



Review

Viral Strategies in Modulation of NF- κ B Activity

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Abstract. Activation of the nuclear factor (NF)- κ B transcription factor family in response to different stimuli, such as inflammatory cytokines, stress inducers or pathogen products, results in host innate and adaptive immunity. NF- κ B plays a central role in promoting the expression of genes involved in inflammatory, immune and apoptotic processes, including those encoding cytokines, chemokines, cytokine receptors and proteins involved in antigen presentation. Although the main function of NF- κ B is to activate specific genes in the cells of the immune system, its role in controlling the host cell cycle makes NF- κ B an interesting target for pathogenic viruses. Some viruses take advantage of the anti-apoptotic properties of NF- κ B to escape host defence mechanisms, others use apoptosis to spread. This review describes the role of the NF- κ B family in immune responses, the mechanism of NF- κ B activation, and different strategies that viruses have developed to modulate the NF- κ B pathway in order to facilitate and enhance viral replication and avoid host immune responses.

Key words: NF- κ B; IKK complex; viral infection; apoptosis.

Introduction

The nuclear factor (NF)- κ B family of transcription factors comprises a group of important regulators of the host innate and adaptive immune response. Discovered by David Baltimore's group in the 80's, it turned out to be an interesting subject of research due to its unusual regulation, the variety of different stimuli that can activate it, as well as the diverse genes and biological responses that it controls. The activation of NF- κ B by many bacterial products, inflammatory cytokines and stress inducers is associated with the induction of the expression of a number of different genes, including those encoding cytokines, chemokines, adhesion molecules, and acute-phase proteins. Moreover, for several reasons this factor comprises an attractive target for many viral pathogens. Its activation is a rapid event that

occurs within minutes after exposure to an appropriate inducer, does not require *de novo* protein synthesis, and results in a strong transcriptional stimulation not only of cellular genes, but also those encoded by viral genetic material. This in turn results in the virus evading the host defense mechanisms and its propagation in the infected organism.

Characteristics of NF- κ B Proteins

The NF- κ B family of transcription factors contains 5 members: NF- κ B₁ (p105/p50), NF- κ B₂ (p100/p52), RelA (p65), RelB and c-Rel. NF- κ B₁ and NF- κ B₂ are synthesized as large polypeptides that are posttranscriptionally cleaved to generate the subunits p50 and p52,

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which can bind to the DNA. All members of the NF- κ B family are characterized by the presence of a Rel homology domain that is involved in sequence-specific DNA-binding, dimerization and interaction with the inhibitory I κ B proteins²¹. The NF- κ B proteins, with the exception of RelB, dimerize to form homo- and hetero-dimers depending on different stimuli; the classic form is a hetero-dimer of p50 or p52, which both lack transcriptional activation domains, and p65, which is able to affect transcription. NF- κ B₁ (p50) and RelA are ubiquitously expressed, while NF- κ B₂, RelB and c-Rel are expressed specifically in lymphoid tissues and cells⁵⁵.

Regulation of NF- κ B Activation

Role of the I κ B regulatory proteins and I κ B kinases

In the cytoplasm of an unstimulated cell, NF- κ B exist in an inactive form as a consequence of association with inhibitory proteins called inhibitors of κ B (I κ B). This family includes I κ B α , I κ B β , I κ B ϵ , Bcl-3 and C-terminal regions of p105 (NF- κ B₁) and p100 (NF- κ B₂) subunits. The I κ B proteins bind with different affinities and specificities to NF- κ B dimers; for example I κ B β inhibits p50-p65 heterodimer stronger than it inhibits p50-RelB and p50-c-Rel complexes, whereas I κ B α similarly affects all these complexes⁶⁰ (Fig. 1).

