

## REPORT

## Bone destruction in arthritis

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Ann Rheum Dis 2002;61(Suppl II):ii84–ii86

Rheumatoid arthritis (RA) is characterised by the presence of an inflammatory synovitis accompanied by destruction of joint cartilage and bone. Destruction of cartilage matrix results predominantly from the action of connective tissue proteinases released by RA synovial tissues, chondrocytes, and pannus tissue. Several lines of evidence in RA and in animal models of arthritis support a role for osteoclasts in the pathogenesis of bone erosions. RA synovial tissues produce a variety of cytokines and growth factors that may increase osteoclast formation, activity, and/or survival. These include interleukin 1 $\alpha$  (IL1 $\alpha$ ) and  $\beta$ , tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), IL11, IL17, and macrophage colony stimulating factor (M-CSF). Receptor activator of NF $\kappa$ B ligand (RANKL) is an essential factor for osteoclast differentiation and also functions to augment T cell-dendritic cell cooperative interactions. CD4<sup>+</sup> T cells and synovial fibroblasts derived from RA synovium are sources of RANKL. Furthermore, in collagen induced arthritis (CIA), blockade with osteoprotegerin (OPG), a decoy receptor for RANKL, results in protection from bone destruction. To further evaluate the role of osteoclasts in focal bone erosion in arthritis, arthritis was generated in the RANKL knockout mouse using a serum transfer model. Despite ongoing inflammation, the degree of bone erosion in arthritic RANKL knockout mice, as assessed by microcomputed tomography and correlated histopathological analysis, was dramatically reduced compared with that seen in arthritic control mice. Cartilage damage was present in both the arthritic RANKL knockout mice and in arthritic control littermates, with a trend toward milder cartilage damage in the RANKL knockout mice. This study supports the hypothesis that osteoclasts play an important part in the pathogenesis of focal bone erosion in arthritis, and reveals distinct mechanisms of cartilage destruction and bone erosion in this animal model of arthritis. Future directions for research in this area include the further investigation of a possible direct role for the RANKL/RANK/OPG system in cartilage metabolism, and the possible role of other cell types and cytokines in bone erosion in arthritis.

## FOCAL BONE EROSION IN RHEUMATOID ARTHRITIS

Although the mechanisms of cartilage destruction in rheumatoid arthritis (RA) are well described, the mechanisms responsible for bone erosion in this disease have only recently been studied. Our laboratory group has been interested in the potential role of osteoclasts in focal bone erosion, as osteoclasts are the principal cell type responsible for bone resorption in states of physiological bone remodelling. The role of osteoclasts in bone erosion in RA has been suspected for many years on the basis of indirect evidence, including the identification of multinucleated cells with phenotypic features of osteoclasts at sites of erosion in human RA.<sup>1–4</sup> Similarly, multinucleated TRAP positive and calcitonin receptor positive cells have been identified at erosive surfaces in arthritic joints in murine collagen induced arthritis (CIA).<sup>5</sup> In addition, sites typical of osteoclastic activity have been demonstrated by electron microscopy in areas of erosion of subchondral bone in metacarpal heads from patients with RA.<sup>7</sup>

A number of cytokines and factors produced by RA synovial tissues and by pannus tissue are known to have the ability to induce the differentiation of monocyte/macrophage lineage cells into functional osteoclasts. These factors act either directly on osteoclast precursor cells or on osteoclasts, (for example, macrophage colony stimulating factor (M-CSF), tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin (IL) 1), or indirectly on bone lining/osteoblast lineage cells (for example, IL1, TNF $\alpha$ , IL11, IL17, parathyroid hormone related peptide (PTHrP)), which in turn contribute to the differentiation of osteoclast precursors. The essential and direct acting factor for osteoclast differentiation has been cloned and identified as receptor activator of NF $\kappa$ B ligand (RANKL), also known as osteoclast differentiation factor (ODF),<sup>8</sup> osteoprotegerin ligand (OPGL),<sup>9</sup> and TNF related activation induced cytokine (TRANCE).<sup>10–12</sup> It has been recommended by the American Society for Bone and Mineral Research (ASBMR) President's Committee on Nomenclature that this factor be designated as "RANKL".<sup>13 14</sup>

