B cell autoimmunity and bone damage in rheumatoid arthritis

S. Bugatti, L. Bogliolo, C. Montecucco, A. Manzo

Rheumatology and Translational Immunology Research Laboratories (LaRIT), Division of Rheumatology, IRCCS Policlinico San Matteo Foundation/University of Pavia, Italy

SUMMARY

Rheumatoid arthritis (RA) is a chronic immune-inflammatory disease associated with significant bone damage. Pathological bone remodeling in RA is primarily driven by persistent inflammation. Indeed, pro-inflammatory cytokines stimulate the differentiation of bone-resorbing osteoclasts and, in parallel, suppress osteoblast function, resulting in net loss of bone. Abating disease activity thus remains the major goal of any treatment strategy in patients with RA. Autoantibody-positive patients, however, often show a rapidly progressive destructive course of the disease, disproportionate to the level of inflammation. The epidemiological association between RA-specific autoantibodies, in particular anti-citrullinated protein autoantibodies, and poor structural outcomes has recently found mechanistic explanation in the multiple roles that B cells play in bone remodeling. In this review, we will summarize the substantial progress that has been made in deciphering how B cells and autoantibodies negatively impact on bone in the course of RA, through both inflammation-dependent and independent mechanisms.

Key words: *Rheumatoid arthritis; bone; B-lymphocytes; anti-citrullinated protein autoantibodies; rheumatoid factor.*

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INTRODUZIONE

In systemic autoimmune diseases, chronic activation of the immune system is classically linked to the development of tissue damage. Both the cellular and the humoral arms of the immune system promote an amplification loop, fueling continuing inflammation, activation of intrinsic resident cells, and final tissue injury. Whilst the close inter-relationship between aberrant autoimmune reactions and organ damage has long been recognized in prototypical settings such as lupus nephritis and ANCA-associated vasculitides (1, 2), a wide range of evidence now suggests that immune signaling cascades also profoundly influence the pathologic remodeling of a tissue previously viewed as immunologically inert, that is to say the skeletal system (3).

Bone loss is a common feature of chronic rheumatic diseases. In particular, rheumatoid arthritis (RA) represents the prototype of a chronic inflammatory disease accompanied by rapid bone loss. In addition to systemic osteoporosis, patients with RA develop periarticular osteopenia and joint erosions early in the disease process. Pro-inflammatory cytokines are traditionally regarded as key drivers of articular and extra-articular bone tissue destruction (4, 5). However, recent experimental evidence indicates that RA-associated autoantibodies can independently stimulate bone remodeling by inducing the differentiation of bone resorbing osteoclasts (OCs) (6), and B cells from RA patients can impact on bone also through non-antibody dependent mechanisms (7).

In this review, we will summarize the current knowledge linking B cell autoimmunity to bone damage in RA. We will discuss the non-autoantibody roles of B cells, as well as the direct and indirect effects of autoantibodies.

BONE REMODELING IN RHEUMATOID ARTHRITIS: INFLAMMATORY MECHANISMS

Bone loss in RA results from an imbalance between bone resorption and bone formation (4, 5).

Corresponding author
Serena Bugatti
Division of Rheumatology
University of Pavia
IRCCS Policlinico San Matteo Foundation
Piazzale Golgi, 19 - 27100 Pavia, Italy
E-mail: serena.bugatti@unipv.it

The marked increase in bone resorption is mediated by bone resorbing OCs at the pannus-bone interface and in subchondral bone marrow, which differentiate locally as a result of over-expression of the osteoclastogenic mediator receptor activator of nuclear factor κB ligand (RANKL) (8-10). Increased OC activity reflects the inflammatory burden in joints. In accordance, both ultrasonography and magnetic resonance imaging studies have shown that the extent of synovitis and subchondral bone marrow inflammation (osteitis) is related to the likelihood of later bone damage (11). Indeed, several pro-inflammatory cytokines present in the inflamed RA synovium, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1, and IL-17, have been shown to stimulate OC differentiation and activation either directly through activating OCs or indirectly by inducing RANKL production by synovial fibroblast-like cells, T-cells, or bone marrow stromal cells (12-16) (Figure 1).

