Tumor necrosis factor-alpha (TNF) is a proinflammatory cytokine with a profound role in many skeletal diseases. The cytokine promotes bone resorption and loss by induction of osteoclast differentiation and activation. Equally important, it was reported that TNF reduces bone formation by inhibiting the production of matrix proteins, including inhibition of type I collagen synthesis and production of osteocalcin. Thus, elevated levels of TNF in inflammatory bone diseases and osteoporosis, not only increase osteoclast activity and bone loss, but also decrease osteoblastic matrix formation. Together, these effects result in pathologic bone loss (1,2).

TNF utilizes two transmembranal receptors and transmits its signals via activation of multiple signaling pathways including NF-kB and MAPK activation (3). Because osteoblast differentiation is tightly regulated by the transcription factor cbfa-1, we hypothesized that TNF utilizes its downstream-signaling molecules to inhibit cbfa-1 activation. Thus, we set out to determine the signal transduction pathway(s) by which TNF inhibits osteoblast differentiation.

Methods:

Primary osteoblast/stromal cells were obtained from the bone marrow of wild type or TNFr1 knockout mice. Long bones of C57Bl/6 mice were dissected and marrow was flushed and strained. Whole marrow was incubated in alpha-MEM media for 4 days to allow adherence of stromal/osteoblast cells. Other cell types which fail to adhere or survive in the absence of growth factors are washed from the culture on the fourth day. The nearly pure population of osteoblast/stromal cells is then utilized according to the various experimental procedures. Osteogenic media contains beta-glycerophosphate (13mM) and ascorbic acid (50mM) to induce osteoblastic differentiation. DNA-protein interactions were determined using electrophoretic mobility shift assays utilizing specific radiolabeled probes.

Results and Discussion:

Using primary marrow stromal cells, ST2, and MC3T3 osteoblastic cells, we first document that TNF inhibits expression of alkaline phosphatase, a marker of osteoblast differentiation, in dose (1-50ng/ml) and time-dependent manners. In this regard, induction of alkaline phosphatase by betaglycerophosphate was moderately reduced in cultures treated with TNF at a late stage of culture (days 8-10), compared to remarkable inhibition in cultures treated with osteogenic media in the absence or presence of 10 ng/ml TNF for 10 days. Cultures were supplemented with fresh media and TNF every 3 days. At the end of the culture period, cells were fixed and stained for alkaline phosphatase expression.

Inhibition of osteoblast differentiation by TNF. Endocrinology 141:3956-3964,2000.

2. Gilbert L., He X., Farmer P., Boden S., Koslowski M., Rubin J., Nanes M. Molecules that mediate the inhibitory effect on alkaline phosphatase expression. In summary, we show in this study that TNF, acting through its type 1 receptor, blocks expression of osteoblast differentiation markers, such as alkaline phosphatase. We also show that TNF inhibits bGP-induced activation of the osteoblast differentiation factor cbfa-1. While speculative, it appears that TNF inhibition of osteoblast differentiation markers might involve activation of NF-kB and ERK MAP kinase pathways.

Figure: TNF inhibits alkaline phosphatase via its type 1 receptor. marrow stromal/osteoblast cells derived from wild type and TNFr1-null mice were cultured with osteogenic media in the absence or presence of 10 ng/ml NF-kB and ERK MAP kinase pathways.

References:
