Mechanisms of joint destruction in rheumatoid arthritis — immune cell–fibroblast–bone interactions

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Abstract | Rheumatoid arthritis (RA) is characterized by inflammation and destruction of bone and cartilage in affected joints. Autoimmune responses lead to increased osteoclastic bone resorption and impaired osteoblastic bone formation, the imbalance of which underlies bone loss in RA, which includes bone erosion, periarticular bone loss and systemic osteoporosis. The crucial role of osteoclasts in bone erosion has been demonstrated in basic studies as well as by the clinical efficacy of antibodies targeting RANKL, an important mediator of osteoclastogenesis. Synovial fibroblasts contribute to joint damage by stimulating both pro-inflammatory and tissue-destructive pathways. New technologies, such as single-cell RNA sequencing, have revealed the heterogeneity of synovial fibroblasts and of immune cells including T cells and macrophages. To understand the mechanisms of bone damage in RA, it is important to clarify how the immune system promotes the tissue-destructive properties of synovial fibroblasts and influences bone cells. The interaction between immune cells and fibroblasts underlies the imbalance between regulatory T cells and T helper 17 cells, which in turn exacerbates not only inflammation but also bone destruction, mainly by promoting RANKL expression on synovial fibroblasts. An improved understanding of the immune mechanisms underlying joint damage and the interplay between the immune system, synovial fibroblasts and bone will contribute to the identification of novel therapeutic targets in RA.

Rheumatoid arthritis (RA) is an autoimmune disease caused by a complex interaction between genetic and environmental factors, and possibly consists of aetiologically heterogeneous subpopulations[1,2]. Autoimmunity is the first step in the pathogenesis of RA and a high serum concentration of autoantibodies, such as anti-citrullinated peptide antibodies (ACPAs), is a hallmark of RA, although some patients are seronegative[1,2]. Immune cells, such as activated T cells, B cells and macrophages, produce pro-inflammatory cytokines and stimulate synovial fibroblasts (tissue-resident mesenchymal cells in the joints) to polarize into pro-inflammatory and tissue-destructive subsets[3–5]. Tissue-destructive synovial fibroblasts express receptor activator of NF-κB ligand (RANKL) and induce osteoclasts to promote bone destruction, and express matrix metalloproteinases (MMPs) that accelerate cartilage degradation (described in detail in BOX 1)[3–6]. Current therapies for RA are not effective in all patients and are associated with adverse effects such as infection; therefore, it is necessary to develop new therapies, ideally targeting joint-specific pathogenic molecules or cells. Moreover, as the existing therapies for RA are not capable of completely preventing structural damage or restoring joints, the time has come to thoroughly elucidate the immune mechanisms of joint destruction in RA in order to establish the scientific basis for novel therapeutic approaches. To that end, it is important to recognize the importance of the interactions among the immune system, fibroblasts and bone.

Extending the concept of immune cell–fibroblast and immune cell–bone interactions, we herein provide an overview of recent advances in these areas and explore the interactivity within the immune cell–fibroblast–bone triad[7]. We provide an overview of the relevant immune mechanisms and mechanisms of structural damage (including bone erosion and periarticular and systemic bone loss), summarize the interplay among immune cells, fibroblasts and bone in both active disease and remission, and provide a perspective on current and future strategies for the treatment of structural damage in RA.

Overview of joint structure and biology

The synovium consists of two layers, an intimal lining layer and a sublining layer. In a healthy joint, the lining layer, which consists of resident macrophages and lining

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Key points

- T helper 17 (T_H17) cells and autoantibodies promote inflammation and tissue destruction in rheumatoid arthritis (RA) by activating other immune cells and synovial fibroblasts, leading to synovitis, bone erosion and cartilage damage.
- Synovial fibroblasts in RA comprise pro-inflammatory and tissue-destructive subsets, the latter of which express RANKL and matrix metalloproteinases that are involved in osteoclastic bone resorption and cartilage degradation, respectively.
- Bone lesions in RA are classified as bone erosion, periarticular bone loss and systemic osteoporosis, which are induced by distinct mechanisms.
- The integration of data from single-cell RNA sequencing and biological studies provides a detailed depiction of the interplay among immune cells, fibroblasts and bone in RA pathogenesis.
- Therapeutic strategies to modulate pathogenic synovial fibroblasts and to achieve a balance between regulatory T cells and T_H17 cells and/or between bone resorption and repair will help achieve structural remission.

synovial fibroblasts, forms a thin barrier at the interface between the sublining and the synovial fluid space. The sublining layer contains endothelial cells and sublining synovial fibroblasts. Synovial fibroblasts produce matrix proteins such as collagen to maintain the structure of the synovium, and lubricate and nourish cartilage surfaces by producing hyaluronic acid and other joint lubricants such as lubricin.

Bone homeostasis is maintained by a balance between bone resorption by osteoclasts and bone formation by osteoblasts[3,8]. Osteoclasts are the exclusive bone-resorbing cells and differentiate from bone marrow-derived monocyte–macrophage lineage cells. Osteoclasts resorb bone via decalcification and matrix degradation that are mediated by the secretion of hydrogen ions and matrix-degrading enzymes, respectively. Bone-forming osteoblasts, which produce bone matrix proteins and mediate mineralization, are of mesenchymal origin[3,8] Some osteoblasts become embedded in the bone matrix where they differentiate into osteocytes, which are thought to orchestrate both osteoclastic bone resorption and osteoblastic bone formation in response to mechanical stress and hormonal cues[3,9,10].

RANKL, a TNF family cytokine, and macrophage colony-stimulating factor (M-CSF) are essential molecules for osteoclastogenesis[3,8,11]. RANKL binds to its receptor RANK and activates downstream signalling pathways such as NF-κB and AP-1, leading to the autoamplification of NFATc1, the master regulator of osteoclastogenesis[12]. Osteoblasts and osteocytes express RANKL and stimulate osteoclastogenesis necessary for the renewal of bone under physiological conditions[3,9,10]. Osteoprotegerin, a decoy receptor for RANKL, inhibits the RANK–RANKL interaction[13]. M-CSF promotes proliferation of osteoclast precursors and activation and survival of osteoclasts[3,8]. Osteoblast differentiation is stimulated by osteogenic cytokines such as Wnt and bone morphogenetic protein (BMP). Sclerostin, an inhibitor of Wnt signalling, is mainly produced by osteocytes. Mechanical loading decreases sclerostin expression in osteocytes and promotes bone formation, whereas mechanical unloading increases RANKL expression in osteocytes and promotes osteoclastogenesis[10,14,15]. Activation of the immune system in autoimmune diseases disturbs bone homeostasis by acting directly on bone cells or by stimulating joint-resident cells such as fibroblasts, as discussed below.

Immune mechanisms in RA

In RA, the immune system stimulates synovial fibroblasts to exert inflammatory and tissue-destructive effects and exacerbate RA pathogenesis[4]. Fibroblasts are the most abundant mesenchymal stromal cells and serve as structural cells that define the architecture of organs; however, attention has increasingly been given to the role of fibroblasts in the pathogenesis of fibrosis, cancer and autoimmunity[16,17]. New technologies, including single-cell RNA sequencing (scRNA-seq) and mass cytometry, have revealed the heterogeneity of synovial fibroblasts and enabled the identification of functionally and phenotypically distinct pro-inflammatory and tissue-destructive subsets of these cells in RA[4,5,18]. Herein we describe the autoimmune responses and the subsequent activation of the distinct fibroblast subsets that mediate inflammation and structural damage in RA.