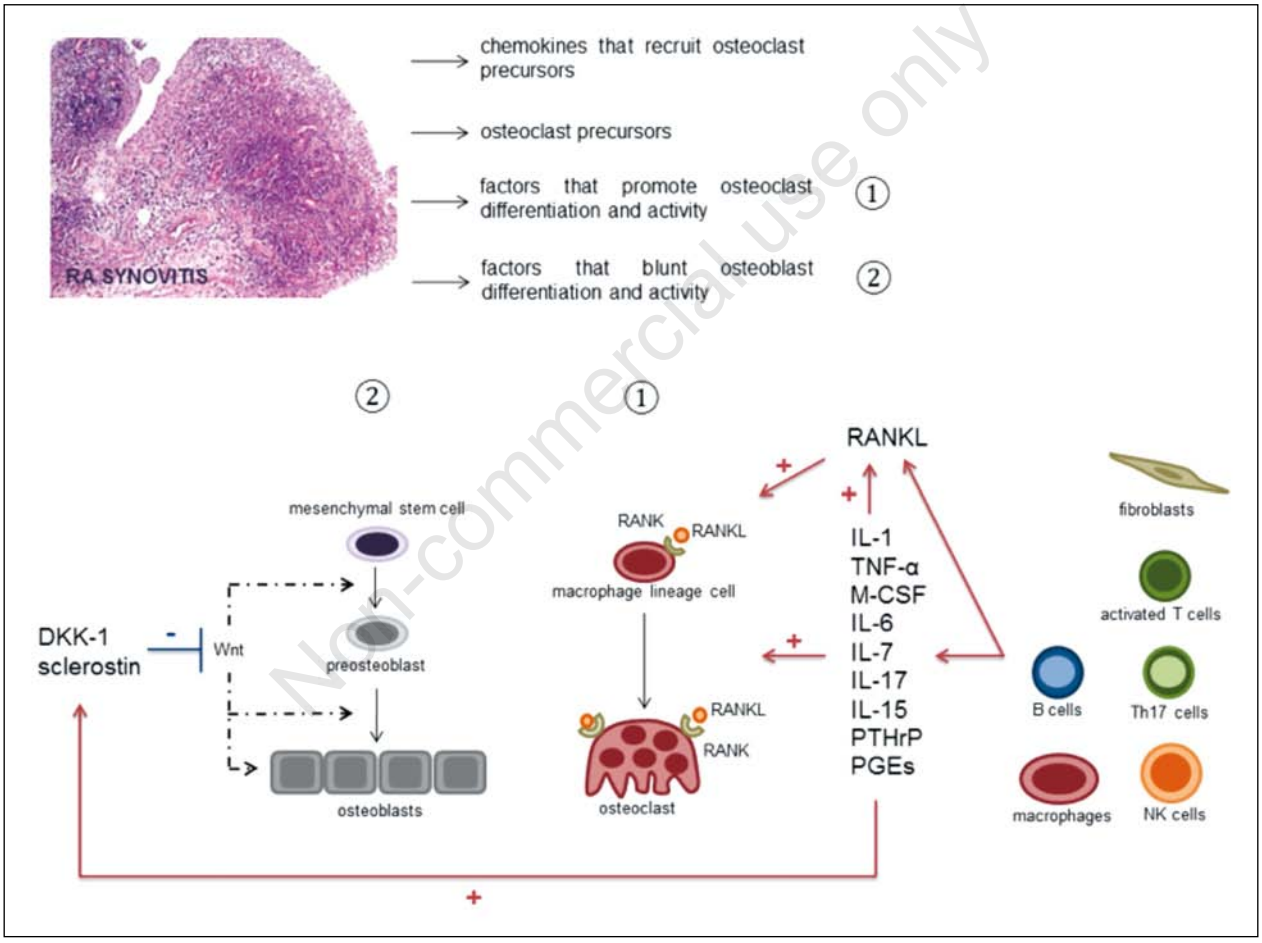

Figure 1 - Inflammatory mechanisms of bone remodeling in rheumatoid arthritis (RA).

