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MINIREVIEW

Virus Targeting of the Tumor Necrosis Factor Superfamily

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Herpesviruses appear to peacefully coexist with their natural hosts, with infection typically manifested as a benign, but lifelong process. However, coexistence depends on active resistance by innate and specific immune defenses as revealed in the striking virulence of herpesviruses when immunity fails. This pattern of infection is characteristic of a viral pathogen, such as cytomegalovirus, that has evolved efficient strategies targeted at host defense systems. Targeting members of the tumor necrosis factor (TNF)/lymphotoxin (LT) superfamily of cytokines is a strategy found in all herpesviruses, which suggests the existence of an intimate evolutionary link in their host–parasite relationship. Here we examine some of the strategies used by herpesvirus that target members of the TNF superfamily and discuss a recent study that revealed a novel mechanism that links LT-related ligands and interferons (IFN) to the establishment of coexistence between herpesvirus and its host cell.

THE TNF SUPERFAMILY

TNF-related cytokines are recognized as crucial regulators and effectors of the innate and adaptive immune defenses (Fig. 1) (Locksley *et al.,* 2001). Signaling pathways initiated by TNF-related cytokines regulate cell death and survival providing strong selective pressures for viruses to evolve counterstrategies. TNF cytokines are type II transmembrane glycoproteins that signal via their cognate receptors, which are single transmembrane domain glycoproteins. The trimeric structure of the ligand clusters the receptor, activating signaling pathways. The cytosolic tails of TNF receptors come in two basic flavors, those that contain a death domain (DD) (a 6 α helix bundle) and others with a TRAF binding region (a short proline-anchored peptide motif) (Wallach *et al.,* 1999). Receptor DD provides sites for binding of DD containing adaptor molecules (such as FADD, Fas-associated DD), which can then recruit initiator caspases (caspase-8) to the receptor through protein–protein interactions mediated by death effector domains. Once recruited to the receptor, procaspase-8 is activated and can then promote cleavage of the executioner caspases (caspase-3 and -9), leading to apoptosis. In contrast, members of the TRAF family of zinc RING finger proteins link their receptors to serine kinase-dependent signaling pathways (such as NIK or RIP), leading to the activation of NFκB and ultimately to the expression of proinflammatory genes, such as those encoding integrins and chemokines. Because NFκB induces the transcription of various antiapoptotic gene products [including the IAPs (inhibitors of apoptosis) and cFLIP (FLICE (caspase-8) inhibitory protein)] of this pathway, it is believed to promote cell survival. Activation of NFκB requires phosphorylation of the inhibitor of κB (IκB) by the IκB kinases (IKKα and β), resulting in the ubiquitination and degradation of IκB and allowing for the translocation of latent, cytosolic NFκB to the nucleus. Adding to the complexity, many TNFR family members (TNFR1, TRAIL-R1 and -R2, and LTβR) can activate both cell survival (NFκB-dependent) and cell death signaling pathways, suggesting that cell-specific factors, for instance the steady-state levels of cFLIP, will effect the eventual outcome of receptor signaling. From these data we can infer that viruses that block host cell protein synthesis may render the infected cell susceptible to the death-inducing activity of TNF family ligands by allowing the apoptotic pathway to predominate in the absence of NFκB-mediated survival.

Although the complexity of receptor cross-utilization by this group of TNF/LT-related ligands suggests functional redundancy, gene deletion studies in mice revealed unique and cooperating roles for these cytokines in the immune responses. Fas ligand is used by cytotoxic T cells as a mechanism to induce apoptosis of virus-infected cells and is also an important mechanism that limits the life span of effector T cells. The lymphotoxin-$\alpha\beta$–LTβR system controls organization of lymphoid tissue, lymph nodes, and Peyer's patches during embryogenesis and tertiary lymphoid structures in the adult,

