Citation: IBMS BoneKEy **9**, Article number: 233 (2012) | doi:10.1038/bonekey.2012.233

www.nature.com/bonekey

COMMENTARY

Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin

Christian Roux

Department of Rheumatology, Université Paris-Descartes Hôpital, Paris, France.

IBMS BoneKEy **9,** Article number: 233 (2012) | doi:10.1038/bonekey.2012.233; published on 12 December 2012

Commentary on: Harre U, Georgess D, Bang H, Bozec A, Axmann R, Ossipova E, Jakobsson PJ, Baum W, Nimmerjahn F, Szarka E, Sarmay G, Krumbholz G, Neumann E, Toes R, Scherer HU, Catrina AI, Klareskog L, Jurdic P, Schett GU. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest* 2012; **122**: 1791–1802.

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by distal and symmetrical synovitis, affecting 0.5% of the general population, with a female preponderance. The disease is responsible for joints destructions, with a high risk of functional disability. Bone complications are the main extra-articular complications of the disease; they are described as three different forms: periarticular bone loss (adjacent to the inflamed joints), bone erosions and systemic osteoporosis. Patients with RA are at increased risk of fractures at the hip, vertebrae, pelvis and humerus. Special attention must be paid to vertebral fractures risk in these patients, as the well-known consequences of recurrent vertebral fractures add a large burden to the underlying disability related to joints destructions. The main risk factors for fractures are disability, long-standing disease, low body mass index, previous non-vertebral fractures and long-term glucocorticoid use. Relevant data have shown that the risk of fractures is increased even in patients who are not receiving steroids, and inflammation itself is the main determinant of bone loss in early RA. Actually the periarticular bone damage and the generalized osteoporosis share common pathways: inflammatory cytokines (interleukin (IL)1, IL6, IL17, tumor necrosis factor (TNF)-α) produced by activated T lymphocytes upregulate receptor activator of nuclear factor-κB ligand (RANKL) and osteoclastogenesis. Recent attention has been paid on the role of Dickopff 1 (DKK1) in radiological progression and absence of healing of bone erosions. Serum assessment of RANKL, and DKK1, in clinical studies, have been used in the prediction of structural damage in RA.[1–6]

Apart from inflammation, a strong risk factor for bone loss and bone erosions in RA in the presence of auto-antibody anticitrullinated protein; their presence must be interpretated in the context of a complex interplay between genotype and environment. Among a long list of genes, an association with human leukocyte antigen–DRB1 has long been established. The genetic risk itself is however different according to the presence of anticitrullinated proteins (ACPA). Citrullination is a post translational modification of proteins, as a stress response of cells to noxious stimuli. Smoking and bacteria involved in periodontitis (*Porphyromonas gingivalis*) induce citrullination in epithelia and mucosa. Smoking and human leukocyte antigen–DRB1 alleles synergistically increase the risk of having ACPA. The citrullination is achieved by enzymes (peptidyl arginine deiminase: PAD), changing arginine into citrulline. The presence of ACPA can be detected in clinical practice with a diagnostic anti-cyclic citrullinated peptide (anti CCP) assay. This is a key issue in clinic, as this anti CCP is used for diagnosis (the ACPA response is highly specific for RA), and prognosis (patients with ACPA response, with a high titer of anti CCP, have a poorer outcome). ACPA presence is a very strong predictor for the occurrence of bone erosions.

Thus, in a recent paper published in the *Journal of Clinical Investigation*, Harre *et al*'s hypothesis was that ACPA influence bone resorption in RA.[7] The demonstration follows six steps.

1. The authors compared values of markers of bone resorption and osteoclasts' number and activity (CTX1, TRAP 5b, cathepsin K) in serum of patients with RA and ACPA, RA without ACPA but seropositive (that is, with presence of rheumatoid factor), seronegative RA, and controls. All the markers were always higher in patients with ACPA than in the three other groups. Even patients with low levels of ACPA had higher CTX1 than did patients without ACPA. In contrast, there was no difference among these groups for alkaline phosphatase, a marker of bone formation.
2. Then the authors demonstrated that ACPAs are related to bone resorption, and isolated ACPAs with specificity to mutated citrullinated vimentin (MCV-ACPA), as vimentin is expressed by osteoclasts and mononuclear cells as well.
3. These MCV – ACPAs were shown to bind to cells of the osteoclast lineage. The PAD2 and PAD4 enzymes (which metabolize arginine into citrullin) were found in osteoclast

precursors (at the RNA and protein levels). The process of differentiation of osteoclast precursors into osteoclasts was parallel to a decrease in the expression of PAD4, an increase in the expression of PAD2, and of vimentin, and finally an increase in the expression of citrullinated vimentin. These data suggest that the target of MCV – ACPAs is inducible in the osteoclast lineage.