The relationship between chronic synovitis and bone remodeling in RA is shown. The inflamed RA synovium produces chemokines that recruit osteoclast (OC) precursors, contains OC precursors and produces pro-inflammatory cytokines able to: promote OC differentiation and activation; inhibit osteoblast (OB) differentiation and function. Inflammatory and resident cells in RA synovium, including T lymphocytes, fibroblasts, macrophages, and others, are a source of the osteoclastogenic mediator receptor activator of nuclear factor κB ligand (RANKL) which, by binding to its cognate receptor RANK, promotes OC differentiation from macrophage lineage cells. The same cells produce large amounts of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-17, and others, which stimulate OC differentiation and activation either directly through activating OCs or indirectly by inducing RANKL production. The same pro-inflammatory cytokines, in particular TNF-α, up-regulate Dickkopf (DKK)-1 and sclerostin production. DKK-1 overexpression blocks new bone formation through antagonism of the Wingless (Wnt) signaling pathway, a critical pathway involved in OB differentiation from mesenchymal stem cells.

In parallel with inducing OC activity, the presence of inflammation also impairs osteoblast (OB) differentiation and function, resulting in a net loss of bone (17). Histopathological studies of synovial tissue of patients with RA have revealed paucity of mature OBs in bone erosions in spite of abundance of OCs, and bone formation rates are markedly reduced in animal models of arthritis (18). OB differentiation from mesenchymal cells is tightly regulated by the Wingless (Wnt) signaling pathway, and Wnt antagonists, including the Dickkopf family members (DKKs) and sclerostin, inhibit bone formation (17). Pro-inflammatory cytokines such as TNF-α have been shown to up-regulate DKK-1 and sclerostin production *in vitro* (19, 20), and DKK-1 overexpression blocks new bone formation in animal models of RA (19) (Figure 1). Confirming that bone remodeling in RA is primarily fuelled by the pro-inflammatory joint *milieu*, therapies that block the action of pro-inflammatory cytokines halt bone resorption and may induce visible, although not clinically relevant, healing processes (21).

BONE REMODELING IN RHEUMATOID ARTHRITIS: THE ROLE OF B CELLS

In some subsets of patients with RA, the chronic inflammatory process has a strong autoimmune component, as highlighted by the recognition of typical autoantibodies, such as antibodies to citrullinated proteins (ACPA) and rheumatoid factors (RFs), and the therapeutic benefit of depleting B cells (22). Whereas it is well established that inflammation constitutes a major risk factor for bone destruction in RA, the impact of B cell autoimmunity on bone remodeling is less well perceived. Epidemiological evidence consistently indicates that the *B cell subset* of RA has a more severe and rapidly progressive destructive course of the disease. Such observation has, however, remained circumstantial rather than causal until very recent years, when data have started revealing that B cells can directly affect the bone by a different mechanism.

Clinical evidence

Since the identification of citrullinated proteins as relevant autoantigens in RA, the association of ACPA with more joint damage progression has been replicated by several independent studies (23-25). Importantly, not only the mere presence of autoantibodies, but also the extent of the overall autoimmune response appears to impact negatively on bone. Syversen and coauthors (25) originally showed that patients with high levels of ACPA were 5 times more likely than patients with low-moderate levels to develop radiographic progression over 10-years. In the Leiden Early Arthritis Clinic and the Norwegian EURIDISS cohort, a high number of ACPA isotypes present at baseline was associated with significantly more radiographic damage during the disease course (26). More recently, anti-carbamylated protein (anti-CarP) antibodies were shown to have a statistically significant additive value to ACPA and RF in predicting radiographic progression in ACPA-positive/RF-negative, ACPA-negative/RF-positive and ACPA-positive/RF-positive patients, with effect sizes ranging 1.05-fold to 1.17-fold rate of joint destruction per year (27). In addition to anti-CarP, other recently identified autoantibodies may potentiate the effect of ACPA. In two large prospective RA cohorts, antibodies against the human isoform 4 of peptidyl arginine deiminase (PAD) were associated with higher radiographic damage scores over time even in the presence of ACPA (28), and high levels of cross-reactive anti-PAD3/PAD4 antibodies appeared in a recent report to increase further the risk of joint destruction (29). Elegant imaging studies have provided quantitative estimation of the addictive effect of autoantibodies on the erosive burden of RA, demonstrating that RF dose-dependently influences the size and number of bone erosions on an ACPA-positive background (30). Intriguingly, autoantibodies emerge as possible determinants not only of joint integrity, but also of the systemic bone mass. Both in long-standing and early RA, the presence of ACPA negatively associates with hip and spine bone

mineral density (31, 32), with increasing effects seen for ACPA in association with high levels of RF (32).

