virus interferes with one of the central regulatory mechanisms of the innate immune system, enabling the virus to suppress the expression of numerous proteins that contribute to the various defense responses against infection. Like many viruses, poxviruses target many of these host proteins, or the processes in which these proteins are engaged, through several mechanisms.

These different mechanisms of poxviral interference with NFκB activation appear to be complementary rather than redundant mechanisms, but we still need to better understand why individual poxviruses express so many inhibitors of the same pathway. Greater understanding of the mechanisms by which poxviruses induce and then inhibit the activation of NFκB will be a cornerstone in the understanding of the molecular aspects of viral pathogenesis and the improvement of strategies for the development of vaccines that are better stimulators of acquired immunity. In addition, NFκB-regulated gene expression is a major contributory factor in the pathogenic processes associated with a variety of infectious and noninfectious diseases, including rheumatoid arthritis, asthma, sepsis, neurodegenerative disorders, and various cancers. Thus, better understanding of the mechanisms by which poxviruses affect the activation of NFκB may assist the development of new therapeutic strategies to control the pathogenic effects of NFκB activation in a number of these diseases. Indeed, there are many more lessons still to be taught by poxviruses about the most effective ways to manipulate NFκB responses in general.

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