4. The next question is whether these MCV – ACPAs can directly stimulate the osteoclasts. The authors show that the well-known formation of osteoclasts pathway using macrophage colony-stimulating factor (MCSF) and RANKL, is actually increased with the addition of ACPA containing serum (and not with RA serum without ACPA). There was a dose dependent increase of resorption (pits) in the presence of MCV-ACPAs. Actually the increase in resorption process is related to an increased formation of osteoclasts (that is, an increased number) and not to an increased activity of the cells.
5. Using Rag $1^{-/-}$ mice (lymphocyte-deficient), the authors assessed the role of ACPAs on bone loss *in vivo*: treated animals elicited lower bone volume/tissue volume, measured at the tibial metaphysis by micro-computerized tomography. This change was the consequence of reduced trabecular number and lower connectivity of trabeculae, without change of thickness. Histomorphometry showed a significant increase in osteoclast number along the metaphysis of ACPA-treated mice.
6. The final question was to elucidate the mechanism by which MCV – ACPA induce bone resorption. In the serum of ACPA-treated mice was measured an increase in TNF-α (although these animals did not have any synovitis). These animals had also an increase of CTX1, without change in osteocalcin level. To confirm the role of TNF-α in this model, spleens of these animals were examined: there was a significant increase of CD11b + CD14 + osteoclast precursors, and higher surface levels of MCSF receptor and RANKL receptor, indicating a higher osteoclastogenic potential, which was furthermore confirmed *in vitro*.

These data demonstrate that autoantibodies (against citrullinated vimentin) increase osteoclast differentiation and bone resorption *in vitro* and *in vivo*.

This paper has several consequences:

1. It shows evidence of a link between a quite common autoantibody and bone loss. So far, only one example has been shown, when autoantibodies against osteoprotegerin were associated with bone loss in patients with celiac disease. This is a new principle, in the current concept on the interactions of immune system and bone.
2. It gives an explanation to the clinical observation of a higher risk of bone loss and bone erosions in RA patients with anti CCP. This is known for many years, without clear reason so far. A tight control of inflammation is the current recommendation of treatment in RA; this study strongly suggests that in patients with high level of anti CCP at baseline, prevention of bone loss is also a priority.
3. However, RA treatments (disease modifying anti rheumatic drugs and biologics) can ameliorate the bone destruction in RA, providing that they are given with the target of tight control of inflammation. Even with this strategy, they do not decrease in parallel the titer of ACPA, suggesting that presence of autoantibodies is not the single determinant of bone involvement in RA.

Conflict of interest

C Roux received research grants and honoraria from Amgen, MSD, Lilly, Servier, Novartis, Roche, Pfizer.

References

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011;**365**: 2205–2219.
2. Schett G, Saag KG, Bijlsma WJ. From bone biology to clinical outcome: state of the art and future perspectives. *Ann Rheum Dis* 2010;**69**:1415–1419.
3. Roux C. Osteoporosis in inflammatory joint diseases. *Osteoporos Int* 2011;**22**:421–433.
4. Klareskog L, Stolt P, Lundberg K, Kalberg H, Bengtsson C, Grunewald J *et al.* A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by circullination. *Arthritis Rheum* 2006;**54**:38–46.
5. De Rycke L, Peene I, Hoffman IE, Kruithof E, Union A, Mehens L *et al.* Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestation. *Ann Rheum Dis* 2004;**63**:1587–1593.
6. Riches PL, McRorie E, Fraser WD, Determann C, Van't Hof R, Ralson SH. Osteoporosis associated with neutralizing autoantibodies against osteoprotegerin. *N Engl J Med* 2009;**361**:1459–1465.
7. Harre U, Georgess D, Bang H, Bozec A, Axmann R, Ossipova E *et al.* Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest* 2012;**122**:1791–1802.