Although the aforementioned studies are overall concordant in establishing the strong prognostic relevance of autoantibodies for structural outcomes, the specificity of such association may not be as simple to ascertain. Indeed, it is theoretically possible that autoantibody-positive RA patients have more severe and refractory synovitis, resulting in accelerated bone loss through conventional inflammatory pathways. Some recent clinical data however speak of a direct link between autoantibodies and bone damage beyond clinically detectable inflammation. According to the 8-year follow-up data of the BehandelStrategieën (BeSt) study, ACPA-positive subjects showed more radiological damage progression despite similar response to steered treatment strategies (33). Analogously, in a pooled analysis of 675 RA patients included in the methotrexate monotherapy randomization arms of several clinical trials, structural progression over 2-years was primarily mediated by higher disease activity in RF-positive compared to RF-negative patients, but high levels of RF conveyed additional risk on bone erosions (34). The strongest and most compelling evidence however comes from imaging studies performed in ACPA-positive healthy subjects with no evidence of arthritis. Using high resolution quantitative peripheral computed tomography, Kleyer and coauthors (35) recently showed that ACPA-positive healthy individuals have a trend towards thinner trabeculae in their metacarpal heads and significant reduction in cortical thickness compared to ACPA-negative matched subjects.

Non-autoantibody dependent mechanisms

From a morphological perspective, inflammatory infiltrates dominated by B and T cells can be recognized in both the synovial membrane and the subchondral bone marrow in a proportion of patients with RA (36, 37), and appear associated with histological and radiographic evidence of bone erosions, a pro-osteoclastogenic molecular *milieu*, and increased bone-resorbing OCs (10, 38, 39). Whilst activated T cells have long been acknowledged as key promoters of pathological bone loss associated with RA and a variety of other conditions through TNF-α and RANKL production (40), recent studies have described RANKL expression also by human B cells (41-46) (Figure 2). Membrane-bound RANKL was shown to be increased on B lymphocytes in the bone marrow of post-menopausal women not on estrogen replacement (41), and B-cell ablation of RANKL partially protected ovariectomised mice from trabecular bone loss (42). Specific to RA, it was recently shown that CD27+IgD- switched memory B-cells produce RANKL in quantities exceeding that produced by T-cells upon stimulation, and synovial RA B cells spontaneously produce RANKL and promote greater osteoclastogenesis than B cells from healthy controls (45). Importantly, RANKL production appears to be triggered via direct engagement of the B cell receptor rather than as a consequence of by-stander inflammatory stimuli (45, 46), pointing to the specific link between immune challenges and pathological bone remodeling.

Autoantibody-dependent mechanisms

If, on the one hand, demonstration that B cells are an additional source of RANKL in RA certainly advances our understanding of the mechanisms of pathological bone remodeling, it does not violate the general rule that assumes inflammation as the *sine-qua-non* condition for bone loss to develop. B cells would indeed *simply* enter the pool of the many cellular players capable of promoting osteoclastogenesis in response to inflammation. More intriguingly, however, recent experimental evidence positions autoreactive B cells as unique triggers of inflammation-independent bone damage through the production of pathogenic autoantibodies (Figure 2).

