INHIBITION OF NF-KB BY DOMINANT-NEGATIVE IKB PROMOTES TNF-INDUCED APOPTOSIS OF OSTEOCLASTS

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Relevance: Osteoclastic activity constitutes a major component of inflammatory bone loss diseases. It follows that strategies designed to block osteoclast differentiation and activity might be efficient in arresting osteolysis.

Introduction: Osteoclast, the sole bone resorbing cell, arises from differentiation of marrow residing hematopoietic cells. These precursor cells, identified as monocytes/macrophages, express a wide range of cell surface receptors that recognize a host of circulating factors. Among these factors are members of the TNF super family, known to induce osteoclast gene transcription. TNF and receptor activator of NF-kB (RANKL) are considered among the potent inducers of osteoclast activation. We have shown previously that TNF plays a key role in recruiting osteoclasts and their committed precursors in sites of inflammatory osteolysis. The cytokine superinduces RANKL-primed precursor cells resulting in a robust osteoclastic response. Examination of intracellular transduction pathways revealed that the effects of RANKL and TNF are superimposed and synergistic (1). One of these pathways is activation of NF-kB, a transcription factor essential for osteoclast development and survival, and plays a key role in inflammatory diseases (2). Given that earlier studies have shown that inhibition of NF-kB leads to TNF induction of apoptosis of a wide range of cell types, we reasoned that TNF would induce rapid apoptosis in osteoclasts depleted of active NF-kB.

Methods: Cell culture - osteoclasts were generated by culturing whole marrow cells in the presence of 1,25-dihydroxyvitamin D3 for 8 days. Cultures were supplemented with fresh media and vitamin D every 3 days. At the end of culture, cells were fixed and processed according to the experimental conditions. Tartrate-resistant acid phosphatase and Hoechst staining was performed according to standard published protocols. Protein transduction – IkB protein was introduced into osteoclasts and their precursors using tat-fusion technology (3).

Results and Discussion: To inhibit NF-kB, we generated a dominant negative form of the NF-kB inhibitory protein, IkB (DN-IkB). This form of IkB lacks its amino-terminal phosphorylation sites, and as such, it binds to NF-kB and retains it in the cytoplasm in its inactive form. DN-IkB is delivered to the cells as a tat-fusion protein, a method that enables protein delivery to the vast majority of cells. Our data indicate that DN-IkB translocates to the cytosolic component of the cells. More importantly, the inhibitory protein significantly inhibits nuclear translocation of NF-kB to the nucleus. Because NF-kB activation manifested by its nuclear translocation is critical for osteoclast development, we examined the effect of DN-IkB on various stages of osteoclastogenesis. We find that when added at early stages of osteoclast differentiation it arrests basal osteoclastogenesis. We next examined the effect of the inhibitory protein on fully formed osteoclasts. In these experiments we examined morphological changes such as membranal, cytoplasm, and DNA integrity. We find that under unstimulated conditions, DN-IkB has a moderate effect on the morphology of mature osteoclasts (partial cell disintegration and integrity. We find that under unstimulated conditions, DN-IkB has a moderate

action, DN-IkB proteins may prove ideal in inducing osteoclast apoptosis and arresting osteoclastic activity.

References:

Figure 1: TNF Induces Osteoclast Apoptosis in Presence of the Suppressor TAT-I6B(46-317), (DN-IkB). Osteoclasts were generated in vitro, as previously described. Cells were then treated with TAT (panels a, b, c) or TAT-I6B(46-317) (d, e, f) for 24 hrs, in the presence of TNF to induce apoptosis. Cells in panels a and d were TRAP-stained (arrows mark large osteoclasts in panel a, which collapse, shrink and undergo apoptosis in the presence of TAT-I6B(46-317), in panel d). Cells in panels b, c, e, f were paraformaldehyde-fixed and stained with Hoechst. Single apoptotic (c, f) vs non-apoptotic (b, c) osteoclasts visualized by phase contrast (top) or UV (bottom) are shown. Note the pycnotic dense chromatin in apoptotic (c, f) compared to normal (b, c) nuclei (arrows).