Immune response activation

Genome-wide association studies have revealed strong genetic associations between RA and the HLA regions, indicating the importance of antigen recognition in RA pathogenesis[1,2]. The citrullination of peptides is mediated by the peptidylarginine deiminases that are upregulated by smoking and periodontitis, suggesting a link between environmental risk factors and RA pathogenesis[1,2]. The combination of genetic and environmental factors contributes to the breakdown of self-tolerance and the onset of autoimmune arthritis.

The cascade of autoimmune responses starts with T cells recognizing self antigens presented by antigen-presenting cells, such as dendritic cells. $CD4^+$ T cells differentiate into T helper (T_H) cells, among which T_H17 cells have a critical role in autoimmune inflammation[3,7]. T follicular helper cells ($CXCR5^+PD\text{-}1^{hi}$), which reside in lymph nodes, as well as newly identified T peripheral helper (T_{PH}) cells ($CXCR5^-PD\text{-}1^{hi}$) that reside in the inflamed synovium, help B cells to produce autoantibodies such as ACPAs and rheumatoid factor[19]. T_H17-derived IL-17 and other cytokines (such as IL-21, IL-22 and TNF) mediate the proliferation of synovial fibroblasts as well as innate immune cells, including neutrophils and macrophages, and induce the expression of pro-inflammatory cytokines (such as TNF, IL-6 and IL-1) and chemokines (such as CCL20 and CCL2) by these cells[3,7]. T_H17 cells also increase the pro-inflammatory activity of autoantibodies via the desialylation of autoantibodies in an IL-21-dependent and IL-22-dependent manner[20]. A decrease in IgG glycosylation in $ACPA^+$ asymptomatic individuals not only parallels the clinical onset of RA, but is also associated with disease activity in $ACPA^+$ patients with RA, suggesting the importance of T_H17 cells and autoantibodies in the immune activation phase of RA[20,21]. A study published in 2021 showed that tissue-resident memory $CD8^+$ T cells cause arthritis flares[22]. Immune complexes activate innate immune cells to further upregulate pro-inflammatory cytokines and chemokines[3,7]. Synovial fibroblasts amplify inflammation in response to these inflammatory mediators as well

as mechanical strain[23]. According to a 2019 scRNA-seq study, IL-6 is produced mainly by synovial fibroblasts, and macrophages are the main producers of IL-1 and TNF; T cells and B cells have also been found to produce TNF in RA[18] (FIG. 1). Another scRNA-seq study revealed that CCL13, CCL18 and MMP3 are upregulated in synovial myeloid cell subsets in ACPA$^-$ RA as compared with ACPA$^+$ RA, which could explain, at least in part, the difference in immune mechanisms between seronegative and seropositive RA[24].

Fibroblast activation

Under arthritic conditions, synovial fibroblasts acquire an aggressive (activated, proliferative and invasive) phenotype and are important in the pathogenesis of RA[17]. This aggressive phenotype of RA synovial fibroblasts is seemingly induced by the inflammatory milieu in the synovium. Analysis of DNA promoter methylation in synovial fibroblasts revealed that the DNA methylation pattern in synovial fibroblasts from patients with very early RA is already different from that in synovial fibroblasts under healthy conditions, suggesting that epigenetic modification is not just a consequence of inflammation, but could also be a cause of disease initiation and progression[25].

Metabolic changes, particularly an increase in glycolysis in synovial fibroblasts, are also linked with the aggressive phenotype of synovial fibroblasts in arthritis[17]. Glycolysis is the source of ATP under hypoxic conditions (which is considered to be a feature of the synovial microenvironment in RA), although this metabolic pathway is less efficient than oxidative phosphorylation. The expression of hypoxia-inducible factor 1α (HIF1α), an inducer of glycolysis, is linked to the aggressive features of synovial fibroblasts[26]. In addition, activation of intracellular complement C3 and C3a receptor on synovial fibroblasts after repeated inflammatory challenges induces metabolic reprogramming that promotes the activation of these cells and the priming of synovial tissue for inflammation[27]. Although these findings suggest that tissue priming occurs independently of adaptive immunity, the efficacy of abatacept (CTLA4-immunoglobulin (Ig), which prevents T cell costimulation) in RA suggests that activation of T cells might be necessary even in the chronic phase of the disease and that the reprogramming of synovial fibroblasts by repetitive priming might require T cell help. Considering that synovial fibroblasts are functionally heterogeneous, as we discuss below, it will be important to clarify how the polarization of fibroblasts into distinct synovial fibroblast subsets with inflammatory or tissue-destructive properties is determined.

Fibroblast heterogeneity

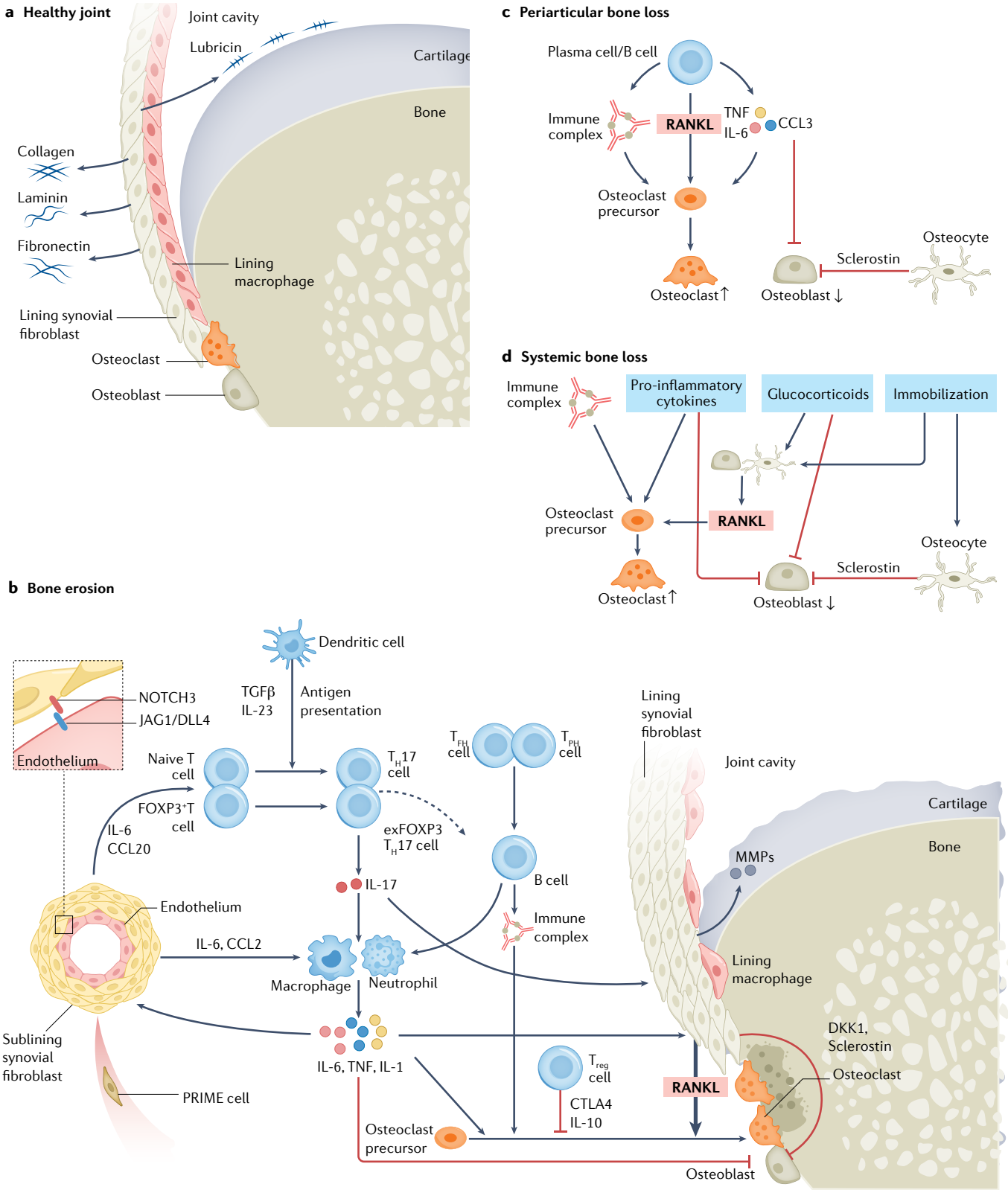
The phenotypic and functional heterogeneity of synovial fibroblasts is attracting increasing attention. Cadherin-11 is one of the most frequently investigated synovial fibroblast surface markers and has been linked to synovial fibroblast activation and inflammation. Cadherin-11 is expressed mainly in lining synovial fibroblasts, although it is also expressed in certain sublining synovial fibroblasts[28]. Cadherin-11 activates mitogen-activated protein kinases and NF-κB, which induce synovial fibroblasts to secrete pro-inflammatory cytokines such as IL-6 (REF.[29]). *Cdh11*-deficient mice have a hypoplastic synovial lining and exhibit reduced inflammation and cartilage erosion in the serum transfer-induced arthritis model[28].

