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Rheumatoid arthritis and osteoimmunology: The adverse impact of a deregulated immune system on bone metabolism

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ABSTRACT

The term osteoimmunology describes an interdisciplinary research field that links the investigation of osteology (bone cells) with immunology. The crosstalk between innate and adaptive immune cells and cells involved in bone remodeling, mainly bone-resorbing osteoclasts and bone-forming osteoblasts, becomes particularly obvious in the inflammatory autoimmune disease rheumatoid arthritis (RA). Besides striking inflammation of the joints, RA causes bone loss, leading to joint damage and disabilities as well as generalized osteoporosis. Mechanistically, RA-associated immune cells (macrophages, T cells, B cells etc.) produce high levels of pro-inflammatory cytokines, receptor activator of nuclear factor κB ligand (RANKL) and autoantibodies that promote bone degradation and at the same time counteract new bone formation. Today, antirheumatic therapy effectively ceases joint inflammation and arrests bone erosion. However, the repair of established bone lesions still presents a challenging task and requires improved treatment options. In this review, we outline the knowledge gained over the past years about the immunopathogenesis of RA and the impact of a dysregulated immune system on bone metabolism.

1. Introduction

RA represents one of the most prevalent inflammatory autoimmune diseases, mainly involving the joints. It is estimated that 0.5–1 % of today's population is affected by RA, presenting a substantial health and economic burden to patients and society [1]. The inflammation of the synovial membrane, named synovitis, portrays the most important feature of RA. Increased vascularization and immune cell infiltration manifests in redness, warmth, swelling, severe pain and stiffness of the affected joints. In addition, the degradation of articular cartilage and the underlying bone is a key characteristic of RA, limiting the mobility of joints and eventually causing total joint destruction [2]. As a systemic disease, RA also provokes several comorbidities, including osteoporosis, cardiovascular disease, lung disease, neurological abnormalities and muscle disorders [3]. Generalized bone loss represents one of the most common conditions in RA. Studies report that the prevalence of osteoporosis in RA patients is approximately twice as high as in the general population. As a consequence, RA patients are at a higher risk to suffer from bone fractures, especially at the vertebrae [4]. The tremendous impact that RA-mediated inflammation has on bone exemplifies the close relationship between the immune system and bone metabolism.

RA treatment has adapted over the past decades. Starting out with a gradual increase of RA medication over time, today, patients are treated with disease-modifying antirheumatic drugs (DMARDs) as soon as the diagnosis is made. Methotrexate is the anchor drug in RA and can be combined with low-dose glucocorticoids or other classical DMARDs. Patients with a poor response to classical DMARDs are recommended to use biological DMARDs, including etanercept (TNF inhibitor), abatacept (T cell co-stimulation blocker), rituximab (B cell depletion) and tocilizumab (IL-6 inhibitor), or synthetic DMARDs like the janus kinase (JAK) inhibitors tofacitinib and baricitinib [5]. To specifically treat systemic bone loss in RA patients, supplemental administration of denosumab (RANKL inhibitor) might be a promising approach. However, combination therapy is often accompanied by stronger side effects, such as an increased susceptibility to infections. Moreover, despite disease remission, some RA patients still suffer from structural bone damage, as established bone lesions in these patients are unable to heal. Hence, the implementation of experimental and clinical studies is important to

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expand our knowledge about the molecular mechanisms underlying RA-associated bone erosion. The results derived from these surveys can help to further optimize bone-directed treatment options in a more personalized approach.

2. Immunopathogenesis of RA

The pathogenesis of RA is a highly complex, multistep process that begins with a breach of self-tolerance and the emergence of autoantibodies in susceptible individuals, who thereby enter a phase of asymptomatic autoimmunity. This stage of pre-articular RA can last for years to decades and is associated with a gradual remodeling of the immune system towards a pro-inflammatory state, before some of the affected individuals develop clinically apparent synovitis. As shown in Fig. 1, acute inflammation often turns into chronic disease that ultimately leads to severe damage targeting cartilage and bone.

2.1. Risk factors

There is a large number of factors that are associated with an increased risk of developing RA (Fig. 1). Genetic predisposition confers 30 to 60 % of risk for the development of RA. Polymorphisms that potentially contribute to this risk were identified in more than 100 different genes, most of which are involved in T cell-mediated immune responses. However, the strongest genetic susceptibility for RA is associated with certain class II human leukocyte antigen (HLA) alleles of the HLA-antigen D related (DR) locus (specifically HLA-DRB1*01 and HLA-DRB1*04). These gene variants carry a specific amino acid motif (known as "shared epitope") that affects the formation of the antigen-binding pocket and is suggested to promote the production of autoantibodies [6]. Identical twin studies have revealed a disease concordance of 12 to 15 % for RA [7], showing that although RA has a strong genetic component, environmental factors also influence the disease development. One of the greatest environmental risk factors for RA is tobacco smoking. The probability of a heavy smoker to develop RA is twice as high as that of a non-smoker, whereas this relationship predominantly concerns genetically predisposed individuals, who carry at least one copy of the shared epitope [8]. Smoking promotes protein citrullination by upregulating the expression of peptidyl arginine deaminases (PADs), possibly facilitating autoantigen production during the early phase of disease development [9]. Other environmental conditions that positively correlate with the development of RA include obesity, certain infectious agents (e.g. Epstein-Bar virus (EBV)) and exposure to air pollution, silica or textile dust [2]. Women generally bear a two to three-fold higher risk of developing RA than men. Nulliparity was described to further increase the RA risk in women, while pregnancy is commonly associated with disease remission [10]. One explanation for the higher incidence of RA in women is the immune-enhancing effect of oestrogen. However, the influence of sex hormones on the initiation and progression of RA is still under investigation [11].

2.2. Loss of self-tolerance

The initial event during the pathogenesis of RA is the loss of tolerance towards endogenous antigens, marked by the appearance of autoreactive antibodies (Fig. 1). There are two main types of RA-associated autoantibodies, namely rheumatoid factors (RFs) and anti-citrullinated protein antibodies (ACPAs). RFs recognize the Fc part of

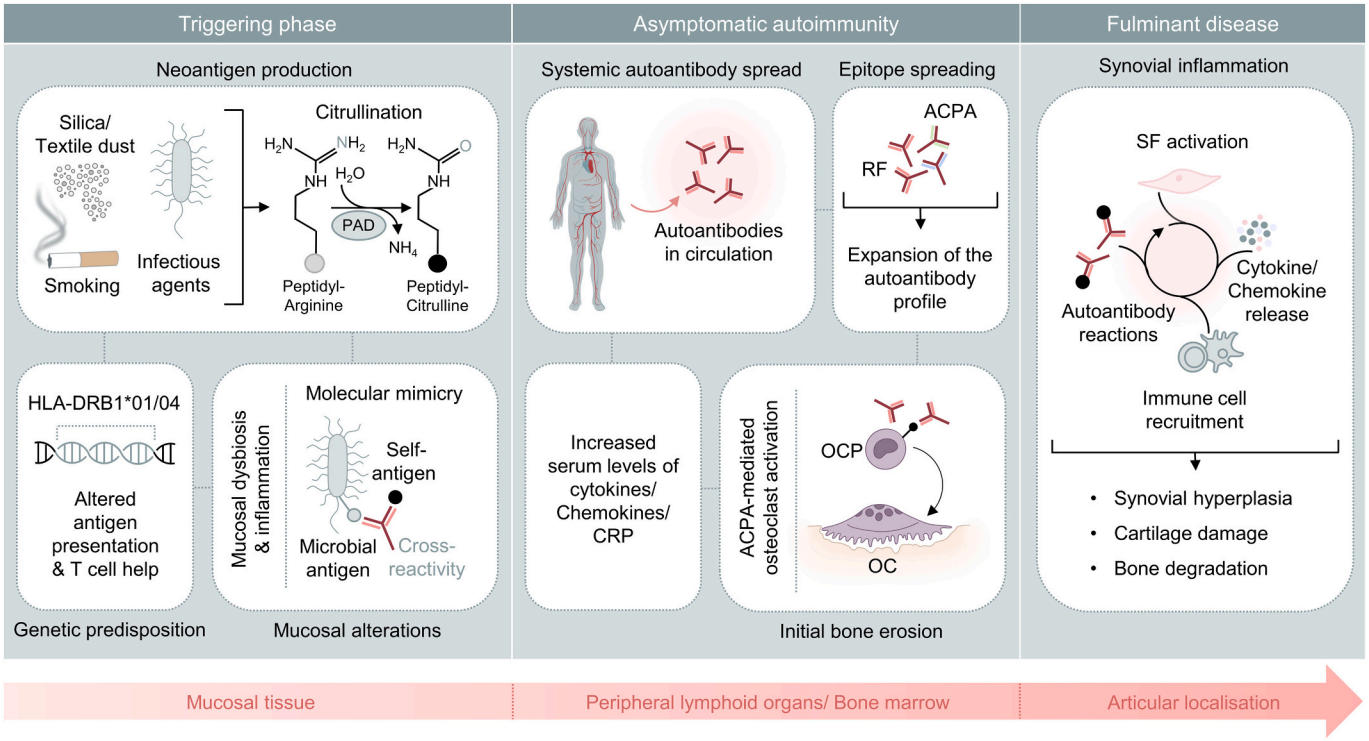

Fig. 1. The developmental stages of RA. Fig. 1 illustrates the progressive development of RA, schematically divided into three phases. The first stage (triggering phase) represents the initial formation of autoreactive antibodies at mucosal sites as a result of accumulating environmental and genetic triggers. These triggers can promote the development of neoantigens, alter the antigen presentation process or create an environment that enables the formation of anti-microbial antibodies that cross-react with modified self-antigens. Phase 2 (asymptomatic autoimmunity) is characterized by the presence of autoantibodies in the circulation, epitope spreading as well as gradually increasing levels of inflammatory markers, indicating the existence of subclinical inflammation. Initial bone erosion can also be observed during preclinical RA. The autoimmune reactions take place in peripheral lymphoid organs. Phase 3 (fulminant disease) describes the establishment of synovitis, associated with the recruitment of immune cells into the joint, stromal cell activation, autoantibody reaction and the release of cytokines and chemokines, amplifying each other in an inflammatory loop that ultimately results in synovial hyperplasia, cartilage and bone erosion. RF, rheumatoid factor; ACPA, anti-citrullinated protein antibodies; CRP, C-reactive protein; PAD, peptidyl arginine deaminase; OC, osteoclast; OCP, osteoclast precursor; SF, synovial fibroblast.