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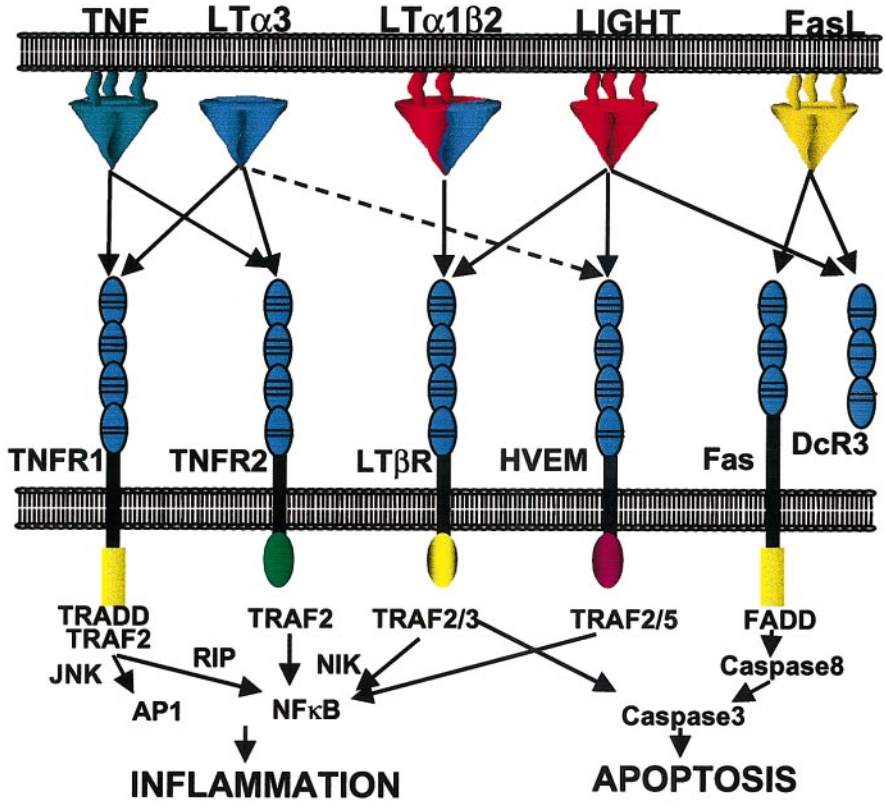

FIG. 1. The immediate members of the lymphotoxin/TNF family. Members of the larger TNF superfamily that are related by their shared specificity for receptors (indicated by arrows) are depicted in the upper diagram. The cognate receptors share a common cysteine-rich motif in their ectodomains. Fas and TNFR1 contain death domains in their cytoplasmic tails (yellow cylinders) and bind FADD and TRADD, death domain-containing adaptors. TNFR2, LTβR, and HVEM bind adaptors of the TRAF family of zinc RING finger proteins. RIP and NIK are serine kinases recruited to these adaptors that activate kinases directly involved in the activation of transcription factors AP1 and NFκB. DcR3 is a soluble decoy receptor that binds both LIGHT and FasL.

which form at sites of infection anywhere in the body. LT$\alpha\beta$ is also essential for the differentiation of NK and NK-T cells, which are critical effector cells of innate defenses and antiviral responses. TNF by contrast is crucial for the activation and recruitment of inflammatory cells, but cooperates with LT$\alpha\beta$ in organizing the white pulp in the spleen. LIGHT signaling via HVEM is one of three evolutionarily related pathways that include the CD27 and 41BB systems, which promote the activation of T cells.

VIRAL STRATEGIES

Given the involvement of TNF superfamily members in immune defenses, it is not surprising that viruses have evolved specific genetic mechanisms that modulate these signaling pathways. Steady progress by several labs has identified examples in DNA and RNA viruses that when viewed collectively target every step of the TNF pathway, from ligand-receptor binding to signal transduction (Fig. 2) (Gooding, 1992; Tortorella *et al.,* 2000). Historically, the first example of a viral gene product inhibiting TNF was gleaned from the poxvirus genome, which encodes a secreted protein with high homology to TNFR2. The poxvirus TNF receptor ortholog binds TNF and LTα with high affinity and acts as a decoy receptor, which competitively blocks these ligands from initiating signaling via their cell surface receptors. Notably, the poxvirus' TNF inhibitor served as the prototype for the successful anti-inflammatory drug Enbrel, a fusion protein of the TNFR2 ectodomain and the Fc region of IgG. Other examples come from adenovirus; indeed the entire E3 gene region of this virus modulates the activity of TNF family members. Adenovirus-infected cells are resistant to apoptosis due to the loss of cell surface expression of Fas and TRAIL receptors. The adenovirus E3-10.4K, 14.5K, and 6.7K proteins serve this function by forming a membrane complex that internalizes Fas and TRAIL receptors-1 and -2 used by cytotoxic T cells and NK cells to kill virus-infected cells. Other examples are found in RNA viruses. HIV nef, through its effect on membrane protein recycling pathways, sustains the expression of cell surface TNF and LIGHT on T cells, potentially prolonging signaling pathways (such as NFκB) that may enhance HIV transcriptional activity as well as regulate surrounding uninfected cells. The core protein of hepatitis C virus binds directly to LTβR, TNFR1, and Fas, potentially regulating the functions of these receptors in a fashion not fully understood.