scRNA-seq studies published in the past few years have identified synovial fibroblast subsets with inflammatory and tissue-destructive properties both in mouse and human arthritis[4,5,18]. Application of scRNA-seq to RA synovial fibroblasts characterized by the expression of podoplanin (PDPN) identified three major subpopulations: CD34$^-$THY1$^-$, CD34$^-$THY1$^+$ and CD34$^+$ cells. Among these subpopulations, the CD34$^-$THY1$^+$ cells, which localize to the perivascular zone in the inflamed synovium, are highly proliferative and secrete pro-inflammatory cytokines[5]. Subsequently, the integration of single-cell transcriptomics and mass cytometry revealed that sublining CD34$^-$CD90$^+$HLA-DR^{hi} synovial fibroblasts are expanded in the RA synovium and constitute a major source of IL-6 and CXCL12 (also known as stromal cell-derived factor 1)[18]. Moreover, a separate single-cell transcriptional analysis found two distinct subpopulations of fibroblast activation protein-α (FAPα)-expressing fibroblasts: inflammatory FAPα$^+$THY1$^+$ cells, located in the sublining, and tissue-destructive FAPα$^+$THY1$^-$ cells, located in the synovial lining layer[4]. Notably, in mice with serum transfer-induced arthritis, adoptive transfer of the FAPα$^+$THY1$^+$ synovial fibroblasts subset induces inflammation, whereas transfer of the FAPα$^+$THY1$^-$ subset induces joint destruction[4].

Box 1 | Cartilage degradation

Cartilage plays an important role in tissue patterning, skeletal development and joint movement[151,152]. Cartilage lacks blood and lymph vessels, and is composed of chondrocytes and extracellular matrix components such as type 2 collagen and aggrecan. Blockade of receptor activator of NF-κB ligand (RANKL) is unable to block cartilage damage in rheumatoid arthritis (RA), suggesting that cartilage erosion is independent of RANKL[126]. Cartilage degradation in RA results from excessive immune responses. Pro-inflammatory cytokines, including IL-1, TNF, IL-6 and IL-17, induce the production of cartilage-degrading enzymes such as matrix metalloproteinases (MMPs) as well as aggrecanases (such as ADAMTS-4 and ADAMTS-5) in synovial fibroblasts and inhibit the production of extracellular matrix by chondrocytes[151,152]. Among the MMPs, MMP1, MMP3, MMP9, MMP13 and MMP14 are upregulated in RA synovial fibroblasts. MMP14 (also known as MT1-MMP) is considered to be important in RA owing to its high level of expression in the RA synovium, following the results of functional analyses, and because treatment with an anti-MMP14 antibody suppressed cartilage destruction in a mouse model of RA[151,152]. MMP3 has been shown to be a useful marker of disease activity and predictor of the progression of cartilage destruction[153]. It is likely that the level of MMP3 reflects the activation state of synovial fibroblasts involved in tissue destruction.

Osteoarthritis (OA), which is the most prevalent age-related cartilage disorder, is caused by a combination of genetic background and environmental factors (such as excessive mechanical stress)[151,152]. The major difference between RA and OA is the source of the enzymes involved: in OA, chondrocytes mainly produce MMP and ADAMTS families, whereas activated synovial fibroblasts are sufficient for joint damage in RA. Nonetheless, shared mechanisms might underlie both OA and RA, as inflammation is also suggested to be involved in OA pathogenesis. For example, gremlin-1, an antagonist of bone morphogenetic protein signalling that is induced by mechanical loading, accelerates the induction of catabolic enzymes (MMPs and ADAMTS-5), inhibits type 2 collagen and aggrecan in OA, and is highly expressed in the RA synovium[154,155]. Thus, studies in OA are likely to contribute to a better understanding of the mechanisms underlying cartilage destruction in RA.



What is the mechanism underlying the generation of inflammatory or tissue-destructive synovial fibroblasts? Activation of Notch signalling induces the production of pro-inflammatory cytokines from RA synovial fibroblasts. In a 2020 study, scRNA-seq revealed that the interaction of Notch ligands expressed by endothelial cells with the receptor NOTCH3 on sublining synovial fibroblasts activates Notch signalling and drives the polarization of THY1⁺ inflammatory synovial fibroblasts[30]. The genetic deletion of *Notch3* or the blockade of NOTCH3 signalling attenuates the inflammation and bone destruction in

◀ Fig. 1 | **Mechanism of structural damage in rheumatoid arthritis. a** | Under physiological conditions, synovial fibroblasts lubricate and nourish the cartilage surface by producing hyaluronic acid and other joint lubricants such as lubricin. **b** | In rheumatoid arthritis, dendritic cells present autoantigens and produce cytokines that induce the differentiation of naive $CD4^+$ T cells into T helper (T_H) cells such as T_H17 cells, T follicular helper (T_{FH}) cells and T peripheral helper (T_{PH}) cells. The conversion of $FOXP3^+$ T cells to exFOXP3T_H17 cells is promoted by IL-6 produced by synovial fibroblasts. IL-17 activates sublining synovial fibroblasts, macrophages and neutrophils, and induces the expression of pro-inflammatory cytokines and chemokines from these cells. T_{FH} and T_{PH} cells help B cells to produce autoantibodies and immune complexes. T_H17 cells upregulate the activity of autoantibodies by regulating antibody glycosylation. Immune complexes activate innate immune cells to further upregulate pro-inflammatory cytokines and chemokines. Pro-inflammatory cytokines upregulate expression of receptor activator of NF-κB ligand (RANKL) in synovial fibroblasts. Pro-inflammatory cytokines and IgG immune complexes directly promote differentiation of osteoclasts through Fc receptors. IL-17 activates lining synovial fibroblasts to express RANKL and matrix metalloproteinases (MMPs), which induce osteoclastogenesis and cartilage degradation. Pro-inflammatory cytokines as well as Wnt inhibitors (such as DKK1 and sclerostin) inhibit osteoblastic bone formation. Regulatory T (T_{reg}) cells inhibit osteoclastogenesis. **c** | Periarticular bone loss. Plasma cells potently induce periarticular bone loss by expressing RANKL, autoantibodies and pro-inflammatory cytokines. B cells inhibit osteoblastic bone formation via TNF, CCL3 and IL-6. **d** | Systemic bone loss. Pro-inflammatory cytokines and immune complexes that circulate from inflamed joints as well as glucocorticoid administration promote osteoclastogenesis and inhibit osteoblastogenesis. Reduced mechanical loading induces expression of sclerostin and RANKL by osteocytes, leading to inhibition of osteoblastogenesis and stimulation of osteoclastogenesis, respectively. PRIME cell, pre-inflammatory mesenchymal cell.