glycation modified IgG molecules and are detectable in 60 to 80 % of RA patients [12]. ACPAs, which bind to citrullinated proteins, can be observed in 80 to 90 % of RA patients [13]. In general, RA-associated autoantibodies are not directed against naïve peptides, but against proteins that have undergone a posttranslational modification, the most common being citrullination. The term citrullination describes the conversion of the amino acid arginine into citrullin by the enzyme PAD. It is a physiological reaction that commonly occurs in the context of cellular stress responses and does not in itself constitute an immunogenic structure that would elicit an antibody response. The loss of tolerance towards posttranslationally modified self-antigens therefore requires additional immunological signals in combination with genetic predisposition and environmental triggers [14].

A substantial body of evidence indicates that mucosal sites (in particular lung, periodontium and the intestine) provide the necessary milieu for the development of RA-associated autoantibodies and therefore might serve as tissues of origin for RA. In line with this, the presence of RA-associated autoantibodies at mucosal sites is a common observation during the pre-articular phase of RA [15]. In this context, mucosal dysbiosis is proposed as one of the main drivers for the initiation of autoimmunity in RA. Accordingly, some infectious agents have been directly linked to increased risk for RA development, including *Porphyromonas gingivalis* (*P. gingivalis*), *Aggregatibacter actinomycetemcomitans* (*Aa*) and EBV. All of these microorganisms are suggested to promote the induction of autoimmunity by boosting the levels of citrullinated neoantigens at mucosal sites [14,16,17].

In addition to that, some microbial antigens exhibit similarities with epitopes from endogenous proteins. This molecular mimicry might be responsible for the development of anti-microbial antibodies that cross-react with self-antigens, providing a possible explanation for the origin of autoantibodies in RA. In support of this hypothesis, it was discovered that ACPAs that recognize human citrullinated α-enolase cross-react with the citrullinated enolase from *P. gingivalis* [18,19]. The ACPA target, citrullinated vinculin, contains an antigen motif that is also found in several microbial organisms [20]. Another study reported similarities between antigenic structures of the alphavirus and type II collagen, another common autoantigen in RA [21]. Notably, the presence of local RFs or ACPAs in mucosal tissues is not in itself pathogenic. The progression of the disease requires a systemic spread of RA-related autoantibodies, which is possibly induced through the disruption of the mucosal barrier function and an unresolved inflammatory reaction at the mucosal site [22].

Besides the induction of autoantibodies, certain microbial stimuli, like LPS and β-glucan, can also mediate long-term immunological memory in innate immune cells, a mechanism that has long been thought to be an exclusive hallmark of the adaptive immune system and is today termed “trained immunity”. Thereby, innate immune cells, primarily monocytes and macrophages, are “rewired” by epigenetic and metabolic adaptations, leading to a hyper-activated state upon second encounter with the microbial ligand, but also upon exposure with an unrelated stimulus [23]. Epigenetic changes in trained immune cells are defined by chromatin restructuring, including elevated levels of H3K4me3, H3K27ac and H3K4me1, as well as DNA demethylation. The metabolic adaptation in trained macrophages is associated with increased cAMP production and activation of the mTOR-HIF1α pathway and aerobic glycolysis [24]. Notably, trained immunity can also be provoked by alarmins like high-mobility group box 1 (HMGB1) and vimentin [25] and is not just limited to cells of the hematopoietic stem cell niche, but can also be observed in stromal and epithelial cells, such as fibroblasts [26].

Since a hyper-functional immune response is linked to chronic inflammation, maladaptive induction of trained immunity is associated with several autoinflammatory diseases, including atherosclerosis, neurodegenerative diseases and systemic lupus erythematosus [27–29]. In RA, macrophages and monocytes from patients might resemble a trained immunity signature. For instance, mTOR signaling and glycolysis are amplified in RA synovium, whereas mTOR neutralization decreases synovial osteoclasts and ameliorates local bone erosion and cartilage loss [30,31]. Moreover, synovial tissue of RA patients presents various epigenetic changes as compared with synovial tissue from osteoarthritis (OA) patients [32]. Recently, a study by Li et al. provided a direct link between microbial-induced trained immunity and exacerbation of arthritis. This work demonstrated that periodontal inflammation reprograms hematopoietic stem and progenitor cells (HSPC) and their myeloid progeny through epigenetic modification towards heightened immune responsiveness in an IL-1 dependent manner, promoting inflammatory arthritis [33].

2.3. Transition from asymptomatic autoimmunity to synovial inflammation

The presence of circulating RA-associated autoantibodies can be observed up to 10 years before the onset of symptoms. As depicted in Fig. 1, this stage of asymptomatic autoimmunity or pre-articular RA is associated with a gradual increase of autoantibody serum levels and epitope spreading, resulting in diversification of the autoreactive immune response. At the same time, pre-RA is often accompanied by rising levels of cytokines and chemokines in the blood, suggesting an ongoing, subclinical inflammatory response [2,13,34]. Considerably, some ACPA positive individuals have been shown to exhibit initial bone loss during the preclinical phase of disease development. This indicates that increased bone erosion in RA does not only occur as a consequence of joint inflammation, but might also be part of the event chain that results in articular involvement [35].

The causes for the transition from asymptomatic autoimmunity to synovial inflammation are still not well understood, but might be initiated by tissue insults like microtraumata or articular infections that would result in vascular activation, innate immune cell hyperactivation and increased access of autoantibodies to the joint [13,24,36]. Changes in the inflammatory properties of the autoreactive antibodies might also facilitate the initiation of clinical synovitis [37]. Once within the joint, circulating autoantibodies might interact with self-antigens as well as Fc receptors on resident cells (e.g. macrophages and osteoclasts), activate complement and initiate an inflammatory cascade that results in the recruitment of innate and adaptive immune cells to the synovium and the activation of synovial fibroblasts (SFs). These in turn produce vasoactive factors, pro-inflammatory cytokines and chemokines, leading to a self-perpetuating synovial inflammation [38,39].

2.4. Pathophysiology of clinical synovitis

The synovial membrane or synovium is a connective tissue structure that lines the inner surface of the joint capsule (Fig. 2). In healthy joints, the synovium is composed of a thin intimal lining that consists of both tissue resident macrophages and fibroblasts as well as a sublining layer containing fibroblasts, interstitial macrophages, adipocytes and blood vessels [40]. As recently uncovered by Culemann et al., synovial macrophages comprise 3 distinct subpopulations: (i) epithelial-like CX_3CR1^+ lining macrophages that shield the intra-articular space and remove waste products from the synovial fluid, (ii) $RELM\alpha^+$ interstitial macrophages that are located in the sub-lining and rather display features of alternatively activated macrophages (CD163 and CD206 expression) and (iii) proliferating $MHCII^+$ interstitial macrophages that give rise to the other two subsets [41]. Lining layer fibroblasts are located directly underneath the lining macrophages and provide lubricants to the joint cavity, while sub-lining fibroblasts are responsible for the maintenance of the extracellular matrix [42]. RA is associated with an expansion of the synovial membrane (Fig. 2), along with the transformation of fibroblasts into autoaggressive, invasive cells that acquire the ability to migrate from joint to joint and are mainly responsible for the generation of cartilage-degrading matrix metalloproteases (MMPs) [3]. Inflammatory arthritis additionally recruits pro-inflammatory monocyte-derived

