periarticular osteolysis is a debilitating complication of inflammatory arthritis. While controversy exists as to whether pannus, itself, is capable of degrading bone, the osteoclast is clearly the principal, if not exclusive, bone resorptive cell in rheumatoid disease and other osteopenic disorders. Thus, the treatment of inflammatory osteolysis depends upon understanding the means by which the osteoclast resorbs bone, and the mechanisms that govern its differentiation from precursor cells.

OSTEOCLAST FUNCTION

The osteoclast is a member of the monocyte/macrophage family and, in fact, macrophages from a variety of organs will commit to the osteoclast phenotype under appropriate culture conditions. This phenotype includes multinucleation, expression of markers such as the calcitonin receptor and tartrate resistant acid phosphatase and most importantly, the capacity to polarise when in contact with bone. The product of such polarisation is generation of the osteoclast ruffled membrane, which is a complex folding of the plasmalemma juxtaposed to bone matrix. The ruffled membrane is the resorptive organelle of the osteoclast and appears only when the cell is in the process of bone degradation. The means by which the osteoclast degrades bone involves its attachment to the target matrix, an event involving the αvβ3 integrin. Once cell/matrix contact is extant, acidifying vesicles, within the osteoclast cytoplasm, move via microtubules, to the bone apposed plasma membrane into which they insert by a process probably involving the small GTPase, Rab3. It is this insertion of acidifying vesicles into the plasmalemma that leads to its profound complexity thus generating the ruffled membrane. Moreover, this process endows the ruffled membrane with an abundance of vesicular proton pumps (H+-ATPases) that acidify an event involving the p75 TNF receptor, which recognises primarily the membrane bound form of the cytokine, is anti-osteoclastogenic. 

While these experiments validate that the osteoclastogenic effects of TNFα are mediated via its p55 receptor, they do not establish the target cell of the cytokine. Specifically, the two cell types essential for authentic osteoclastogenesis—namely, the macrophage and the marrow stromal cell or its derivatives, each express TNF receptors. Thus, either cell type (or both) may theoretically transmit the primary TNF signal. The potential role of stromal cells as the target is buttressed by the fact that TNFα increases expression of the key osteoclastogenic cytokine, RANKL, by these cells. Alternatively, the possibility existed that TNF, independent of RANKL would directly stimulate macrophages to undergo osteoclastogenesis. This second hypothesis was held by a number of previous reports. 

We investigated this issue by affinity purifying osteoclast precursors in the form of bone marrow macrophages to greater than 99% homogeneity. Regardless of the concentration of TNFα to which these cells are exposed, they fail to differentiate into osteoclasts. On the other hand, addition of less than one percent of RANKL sufficient to induce osteoclastogenesis to minuscule amounts of TNFα prompts exuberant osteoclast differentiation. This synergy of the two cytokines is reflected by a similar effect on activation of the downstream signalling molecule, NF-kB, an essential osteoclastogenic transcription complex. These experiments led us to postulate that while RANKL is necessary for TNFα induced osteoclastogenesis, the former cytokine need not be present in more than constitutive levels. To investigate this issue, in vivo, we generated chimeric mice consisting of bone marrow macrophages responsive to TNFα while their stromal cells are not. The marrow stromal cells were therefore capable of expressing only basal, non-induced quantities of RANKL despite the presence of TNFα. Confirming that basal, non-induced

## Abbreviations:
- TNF: tumour necrosis factor
- RANKL: receptor activator of NF-κB ligand
- LPS: lipopolysaccharide

THE TNF SUPERFAMILY AND THE OSTEOCLAST

The local bone loss attending rheumatoid arthritis is associated with a plethora of inflammatory cytokines, among the most prominent being TNFα. This being the case, we asked if TNFα is osteoclastogenic and if so, could we detail the mechanism by which the cytokine commits macrophages to the osteoclast phenotype. Our initial experiments involved the use of lipopolysaccharide (LPS) a potent inducer of TNFα expression. We find that LPS induces osteoclastogenesis, in vitro, and does so through a process involving the p55, but not p75 TNF receptor. Mirroring these in vitro studies, LPS is a potent osteoclastogenic agent in wild type mice but does not affect those lacking the p55 TNF receptor. This finding is in keeping with subsequent experiments establishing that TNFα, in itself, induces osteoclast differentiation in vitro and in vivo. Interestingly and in contrast to p55 TNF receptor, the p75 receptor, which recognises primarily the membrane bound form of the cytokine, is anti-osteoclastogenic. 

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The past five years have witnessed great advances into understanding the mechanisms by which macrophages differentiate into osteoclasts. It had been appreciated for some time that while RANKL is necessary for TNFα induced osteoclastogenesis, the former cytokine need not be present in more than constitutive levels. To investigate this issue, in vivo, we generated chimeric mice consisting of bone marrow macrophages responsive to TNFα while their stromal cells are not. The marrow stromal cells were therefore capable of expressing only basal, non-induced quantities of RANKL despite the presence of TNFα. Confirming that basal, non-induced...
amounts of RANKL allow TNFα to enhance osteoclast differentiation, administration of TNFα to these chimeric animals prompts osteoclastogenesis at a level commensurate with that seen in wild-type animals receiving the cytokine. The critical message of these studies is that although TNFα alone cannot induce osteoclast differentiation, it is very effective in the presence of permissive levels of RANKL.

CRYSTAL STRUCTURE OF RANKL

Interestingly, RANKL is a member of the TNF superfamily and shares much homology with TNFα. The fact, however, that only RANKL can engage RANK and hereby induce osteoclast differentiation indicates that it possesses unique structural features. To investigate this issue, we solved the structure of RANKL to a resolution of 2.6 angstroms by X-ray crystallography. Like other members of the TNF superfamily, RANKL monomers assume a prototypical “jellyroll” topology and spontaneously associate as homotrimers. While the β-strand core scaffold of RANKL is similar to that of other TNF cytokines, the solvent-accessible loops connecting these β-strands are unique in residue composition and surface topology. The significance of this observation lies in the fact that it is the unique surface elements of the TNF family cytokines that underlie receptor-binding specificity. Given this fact, and the structural congruence of the ligand binding domains of DR5 and the p55 TNF receptor, we modelled the probable receptor contact points on RANKL by superimposition of these two TNF superfamily receptors. This exercise showed that prospective RANK contact sites on RANKL, are structurally unique in comparison with other TNF cytokines, encompassing residues in the AA”, CD, and DE loops. To determine if these putative RANK contact sites on RANKL endow the cytokine with its unique osteoclastogenic properties, we performed structure based mutagenesis experiments. Specifically, we (1) replaced Ile248 with Asp in the DE loop, (2) deleted most of the AA” loop, and (3) replaced the AA” loop of RANKL with that of TNF. Confirming our hypothesis that the mutated structures engender the capacity of RANKL to promote osteoclast differentiation, deletion or TNF swap of the AA” loop completely abrogates osteoclastogenesis and the DE loop point mutation dampens the process eightfold. Thus, TNFα is clearly central to the pathogenesis of inflammatory osteolysis but it requires the presence of RANKL. While both cytokines are homotrimERIC members of the same superfamily, RANKL contains distinct structural features which explain its unique osteoclastogenic properties.

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