serum transfer-induced arthritis[30]. TNF signalling is also important for the polarization of inflammatory synovial fibroblasts, as the activation of TNF signalling exclusively in fibroblasts induces arthritis in mice[31,32]. Moreover, the IL-6 family cytokine leukaemia inhibitory factor (LIF), which is upregulated in synovial fibroblasts under arthritic conditions, acts in an autocrine manner via LIF receptor to promote STAT4 activation, which increases production of important pro-inflammatory factors, including IL-6, leading to the polarization of inflammatory synovial fibroblasts[33].

Although inflammation is known to also promote tissue-destructive synovial fibroblasts, the stimulatory factors, intracellular signal transduction pathways and transcriptional machinery underlying the generation of these cells remain to be elucidated. The most important feature of tissue-destructive synovial fibroblasts is the production of RANKL, but they also produce tissue-destructive factors such as MMPs, thus orchestrating a variety of mechanisms that are involved in bone and cartilage destruction[4,5]. Understanding how RANKL production is induced in synovial fibroblasts will be helpful for determining the main mechanisms driving these cells.

Whether these distinct populations of synovial fibroblasts are subsets with fixed phenotypes or whether they have phenotypic plasticity also remains unclear. Fate mapping and/or tracing experiments in vivo are necessary to address this question.

Structural damage in RA

Structural abnormalities in RA involve bone erosion as well as periarticular and systemic bone loss. Osteoclasts were first observed at the interface between the inflamed synovium and bone in the 1980s, although it was initially unclear why autoimmunity increased osteoclastic bone resorption[34]. The generation of osteoclasts by culturing synovial cells from patients with RA indicated that both osteoclast precursor cells and osteoclastogenesis-supporting fibroblasts were present in the RA synovium[35]. Moreover, synovial fibroblasts were found to express a high level of RANKL, a molecule that is important for osteoclast differentiation[36,37]. This finding suggested that the immune system induced osteoclastogenesis mainly by stimulating synovial fibroblasts, rather than by acting directly on osteoclast precursor cells, and indicated the importance of tissue-destructive fibroblasts in arthritis.

Bone erosion

How does inflammation cause bone erosion? Protein-degrading enzymes were originally thought to sufficiently explain the bone erosion that occurs in arthritis; however, the importance of osteoclast-mediated bone resorption was suggested by the essential role of these cells in bone resorption in physiological bone remodelling and by the observation that osteoclasts are numerous at the synovium–bone interface in RA. Indeed, mice lacking osteoclasts are protected from bone erosion in TNF-transgenic (TNF-Tg) and serum transfer-induced models of arthritis, indicating the primacy of osteoclasts in bone erosion in RA[38,39]. The increased osteoclastogenesis observed in arthritis is attributed mainly to the increased expression of RANKL, as RANKL-deficient mice exhibit much less severe bone damage in arthritis than their matched littermates[38].

Synovial fibroblasts, as well as T cells and B cells, express RANKL when activated[36,37,40–43]. A long-standing question was which cell type induces osteoclastogenesis in the inflamed synovium. In the collagen-induced arthritis (CIA) model, mice lacking RANKL expression in synovial fibroblasts, but not those with T cell-specific or B cell-specific RANKL deficiency, are protected from bone erosion, indicating that synovial fibroblasts are the primary RANKL-producing cells in the synovium in autoimmune arthritis[6,44]. These findings lend support to the concept of 'tissue-destructive fibroblasts'[6,44].

Pro-inflammatory cytokines such as TNF, IL-6 and IL-1, which are abundant in the synovium and synovial fluid in RA, promote RANKL expression by synovial fibroblasts. In addition, the immune system enhances osteoclastogenesis by activating osteoclast precursor cells in several ways. Pro-inflammatory cytokines act directly on osteoclast precursor cells to enhance signalling downstream of RANK as well as to increase the expression of co-stimulatory receptors for RANK[45–47]. Moreover, IgG immune complexes directly promote osteoclast differentiation through Fc receptors[48] (FIG. 1). Antibodies have been shown to stimulate the production of IL-8 and TNF as well as to promote osteoclastogenesis[49,50]. Serum concentration of soluble RANKL is associated with disease activity in RA[51]. However, studies in mice selectively lacking soluble RANKL have demonstrated that soluble RANKL does not contribute to physiological bone remodelling or to a model of postmenopausal osteoporosis[52]. The question of how soluble and membrane-bound RANKL contribute to bone

destruction in RA will be an interesting issue to explore in future investigations.

How are T cells involved in bone erosion in RA? Activated T cells express not only RANKL but also effector cytokines with either stimulatory or inhibitory effects on osteoclastogenesis[40,41]. T_H1 and T_H2 cells inhibit osteoclastogenesis through the expression of IFNγ and IL-4, respectively. T_H17 cells comprise an exclusively osteoclastogenic T cell subset that induces RANKL expression on synovial fibroblasts via production of IL-17, IL-21 and IL-22 (REFS[7,53,54]). Pro-inflammatory cytokines from IL-17-activated innate immune cells further induce RANKL expression on synovial fibroblasts and act on osteoclast precursor cells to activate the downstream pathway of RANK[3,7]. Desialylation of IgG by T_H17 cells also increases the osteoclastogenic capacity of immune complexes[20,55] (FIG. 1).

Regulatory T (T_{reg}) cells are pivotal in the suppression of immune responses and the prevention of autoimmunity[56]. FOXP3 functions as the master transcription factor for the development and function of T_{reg} cells[57]. Humans and mice deficient in FOXP3 exhibit lethal autoimmune diseases owing to a lack of T_{reg} cells[56]. Evidence from a combination of genome-wide association studies with epigenetic analysis or expression quantitative trait locus analysis suggests that T_{reg} cells are strongly associated with RA[58,59]. T_{reg} cells express high amounts of CTLA4 and IL-10, which act on osteoclast precursor cells and inhibit osteoclastogenesis[60,61]. Thus, T_{reg} cells not only regulate inflammation in arthritis, but also directly inhibit bone destruction. Considering that T_{reg} cells recognize autoantigens, the loss of FOXP3 expression in T_{reg} cells could exacerbate autoimmune arthritis. Indeed, some $FOXP3^+$ T cells are plastic, and they lose FOXP3 expression and convert to arthritogenic T_H17 cells, which exacerbate autoimmune arthritis[62]. These T_H17 cells of $FOXP3^+$ T cell origin (called exFOXP3T_H17 cells) induce osteoclastogenesis more efficiently than T_H17 cells derived from naive $CD4^+$ T cells. exFOXP3T_H17 cells produce copious amounts of effector molecules such as IL-17, CCR6, CCL20 and RANKL, and induce RANKL expression on synovial fibroblasts[62,63]. Thus, exFOXP3T_H17 cells are the osteoclastogenic T cell subset that most potently induces bone erosion.