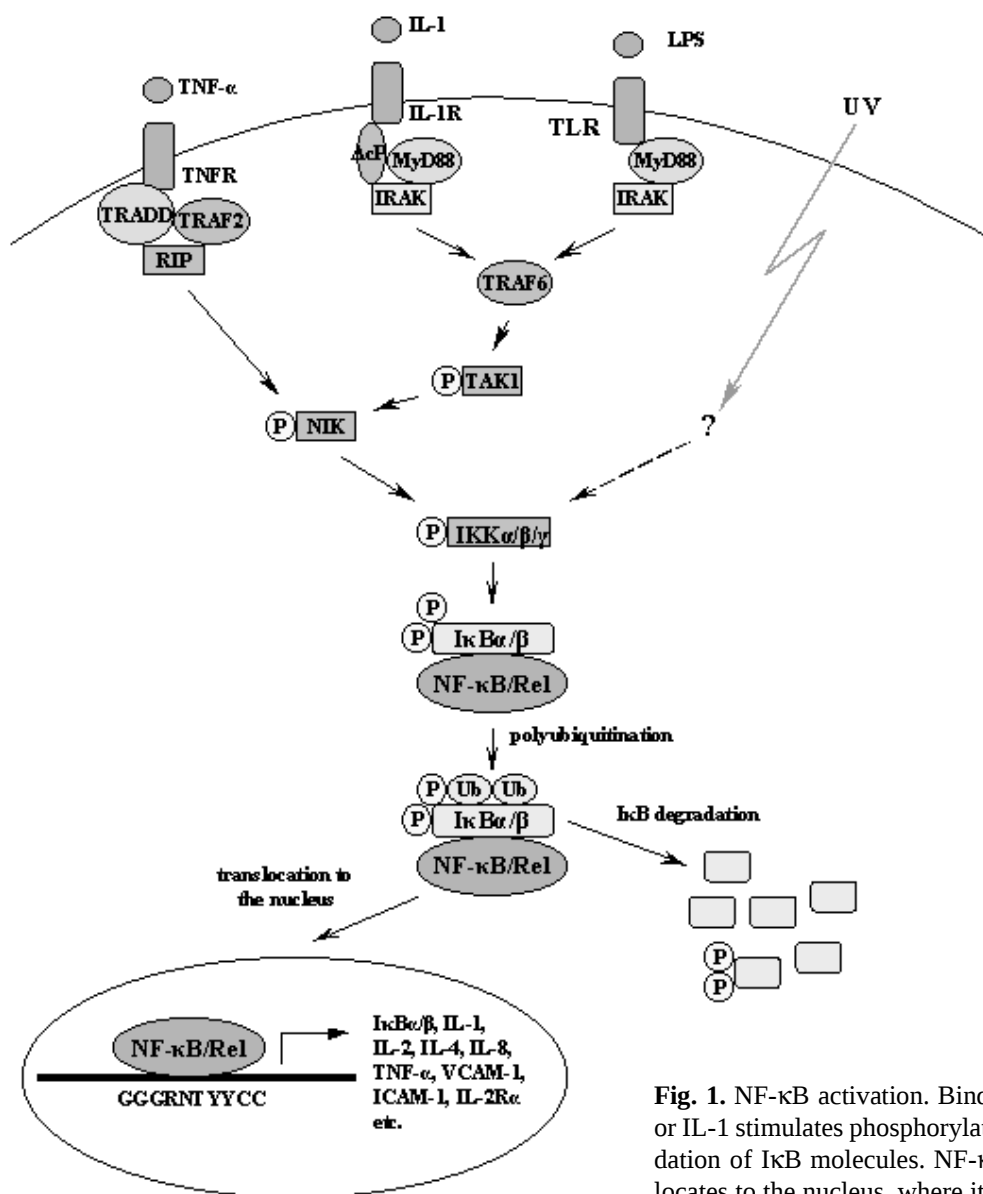


Fig. 1. NF- κ B activation. Binding of agents such as TNF- α or IL-1 stimulates phosphorylation, ubiquitination and degradation of I κ B molecules. NF- κ B is then released and translocates to the nucleus, where it binds to κ B motifs present in the promoters, inducing transcription of target genes

Phosphorylation of these inhibitors is an important step in NF- κ B activation. The binding of a ligand (e.g. tumor necrosis factor (TNF)- α , interleukin (IL)-1, or CD40 ligand) to its receptor on the cell surface and transduction of a signal generated in that way results in the activation of the I κ B kinases (IKKs). The IKK complex consists of 3 components: IKK α , IKK β ⁷⁰ and the regulatory subunit IKK γ ⁶⁸. These kinases phosphorylate specific serine residues at the N-terminal region of I κ B molecules, the phosphorylated I κ Bs are then ubiquitinated, which is a signal for their degradation by the 26S proteasome³³. NF- κ B is then released and translocates to the nucleus, where it binds to κ B motifs present in the promoters, inducing transcription of target genes. In some cells I κ B α protein can be degraded by calpains, a calcium-dependent group of cytoplasm proteases, instead of or in addition to the proteasome pathway^{24, 40}.