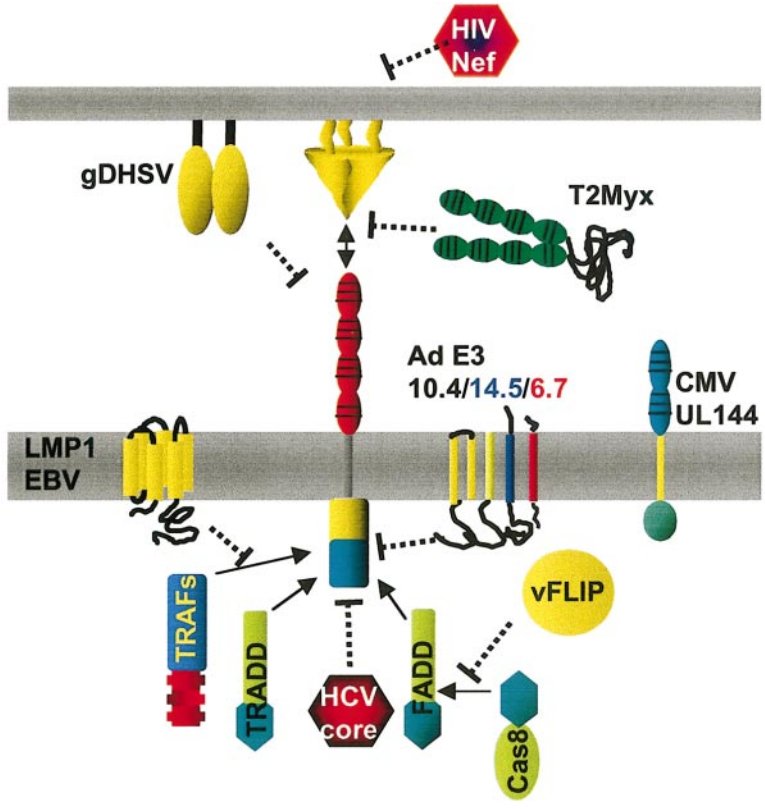

FIG. 2. Virus modulators of the TNF superfamily. Some examples of viral proteins that modulate ligand-receptor signaling pathways are given. Nef protein expressed by HIV (HIV Nef) sustains the expression of TNF and LIGHT on the membrane of activated T cells as it downregulates MHC class I and CD4 (Lama and Ware, 2000). Herpes simplex virus envelope glycoprotein D (gD HSV) binds to HVEM, which blocks binding to its cellular ligand LIGHT (Mauri *et al.,* 1998). The myxoma T2 protein encodes a soluble TNF receptor that binds TNF and LTα with high affinity (McFadden *et al.,* 1997). T2 is a virulence factor for rabbit poxvirus and similar gene products are found in other poxviruses. The latent membrane protein-1 of Epstein–Barr virus (LMP-1 EBV) is a six-transmembrane protein that binds the TRAFs and TRADD adaptors activating NFκB pathways, characteristic of a constitutively active receptor, most similar to CD40 (Mosialos *et al.,* 1995). LMP1 is the dominant oncogene of EBV. Adenovirus E3 region proteins 10.4K, 14.5K, and 6.7K (AdE3-10.4/14.5/6.7) form a multimeric membrane complex. E3-10.4K and 14.5K are sufficient to internalize Fas; however, 6.7K is necessary for downregulation of TRAIL receptors 1 and 2, but not death domain receptors DR3 and TNFR1. Apoptosis is blocked by this AdE3 complex (Benedict *et al.,* 2001b). The UL144 open reading frame in human cytomegalovirus (UL144 CMV) encodes an ortholog of HVEM and TRAIL receptor 2 containing two cysteine-rich domains (Benedict *et al.,* 1999). Hepatitis C virus core protein (HCV core) binds to LTβR, TNFR1, and Fas and modulates signaling (Matsumoto *et al.,* 1997). Viral FLIP, found in some γ herpesviruses, blocks recruitment of caspase-8 (cas8) to FADD, thus blocking apoptosis signaling by Fas and TRAIL receptors (Bertin *et al.,* 1997).

Targeting members of the TNF superfamily did not escape the attention of herpesviruses. One of the routes of entry used by herpes simplex virus is a TNFR family member, HVEM. This discovery by Spear's lab showed that HSV envelope glycoprotein-D of herpes simplex virus (α-herpesvirus) binds HVEM and acts as a competitive antagonist of LIGHT binding to HVEM (Mauri *et al.,* 1998). The prominent expression of HVEM on T cells may provide HSV with a direct route to infect and inactivate cells important in mounting an adaptive immune response. Support for this hypothesis comes from the fact that HSV infection of T cells induces their death by a Fas ligand-dependent pathway. Additionally, infection of dendritic cells by HSV blocks their maturation into competent antigen-presenting cells. Thus, HSV may achieve localized immune suppression by these mechanisms. The latent membrane protein-1 of Epstein–Barr virus (γ-herpesvirus) usurps the signaling adaptors TRAF2 and -3 and TRADD, leading to potent activation of NFκB in B cells. The virus-encoded FLIP (vFLIP) of equine γ-herpesvirus, an antagonist of caspase-8 activation, blocks apoptosis by Fas and TRAIL receptors similar to its cellular homologue cFLIP. Additional evidence for targeting of the TNF family by herpesvirus is the UL144 open reading frame present in human cytomegalovirus (β-herpesvirus; HCMV), which encodes an orthologue of HVEM and TRAIL receptor type 2. A ligand for this receptor has not been identified.