In a seminal work published in 2012, Harre and coworkers (47) elegantly demonstrated that OCs and OC precursors express large amounts of citrullinated vimentin on their

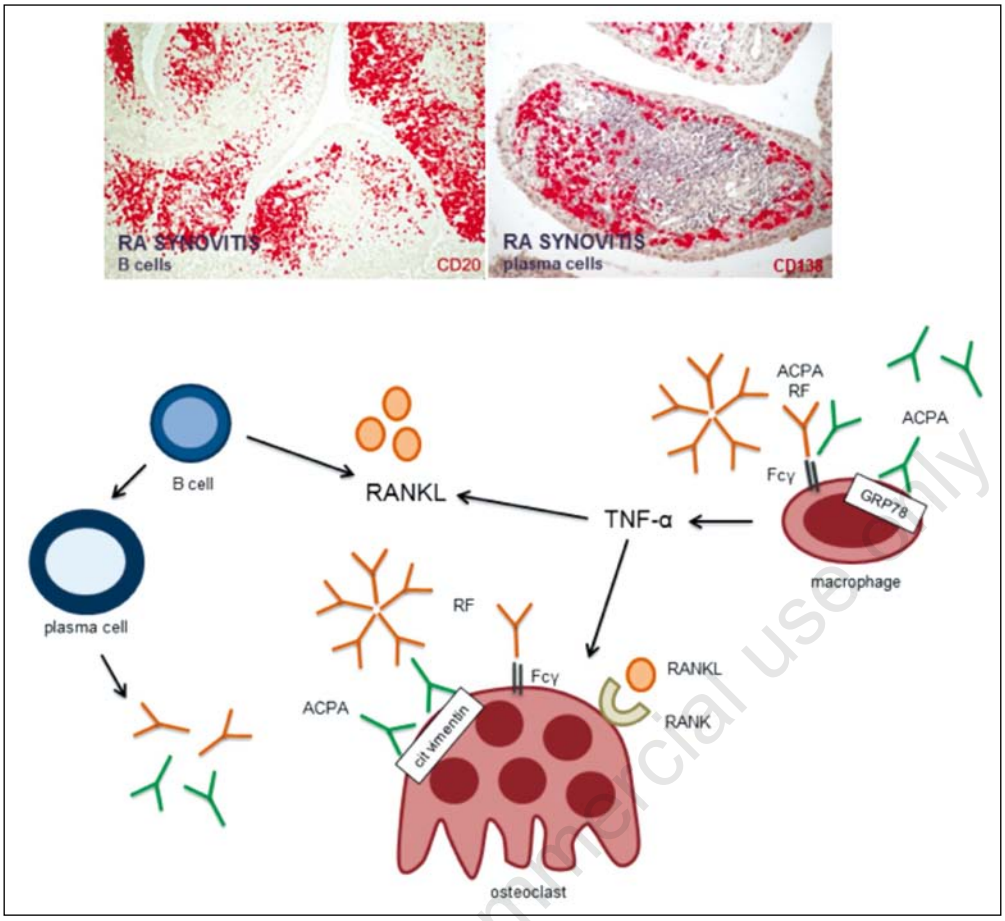

Figure 2 - B cell contribution to bone remodeling in rheumatoid arthritis (RA).

The autoantibody-independent and autoantibody dependent contribution to bone remodeling in rheumatoid arthritis (RA) is shown. B cells infiltrating the RA synovium are a source of the osteoclastogenic mediator receptor activator of nuclear factor κB ligand (RANKL) which, by binding to its cognate receptor RANK, promotes OC differentiation and activation. Furthermore, plasma cells produce autoantibodies such as anticitrullinated protein autoantibodies (ACPA) and rheumatoid factor (RF). OC precursors express citrullinated vimentin on their surface, and ACPA directed against mutated vimentin increase the formation and differentiation of OCs. Furthermore, ACPA trigger tumor necrosis factor (TNF)-α production by macrophages through engagement of the Fcγ receptor and by direct binding to a citrullinated GRP78 cell-surface receptor. In turn, TNF-α promotes OC differentiation and activation both directly and by up-regulating RANKL production. RF can amplify OC activation by inducing TNF-α production by macrophages through engagement of the Fcγ receptor. Furthermore, OCs themselves can be activated through the Fcγ receptor, and immune complexes containing RF and ACPA can amplify TNF-α production and OC activation.