Toll-like receptor-mediated NF- κ B activation

Toll-like receptors (TLRs) represent a family of transmembrane proteins that are characterized by the presence of multiple copies of leucine-rich repeats (LRRs) in the extracellular domain and a Toll/IL-1R (TIR) motif in the cytoplasmic domain. Many bacterial products, including lipopolysaccharide (LPS), lipoproteins and other cellular components, can activate NF- κ B thanks to the ability of TLRs to recognize and distinguish different pathogens²⁸. Engagement of these receptors leads to the activation of the innate immune response³ by inducing NF- κ B, a main regulator of immune and inflammatory responses. Several TLRs have been found, distributed in various cells of the human immune system (macrophages, dendritic cells, B and T cells)^{42, 48}, which can explain the recognition of so many different classes of pathogens by these receptors. For instance, TLR4 is known as a ligand for LPS²⁷, whereas TLR2 recognizes a larger group of microbial products, including lipoproteins and peptidoglycans⁶¹. The consequence of signaling through TLRs is linked to the expression of a number of genes known to be regulated by NF- κ B, including those encoding cytokines, costimulatory molecules, nitric oxide synthase and proapoptotic proteins^{2, 13}.

Role of the NF- κ B Pathway in Immune Responses

NF- κ B can be activated by a variety of inducers, including proinflammatory cytokines such as TNF- α and IL-1, T cell activation signals, growth factors, stress inducers and pathogens invading the organism²¹.

This results in binding of this transcription factor to specific κ B motifs present in the promoters of target genes. Genes controlled by NF- κ B are involved in immune and inflammatory responses, cell growth control and apoptosis. Those encoding cytokines, cytokine receptors, adhesion molecules and growth regulators are positively controlled by this factor and include genes encoding IL-2, IL-6, IL-8, the IL-2R, the IL-12 p40 subunit, VCAM-1, ICAM-1, TNF- α , interferon (IFN)- γ and c-Myc^{5, 6, 21}. Activation of IL-12, one of the most important cytokines involved in the induction of innate and adaptive response to intracellular infection that leads to activation of natural killer cells, enhances their cytolytic activity, and the production of IFN- γ is considered one of the essential activities of NF- κ B⁵¹.

The expression of most of the NF- κ B family members in T lymphocytes points to involvement of these factors in the regulation of T cell functions. There are many studies which suggest direct links between NF- κ B and T cell proliferation. First, activation of these transcription factors induces the expression of cyclin D1, which is the main positive regulator of G1-to-S phase progression. NF- κ B activates cyclin D1 expression by direct binding to multiple sites in its promoter^{23, 31}. Interestingly, until very recently the involvement of cyclin D1 in the proliferation mechanisms of T cells was negated at the expense of other members of the cyclin D group – cyclins D2 and D3^{1, 7}; however, a recently published gene array study of activated human CD4⁺ cells has demonstrated the transcriptional activity of the D1 gene³⁸. Our group has also recently observed the cyclin D1 protein in rapidly dividing human CD4⁺ lymphocytes (WITKOWSKI and BRYL, in preparation). Secondly, signaling through the T cell receptor (TCR) leads to protein kinase C θ activation and consequently to the induction of NF- κ B⁵⁹, which may be essential in TCR-mediated proliferative signals. Ligation of CD28, a critical costimulatory molecule, provides an additional I κ B kinase-targeting signal during T cell activation²⁵ and leads to activation of c-Rel and NF- κ B₂³², which regulate the expression of IL-2 and the antiapoptotic molecule Bcl-x_L^{11, 37}.