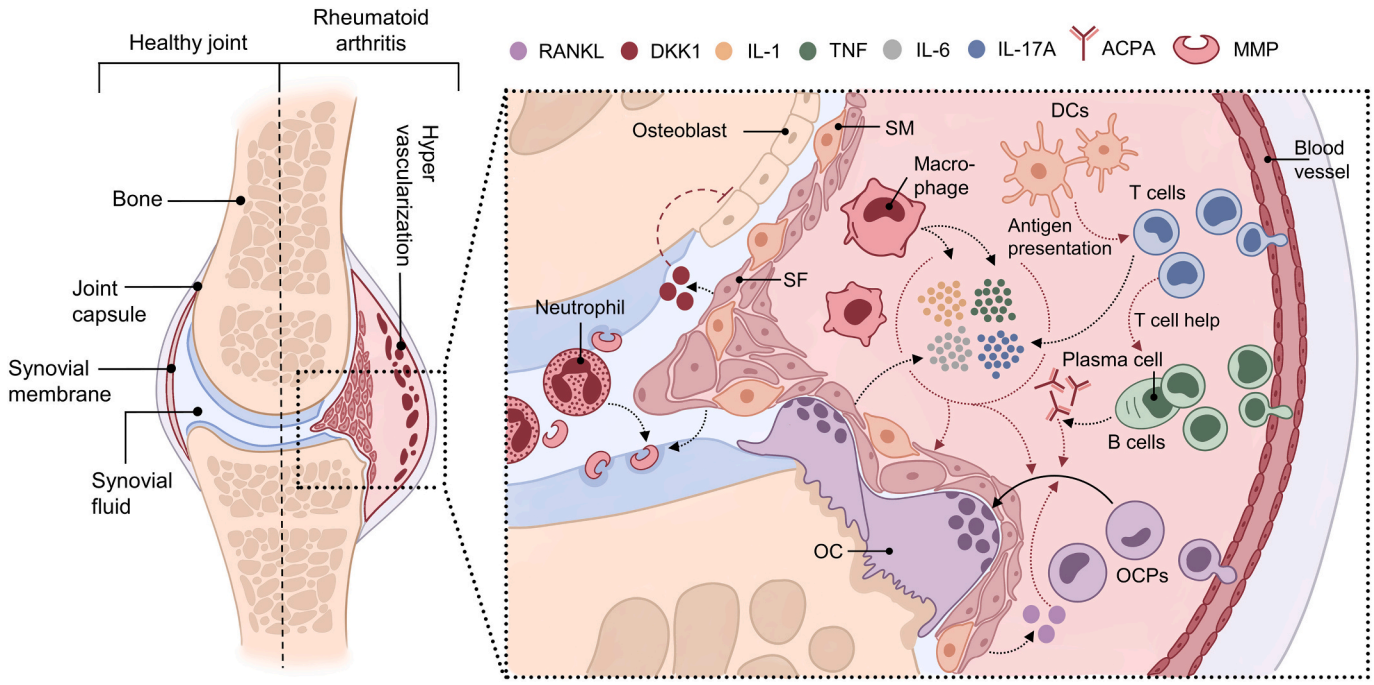

Fig. 2. RA-associated synovitis and joint damage. The synovial membrane is infiltrated by cells of the innate and adaptive immune system (DCs, T cells, B cells, plasma cells and macrophages). The inflammatory process within the RA joint includes autoantigen presentation by dendritic cells (DCs), followed by T cell-mediated activation of B cells and subsequent production of autoantibodies by autoreactive plasma cells. The interaction of macrophages with autoantibodies activates them to produce pro-inflammatory cytokines, such as TNF and IL-1. T cells additionally produce the cytokine IL-17A. Pro-inflammatory cytokines reprogram SFs into an aggressive phenotype, leading to synovial fibroblast hyperplasia and the characteristic pannus formation, while synovial lining macrophages (SMs) get disrupted. Degenerated SFs participate in the pathogenesis of RA through the production of IL-6 and receptor activator of NFκB ligand (RANKL). SFs and neutrophils (found in the synovial fluid) release matrix metalloproteinases (MMPs) that degrade the joint cartilage. Articular bone erosion is caused by an enhanced differentiation of OCPs into bone-resorbing osteoclasts that is provoked by RANKL, TNF, IL-1, IL-6 and IL-17A. Osteoclast development can also be induced by an inflammation independent pathway through autoantibodies secreted by plasma cells. The inflammatory setting hampers new bone formation by osteoblasts, as SFs overexpress the Wnt signaling antagonist dickkopf1 (DKK1) upon exposure to TNF. Arrows with dotted, back lines indicate the production of soluble mediators. Dotted, red lines indicate activation, while dashed, red lines indicate suppression. Arrows with a solid, black line indicate enhanced differentiation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

macrophage subsets (expression of IL-1β, CCR2 and Ly6C) into the synovium, while CX_3CR1^+ lining macrophages become disrupted (Fig. 2). This inflammation-mediated disintegration of lining macrophages is most likely induced by autoantibody-containing immune complexes, leading to an impaired barrier function that causes an early and exacerbated onset of arthritis and enhanced neutrophil influx into the joint [41].

Established synovitis is characterized by neovascularization and significant infiltration of the synovial membrane with innate and adaptive immune cells (e.g. macrophages, dendritic cells, natural killer cells, mast cells, T and B cells as well as plasma cells), while the synovial fluid contains neutrophils (Fig. 2). Macrophages strongly contribute to RA pathology through the production of cytokines (TNF, IL-1 and IL-6), promoting the dedifferentiation of SFs. Besides that, they release chemokines, vasoactive mediators, oxygen and nitrogen intermediates as well as matrix-degrading enzymes and are involved in antigen presentation [13]. Another major contributor to the pathophysiology of synovial inflammation are $CD4^+$ T cells. Type 1 helper T cells (Th1) were originally viewed as the main pathogenic $CD4^+$ T cell subset in RA. However, more recent studies have shifted the attention towards type 17 helper T cells (Th17). The cytokine milieu within the inflamed joint favors the differentiation of Th17 cells, while the development of anti-inflammatory regulatory T cells (Tregs) is inhibited. Th17 cells secrete the cytokine IL-17A that further enhances fibroblast activation and perpetuates synovial inflammation [43]. Other immune cells that promote the inflammatory reactions within the joint are B cells and mature plasma cells, which produce autoantibodies and are involved in antigen presentation as well as cytokine production, while neutrophils produce reactive oxygen species, prostaglandins and proteases [3]. The pathological events described in this paragraph occur partially simultaneously, amplifying each other in a vicious cycle, resulting in tissue damage, as visualized in Fig. 2. Besides cartilage degradation, this involves osteoclast-mediated bone erosion, which will be outlined in the following section.

3. Interaction between the immune system and bone in RA

Bone remodeling is the predominant metabolic process to maintain a healthy skeleton throughout adult life. Up to 10 % of the calcified bone is renewed every year, which depends on a fine balance between bone-resorbing osteoclasts and bone-forming osteoblasts. In a healthy setting, local bone damages, e.g., micro-fractures, recruit osteoclasts that resorb the affected bone. Afterwards, the bone cavity is refilled with new bone matrix by osteoblasts. In RA the close interaction between the immune system and bone is evident as the inflammatory milieu deeply disrupts this bone homeostasis, favoring bone resorption over bone formation. This leads to substantial bone loss, manifesting at different sites during the course of RA, including local bone loss in articular and periarticular site and generalized bone decline.

3.1. Bone phenotype in RA

Articular bone erosion is a radiographic hallmark of RA, visualized as a break in cortical bone with destruction of the natural barrier between the extra skeletal tissue and the bone marrow compartment [44]. Since the detection of these bone changes has been shown to have

considerable clinical utility in diagnosis and prognosis of RA, articular bone erosion has been included as a main characteristic of RA by the American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) collaborative initiative [45]. Moreover, radiography is used to monitor the efficacy of DMARDs in retarding or even arresting bone damage in daily clinical practice as well as in all major clinical trials. Although conventional radiography is still widely used to validate bone erosion in RA patients, improved imaging techniques, such as computed tomography (CT), high-resolution ultrasonography and magnetic resonance imaging (MRI) nowadays allow the detection of even small bone erosions [46,47]. Articular bone erosion manifests early in RA, sometimes even before disease onset in still asymptomatic ACPA-positive subjects [35]. The occurrence of bone erosions predicts a more severe course of RA with a greater extent of joint disability and a higher mortality rate [44,48].

Osteoporosis is an important co-morbidity of RA and occurs in two forms: periarticular osteopenia in close proximity to inflamed joints and generalized osteoporosis, which affects the axial and appendicular bones. Indeed, periarticular bone lesions are one of the earliest structural bone alterations presented in RA patients, emerging even before bone erosion and joint space narrowing [49]. Morphologically, bone marrow fat is replaced by inflammatory infiltrates composed of T cell and B cell aggregates that can be visualized by MRI as regions with increased water content [50,51]. The mechanisms that cause bone marrow lesions are still incompletely understood. During established arthritis, periarticular bone loss is most likely induced by proinflammatory cytokines released from the inflamed synovial tissue [52], while the early onset of periarticular bone loss in RA patients may be attributed to autoantibodies against citrullinated vimentin [53]. Since RA also has a strong systemic effect on bone, patients with RA are at an increased risk of osteoporosis and osteoporotic bone fractures [54]. The main reason for a generalized reduction of bone mineral density (BMD) in RA patients is the systemic impact of RA-associated inflammation on bone, including the universal distribution of pro-inflammatory cytokines via the blood stream. However, also many inflammation-independent factors can influence bone homeostasis in RA, such as treatment with corticosteroids and reduced weight-bearing due to immobility. Moreover, RA more commonly affects people at higher age and of female gender, and is strongly associated with smoking, all conditions that are also considered as standard risk factors for osteoporosis [55].