We reasoned that these diverse molecular links reflect a specific evolutionary history between the LT/TNF cytokines and herpesviridae. Herpesviruses establish a lifelong coexistence with their hosts that requires active participation of the immune system to control the primary infection and prevent reactivation from latency. The ability of proapoptotic TNF family ligands (TNF, Fas ligand, and TRAIL) to induce premature death of infected cells,

thereby interrupting completion of the viral replication cycle, may provide an effective antiviral defense against some pathogens. However, rapid or large-scale destruction of crucial tissues harboring infected cells could be detrimental to host survival, for example in the liver or brain. Noncytolytic mechanisms exist to clear or at least control viral replication such as seen in the control of hepatitis B virus infection in the liver (Guidotti and Chisari, 2000).

HOST VIRUS DÉTENTE

These observations prompted an examination of whether LT/TNF-related cytokines elicit nonapoptotic resistance to herpesvirus infection (Benedict *et al.,* 2001a). As a model, human CMV infection was examined in dermal fibroblasts, one of the few cell types that allows a productive infection in culture. The investigation revealed the ability of LT-related cytokines to mediate a nonapoptotic block of viral replication that requires both HCMV and host factors to arrest virus spread, yet viral genome is preserved without detriment to the cell. Treatment of HCMV-infected fibroblasts with the LT-related ligands LTα1β2 and LIGHT, which activate LTβR, or LTα3, which binds TNFR1, inhibited cytopathicity and spread of HCMV in cultures of dermal fibroblasts. Neither Fas ligand nor TRAIL exhibited this antiviral activity. Dominant negative mutants of FADD or TRAF3, adaptors required for apoptosis mediated by these receptors, showed no inhibition of the antiviral effects of LT/LIGHT. The antiviral effect of LT/LIGHT was reversible and, as deduced from studies using dominant negative signaling mutants, required the NFκB-dependent activation of interferon-β (IFN-β) gene expression. However, efficient induction of IFN-β required virus infection and LT receptor signaling, demonstrating the need for both host and viral factors in the curtailment of viral replication without cellular elimination. Neither virus infection alone nor activation of the LT receptors induced significant IFN-β production. The viral genome was maintained without virion production for 2 weeks without any evidence of cytopathic effects toward the fibroblasts; however, removal of LT or neutralization of IFN-β reversed the antiviral effect and reinitiated virus replication and virion production. The dependence on virus and lymphotoxin to induce IFN-β may provide, in part, a molecular basis for the ability of HCMV to establish a state of coexistence, or détente, in immunocompetent hosts. Furthermore, the ability of LT$\alpha\beta$, a product of activated TH1, CD8, and NK cells, to selectively induce IFN-β gene expression may reflect an additional component of the IFN amplification pathway. The potent immunoregulatory actions of IFN-$\alpha\beta$ may then contribute to shaping the immune response (Biron, 2001).

Interestingly, LTα-deficient mice showed a profound susceptibility to murine CMV, as did animals that expressed a decoy receptor for LTα1β2 and LIGHT. This observation appears to substantiate the idea that CMV and lymphotoxins share significant evolutionary history. Yet, we cannot say whether the mechanisms of action of LT in controlling the two species of CMV are similar, because the species-restricted evolutionary divergence of MCMV and HCMV is substantial. Indeed, the molecular mechanisms of immune evasion strategies diverge between human and mouse CMV. Although their genomes are colinear and highly conserved, the recognized mechanisms used to modulate immunity, including those we suspect are involved in altering antigen presentation pathways, chemokines, and cytokines, are not conserved. For example, there is no UL144 homolog in MCMV, and although both human and mouse CMV encode chemokine homologs, the viral chemokines are of different subtypes. The species specificity presents an important impediment to understanding the significance of viral evasion mechanisms. Are these evasion mechanisms important for viral pathogenesis? In the case of the poxvirus TNFR decoy, deletion of this gene substantially attenuates the lethal pathogenesis of myxoma in rabbits. Frankly, however, our knowledge is limited, as many of the viruses are unique human pathogens that force reliance on tissue culture models. Nonetheless, we take the view that understanding these processes may reveal further insight into viral pathogenesis and hope the lessons learned from various viral strategies will provide new approaches for modulating cytokine pathways.

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