## THE RANKL/RANK/OPG SYSTEM

RANKL is a member of the TNF ligand superfamily of cytokines that binds to its signal transducing receptor, receptor activator of NF $\kappa$ B (RANK).<sup>15–19</sup> In addition to its required role in the differentiation of osteoclasts from their precursor cells, RANKL also augments osteoclast activity and survival.<sup>9 16 20–25</sup> Osteoprotegerin (OPG) is a naturally occurring decoy receptor for RANKL.<sup>26</sup> When bound to RANKL, OPG prevents the binding of RANKL to RANK and thus inhibits the biological activity of RANKL. The relative local expression levels of RANKL and OPG (often represented as the RANKL/OPG ratio), is instrumental in determining the degree of osteoclast mediated bone resorption.<sup>16 27–29</sup>

Synovial tissues provide a source of RANKL that could influence osteoclastogenesis. Synovial fibroblasts from patients with RA produce mRNA<sup>2 30</sup> and protein<sup>30</sup> for RANKL. RANKL is also expressed by T lymphocytes from RA synovial tissues.<sup>2 31 32</sup> Co-culture experiments using RA synovial fibroblasts and peripheral blood mononuclear cells as a source of osteoclast precursors demonstrate that osteoclast-like cells are generated, and the generation of these cells is inhibited by the addition of OPG.<sup>30 33</sup> Similarly, activated T cells expressing RANKL induce osteoclasts from autologous peripheral blood monocytes, a process that is also inhibited by OPG.<sup>34</sup> Synovial tissues may also provide a source of osteoclast precursor cells, as macrophages isolated from RA synovial tissues differentiate into osteoclasts in the presence of M-CSF plus RANKL.<sup>35</sup> More recent studies have extended these findings. Cells digested from RA synovial tissue samples generate TRAP positive multinucleated cells that form resorption pits on dentine slices,<sup>36 37</sup> a definitive demonstration that these cells are osteoclasts. Pit

**Abbreviations:** RA; rheumatoid arthritis; IL, interleukin; CIA, collagen induced arthritis; TNF $\alpha$ , tumour necrosis factor  $\alpha$ ; RANKL, receptor activator of NF $\kappa$ B ligand; OPG, osteoprotegerin; OPGL, osteoprotegerin ligand; ODF, osteoclast differentiation factor; M-CSF, macrophage colony stimulating factor; PTHrP, parathyroid hormone related peptide; TRANCE, tumour necrosis factor related activation induced cytokine

formation is inhibited by OPG. Furthermore, the number of resorption pits produced strongly correlates with the ratios of RANKL/OPG mRNA levels in the samples studied.<sup>36</sup> Taken together, these studies support a role for the RANKL/RANK/OPG system in osteoclastic bone resorption in RA.

### “BLOCKADE” OF OSTEOCLASTS IN ANIMAL MODELS OF ARTHRITIS

Perhaps the most important evidence for the role of osteoclasts in bone erosion in arthritis, and for the critical role of RANKL in osteoclastogenesis in this setting, is the study of OPG blockade in the adjuvant model of arthritis in rats.<sup>31</sup> Expression of RANKL was demonstrated in T cells and in synovial cells in rats with adjuvant arthritis. After treatment with OPG, initiated at disease onset, almost complete preservation of cortical and trabecular bone was noted in arthritic joints from treated rats as compared with severe bone loss in joints from untreated rats, with a similar dramatic decrease in osteoclast numbers in the treated animals. Cartilage destruction was also prevented in OPG treated arthritic rats. OPG treatment in this animal model was most effective when given early in disease, when erosions could be prevented.<sup>38</sup>

As adjuvant arthritis is a T cell driven experimental arthritis in which RANKL blockade could potentially influence immune responses, our group sought to extend these findings in an animal model that would isolate the osteoclastogenic effects of RANKL from its potential effects on T cell-dendritic cell interactions. We studied a serum transfer model of arthritis developed by Mathis and Benoist, a variant of the T cell transgenic K/BxN spontaneous arthritis model developed by this same research group.<sup>39-41</sup> In the spontaneous arthritis model, T cell-B cell interactions result in the production of pathogenic anti-glucose-6-phosphate isomerase (GPI) antibody.<sup>39-42</sup> Serum containing anti-GPI antibody can then be transferred from arthritic mice, leading to the development of arthritis in recipient mice.<sup>41-43</sup> It has been demonstrated that T cells and B cells are not required for the serum transfer variant of this model.<sup>41</sup> Arthritis was generated in the RANKL knockout mouse using this serum transfer model.<sup>44</sup> RANKL knockout mice are characterised by an osteopetrotic bone phenotype, including the complete absence of osteoclasts. Inflammation was assessed clinically and histologically and was comparable in arthritic RANKL knockout mice and arthritic control littermates. Despite ongoing inflammation, the degree of bone erosion in arthritic RANKL knockout mice, as assessed by microcomputed tomography and correlated histopathological analysis, was dramatically reduced compared with that seen in arthritic control mice. Multinucleated TRAP positive osteoclast-like cells were abundant in areas of bone erosion in arthritic control mice, and were completely absent in arthritic RANKL knockout mice, demonstrating the requirement for RANKL in osteoclastogenesis in this model of arthritis. Cartilage damage was present in both the arthritic RANKL knockout mice and in arthritic control littermates, with a trend toward milder cartilage damage in the RANKL knockout mice. This effect was attributable at least in part to loss of articular cartilage in areas of subchondral bone erosion where the “scaffolding” bone was lost in arthritic control mice, but not in arthritic RANKL knockout mice.