3.2. Osteoclast origin and differentiation in RA

As the only bone degrading cells in the body, osteoclasts are primarily responsible for RA-associated bone loss. In 1984, a study by Bromley and Woolley provided first evidence for the localization of polynucleated osteoclasts at sites of bone erosion in rheumatic joints [56]. Later on, the use of genetically modified mice elucidated the central role of osteoclasts in the pathogenesis of bone erosion in arthritis, as osteoclast-deficient RANKL and c-Fos knockout mice were completely protected from bone erosion despite joint inflammation [57,58].

Excessive bone erosion on one hand requires amplified recruitment of osteoclast precursors (OCPs) into the synovial compartment and on the other hand elevated pro-osteoclastogenic signals that mediate the differentiation of OCPs into mature osteoclasts. At fetal stage, osteoclasts that are involved in the formation of bone and the bone marrow cavity develop from the embryonic erythro-myeloid progenitor (EMP) lineage [59]. Postnatally, hematopoietic stem cell (HSC) lineage-derived OCPs become essential for osteoclast maintenance and function. These OCPs belong to the monocyte/ macrophage lineage and exhibit high cellular plasticity, as they are able to differentiate into macrophages, dendritic cells, osteoclasts and other more organ-specific cell lineage types, such as Langerhans cells in the skin, Kupffer cells in the liver or microglia in the brain [60,61]. In this regard, synovial osteoclasts may not only arise from the fusion of peripheral monocytes, but also from immature dendritic cells (DCs), since several studies have proven the ability of DCs to transdifferentiate into osteoclasts [62,63]. Notably, the occurrence of DC-derived osteoclasts is linked to pathologies, such as inflammation or cancer, while these cells were never detected under healthy conditions [64]. For instance, the formation of osteoclasts from human DCs could be specifically induced using synovial fluid from patients with arthritis [62].

OCPs most likely migrate into synovial sites through chemotaxis. In that respect, the monocyte chemoattractant protein-1 (MCP-1), also known as chemokine ligand 2 (CCL2), was found upregulated in synovial tissue and fluid of RA patients compared with healthy controls [65]. A study by Charles and colleagues has proven that $Ly6C^{hi}$ monocytes that serve as an osteoclast precursor population increase upon arthritis [66], supporting the view that the peripheral monocytic pool changes during inflammation. In addition, Hasegawa et al. recently uncovered that upon migration into the synovium the $Ly6C^{hi}$ monocytic population further differentiates into a special subset of macrophages defined as $CX_3CR1^{hi}Ly6C^{int}$ arthritis-associated osteoclastogenic macrophages (AtoMs). These pathogenic AtoMs are distinct from physiological OCPs in the bone marrow, because they present unique surface markers like CD80/ 86, I-A/ I-E and CD11c, are regulated by the transcription factor forkhead box protein M1 (FOXM1) and possess a higher potential to differentiate into osteoclasts [67]. Generally speaking, the pro-inflammatory environment in RA favors the accumulation of OCPs in the joints, paving the way for increased osteoclast differentiation and bone erosion.

As illustrated in Fig. 3, osteoclast biology is controlled by the cytokines, macrophage colony-stimulating factor (M-CSF) and RANKL. The interaction of M-CSF with its ligand, colony stimulating factor 1 receptor (CSF1R), leads to the downstream activation of the phosphoinositide 3-kinase (PI3K)/Akt and extracellular signal-regulated kinase (ERK) signaling pathway and is central for the proliferation and survival of osteoclastogenic cells (Fig. 3) [68]. The differentiation of OCPs to mature bone resorbing osteoclasts critically depends on RANKL. During the process of physiologic bone remodeling, RANKL is mainly released by osteoblasts and osteocytes. However, in the arthritic joints, RANKL is dramatically increased, as it is also produced by degenerated SFs and infiltrating immune cells [69,70]. The binding of RANKL to its receptor RANK causes the recruitment of the adaptor molecule, TNF receptor-associated factor 6 (TRAF6), which in turn induces MAP kinases and the transcription factors nuclear factor k-light-chain-enhancer of activated B cells (NFκB) and activator protein 1 (AP-1) (Fig. 3) [71].

Similarly to other immune cells, osteoclasts require co-stimulatory signals besides RANKL-RANK interaction for their complete activation (Fig. 3). This is mediated by the stimulation of the immunoglobulin-like receptors, osteoclast-associated receptor (OSCAR) and triggering receptor expressed on myeloid cells 2 (TREM-2), which are associated with the immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor proteins FcRγ and DNAX activating protein of 12 kDa (DAP12), respectively, leading to the induction of calcium signaling [72]. Indeed, the OSCAR/ TREM-2 signaling pathway is enhanced in RA. For instance, OSCAR expression is higher in peripheral blood monocytes from RA patients compared with controls and is associated with a more severe disease activity [73]. Moreover, the percentage of cells expressing OSCAR, TREM-2, FcRγ and DAP12 is significantly increased in RA tissue compared with healthy synovial tissue [74]. OSCAR mainly binds to type I and type II collagen, which are the most abundant collagen elements in bone and cartilage tissue [75]. However, the ligands for TREM-2 within the arthritic joint are still poorly described.

The two pathways that are activated by RANK and OSCAR/ TREM-2 culminate in the activation of the master regulator of osteoclastogenesis, nuclear factor of activated T cells 1 (NFATc1) (Fig. 3). As a transcription factor, NFATc1 translocates into the nucleus, where it induces numerous osteoclast-specific target genes that are essential for osteoclast maturation and bone resorption [76]. Upon full maturation, osteoclasts resorb

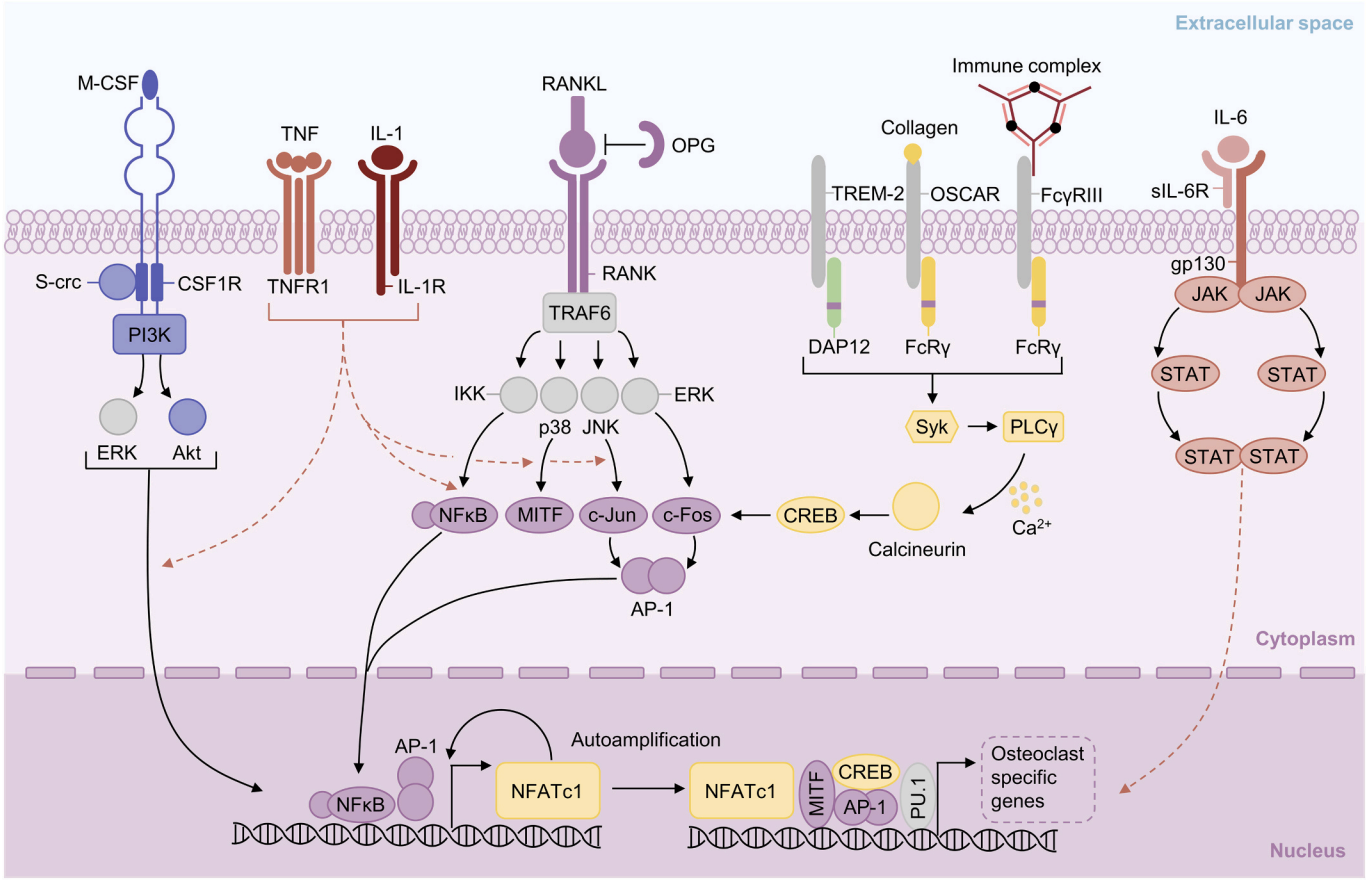

Fig. 3. Signaling pathways that induce osteoclast differentiation in RA. The interaction of M-CSF with its receptor CSF1R and the downstream activation of the PI3K/ Akt and ERK pathway is essential for the proliferation and survival of osteoclasts. This signaling cascade is further induced by the pro-inflammatory cytokines TNF and IL-1 that bind to the transmembrane receptors TNFR1 and IL-1R on OCPs, respectively. The differentiation of osteoclasts is controlled by the RANK/ RANKL/ OPG triad. Hereby, the interplay of RANKL with its receptor RANK leads to the recruitment of the adaptor protein TRAF6 that in turn induces a signaling network, promoting the translocation of the transcription factors NFκB, MITF and AP-1 into the nucleus to enable the expression of NFATc1, the master regulator of osteoclastogenesis. As a decoy receptor of RANKL, OPG counteracts this signaling pathway. However, the abundance of RANKL is increased within the inflamed joint. Moreover, TNF and IL-1 further amplify RANKL signaling. For complete activation, osteoclasts also need the costimulatory signal mediated by the transmembrane receptors OSCAR and TREM-2 and their respective adaptor proteins FcRγ and DAP12. Downstream of this interaction the calcium pathway involving Syk/ PLCγ/ Ca^{2+} flux and calcineurin strengthens the transcription of NFATc1 via CREB. Autoantibodies binding to the FcγRIII on osteoclasts reinforces osteoclast differentiation through the same signaling cascade. Finally, NFATc1, in combination with other transcription factors, elicits the expression of osteoclast specific genes. In addition, IL-6 enhances osteoclast formation via JAK-STAT signaling. AP-1, activator protein 1; CREB, cAMP-response element binding protein; CSF1R, colony stimulating factor 1 receptor; DAP12, DNAX-activating protein of 12 kDa; ERK, extracellular signal-regulated kinase; FcRγ, Fc receptor gamma-chain; IL-1R, interleukin-1 receptor; JAK, janus kinase; M-CSF, macrophage colony-stimulating factor; MITF, microphthalmia-associated transcription factor; NFATc1, nuclear factor of activated T cells 1; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; OPG, osteoprotegerin; OSCAR, osteoclast-associated receptor; PI3K, phosphoinositide 3-kinase; PLCγ, phospholipase Cγ; RANK, receptor activator of nuclear factor κ B; RANKL, RANK-ligand; STAT, signal transducer and activator of transcription; Syk, spleen tyrosine kinase; TNFR1, tumor necrosis factor receptor 1; TRAF6, TNF receptor-associated factor 6; TREM-2, triggering receptor expressed on myeloid cells 2.

bone through the secretion of protons, which acidify the extracellular compartment in order to solubilize calcium phosphate, and proteases like cathepsin-K, which thereafter degrade the exposed organic bone matrix [77]. Recently, a group from Australia unveiled that osteoclasts fission into osteomorphs after bone resorption and can be recycled again by fusion with neighboring osteoclasts. Since osteomorph-related genes were associated with human skeletal diseases like osteoporosis, osteomorphs may also play a relevant role in RA-associated bone loss [78].

Under physiological conditions, osteoclast activity is balanced by the expression of osteoprotegerin (OPG) through stromal cells (Fig. 3). As a decoy receptor of RANKL, OPG can counteract the binding of RANKL to its receptor RANK, thereby arresting osteoclast differentiation and further bone resorption [79]. As demonstrated in a 2010 published cohort study, the RANKL:OPG ratio may be used as a predictor of long-term radiological damage progression in RA patients [80]. Several studies examined the efficacy of OPG as an anti-bone resorptive agent, showing that the experimental administration of OPG slows down or even completely blocks bone erosion in animal models of arthritis [81,82].

In line with this, the therapeutic inhibition of RANKL with the monoclonal antibody denosumab holds strong potential to cease bone erosion in patients with RA, although it has no influence on the inflammation status [83,84]. In that regard, the recently conducted placebo-controlled phase 3 clinical study named DESIRABLE demonstrated that denosumab significantly reduces the progression of joint destruction and increases the BMD in RA patients receiving concomitant conventional DMARD therapy [85]. However, it should be kept in mind that anti-resorptive drugs, including denosumab and bisphosphonates, not only target pathological osteoclasts, but may also impair physiological bone remodeling.

3.3. *Osteoclast-promoting immune responses*

Bone homeostasis is strongly disturbed by the inflammatory

environment in RA. Hence, RA-related immune cells and their secretion products activate a number of pathways that add up to an overall reinforcement of osteoclast differentiation (Figs. 3 and 4).

3.3.1. B cells

B cells are significantly involved in RA-associated bone erosion, which in large part can be attributed to the effects of autoreactive antibodies. Thus, high serum levels of ACPA and RF correlate with more pronounced bone erosion in RA [44]. Interestingly, ACPA positivity was also associated with decreased systemic cortical bone density in individuals without clinical synovitis, indicating that ACPAs could promote bone loss even in the absence of inflammation [35]. In accordance with this assumption, it was discovered that ACPAs are able to directly interact with citrullinated epitopes on osteoclasts and osteoclast progenitors. Differentiating osteoclasts upregulate the expression of the enzyme PAD type 2 (PAD2), which catalyzes the citrullination of proteins, in this case vimentin. Osteoclast progenitor cells therefore display high levels of citrullinated vimentin on their cell surface. Binding of ACPAs to citrullinated vimentin on OCPs stimulates the production of TNF, resulting in enhanced osteoclast cell differentiation through autocrine signaling (Fig. 4) [39,44]. Aside from direct binding to citrullinated vimentin, ACPAs can also promote osteoclastogenesis through the formation of immune complexes and interaction with Fcγ receptors on the cell surface of OCPs (Figs. 3 and 4). Remarkably, the pro-osteoclastogenic potential of ACPA immune complexes strongly depends on their sialylation status. Thus, it was demonstrated that osteoclast differentiation is only enhanced upon interaction with non-sialylated immune complexes [86,87]. Another, rather indirect effect of RA-associated autoantibodies on osteoclast activity is mediated by the interaction of ACPAs and RFs with Fcγ receptors on macrophages, resulting in secretion of pro-osteoclastogenic cytokines (e.g. TNF, IL-1, IL-6 and IL-8) [88]. The significant role of B cells for pathogenic bone resorption was further substantiated when pro-inflammatory B cells were identified as an important source of RANKL in RA (Fig. 4) [89,90].