The identification of the κ B site in the immunoglobulin κ light chain enhancer and the observation of the activity of NF- κ B in B cells indicate that this transcription factor is also very important in the control of B cell functions⁵². During B lymphocyte maturation the composition of proteins in complex binding to the κ B motifs changes, while in mature cells p50/c-Rel is observed to be the dominant dimer. This observation indicates that the NF- κ B molecules participate in immunoglobulin class switching⁵⁷. NF- κ B₁ is required for

the survival of quiescent B cells and, in combination with c-Rel, prevents apoptosis of mitogen-stimulated cells. Similarly, c-Rel, RelA and RelB are necessary for proliferative responses upon stimulation through the CD40 receptor^{18, 22, 56, 64}.

The NF- κ B family members also participate in the regulation of apoptosis, an important process in the development and selection of T and B lymphocytes that is critical for the maintenance of immunological homeostasis. NF- κ B activation is strongly involved in the inhibition of apoptosis⁸ through regulation of the expressions of antiapoptotic genes, e.g. TRAF1, TRAF2, c-IAP1, c-IAP2, IEX-1L, Bcl-x_L and Bfl-1/a1^{35, 65, 67, 71}. Moreover, this transcription factor is also associated with proapoptotic functions such as the apoptosis of double-positive thymocytes, which is associated with the down-regulated expression of the gene *bcl-x_L*²⁶. There are also studies suggesting a direct link between proteasome-dependent NF- κ B activation and activa-

tion-induced cell death through FasL gene activation and up-regulation of the Fas gene^{17, 63}. However, it remains unclear whether translocated NF- κ B influences FasL gene expression by binding directly to its promoter region or indirectly through immediate genes, whose expressions hinge on NF- κ B activity. Consensus has been reached that NF- κ B binding sites in the Fas promoter^{9, 44} indicate that the activation of Fas gene expression is mediated by RelA⁴⁴.

Mechanisms of Virus-Dependent Activation and Inhibition of the NF- κ B Pathway

Although activation of NF- κ B in response to the appearance of viral and bacterial pathogens is associated with the development of protective immunity, some pathogens have developed strategies that can interfere with the host NF- κ B pathway (Fig. 2). The best

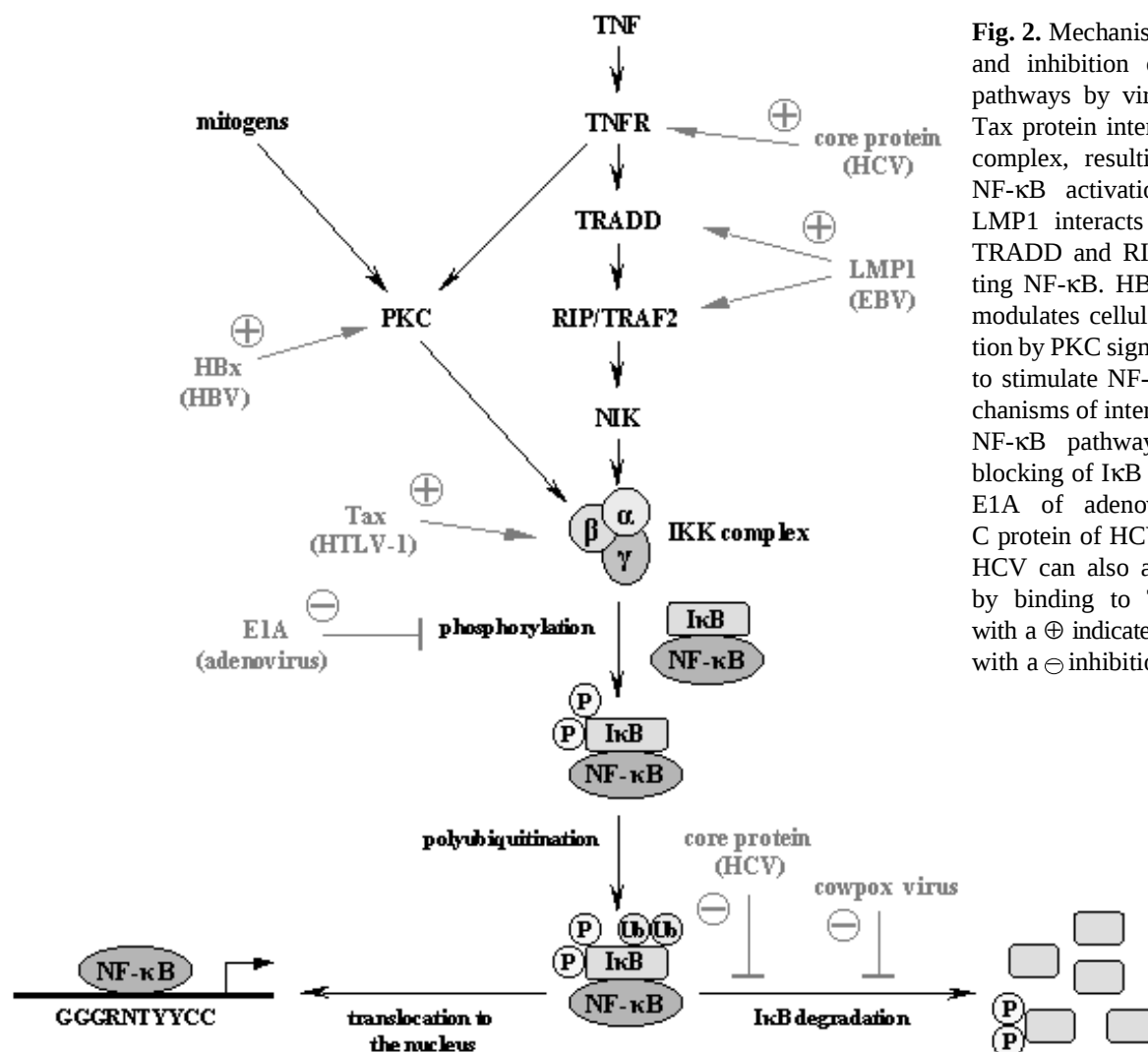


Fig. 2. Mechanism of activation and inhibition of the NF- κ B pathways by viruses. HTLV-1 Tax protein interacts with IKK complex, resulting in chronic NF- κ B activation. The EBV LMP1 interacts with TRAF2, TRADD and RIP, thus activating NF- κ B. HBx of the HBV modulates cellular gene activation by PKC signaling pathways to stimulate NF- κ B. Other mechanisms of interacting with the NF- κ B pathway include the blocking of I κ B degradation by E1A of adenovirus or core C protein of HCV. C protein of HCV can also activate NF- κ B by binding to TNFR1. Lines with a $\oplus$ indicate activation, and with a $\ominus$ inhibition of NF- κ B

examples of this phenomenon are viruses that make use of NF- κ B activation to directly enhance viral replication or to avoid the cell apoptosis the host mechanism employs to limit it. Furthermore, the persistent activation of this transcription factor maintained by some viruses is conducive to oncogenic transformations⁴⁶. NF- κ B can be activated by several families of viruses, including human immunodeficiency virus type 1 (HIV-1), human T cell lymphotropic virus type 1 (HTLV-1), Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), influenza virus and others. There are known viral products that activate the NF- κ B molecule, such as the HTLV-1 Tax protein or the HIV-1 Tat protein, which act through several different mechanisms to enhance viral replication. For example, in the case of HIV the fusion of cellular and viral membranes that induces NF- κ B activity requires the presence of the CD4 molecule. Moreover, NF- κ B can also be induced by synthetic dsRNA, suggesting that viruses, which generate dsRNA replicative intermediates, engage common mechanisms enhancing their replication. Below we will shortly summarize current knowledge on the interaction between the NF- κ B activation machinery and the viruses mentioned above.

Intracellular efficiency of HIV-1 gene expression and replication is a result of the ability of this pathogen to use host signaling pathways. Two NF- κ B binding sites has been identified in the enhancer region of the viral promoter of the long terminal repeat (LTR)¹⁵, a finding which supports a central role of this transcription factor in mediating the expression of viral genes. This observation confirms that these elements play an important part in the pathogenesis of AIDS by facilitating the propagation of the virus. Stimulation by TNF- α , IL-1 or LPS visibly enhances transcription of the HIV-1 LTR through the activation of NF- κ B⁴⁹. What is more interesting, differences in the number of κ B motifs in different HIV-1 subtypes have been observed. For example, HIV-1 subtype E contains a single NF- κ B binding site in the enhancer and exhibits reduced TNF- α responsiveness^{30, 41}, while the enhancer of HIV-1 subtype C contains three NF- κ B binding sites and has the highest transcriptional activity of all the subtypes³⁰. Synergism between NF- κ B and another transcription factor, SP-1, which binds within three sites adjacent to the κ B motifs, enhances the transcriptional activity of LTR. Additionally, NFAT1 (NFATp), a molecule from the NFAT family, competes with NF- κ B for its binding site and negatively regulates HIV-1 LTR, whereas NFAT2 (NFATc) positively regulates this region, also by binding to the κ B site³⁶. These studies indicate that these transcriptional modifications alter viral replication

and can possibly affect the pathogenesis of the disease³⁰.

Another very important fact is that in HIV-1-infected cells the IKK complex is constitutively activated⁴. It is still unclear how IKKs are activated, but this observation points to multiple mechanisms that are involved in the NF- κ B-dependent induction of viral gene transcription, and several HIV proteins can regulate this transcription factor. Binding of the envelope glycoprotein 120 to the CD4 receptor generates a signal that involves p56^{lck} kinase, the signaling molecule Ras and Raf kinase^{20, 62} and activates NF- κ B in much the same way as during the response of a lymphocyte to presented antigen. CD4 cross-linking also activates the phosphatidylinositol 3-kinase, which through Akt kinase stimulates the IKK complex¹².

Many HIV regulatory and accessory proteins have been identified as involved in NF- κ B induction. One of them, Tat protein, the primary role of which is to regulate productive transcription from the HIV-1 LTR, induces a variety of cellular responses, including caspase activation and accumulation of reactive oxygen intermediates. This viral protein stimulates NF- κ B in a p56^{lck}-dependent manner³⁹: it induces serine phosphorylation of one of the I κ B proteins only in cells expressing p56^{lck}, which suggests that this kinase can indirectly modulate the function of IKK. Yet another protein, called Vpr, activates IL-8 expression in T cells and macrophages through NF- κ B- and NF-IL-6-dependent mechanisms⁵⁰. An elevated level of this molecule also increases the transcriptional activity of other viral promoters (e.g. cytomegalovirus, SV40 genes) and several proinflammatory cytokine genes⁵⁰. Finally, Nef protein increases IL-2 secretion in CD3- or CD28-stimulated T lymphocytes, which results in activation of both NFAT and NF- κ B. It has been shown to bind to p56^{lck}¹⁹, which indicates that this T cell-specific kinase plays a critical role in HIV-Tat and -Nef signaling. In this way, Nef enhances the transcription of viral genes, promoting HIV-1 replication and virus spread⁶⁶.

HTLV-1 is the etiological agent of adult human T cell leukemia, an acute malignancy of CD4⁺ T lymphocytes. Its component protein, called Tax, is an oncoprotein that is required for the initiation of transcription directed from the 5' LTR of the proviral genome and is necessary for the oncogenic activity of the retrovirus. The Tax protein also influences NF- κ B activity, which results in the up-regulation of cellular genes that are normally responsible for growth signal transduction, such as those encoding cytokines and growth factors (IL-2, IL-6, IL-15, TNF and GM-CSF), cytokine receptors (IL-2 and IL-15 receptor α chains), proto-on-

cogenes (c-Myc) and antiapoptotic proteins (Bcl-x_L)²⁹. The persistent activity of Tax results in the constitutive activation of NF- κ B, which leads to the initiation and/or maintenance of the malignant phenotype⁵⁸. Interactions between the IKK complex and the viral protein lead to chronic IKK activation, which results in persistent expression of the NF- κ B^{16, 58}. Another mechanism by which the Tax protein promotes T cell oncogenic transformation requires inactivation of the p53 tumor suppressor by phosphorylation of its serine residues. This process is a result of Tax-related activation of cellular pathways that lead to NF- κ B gene expression⁴⁵.

