**Introduction:** Rheumatoid arthritis (RA) is an immune-inflammatory response associated with a joint inflammation, which often leads to matrix degradation and focal bone erosion. Several pro-inflammatory cytokines, primarily members of the TNF family, possess osteoclastogenic properties and contribute to exacerbation of bone loss associated with this disease. A primary pathway utilized by TNF family members is activation of the NF-kB pathway. The NF-kB family is comprised of several inducible transcription factors that regulate inflammatory responses and osteoclastogenesis. NF-kB regulates a large number of genes including TNF, which under pathologic conditions forms an autocinorm loop that amplifies the inflammatory response. The role of NF-kB as a mediator of inflammation and bone erosion has been established and therapeutic strategies to inhibit NF-kB and subsequent events have been proposed (1). Additional studies revealed that the MAP kinase p38 is also a mediator of osteoclastogenesis, plays a role in inflammation and regulates components of the NF-kB machinery (2). These findings strongly suggest that NF-kB and p38 MAP kinase are major mediators of inflammatory arthritis and osteolysis and position these pathways as targets for therapeutic intervention.

**Methods:** We utilized a mouse model of T-cell transgenic K/BxN mice, which develop spontaneous joint RA. Serum-transfer from these mice triggers a rapid autoreactive Ig response in host mice that results in a highly joint-specific inflammatory arthritis. BALB/c mice were injected (i.p) with serum (150 ul) from normal or K/BxN mice. Some mice were injected with IkB protein (i.p. 250ul) on days 4, 5, and 6. Mice were sacrificed on day 7 for evaluation by histology (standard techniques) or tissue collection for cellular and molecular examination. NF-kB and p38 activity was measured in cells retrieved from control and infected joints. Measurements include retardation assays for NF-kB and p38 using specific oligonucleotide from the TNF promoter and MEF2c, respectively. Dominant-negative IkB was generated by deleting the N-terminal 45 amino acids that contains the phosphorylation sites. The protein was then expressed as a TAT-fusion protein to facilitate cellular transduction.

**Results and discussion:** In this study, we set out to investigate molecular events that contribute to exacerbation of bone lesions in RA. The onset of arthritis in serum-induced mice was visible in joints and ankles within 48 hours. Swelling (2.5 fold increase) and limb deformation progressed thereafter. Histological examination of joint and ankle sections revealed a dramatically eroded bone surfaces which includes both cortical and trabecular bone. Bone erosion was maximal as early as 7 days post-serum transfer and was sustained thereafter. Eroded surfaces (lacunae) were 5-10 times larger in affected animals compared to controls and contained over 10 fold osteoclasts. Next we examined osteoclast recruitment in long bones, ankles and joints. The data indicate a striking increase of osteoclast number (8-12 fold) and size (2-5 fold) in cortical and trabecular bone cavities of arthritic mice compared to controls, with osteoclasts present throughout the arthritic cartilage. Articular cartilage and subchondral bone thickness in arthritic mice was reduced to approximately third of that in control mice. We next turned to examine molecular events that mediate this inflammatory osteolysis. Given that NF-kB and p38 MAP kinase are central to inflammatory processes and both are essential for osteoclast development, activity of these two factors was investigated. Nuclear extracts from healthy or diseased joint tissue were subjected to DNA binding assays using specific probes. The data indicate that DNA binding activity of NF-kB and p38 was significantly increased within 48 hours post serum-induction of arthritis and maximal at 7 days. Thus, we reasoned that inhibition of NF-kB and/or p38 may reduce severity of the disease. First, we show that SB203580, a selective inhibitor of p38, inhibits (75%) DNA binding activity of NF-kB. More importantly, we find that administration of dominant-negative form of IkB significantly reduced NF-kB DNA-binding activity (70%) and recruitment of osteoclasts in vivo (60%). We also observed reduced clinical signs such as joint swelling and ankle thickening (50%) in the presence of the inhibitor. In summary, our study describes a rapid and sustained autoimmune RA confined to joints. Development and progression of the disease are rapid and highly reproducible. More importantly, we document that severe osteolysis and bone erosion in this model is mediated, at least in a major part, by NF-kB and p38 activities. This conclusion was based on the fact that in vivo blockade of NF-kB by a dominant-negative form of its inhibitory protein IkB diminished the severity of inflammation and bone erosion. Taken together our data suggest that the NF-kB signaling pathway is an essential mediator of rheumatoid inflammatory osteolysis and as such is a good target for therapy.

**Figure:** Intact limbs were collected from control and arthritic (serum-induced) mice. Bones and paws were carefully skinned, fixed and processed for histology. Longitudinal sections were stained with tartrate-resistant acid phosphatase (TARP) to detect osteoclasts. Asterisks denote bone tissue and arrows point to osteoclasts. (red color indicates TRAP-positive multi-nucleated osteoclasts).

**References:**