INTERLEUKIN-4 ABROGATES OSTEOCLASTOGENESIS VIA STAT6-INHIBITION OF NF-KB TRANSACTIVATION.

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Relevance: Subsets of T-cell lymphocytes of the immune system regulate differentiation and functions of bone cells through secreted cytokines. Thus, understanding regulatory mechanisms of T-lymphocyte functions and their secreted products will advance therapies targeting autoimmune-linked bone diseases, such as various forms of arthritis.

Introduction: Interleukin-4 (IL-4), an anti-inflammatory cytokine secreted by T-helper type-2 (Th2) lymphocytes, has been shown as a potent inhibitor of osteoclastogenesis. However, the molecular mechanism(s) of its action remains unknown. Osteoclast differentiation requires activation of the receptor activator of NF-κB (RANK) pathway. RANK is a member of the TNF receptor family and upon its ligation, TNF receptor associated factors (TRAFs), notably TRAF2 and TRAF6 are recruited to its intracellular domain. Acting as adaptor proteins TRAFs facilitate the recruitment of a cascade of kinases leading to NF-κB activation manifested by its nuclear translocation and dissociation of its cytosol-residing inhibitory protein IκB. Nuclear NF-κB dimer then binds to DNA response elements and activates target genes. The transcription factor is essential for osteoclast differentiation as combined deletion of its dimer p50/p52 led to osteopetrosis. The goal of this study is to define the molecular steps by which IL-4 inhibits osteoclastogenesis.

Methods: NF-kB translocation and activation were measured by immunoblots and electrophoretic mobility shift assay (EMSA), respectively. Osteoclast phenotype was assessed by tartrate-resistant acid phosphatase (TRAP). STAT6-null and control Balb/c mice were used. RANK ligand (RANKL) and IL-4 were used at 20 and 10 ng/ml, respectively. All experiments were repeated at least three times.

Results and Discussion: Using pure population of osteoclast precursor cells in the form of bone marrow macrophages we first document that IL-4 blocks RANKL-induced osteoclast differentiation by targeting these cells. We next turned to delineate the molecular mechanism(s) by which IL-4 attenuates osteoclastogenesis. Examination of NF-κB by immunoblots and EMSA revealed that IL-4 partially inhibits NF-κB nuclear translocation and markedly reduces its DNA binding activity, respectively. Supporting NF-κB inactivation, we find that IL-4 blocks RANKL-induced IκB phosphorylation. However, western blot analysis of nuclear extract indicated that IL-4 did not entirely block NF-κB nuclear translocation, leading to the hypothesis that IL-4 may be also preventing NF-κB binding to its DNA response elements. To address this issue, we examined the role of IL-4-induced STAT6, a transcription factor (signal transducer and activators of transcription) that mediates IL-6 functions. We reasoned that STAT6 may either bind to NF-κB rendering it inaccessible for DNA binding or inhibit NF-κB binding to DNA sequences. Co-immunoprecipitations of NF-κB and STAT6 revealed that the two transcription factors do not interact. Next, NF-κB EMSA was performed using 32P-end labelled κB oligonucleotide in the presence of excess unlabelled STAT6 oligonucleotide or its mutated form. Our findings point out that consensus sequence STAT6 inhibits NF-κB DNA binding while mutant STAT6 fail to do so. These results indicate that STAT6 when present prevents NF-κB-DNA interaction, presumably by binding to shared or overlapping DNA sequences. To extend this finding to the in vivo state, osteoclast precursors lacking STAT6 were used. We find that high doses of IL-4 (100 ng/ml) fail to block RANKL-induced osteoclastogenesis in STAT6-deficient cells, yet potently obliterates osteoclast differentiation in wild type cells at concentrations as low as 0.1 ng/ml. Taken together our data indicate that IL-4 inhibits osteoclast differentiation by at least two cooperative mechanisms; 1) inhibition of NF-κB release and nuclear translocation via stabilization of IκB, and 2) by antagonizing NF-κB DNA binding activity in STAT6-dependent manner.