How is osteoblastic bone formation impaired in RA? In arthritic joints, osteoblast function is impaired, especially in the bone adjacent to the inflammatory synovium[64]. Pro-inflammatory cytokines inhibit osteoblastic bone formation via several mechanisms[8].

TNF suppresses osteoblast differentiation by suppressing expression of the transcription factor RUNX2 and by upregulating inhibitors of Wnt signalling[65]. In the RA synovium, endogenous Wnt inhibitors such as Dickkopf-related protein 1 (DKK1), sclerostin, and Frizzled-related proteins are upregulated[8]; DKK1 and sclerostin inhibit Wnt signalling by binding to LRP5 and LRP6, which are receptors for canonical Wnt signalling, whereas Frizzled-related proteins bind directly to Wnt ligands. DKK1 is produced mainly by TNF-stimulated synovial fibroblasts. In patients with RA, variants of *Dkk1* variants are associated with severe joint destruction[66]. Sclerostin is produced not only by osteocytes, but also by TNF-stimulated synovial fibroblasts[67]. TNF also inhibits BMP signalling by inducing the production of BMP3, an endogenous BMP inhibitor, by osteoblasts[68].

IL-1 inhibits osteoblast differentiation, whereas IL-6 promotes osteoblast differentiation and bone formation under certain conditions[69,70]. Administration of IL-6 stimulates bone formation in mice via trans-signalling, but treatment with an antibody targeting IL-6 receptor has no negative effects on bone mass[71], possibly because IL-6 blockade has a positive influence on bone mass by suppressing inflammation mediated by IL-6 and other cytokines such as TNF as well as by inhibiting osteoclastogenic bone resorption.

The role of IL-17 in osteoblast differentiation is controversial[72]. Spondyloarthritis, including psoriatic arthritis and ankylosing spondylitis, is characterized by inflammation and new bone formation in entheses, both of which are known to be reduced by IL-17 blockade[73,74]. Conversely, IL-17 deficiency promotes bone formation without influencing inflammation and bone erosion in the joints of mice with serum transfer-induced arthritis[75]. IL-17 inhibits calvarial osteoblast differentiation in vitro by inducing osteoblast expression of secreted Frizzled-related protein 1 (REF.[75]). Reportedly, IL-17 from γδ T cells promotes bone formation and facilitates bone fracture healing[76]. It is likely that the effect of IL-17 on bone formation is dependent on the type of osteoblast precursors and the microenvironments of the affected sites[72].

Pro-inflammatory cytokines can modulate osteoblasts by regulating the expression of semaphorins, which are known to act as osteoimmune factors. Under physiological conditions, semaphorin 3A (Sema3A) promotes osteoblastic bone formation, whereas Sema4D suppresses it[77,78]. TNF and IL-6 promote the expression of ADAMTS-4, which cleaves cell-surface Sema4D to generate soluble Sema4D[79]. In RA, serum levels of Sema3A are negatively correlated with disease activity, whereas levels of Sema4D are positively correlated with disease activity, suggesting that altered expression of Sema3A and Sema4D under inflammatory conditions might lead to impaired osteoblastic bone formation in arthritis[79,80].

Whereas immune regulation of osteoblasts has been extensively studied, the regulation of the immune response by osteoblasts in RA remains largely unclear. Within the past few years, it has been reported that osteoblasts produce PLEKHO1, a negative regulator of osteoblastic bone formation that promotes the production of pro-inflammatory cytokines in osteoblasts[81]. Osteoblast-specific inhibition of PLEKHO1 ameliorated inflammation and promoted bone formation in a mouse model of arthritis[81]. Elucidation of osteoblast–immune interactions will contribute to the development of future therapeutic strategies for restoring joint structure.

Periarticular bone loss

Periarticular bone loss in RA is an osteoporotic lesion observed in the bone adjacent to joints[82]. Periarticular bone loss has been attributed to joint inflammation but

the precise mechanism remains unclear. Periarticular bone loss is already present in the pre-RA state in ACPA$^+$ individuals[83]. Indeed, ACPAs were shown to induce osteoclastogenesis and periarticular bone loss in a model of antigen-induced arthritis[84].

Plasma cells are specialized B lineage cells that produce antibodies and reside primarily in the bone marrow[84,85]. Under arthritic conditions, plasma cells accumulate in the bone marrow proximal to inflamed joints and express RANKL at high levels[44]. Plasma cells efficiently induce osteoclastogenesis in vitro in a RANKL-dependent manner[44]. The ability of plasma cells to induce osteoclasts is much greater than that of B cells. Mice deficient in RANKL in the B cell lineage are protected from periarticular bone loss, although not from bone erosion[44]. Plasma cells also produce antibodies and pro-inflammatory cytokines such as IL-6 (REFS[85,86]). Taken together, these findings show that bone marrow plasma cells promote osteoclastogenesis and thereby periarticular bone loss by expressing RANKL, pro-inflammatory cytokines and autoantibodies (FIG. 1). The contribution of RANKL derived from osteoblasts or osteocytes to periarticular bone loss needs to be further explored.

Impaired osteoblastic bone formation results in periarticular bone loss because bone mass that is removed by osteoclasts is not fully replaced. Subchondral bone marrow B cells inhibit osteoblast function by expressing CCL3 and TNF[87]. Expression of sclerostin is increased and expression of RUNX2 is decreased in periarticular bone before the onset of adjuvant-induced arthritis, suggesting that osteocytes may contribute to the impaired bone formation in periarticular bone loss[88].

The bone marrow in proximity to the inflamed joints where periarticular bone loss occurs might be called 'draining' bone marrow, by analogy with draining lymph nodes, which are essential for the initiation and progression of immune responses at inflammatory sites. The immune dysregulation that elicits periarticular bone loss can also trigger joint damage. Patients with RA develop cortical microchannels in the bare area of the joint, where bone is not covered by articular cartilage within the joint capsule at an early stage of the disease, suggesting that the microchannels might facilitate communication between the draining bone marrow and the synovium, leading to the clinical onset and progression of RA[89].

Systemic bone loss

Systemic bone loss in RA is observed as widespread osteoporosis, typically in the vertebrae and femurs, which have an increased risk of fracture compared with those in individuals without RA[8,90,91]. In general, osteoporosis is caused by diverse factors such as ageing, menopause and vitamin D deficiency[11]. The incidence of osteoporosis in patients with RA is approximately two times higher than in the general population[92]. This increased incidence is possibly attributable to RA-specific factors such as activation of the immune system, glucocorticoid treatment and loss of mobility.

Inflammatory factors such as immune complexes and pro-inflammatory cytokines contribute to systemic bone loss, but additional factors, such as glucocorticoid treatment and immobility, are also important. Glucocorticoid treatment inhibits osteoblast differentiation, induces osteoblast apoptosis and activates osteoclast differentiation[90,91]. Pro-inflammatory cytokines, which are produced in the inflamed joints, can induce systemic bone loss by activating osteoclastic bone resorption and inhibiting osteoblastic bone formation in bones at distant sites[90,91]. Immune complexes circulating in the bloodstream stimulate osteoclastic bone resorption systemically[48–50]. As for the source of RANKL in systemic bone loss in RA, plasma cell-derived RANKL is reportedly dispensable, suggesting the importance of RANKL derived from osteoblasts and osteocytes[44]. As mentioned above, a reduction in mechanical loading increases RANKL expression in osteocytes, leading to enhanced osteoclastic bone resorption[10,14]; in addition, mechanical unloading increases sclerostin expression in osteocytes, thereby decreasing osteoblastic bone formation[15]. These mechanisms could explain the immobility-related bone loss in RA (FIG. 1).

