**NF-κB Inhibition in EBV-Transformed Lymphoblastoid Cell Lines**

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**Abstract**

Epstein-Barr virus (EBV) transforms B-lymphocytes into lymphoblastoid cell lines usurping multiple signaling pathways including NF-κB activation. To determine whether NF-κB activity is essential in the growth and survival of EBV-transformed lymphoblastoid cell lines, a non-degradable IκBa mutant was expressed under tetracycline regulation in IB4 cells. NF-κB inhibition caused caspase 3 and 8 activation, PARP cleavage, and DNA fragmentation indicative of apoptosis. Mitochondrial membrane potential was diminished without release of cytochrome c or apoptosis initiating factor. z-VAD.FMK, a general caspase inhibitor, failed to block apoptosis, indicating a distinct pathway contributes to cell death. Bfl-1 expression, an anti-apoptotic Bcl-2 family member, is diminished after NF-κB inhibition whereas Bcl-2 and Bcl-x/L expression is unaffected. These studies suggest that NF-κB itself, or NF-κB-regulated genes, will be successful molecular targets for the treatment of EBV-associated diseases.

**Introduction**

Latent Epstein-Barr virus (EBV) infection is associated with a number of human malignancies such as Hodgkin’s disease, nasopharyngeal carcinoma, Burkitt’s lymphoma and most lymphoproliferations associated with immune suppression (Reviewed in [1]). EBV transforms B-lymphocytes in vitro into lymphoblastoid cell lines (LCLs). The full complement of latent gene expression, that of EBNA1, -2, -3A, -3B, -3C, -LP and LMPs -1, -2A, and -2B, is established in LCLs, and is
similar to that seen in infectious mononucleosis (caused by primary infection) and to that seen in post-transplant lymphoproliferative disorders (PTLD). Thus, LCLs function as a model of EBV-associated disease in vitro.

LMP1 is an oncogene, expression of which is required for the transformation of LCLs. It functions as an activated tumor necrosis factor (TNF) receptor constitutively stimulating the NF-κB and stress-activated kinase pathways. Using EBV recombinants and transformation assays, it is apparent that any mutant that affects the ability of LMP1 to activate NF-κB and JNK/p38 kinase pathways is defective in the ability to transform B-lymphocytes. Thus, the establishment of LCLs is genetically and biochemically linked to LMP1-mediated NF-κB activation (reviewed in [2]). It is now clear that NF-κB activity, like LMP1 expression, is also important for LCL survival after an LCL has been established.

The family of NF-κB proteins hetero- and homodimerize and are bound to an inhibitor, IκB, in the cytoplasm. Integration of the appropriate signals triggers phosphorylation, ubiquitination and degradation of IκB followed by translocation of NF-κB to the nucleus where it affects transcription (reviewed in [3]). NF-κB activity is an important regulator of apoptosis (reviewed in [4]). NF-κB inhibition renders fibroblasts and many other cell types sensitive to DNA damaging agents and TNFα [5–8]. The transcriptional activation of genes such as c-IAPs and TNF-receptor associated factors (TRAFs) in necessary to protect fibroblast from TNFα-mediated apoptosis [9]. Similarly, mice deleted for NF-κB family members die from TNFα-mediated apoptosis of the liver [10] (reviewed in [11]).

We used tetracycline-regulated expression of a deletion mutant of IκBa, ΔN-IκBa, in IB4 cells to test the function of NF-κB in an established LCL. ΔN-IκBa is deleted for the first 36 amino acids of IκBa making it resistant to degradation. Therefore it retains NF-κB in the cytoplasm [12]. In this system, inclusion of tetracycline represses ΔN-IκBa expression. Withdrawal of tetracycline results in the accumulation of ΔN-IκBa. NF-κB activity declines between 12 and 24 h after removal of tetracycline. Subsequently, 2 or 3 days later IB4 cells with low NF-κB activity undergo spontaneous apoptosis [13].

Apoptosis mediated by NF-κB inhibition is unlike that of growth factor withdrawal, TNF-receptor engagement or Fas engagement. Caspases are activated in an unusual cascade with caspase 3 preceding caspase 8 activation and no measurable caspase 9 activity. Furthermore, treatment of cells undergoing apoptosis mediated by NF-κB inhibition with z-VAD.fmk, a potent general caspase inhibitor, fails to