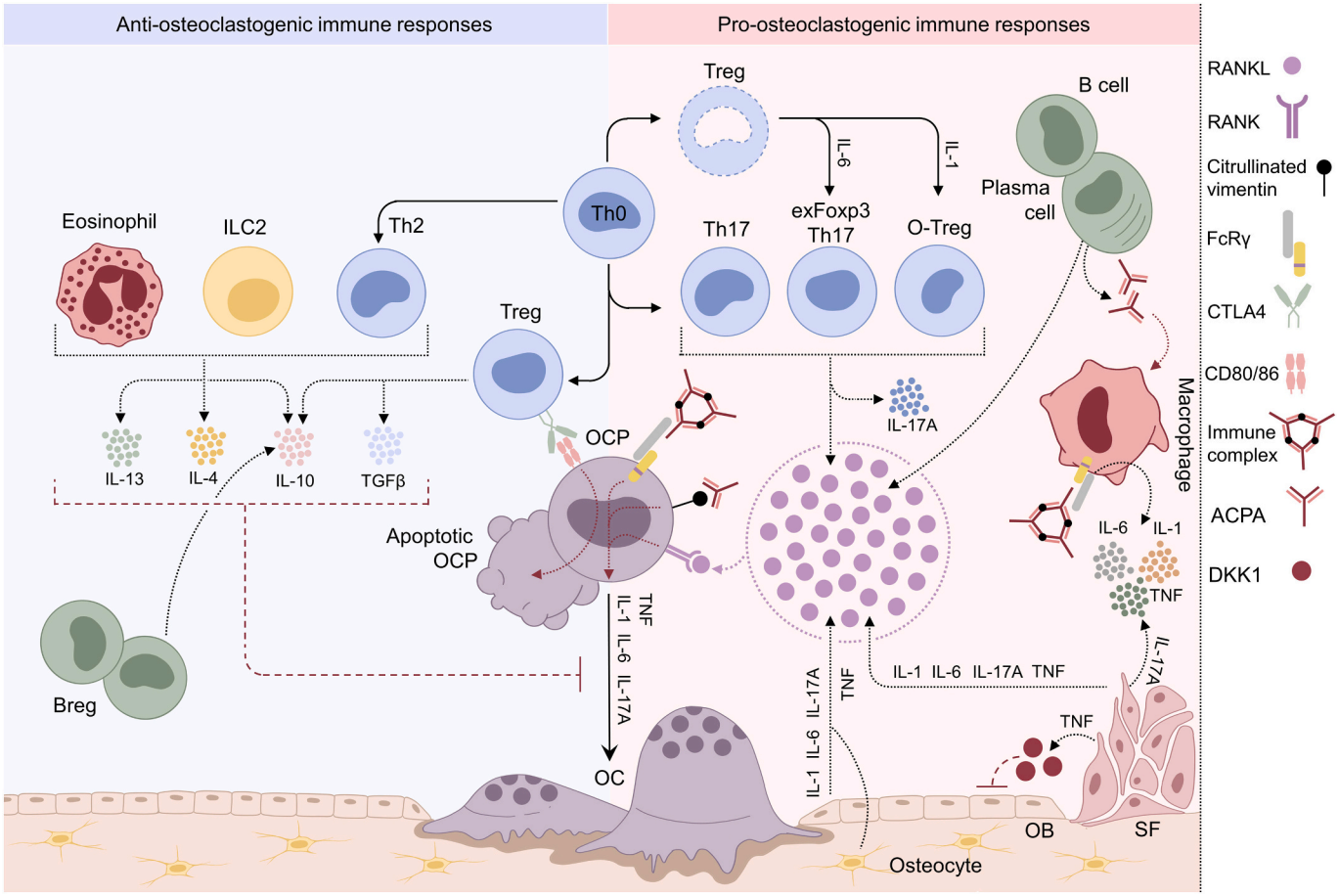

Fig. 4. Pro- and anti-osteoclastogenic immune responses in RA. Macrophages are a major source of the pro-inflammatory cytokines IL-6, TNF and IL-1. While these cytokines can directly provoke osteoclast differentiation, they also reprogram SFs and osteoblasts to produce higher amounts of RANKL. RANKL is also provided by Th17 cells that are abundantly present in the inflamed synovium. Particularly pathogenic are exFoxp3Th17 cells that transdifferentiate from former Tregs through the influence of IL-6. Tregs can also lose their regulatory identity through exposure to IL-1, leading to the formation of highly osteoclastogenic Tregs (O-Tregs). Another source of RANKL within the arthritic joint are B cells. In addition, plasma cells secrete ACPAs that promote osteoclastogenesis either through immune complex binding to FcRγ on OCPs or via binding to citrullinated vimentin on osteoclasts, activating a TNF-dependent autocrine signal. Altogether, the over-activation of osteoclasts causes inflammatory bone loss. Moreover, the inflammatory environment blunts osteoblast-dependent bone formation, as TNF reprograms SFs to upregulate DKK1. In contrast, certain immune cells possess anti-osteoclastogenic properties. Most importantly, classical Tregs inhibit osteoclast formation through the production of osteoclast-suppressing cytokines, such as IL-10 and TGF-β, and through cytotoxic T-lymphocyte-associated protein 4 (CTLA4) that interacts with CD80/ 86 on OCPs, inducing apoptosis. Another relevant source of anti-osteoclastogenic IL-10 are regulatory B cells (Bregs), in particular B10 cells. In addition, the type 2 immune response-associated immune cells Th2 cells, innate lymphoid cells type 2 (ILC2s) and eosinophils inhibit excessive osteoclast formation through the release of the osteoclast-inhibiting cytokines IL-4, IL-10 and IL-13. Thus, the priming of a rather regulatory immune response in RA might be beneficial for protecting the bone from inflammation-mediated bone erosion. Arrows with dotted, back lines indicate the production of soluble mediators. Dotted, red lines indicate activation, while dashed, red lines indicate suppression. Arrows with a solid, black line indicate enhanced differentiation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

This finding was further confirmed through the observation that the treatment of RA patients with the B cell depleting monoclonal anti-CD20 antibody (rituximab) reduces synovial RANKL levels [91]. Consistently, it was demonstrated that activated human B cells are able to enhance in vitro osteoclast differentiation in a RANKL-dependent manner, whereby the osteoclastogenic function of B cells from RA patients was higher as compared to B cells from healthy control subjects [92].

3.3.2. Th17 cells

During RA pathogenesis, $CD4^+$ T cells are among the most predominant immune cell types within the synovial lesion, where they actively shape the inflammatory response and influence osteoclast-mediated bone loss (Fig. 4). The osteoclastogenic activity of $CD4^+$ T cells in RA can mainly be attributed to the Th17 subset. The most important mediator of Th17-induced osteoclast activation is the pro-inflammatory cytokine IL-17A, which stimulates the expression of RANKL by osteoblasts and SFs and enhances the expression of RANK on OCPs [70,93,94]. IL-17A additionally promotes the production of the osteoclastogenic cytokines TNF, IL-1 and IL-6 by synovial macrophages or fibroblasts, respectively, whereas it suppresses the activity of anti-osteoclastogenic factors, such as IL-4 and Tregs [95,96]. In addition to that, Th17 cells further raise the local RANKL level by producing the cytokine themselves [70]. Komatsu et al. have discovered a particular type of Th17 cells in the inflamed joints of arthritic mice, called exFoxp3Th17 cells. These cells originally derive from $Foxp3^+$ Tregs that lose the expression of their hallmark transcription factor Foxp3 and are transformed into IL-17A producing cells in response to the IL-6 rich synovial microenvironment. Interestingly, exFoxp3Th17 cells were shown to produce higher amounts of RANKL and exhibit stronger pro-osteoclastogenic activity than conventional Th17 cells (Fig. 4). Furthermore, $CD4^+$ T cells that co-express Foxp3 and IL-17A were detected in the synovial tissue of patients with active RA. Since $Foxp3^+IL\text{-}17A^+$ T cells represent a transitional stage during the conversion of $Foxp3^+$Tregs into exFoxp3Th17 cells, exFoxp3Th17 cells might also be involved in the pathogenesis of human RA [97].

3.4. Pro-inflammatory cytokines

The pro-inflammatory cytokines TNF, IL-1 and IL-6 are excessively produced during synovitis and are considered to be major drivers of the pathogenic immune reaction as well as the inflammation-related osteoporosis in RA. All three cytokines can be secreted by a number of different cell types within the arthritic joint (e.g. macrophages, T cells and stromal cells) and have been repeatedly proven to influence the development as well as the activity of osteoclasts through multiple autocrine and paracrine mechanisms (Figs. 3 and 4) [98]. TNF can promote osteoclast differentiation by direct interaction with the TNF receptor TNFR1 (also known as TNFRSF1A or p55) on OCPs and subsequent activation of the downstream signaling via NFκB and c-Jun N-terminal kinase (JNK) (Fig. 3). This mechanism most likely acts in synergy with RANKL activity, meaning that although TNF is known to strongly enhance osteoclastogenesis, it is unable to induce the differentiation of osteoclasts in the complete absence of RANKL [99,100]. In addition to that, TNF has been shown to further enhance osteoclast activity by increasing the expression of OSCAR on OCPs and mature osteoclasts [73,99]. Besides direct interaction with OCPs, TNF has also been shown to boost osteoclast development by inducing the expression of M-CSF and RANKL in mesenchymal cells [101].

Like TNF, IL-1 also has the capacity to stimulate osteoclastogenesis through direct binding to its receptor (type I IL-1 receptor, IL-1RI) on OCPs as well as through the upregulation of RANKL production in stromal cells (Figs. 3 and 4). Thus, it was demonstrated that IL-1 enhances RANKL production in a p38 MAPK-dependent manner, while the IL-1 mediated induction of characteristic osteoclast genes in OCPs is related to the activation of the microphthalmia transcription factor (MITF) as well as NFκB and JNK signaling (Fig. 3). [102–104]. Moreover, IL-1 activates the PI3K/ Akt and ERK signaling pathways, resulting in the inhibition of apoptotic cell death and increased survival in osteoclasts (Fig. 3) [105]. Since TNF also induces the expression of IL-1 and its receptor, it was proposed that the osteoclastogenic function of TNF might partially be mediated by IL-1. In line with this assumption, it was demonstrated that the abrogation of IL-1 signaling can strongly diminish TNF-induced RANKL expression [102,103]. A recent study furthermore reported that IL-1 is responsible for the induction of an osteoclastogenic $CD4^+Foxp3^+$Treg subset in arthritic mice. These so called osteoclastogenic Tregs (O-Tregs) have been shown to accelerate bone erosion due to their particularly high RANKL expression (Fig. 4) [106].

The significant role of IL-6 as a mediator of pathological bone destruction is illustrated by the fact that IL-6 deficient mice are protected from osteoporosis in the presence of inflammatory arthritis as well as oestrogen deficiency. Furthermore, blocking the IL-6 signaling via a monoclonal antibody against the IL-6 receptor (tocilizumab) has proven to be one of the most efficient therapeutic strategies to reduce bone erosion in RA patients. In line with these observations, IL-6 was shown to promote osteoclastogenesis by enhancing the expression of RANKL in mesenchymal cells as well as by direct interaction with OCPs (Fig. 4) [107–109]. However, the IL-6 mediated induction of osteoclast formation was only observed in the presence of its soluble receptor (sIL-6R) (Fig. 3), whereas classical IL-6 signaling via the membrane-bound IL-6R has strikingly been linked to inhibited osteoclast differentiation [110]. Another observation that emphasizes the role of IL-6 as a pro-resorptive factor includes the necessity of this cytokine for the differentiation of Th17 as well as $exFoxp3^+$Th17 cells in the context of arthritis [97].