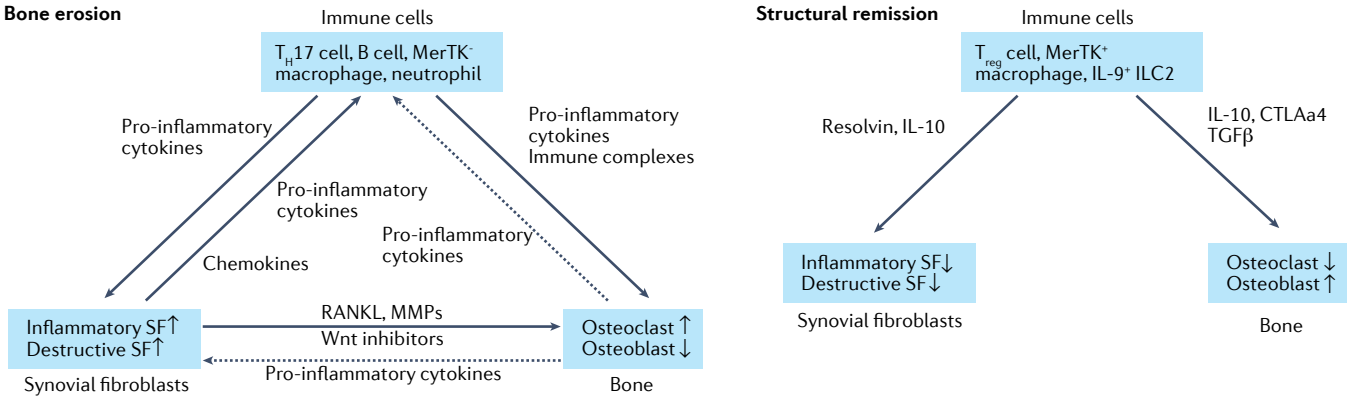
Immune cell–fibroblast–bone interactions

Active disease

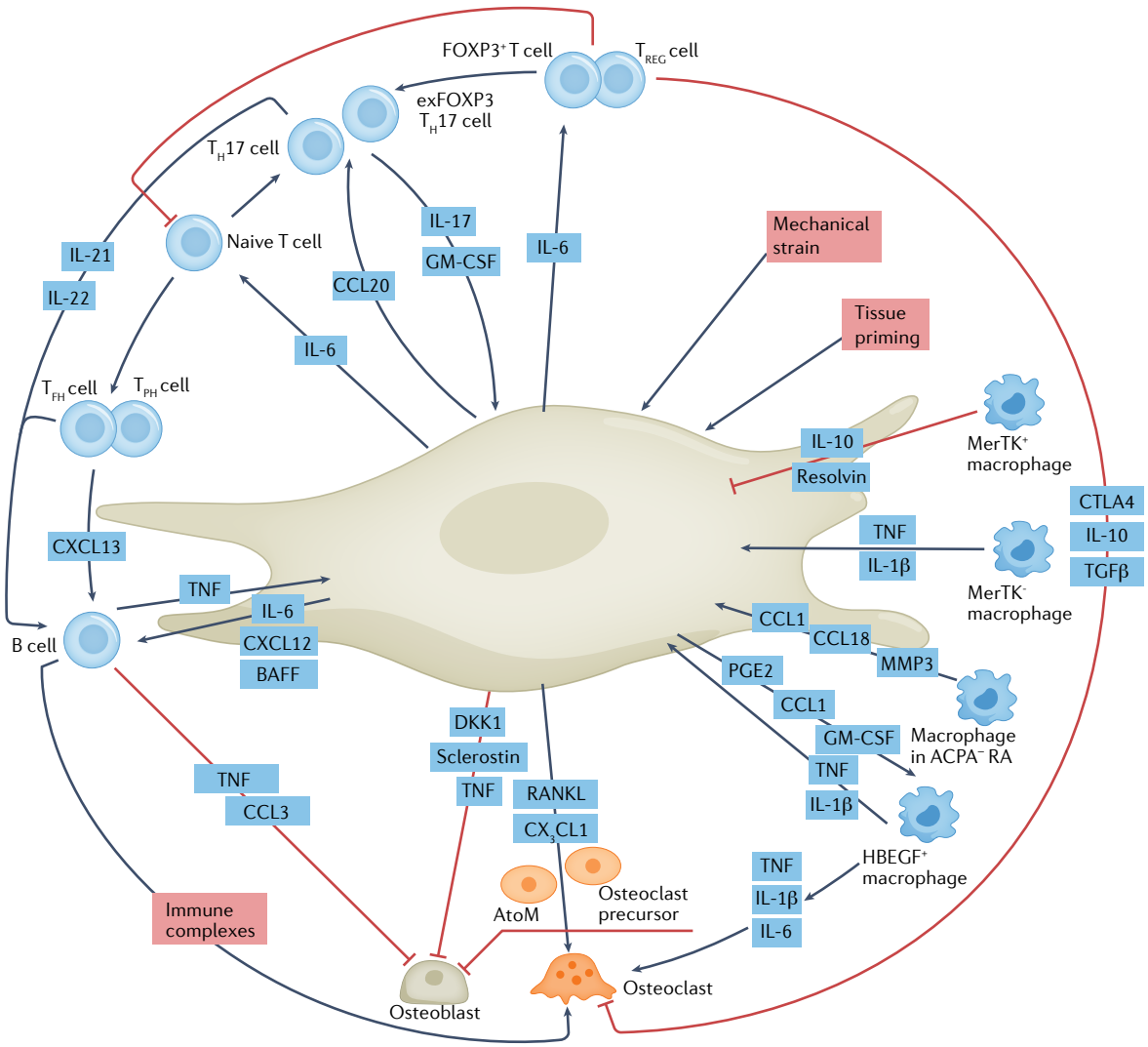
As tissue-destructive synovial fibroblasts are important mediators of joint destruction, it is important to clarify the immune mechanism(s) that promotes the expression of genes encoding tissue-destructive molecules (such as RANKL) in synovial fibroblasts. Early in vitro experiments showed that pro-inflammatory immune mediators, such as IL-17, IL-6, IL-1, TNF, oncostatin-M and prostaglandin E_2, or a combination thereof, increase RANKL expression in synovial fibroblasts[93]. Subsequent studies have provided more information regarding the interactions among immune cells, fibroblasts and bone at the cellular and molecular level.