surface, and ACPA directed against mutated vimentin and purified from serum of patients with RA increase the formation and differentiation of OCs *in vitro* and induce bone resorption *in vivo* after transfer to mice. Applying these findings to human arthritis, serum markers of bone resorption and OC activity, such as C-terminal cleavage products for collagen type I, tartrate-resistant acid phosphatase 5b, and cathepsin K, were found to be specifically increased in association with ACPA-positivity in patients with early RA (47). Further studies have confirmed that polyclonal ACPA obtained from the peripheral blood or synovial fluid of patients with RA are effective in inducing osteoclastogenesis from human monocytes (48). The process of ACPA-induced OC differentiation appears dependent on a combination of intracellular protein citrullination in the OC as well as autocrine preferential production of

IL-8 (48). The finding that, in lymphocyte-deficient mice, the administration of ACPA induced trabecular bone loss in the absence of synovial inflammation, and bone loss was inhibited by IL-8 but not TNF blockade (48), further reinforces the concept that antibodies may mediate inflammation-independent tissue damage. Much work needs to be done to identify more precisely the antigenic targets and the fine characteristics of ACPA before definitive conclusion on their direct and causal involvement in bone remodeling can be drawn. Indeed, not all ACPAs are pathogenic, and characteristics such as avidity and IgG glycosylation seem important in determining the strength of OC activation and the amount of joint destruction (49, 50). The direct effect of ACPA would, however, offer compelling explanation of the disproportionate amount of joint and systemic bone damage observed in autoantibody-positive patients (24, 31, 32) and of the occurrence of bone loss despite the disease being inactive (51) or not yet clinically apparent (35).

While research interest in recent years has, to a very large extent, revolved around the direct roles of ACPA, it is worth recalling that antibodies have long been recognized as possible regulators of OC function through classic, inflammation-dependent pathways. As with any antibody to a self-antigen, ACPA can form immune complexes that trigger TNF-α secretion through engagement of a Fcγ receptor on macrophages *in vitro* (52-55). Furthermore, independently from the formation of immune complexes, ACPA purified from RA sera have been shown to activate monocytes directly by binding to a citrullinated GRP78 cell-surface receptor, driving cytokine production (56). Other autoantigens recently identified in patients RA, including malondialdehyde-acetaldehyde adducts and 14-3-3 proteins, may also promote robust pro-inflammatory responses and metalloproteinase-mediated tissue destruction (57-60), further contributing to joint damage. The relevance of the pro-inflammatory role of ACPA *in vivo* remains questionable, however, and recent lines of research assign most of the inflammatory activity to the concomitant presence of RF. Incorporation of IgM and IgA RF into ACPA immune complexes substantially enhanced their capacity to induce TNF-α by human monocytes-macrophages (61-63), and ACPA-positive/RF-positive concordant patients displayed higher levels of multiple circulating inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-17A, compared to double-negative and single ACPA-positive patients in a large (nearly 1500) registry across the U.S. (61).

Although full understanding of the relative contribution of ACPA and RF to RA pathology is hampered by their substantial overlap, it is noteworthy to mention some specific differences that seem to emerge from recent clinical studies. In the U.S. registry, single RF-positive patients exhibited small, albeit significant, elevations in cytokines and some clinical measures of disease activity compared to the double-negative and single ACPA-positive subgroups (61). Analogously, baseline data from the rituximab and golimumamb trials (2118 patients) have revealed that RF-positive patients demonstrate the highest disease activity levels regardless of ACPA status, whilst disease activity is lower in single ACPA-positive patients (64). The opposite scenario would instead characterize the relationship with bone remodeling, where ACPA appear sufficient, and RF confers additional risk (30, 32).

Based on these findings, some Authors have put forward the hypothesis that ACPA would primarily trigger bone damage through direct binding to OCs independently of disease activity, whilst the effects of RF would be predominantly mediated by the induction of local and systemic inflammation (30, 65).

CONCLUSIONS

As pathological bone remodeling is primarily driven by inflammatory stimuli, abating disease activity remains the major goal of any treatment strategy in patients with RA. However, the recent demonstration that B-cells may directly boost OC activity *via* antibody-independent and -de-

pendent mechanisms provides mechanistic explanation of the increased rates of local and systemic bone loss observed in patients with ACPA-positive RA irrespective of disease duration and activity. Direct targeting of autoreactive B-cells and pathogenic autoantibodies may thus represent additional weapons in the war on autoimmune-mediated bone loss.

Competing interests: none to declare.

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