3.5. Immune functions of osteoclasts

The phenotypic and functional heterogeneity of osteoclasts shows a strong resemblance with that of immune cells. In fact, osteoclasts can be regarded as a bone-specific type of innate immune cells that are involved in phagocytosis and immune modulation. In particular, osteoclasts appear to share crucial features with professional antigen presenting cells (APCs), especially DCs [111]. Professional APCs are characterized by the ability to process and present exogenous antigens to T cells via the major histocompatibility complex II (MHC-II), which is therefore constitutively expressed on these cells. After the binding of the T cell receptor (TCR) to the MHC-II antigen complex, APCs provide co-stimulation through CD80 and CD86 and produce cytokines, which guide the differentiation of the activated T cells towards one of the effector T cell subtypes [112]. Interestingly, recent studies indicate that osteoclasts exhibit a number of features that are typically attributed to professional APCs. Not only was it demonstrated that human osteoclasts express MHC-I, MHC-II and the co-stimulatory molecules CD80, CD86, and CD40, they were also shown to engulf and present soluble antigen [113,114]. In addition, osteoclasts are able to produce chemokines as well as the cytokines IL-1, IL-6, IL-10, transforming growth factor-beta (TGF-β) and TNF, thereby influencing T cell migration and differentiation [113–116]. Similarly to regular APCs, osteoclasts release different sets of cytokines, depending on the environmental context. Thus, osteoclasts were found to secrete the immunosuppressive cytokine IL-10, promoting the development of $CD4^+$ Tregs in healthy mice, while chronic inflammation was associated with osteoclast expression of IL-1, IL-6 and TNF as well as the induction of pro-inflammatory TNF^+ $CD4^+$ T cells [113]. Seifert et al. additionally revealed that osteoclasts have the capability to induce $CD8^+$ T cell proliferation and activation upon antigen cross-presentation via MHC-I, which was originally considered a unique function of DCs [115,117]. These findings were further supported by genome-wide expression analyses, which demonstrated that the transcriptome of in vitro generated human osteoclasts shows strong similarity with that of DCs [111].

A possible explanation for the antigen presenting function of

osteoclasts might be connected to the constant release of self-peptides during physiological bone resorption, requiring a mechanism that can prevent a potentially harmful immune reaction to these remnants of bone degradation. Under healthy conditions, osteoclasts would therefore internalize and present the self-peptides, while releasing immunosuppressive cytokines to induce a regulatory T cell response [113,115,118]. However, pathological conditions, such as RA, favor the development of inflammatory osteoclast subsets that lack the ability to induce tolerogenic immune responses and instead promote T-cell mediated inflammation [113]. Overall, osteoclasts not only respond to immunological stimuli, but actively modulate immune reactions themselves, meaning that the role of osteoclasts in inflammatory bone erosion is not restricted to its unique ability to resorb bone. Instead they create a link between bone destruction and inflammation in a perpetuating feedback loop.

3.6. Osteoblast-suppressing immune responses

Usually, bone resorption is directly followed by new bone formation through osteoblasts. Thus, in inflammatory rheumatic diseases excessive bone degradation is often coupled to increased bone formation as it is the case in spondyloarthritis (SpA), characterized not only by osteoporosis, but also by ectopic bone formation that in some cases can even lead to bone ankylosis [119]. However, this is not observed in RA, which is a purely erosive disease. The inflammatory setting in RA actively suppresses new bone formation by osteoblasts (Figs. 2 and 4), presenting a challenge to heal already existing bone lesions despite drug-mediated disease remission [120].

Just as osteoclasts are necessary for bone resorption, osteoblasts are indispensable for bone formation, also called ossification. Osteoblasts develop from mesenchymal stem cells by two independent processes: intramembranous ossification and endochondral ossification. Intramembranous ossification only takes place in certain parts of the skull, where osteoblasts directly rise from mesenchymal progenitors. In endochondral ossification, osteoblasts transdifferentiate from primarily perichondrial cells, a mechanism that generates osteoblasts in the rest of the skeleton [121]. Osteoblast differentiation strongly depends on the transcription factor runt-related transcription factor 2 (Runx2). Thus, homozygous *Runx2* gene deletion manifests in a complete lack of bone formation due to maturational arrest of osteoblasts [122]. Runx2 synergizes with other nuclear factors to execute the transcription of osteoblast-specific genes. Notably, an inflammatory environment (TNF stimulation) has been shown to promote Runx2 degradation in osteoblasts in a Smurf1 (E3 ubiquitin ligase Smad ubiquitin regulatory factor 1) and Smurf2-dependent manner [123]. Upon full maturation, osteoblasts synthesize a unique combination of extracellular proteins, including the small accessory proteins, osteocalcin (OCN) and osteopontin (OPN), alkaline phosphatase (ALP) and a large amount of type I collagen. First, the unmineralized osteoid is formed, which is an extracellular matrix, rich in type I collagen. Later on, the osteoid becomes mineralized through the accumulation of calcium phosphate in the form of hydroxyapatite that is linked to the collagen fibrils by OCN and OPN. It has been shown that areas with RA-associated bone erosions present less maturated osteoblasts and a reduced amount of mineralized bone [124]. In line with this, a pro-inflammatory setting (presence of TNF) reduces the expression of proteins that are crucial for bone matrix generation and mineralization in osteoblasts, such as collagen type I, ALP and OCN [125].

Osteoblast development depends on various extracellular signals that activate specific transcription factors in mesenchymal progenitors, provoking the commitment towards osteoblastic lineage. For instance, osteoblastic gene expression is positively regulated by bone morphogenetic protein (BMP) signaling. Hereby, ligands of the BMP superfamily, the best studied among them being BMP-2, −4 and −7, interact with their receptors, resulting in the activation of the SMAD protein complex that enters the nucleus to govern the expression of BMP responsive genes [126]. In the synovial tissue of RA, the expression of BMP-4 and BMP-5 is significantly decreased as compared to that of healthy controls [127]. In contrast, BMP-3 functions as an inhibitor of the BMP pathway. Matzelle et al. demonstrated that the expression of BMP-3 is upregulated upon arthritic inflammation [128], suggesting that alterations in BMP signaling may be responsible for the reduced bone formation at sites of bone erosion.

Beside these factors, hedgehog signaling [129], fibroblast growth factor (FGF) [130], parathyroid hormone (PTH) [131] and vitamin D [132] are additional mediators of osteoblastogenesis. In a human study, serum levels of PTH and especially vitamin D metabolites were negatively correlated with the disease activity in RA patients, indicating that changes in these parameters may also be linked to reduced bone formation [133]. Moreover, experimental treatment with PTH in combination with OPG and anti-TNF yields better outcome in the regression of local bone erosions in TNF-transgenic mice than OPG and anti-TNF alone [134].

The Wnt pathway is one of the most important players in osteoblast differentiation and can act dependently or independently of β-catenin. During the β-catenin-dependent Wnt signaling, the binding of Wnt ligand to its receptor Frizzled and the co-receptor low-density lipoprotein receptor-related protein 5 (LRP5) or LRP6 leads to the stabilization of cytosolic β-catenin and the transcription of β-catenin target genes [135]. This pathway can be inhibited by a set of physiological factors, e.g., dickkopf1 (DKK1) and sclerostin (encoded by *SOST* gene) that function as antagonists of the co-receptor LRP5/ 6 [136]. Diarra et al. identified TNF as an important inducer of DKK1 in SFs in a murine model of arthritis (Fig. 4) [137]. In human, the serum level of DKK1 is elevated in RA patients in comparison to healthy individuals and is positively correlated with the disease activity [138]. Moreover, polymorphisms (SNPs) of the *SOST* gene have been associated with bone destruction in the joints of patients with RA [139]. Therefore, several murine studies have evaluated the effect of Wnt-inhibitor neutralization on bone during RA. In that regard, DKK1 inhibition indeed protects from bone erosions by inducing osteoblast differentiation and bone formation [137,140]. In line with this, therapeutic inhibition of sclerostin is able to reverse systemic, periarticular and local bone loss in arthritis [141]. The relevance of the Wnt signaling was further elucidated by Adam et al., demonstrating for the first time that the clinically approved JAK inhibitors tofacitinib and baricitinib are able to increase osteoblast function by activating the Wnt pathway. Remarkably, the treatment with JAK inhibitors elicited the repair of articular bone erosions in RA patients [142]. Hence, targeting Wnt signaling in combination with conventional DMARD might represent an effective treatment option against RA, where synovial inflammation and bone erosion is arrested and new bone formation is reinforced to heal remaining bone lesions.

3.7. Osteoprotective role of immune cells

While the majority of RA-associated innate and adaptive immune cells reinforces inflammation and bone erosion, the presence of a special group of regulatory immune cells, including Tregs, B regulatory cells (Bregs) and type-2 immune response-related cells, e.g., Th2 cells, innate lymphoid cells type 2 (ILC2s) and eosinophils, rather display an osteoprotective role (Fig. 4).

3.7.1. T regulatory cells

The establishment of a Th17-predominating immunological environment is generally associated with high disease activity and bone loss in overall rheumatic diseases, comprising RA and different forms of SpA [143]. However, despite their overall pro-inflammatory functions, Th1 cells secrete high amounts of IFN-γ, which is a strong inhibitor of osteoclasts [144]. Nonetheless, Th1 cell accumulation is frequently observed in RA, being associated with an increased disease activity [145], supporting the view that the function of Th cells greatly depends on the context, namely physiological or pathological conditions.