CD40L on activated T cells was shown in the early studies to induce the proliferation and expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), as well as pro-inflammatory cytokines such as IL-6 by synovial fibroblasts[94]. ICAM1 and VCAM1 expressed by synovial fibroblasts further promote the interaction between T cells and synovial fibroblasts and CD4$^+$ T cell activation[95,96]. T cells and synovial fibroblasts activate each other by producing pro-inflammatory cytokines and chemokines. For instance, T cells induce the expression of pro-inflammatory cytokines such as IL-6 and IL-8 by synovial fibroblasts[97], and IL-7 from synovial fibroblasts promotes a homeostatic proliferation of T cells under arthritic conditions[98]. CX_3CL1 (also known as fractalkine) expressed by synovial fibroblasts promotes the recruitment and activation of CX_3CR1-expressing T cells[99]. Considering the high level of expression of CX_3CR1 on T_{PH} cells, it is possible that T_{PH} cells and synovial fibroblasts might interact with each other[19]. CXCL10 expressed by synovial fibroblasts enhances the recruitment of CXCR3-expressing T cells, including T_H1 cells, and CCL20 from synovial fibroblasts promotes the recruitment of CCR6-expressing T_H17 cells to inflammatory joints[100,101]. Of note, IL-6 produced by synovial fibroblasts promotes the differentiation of T_H17

a Immune cell-fibroblast-bone interplay in rheumatoid arthritis



b



cells, and IL-17 together with IL-6 further enhances IL-6 production by synovial fibroblasts, forming a positive feedback loop referred to as the IL-6 amplifier[102]. IL-6 is also important for the pathogenic conversion of FOXP3⁺ T cells into exFOXP3T_H17 cells. IL-6 produced by synovial fibroblasts determines the fate of plastic FOXP3⁺ T cells and thereby promotes an imbalance between T_{reg} cells and T_H17 cells[62]. In line with this concept, in a clinical study an increase in peripheral blood T_{reg} cells was correlated with clinical response in patients with RA treated with IL-6 blockade[103]. T_H17 cells promote the production of not only RANKL, but also many pro-inflammatory mediators including granulocyte–macrophage colony-stimulating factor (GM-CSF) and

Fig. 2 | **Immune cell–fibroblast–bone interplay in rheumatoid arthritis.** Overview of the interactions among immune cells, fibroblasts and bone in bone erosion and remission. **a** | *Left*: Immune cells and pro-inflammatory synovial fibroblasts interact and activate each other via the production of pro-inflammatory cytokines and chemokines. Tissue-destructive synovial fibroblasts induce osteoclastogenesis by expressing receptor activator of NF-κB ligand (RANKL) and inhibit osteoblastogenesis by expressing Wnt inhibitors. Immune cells can directly promote osteoclastogenesis and inhibit osteoblastogenesis via pro-inflammatory cytokines and immune complexes. Bone cells reportedly activate immune cells and synovial fibroblasts via pro-inflammatory cytokines, but the effects of bone cells on the other cells are not well investigated. *Right*: Immune cell subsets including regulatory T (T_{reg}) cells, MerTK$^+$CD206$^+$ macrophages and IL-9-producing innate lymphoid cells (ILCs) are thought to be involved in structural remission. Regulatory subsets of synovial fibroblasts and bone cells remain to be identified. **b** | Details of the interplay within the immune cell–fibroblast–bone interplay. Integration of findings from single-cell RNA sequencing and biological studies enables us to depict the interactions among immune cells, fibroblasts and bone at the cellular and molecular levels. ACPA, anti-citrullinated peptide antibody; AtoM, arthritis-associated osteoclastogenic macrophage; GM-CSF, granulocyte–macrophage colony-stimulating factor; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; SF, synovial fibroblast; T_{FH} cell, T follicular helper cell; T_H17 cell, T helper 17 cell; T_{PH} cell, T peripheral helper cell.

cadherin-11, by synovial fibroblasts[104,105]. Thus, synovial fibroblasts and T cells activate each other to amplify inflammation and bone erosion in arthritis[7]. RA synovial fibroblasts can reportedly act as antigen-presenting cells by internalizing neutrophil extracellular traps that contain citrullinated peptides and presenting them as antigens to T cells[106,107]. Considering that the proportion of HLA^{hi}THY1$^+$ sublining synovial fibroblasts is increased in the RA synovium[18], it is possible that these synovial fibroblasts may amplify inflammation as antigen-presenting cells.

RA synovial fibroblasts promote the survival of B cells by producing VCAM1 and CXCL12 (REF.[108]). In addition, synovial fibroblasts stimulated with Toll-like receptor 3 ligands promote the differentiation and activation of B cells by producing TNF ligand superfamily members 13 and 13B (also known as APRIL and BAFF, respectively) as well as IL-6, thereby promoting the production of antibodies, including ACPAs[109]. In turn, immune complexes exacerbate inflammation and osteoclastogenesis[3,48–50]. In a study published in 2020, longitudinal genomic analysis of blood from patients with RA revealed that just before a flare of RA the activation of B cells is followed by an expansion of circulating CD3$^-$CD45$^-$PDPN$^+$ pre-inflammatory mesenchymal (PRIME) cells, which resemble pathogenic sublining inflammatory synovial fibroblasts[110]. Levels of PRIME cells then decrease in the blood just after symptom onset and are considered to expand in inflammatory synovium, suggesting that they might migrate from the blood to the synovium. This suggests a possible contribution of an interaction between B cells and synovial fibroblasts to the recurrence of RA symptoms[110]. It would be interesting to clarify where PRIME cells come from and how they are activated, in order to better understand the mechanism of RA flare.

As mentioned above, RA synovial fibroblasts promote the recruitment of monocytes into joints by secreting chemokines such as CCL2 and CXCL10. Mechanical strain-mediated exacerbation of arthritis is dependent on these chemokines, suggesting that the interaction between monocytes and mechanosensitive synovial fibroblasts might underlie the joint specificity of RA pathogenesis[23]. Obviously, the recruitment of RANK$^+$ monocytes and macrophages enhances osteoclastic bone erosion through their interaction with RANKL$^+$ synovial fibroblasts. A study published in 2019 identified a $CX_3CR1^{hi}Ly6C^{int}F4/80^+$I-A/I-E$^+$ macrophage subset, termed arthritis-associated osteoclastogenic macrophages (AtoMs), as the pathogenic osteoclast precursor population in arthritis[111]. Because CX_3CL1 is highly produced by endothelial cells and synovial fibroblasts, the CX_3CR1–CX_3CL1 axis is important for the migration of pathogenic osteoclast precursors to the inflamed synovium. In addition, prostaglandins produced by RA synovial fibroblasts drive the polarization of proheparin-binding EGF-like growth factor (HBEGF)-expressing macrophages[112]; in turn, these HBEGF$^+$ macrophages promote synovial fibroblast invasiveness.

Aside from their interaction with immune cells, synovial fibroblasts interact with mesenchymal cells such as endothelial cells and osteoblasts in arthritis. As mentioned above, the differentiation of inflammatory synovial fibroblasts requires Notch signalling triggered by endothelial cells[30]. Moreover, RA synovial fibroblasts suppress osteoblastic bone formation via the expression of DKK1 (REF.[113]). Although the effects of immune cells on synovial fibroblasts and bone cells have been extensively studied, the influence of bone cells on synovial fibroblasts and/or immune cells has not been fully clarified and needs to be explored in further studies (FIG. 2).

Remission

In patients with clinical remission, joint structural damage typically does not proceed. However, certain patients can be in a state of clinical remission in terms of signs and symptoms of inflammatory joint disease, but can have subclinical synovitis detectable by ultrasonography, which is associated with a high risk of bone erosion[114]. Thus, complete resolution of joint inflammation could be important for the achievement of structural remission with no further bone loss. Conversely, however, treatment with TNF blockade has been reported to suppress joint destruction even in patients who experience no or little clinical improvement, suggesting that joint destruction sometimes proceeds independently of inflammation[115]. Identifying the specific mechanism by which joint destruction occurs would help in the development of a method for establishing structural remission.