Further evidence for the role of osteoclasts in bone erosion in arthritis comes from a recent study using the TNF $\alpha$  transgenic mouse model in which mice develop a spontaneous, destructive polyarthritis at an early age.<sup>45</sup> Osteoclast targeted therapies were used to treat TNF $\alpha$  transgenic mice, and inflammation and tissue destruction were evaluated.<sup>46</sup> Mice treated with pamidronate alone, OPG alone, or OPG plus pamidronate failed to show any improvement in inflammation. However, quantitative histological analysis showed a 53% reduction in the size of bone erosions in the pamidronate treated mice, a 56% reduction in the OPG treated mice, and an 81% reduction in mice treated with OPG plus pamidronate.

There was also a significant reduction of the number of osteoclasts present in the joints of animals treated with OPG alone, and a further reduction in those animals treated with OPG plus pamidronate. Finally, in a preliminary study, Romas *et al* treated rats with CIA at the onset of inflammation using an OPG fusion protein to target osteoclast mediated bone erosion.<sup>47-48</sup> Clinical measures of inflammation were unchanged in OPG treated arthritic rats compared with arthritic control rats. However, osteoclasts were eliminated at the synovial/bone attachment, and there was a greater than 75% reduction in osteoclasts in juxta-articular bone. Protection from bone erosion and preservation of cartilage integrity was demonstrated in the OPG treated arthritic rats compared with arthritic control rats. These studies provide evidence for the role of osteoclasts (and of RANKL) in the pathogenesis of bone erosion in arthritis in several animal models of arthritis with distinct pathogenetic mechanisms.

### QUESTIONS REMAINING

A number of interesting questions remain to be answered in this area of active research. Firstly, the role of the RANKL/RANK/OPG system in cartilage destruction in arthritis is an open question. RANKL “blockade” has resulted in some degree of protection from cartilage destruction in all of the animal models of arthritis reviewed above, but to varying degrees. Some of the effects on cartilage are clearly indirect, as protection from erosion of subchondral “scaffolding” bone also protects the cartilage attached to that bone from dissolution. Whether the RANKL/RANK/OPG system has a direct effect on cartilage is an area of active investigation. RANKL, RANK, and OPG are expressed by chondrocytes, and expression of these factors may be upregulated in osteoarthritic cartilage.<sup>49</sup> However, RANKL treatment did not change the production of proinflammatory mediators, collagenase, or nitric oxide in cultured chondrocytes in one published study.<sup>49</sup>

Although osteoclasts seem to play an important part in mediating bone erosion in arthritis, none of the evidence thus far presented excludes the possibility that other cell types may contribute to bone erosion. Synovial fibroblasts and macrophages are a source of a number of enzymes and factors that could contribute to this process. For example, the production of matrix metalloproteinases by these and other cell types may directly increase bone erosion and may play a part in the preparation of the bone surface for osteoclast attachment. Our laboratory group and others<sup>50</sup> have demonstrated that these cells, as well as macrophages within synovial tissues, are sources of cathepsin K, an important proteinase released by osteoclasts themselves in states of physiological bone remodelling. In addition, there may be synergistic effects between osteoclasts and other cells types in the process of bone erosion in arthritis.

Finally, studies in *in vitro* models of osteoclastogenesis and in animal models of arthritis demonstrate that RANKL is required for osteoclastogenesis, and the absence of osteoclasts in arthritic RANKL knockout mice supports this hypothesis. However, whether changes in the RANKL/OPG ratio directly modulate osteoclastic bone resorption in arthritis needs to be further investigated. In the presence of RANKL, other cytokines known to increase osteoclastogenesis, such as TNF $\alpha$ , may be important factors in driving the process of osteoclastogenesis. Further elucidation of each of these mechanisms will be important to direct new therapeutic interventions in this area.

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