In contrast to this, Tregs have a relatively strict anti-inflammatory and anti-osteoclastogenic function (Fig. 4). Tregs deploy a wide repertoire of immune-suppressive mechanisms that are dependent or independent of cell contact. Just to name some, they (i) secrete inhibitory cytokines (IL-10, IL-35, TGF-β), (ii) induce cytolysis by the release of granzymes and (iii) express the surface proteins cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and lymphocyte activation gene 3 (LAG3) that interact with CD80/ 86 and MHC class II on APCs, respectively, impairing antigen presentation and T effector cell priming [146]. The adverse effect of Tregs on the formation of T effector cells, including the osteoclastogenic Th17 cells, indirectly reduces osteoclast differentiation and function. Moreover, Tregs can directly inhibit osteoclastogenesis, on one hand, via the release of anti-osteoclastogenic cytokines like IL-4, IL-10 and TGF-β [147,148], and on the other hand through the expression of CTLA4 that interacts with CD80/86 on OCPs, inducing indoleamine-2,3-dioxygenase (IDO)-dependent apoptosis (Fig. 4) [149]. Hence, overexpression of Foxp3, which is the most important transcription factor of Tregs, leads to an osteopetrosis-like bone phenotype due to impaired osteoclast differentiation and bone resorption [150].

Interestingly, the frequency of Tregs in the peripheral blood and synovial fluid of patients with RA is elevated as compared to that of healthy individuals [151–153]. This may be partially explained by a disrupted function of Tregs in RA patients. In this context, a study by Flores-Borja et al. unveiled a defect in CTLA4 surface expression on Tregs from RA patients [154]. In line with this, CTLA4 polymorphisms are characterized as a genetic risk factor for RA and are associated with RA susceptibility [155]. Another study reported that Tregs from RA patients fail to suppress effector T cell proliferation in a TNF-dependent manner [156]. However, these Tregs show normal inhibitory activity in vitro [157], suggesting that the alteration in Treg function is not predisposed, but is rather primed by the inflammatory environment in RA. In mice with collagen-induced arthritis (CIA), the inflammatory milieu (IL-6 produced by SFs) induces the transdifferentiation of $Foxp3^+$ Tregs towards exFoxp3Th17 cells that accumulate in inflammatory joints and show a stronger pro-osteoclastogenic potential (Fig. 4) [97]. In addition, TNF exposure upregulates the expression of TNF-receptor II (TNFRII) on Tregs and the binding of TNF to TNFRII causes the downregulation of Foxp3, leading to a reduced capacity of these cells to suppress effector T cells [156]. In addition, a recent study by Levescot et al. showed that also IL-1 has the ability to reprogram $CD4^+Foxp3^+$ Tregs into O-Tregs presenting high levels of RANKL (Fig. 4) [106]. Therefore, several approved immune-modulating anti-rheumatic drugs, including anti-TNF (infliximab), anti-IL-6 (tocilizumab), CTLA4–Ig fusion protein (abatacept) and mTOR inhibitor (rapamycin) imply a positive impact on Treg numbers and function [158].

Multiple studies pointed out that the adoptive transfer of Tregs ameliorates inflammation and bone erosion in the CIA mouse model [159,160], assuming that Treg-based immunotherapy might also be a promising tool in the treatment of RA patients. Not only the expansion of Tregs, but also the maintenance of Treg stability and plasticity should be an important therapeutic goal in RA. In recent years, genetic and epigenetic studies improved the understanding about the phenotypic stability of Tregs [161]. Moreover, genome editing technologies, such as CRISPR/Cas-9 will soon permit the generation of chimeric antigen receptor (CAR) Tregs that specifically migrate to target sites and show more precise antigen-specific inhibitory activity [162].

3.7.2. B regulatory cells

Newest studies indicate that an additional regulatory cell type belonging to the B cell lineage might be involved in the regulation of osteoclast-dependent bone loss (Fig. 4). These B cells mediate their regulatory function through the release of the inhibitory cytokines TGF-β [163], IL-35 [164] and IL-10 [165]. Different subsets of B cells like transitional 2 B cells, plasmablasts and B1a cells can exert immune-regulatory functions through the production of IL-10, in the following referred to as B10 cells [166–168]. Several studies have reported an anti-inflammatory role of B10 cells in the CIA mouse model based on the priming of immuno-regulatory T cells over pro-inflammatory Th1 and Th17 cells [166,168,169]. The work of Meng et al. demonstrated that the expression of IL-10 by B10 cells critically depends on the transcription factor hypoxia-inducible factor-1α (HIF-1α). Thus, the specific depletion of HIF-1α in B cells exacerbates CIA-mediated inflammation and osteoclastogenic bone erosion due to a reduced frequency of B10 cells [168]. The osteoprotective function of B10 cells in CIA is most likely induced by a general reduction of the inflammatory status. Nevertheless, IL-10 is a well-established anti-osteoclastogenic cytokine [170]. In line with this, a recently published survey by Sapra et al. described that B10 cells are able to directly inhibit in vitro osteoclast differentiation and function in an IL-10 dependent manner (Fig. 4) [171]. In humans, B10 cells generated from peripheral blood mononuclear cells of RA patients are decreased as compared to healthy subjects and inversely correlated with the disease activity [172,173]. Still the knowledge about B10 cells as potential regulators of RA-mediated bone loss especially in humans is limited and demands further investigation.

3.7.3. Type-2 immune response

Another Th subtype that is associated with resolution of inflammatory arthritis and decreased bone erosion are Th2 cells (Fig. 4). The Th2-related cytokine IL-4 directly suppresses osteoclast differentiation through PPARγ (peroxisome proliferator activated receptor γ) and STAT6 (signal transducer and activator of transcription 6) activation [174,175] and indirectly by inhibiting RANKL, but enhancing OPG expression in stromal cells [176,177]. However, IL-4 transgenic mice display an osteoporotic bone phenotype that is most likely attributed to a more dominant suppression of osteoblast formation in vivo relative to its role in impairing osteoclastogenesis [178]. Nevertheless, other Th2-associated cytokines like IL-10 and IL-13 were also demonstrated to efficiently inhibit osteoclast differentiation and function (Fig. 4) [179]. The level of Th2-related cytokines is elevated in early RA patients before disease manifestation [180]. The induction of a Th2 immune response by *Nippostrongylus brasiliensis* infection significantly suppresses inflammation as well as articular bone erosion in a murine model of inflammatory arthritis, which is dependent on the IL-4/ IL-13/ STAT6 signaling pathway. Moreover, the expression of the Th2-related transcription factor Gata-3 is upregulated in the synovial tissue of RA patients as compared to OA patients, indicating that Th2 cells may also play a role in human RA [181].

Also other type-2 immune response-associated immune cells have been shown to induce resolution of arthritic inflammation, accompanied by reduced structural damage of the joints. In particular, several studies examined the role of ILC2s during the resolution process of RA. In this context, the IL-9/ ILC2/ Treg axis strongly promotes arthritis resolution as displayed by reduced synovial inflammation and tissue damage, comprising bone and cartilage loss [182]. In addition, the expansion of ILC2s during arthritis is associated with mitigated inflammation and reduced osteoclast-mediated bone degradation (Fig. 4) [183]. The study by Omata et al. unveiled a direct, inhibitory effect of ILC2s on osteoclasts via IL-4 and IL-13. In this way, ILC2s control steady state bone remodeling as well as oestrogen deficiency-induced bone loss [184]. Moreover, a recently published study showed that downstream of ILC2 activation, eosinophils migrate into the inflamed synovium, where they exhibit pro-resolving and bone-tissue regenerating properties (Fig. 4). Regulatory eosinophils are specifically upregulated in synovial tissue of RA patients undergoing remission [185]. Thus, the priming of a rather type-2 related immune response in RA may have considerable clinical utility in initiating the resolution of inflammation and preventing an osteoclast-promoting environment. However, despite some evidence from animal models of arthritis, appropriate clinical studies must be undertaken to further address this issue.

4. Conclusion

Bone loss represents a key characteristic of RA and is used as a main criteria for disease progression. The combination of immunology and bone research gave us a deeper insight into the molecular mechanisms underlying RA-associated bone erosion. Today, it is well recognized that the inflammatory environment in RA provokes the over-activation of osteoclasts, the crucial driver of RA-mediated bone loss. Hitherto, RA therapy primarily aims for the alleviation of the inflammatory burden, which improves RA symptoms, but not always yields the best outcome for bone healing. As we learned from experimental and clinical studies, bone loss can already occur before the clinical onset of RA through autoimmune mechanisms. Moreover, the inflammatory setting also alters osteoblast differentiation and function, arresting new bone formation and bone repair. Patients with this background need special treatment beside inflammation-modulating drugs. A promising approach may be a combined therapy of DMARDs that inhibit inflammation and accompanying bone erosion with the neutralization of Wnt signaling antagonists, which restores osteoblast function. Latest preclinical studies pose cell-based immunotherapy with Ag-specific Tregs as a potential tool to prevent the auto-inflammatory events that, in the first place, lead to the development of RA. Until then there is still a long way to go, but the constant progress in osteoimmunology research gives hope for improvements in bone-directed RA treatment in the near future.

Author contributions

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

Declaration of competing interest

The authors declare that no conflict of interest exists.

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