EBV is a human γ herpesvirus that targets B lymphocytes and is associated with a number of human malignancies. In this case, the viral oncoprotein latent membrane protein 1 (LMP1), which is a transmembrane protein, is essential for B cell oncogenic transformation and is expressed in most EBV-associated carcinomas and in Hodgkin's lymphoma. LMP1 is a source of continuous signal, which activates NF- κ B protein by usurping the TNF signaling pathway. Normally, TNF- α activates NF- κ B within a few minutes of ligand binding and induces apoptosis if stimulation prolongs. TNF- α binds to the receptor TNF receptor 1 (TNFR1), inducing its oligomerization. The cytoplasmic region of TNFR1 contains a motif which is recognized by the TNFR-associated death domain (TRADD) protein, the over-expression of which activates NF- κ B and induces apoptosis. TRADD can also interact with another protein, TNFR-associated factor 2 (TRAF2), and with receptor-interacting protein (RIP), both of which can activate NF- κ B. Viral LMP1 interacts with TRAF2, TRADD and RIP, thus activating transcription factor without inducing apoptosis. Just as in case of TNF- α , NF- κ B activation by the LMP1 signaling cascade is mediated by the IKK complex that phosphorylates I κ B proteins¹⁴.

HBx protein is a 17 kDa polypeptide encoded by the DNA of the HBV (responsible for acute and chronic infections of the liver and promoting hepatocellular carcinoma). This protein can modulate cellular gene transcription by modulating the Ras and protein kinase C signaling pathways to stimulate NF- κ B^{10, 34}. HBx is also involved in interactions with other transcription factors, such as AP-1, AP-2, NFAT and C/EBP. In the case of HCV, its multifunctional core protein competes with TRADD for binding to the cytoplasmic domain of the TNFR1 – receptor for TNF- α and also binds to the cytoplasmic region of the lymphotoxin receptor, which results in dysregulation of I κ B proteins followed by enhancement of NF- κ B activation⁶⁹. Thus, HCV core

protein can modulate both apoptotic and antiapoptotic pathways in infected cells, which in turn can contribute to an oncogenic transformation.

Most known viruses, like EBV or HCV, make use of NF- κ B activation to block apoptosis of the infected cell, but in some cases viral proteins can inhibit the activity of this transcription factor. For example, the African swine fever virus, which replicates in macrophages, encodes an I κ B-like protein which lacks the classic serine residues necessary for signal-induced phosphorylation and binds to RelA, blocking its activity⁴⁷. Cowpox virus, a member of the orthopoxviruses, interferes with the regulation of NF- κ B; although the I κ B are phosphorylated, the virus is capable of blocking the further process of their degradation⁴³. The core protein of HCV, although it is known to trigger the NF- κ B pathway in order to promote cell death, is also capable of suppressing NF- κ B activation induced by TNF- α or phorbol esters through inhibition of I κ B degradation⁵⁴. The adenovirus E1A protein sensitizes infected cells to γ -radiation and TNF- α by inhibiting NF- κ B activity by blocking IKK complex activity and I κ B phosphorylation⁵³.

Conclusions

In view of the highly selective pressure of the immune system, viruses have had to develop special mechanisms of cell control to facilitate their survival and expansion in the host organism. As many types of viruses exist, they utilize the ability of NF- κ B to promote cell proliferation and to suppress or induce apoptosis in many different ways. Viral strategies use modulation of NF- κ B activity to promote or facilitate viral replication, infected cell survival, and evasion of immune responses. In addition, some viruses use the NF- κ B pathway to control apoptosis in two different ways: they use its antiapoptotic features to avoid the host defense mechanisms that could limit viral replication, then they make use of apoptosis as a mechanism of virus spread. Another important thing is that continuous activation of NF- κ B as a consequence of viral infection often leads to oncogenic transformation. These observations indicate how essential it is to investigate the molecular basis of interactions between the NF- κ B pathway and viral pathogenesis.

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