As mentioned above, T_{reg} cells have an important role in immune suppression[56]. T_{reg} cells also regulate bone homeostasis by decreasing osteoclastogenesis and increasing osteoblastic bone formation via CTLA4, IL-10 and transforming growth factor-β[61,116–118]. Under physiological conditions, the adoptive transfer of T_{reg} cells suppresses osteoclastogenesis and increases bone volume[119]. Moreover, Foxp3-Tg mice are protected from bone erosion in a model of TNF-Tg arthritis[120]. Impaired T_{reg} cell function or the emergence of exFOXP3T_H17 cells has a pathological role in both autoimmune inflammation and bone resorption[62]. IL-9-deficient mice exhibit

delayed resolution of antigen-induced arthritis as well as impaired activation of T_{reg} cells and impaired proliferation of type 2 innate lymphoid cells (ILC2s)[121]. Administration of IL-9 in a serum transfer-induced arthritis model led to resolution of inflammation and joint destruction. IL-9 induces proliferation of ILC2s, which activate T_{reg} cells in a manner dependent on inducible T cell costimulator (ICOS) and TNF receptor superfamily member 18 (GITR), supporting the importance of the interaction between T_{reg} cells and ILC2s in the resolution phase of arthritis[121]. These findings suggest that controlling T_{reg} cells would be a powerful approach to achieving structural remission.

As for anti-inflammatory subsets of macrophages, a 2019 study identified a population of CX_3CR1^+ resident synovial macrophages that restrict inflammatory reactions by providing a tight-junction-mediated protective barrier for the joint[122]. Determining how CX_3CR1^+ resident synovial macrophages and lining synovial fibroblasts interact with each other under physiological and arthritic conditions would be of interest. Another protective macrophage subset was identified by scRNA-seq analysis of synovial tissue macrophages from patients with early active RA, treatment-refractory active RA or treatment-sensitive RA in remission. These $MerTK^+CD206^+$ macrophages, which are enriched in the synovium of patients with RA in a state of sustained remission, resolve inflammation and induce a 'repair' phenotype of synovial fibroblasts via the production of lipid mediators[123]. Having a low proportion of $MerTK^+$ macrophages is associated with an increased risk of disease flare after treatment cessation. This approach using scRNA-seq analysis will be important for the further identification of regulatory cell subsets that are necessary for inhibiting structural damage in RA (FIG. 2).

Treating structural damage

RA treatment is generally focused on immunomodulatory therapy to address joint inflammation. DMARDs, which are widely used for RA treatment, are now classified into three groups: conventional synthetic DMARDs, such as methotrexate; biologic DMARDs (bDMARDs), including anti-TNF, anti-IL-6 and anti-CD20 agents and CTLA4-Ig; and targeted synthetic DMARDs (tsDMARDs), such as Janus kinase (JAK) inhibitors[8,90].

bDMARDs and JAK inhibitors are effective in preventing both joint inflammation and bone erosion. However, the effects of DMARDs on periarticular and systemic bone loss in RA are limited or have been poorly investigated[90,91]. The anti-RANKL antibody denosumab decreases bone erosion in RA[124–126] and is approved in Japan for the treatment of bone erosion of RA. Anti-RANKL antibodies and bisphosphonates are effective for treating systemic osteoporosis and in reducing the risk of fracture in patients with RA, although they do not exert effects on inflammation or cartilage degradation[126].

Current therapies effectively inhibit the progression of bone destruction in the majority of patients with RA, but in certain cases the response to even multiple DMARDs is inadequate, and it is thus difficult to completely prevent bone destruction. Therefore, we urgently need to fully elucidate the cellular and molecular network underlying structural damage in RA. In this section we provide an overview of the effects of bDMARDs and JAK inhibitors on joint structure and discuss novel candidates for future therapies to treat structural damage.

Biologic DMARDs

TNF inhibitors, IL-6 inhibitors and CTLA4-Ig are widely used bDMARDs. These bDMARDs effectively inhibit inflammation, bone erosion and cartilage degradation by suppressing the local inflammation mediated by synovial fibroblasts and macrophages as well as by inhibiting RANKL induction and RANK signalling pathways[3,8,11]. One might consider that structural protection is achieved mainly by the inhibition of inflammation, but inflammation-independent effects of TNF blockade on bone could exist, given that bone erosion is ameliorated in certain cases without any improvement in inflammation[115]. CTLA4-Ig inhibits inflammation by binding to CD80/CD86 on dendritic cells and suppressing T cell activation, and directly inhibits osteoclast differentiation by inducing apoptosis of osteoclast precursor cells in a CD80/CD86-dependent manner[118,127].

In patients with established RA, inhibitors of IL-17A or IL-23 are less effective than other bDMARDs[128], even though it has been well documented that T_H17 cells are critical to arthritis pathogenesis (both inflammation and bone damage) and IL-17-deficient mice have been shown to be resistant to inflammation and bone destruction in various mouse arthritis models[7]. This reduced efficacy could be attributable to the heterogeneity of RA, with T_H17-dependent mouse models reflecting the disease of only some patients with RA. Alternatively, it is possible that T_H17 cells are important only for the early phase of RA rather than the established phase[20]. In line with this idea, an anti-IL-17 antibody was shown to be effective in the early phase rather than the late phase in a T_H17-dependent mouse model[129]. Notably, dual blockade of IL-17A and IL-17F with bimekizumab produced a favourable result in a clinical trial involving patients with RA who had an inadequate response to a TNF inhibitor, suggesting that IL-17 blockade remains a promising approach if IL-17 family cytokines are fully blocked[130]. The current therapies for RA target pro-inflammatory cytokines mainly produced by innate immune cells and synovial fibroblasts, and thus target bystander pathways rather than antigen-specific pathways. Understanding the autoimmune mechanisms in RA could lead to the establishment of new therapeutic strategies in the future.

JAK inhibitors

JAKs (including JAK1, JAK2, JAK3 and TYK2) are widely expressed in immune and stromal cells in joints and are involved in various cellular responses initiated by cytokines. JAKs phosphorylate signal transducer and activator of transcription proteins (STATs), which then translocate to the nucleus to regulate gene transcription. JAK inhibitors suppress joint inflammation to an extent similar to the suppression produced by bDMARDs[8,90]. Which type of cells and signalling

pathways are the specific targets of JAK inhibitors in vivo remains unclear, as most immune and bone cells are influenced by cytokine signalling that utilizes JAK–STAT pathways. In vitro studies have shown that JAK inhibitors suppress the production of IFNγ and IL-17 as well as the proliferation of $CD4^+$ T cells[131]. JAK inhibitors also inhibit the expression of CD80/CD86 as well as pro-inflammatory cytokines such as IL-6 and TNF in dendritic cells[132].

Certain JAK inhibitors inhibit bone erosion in patients with RA more potently than TNF blockade, suggesting that some JAK inhibitors might protect against structural damage through distinct mechanisms[133,134]. Although JAK inhibitors have no direct effects on osteoclast precursors, they suppress osteoclastogenesis by inhibiting the expression of RANKL on osteoclast-supporting mesenchymal cells[135,136]. In vitro, JAK inhibitors promote osteoblastogenesis in part by increasing the expression of anabolic proteins such as Wnt1 and β-catenin in osteoblasts[135]. In addition, it seems that JAK inhibition reverses bone erosion in RA by promoting the restoration of bone mass[135]. Further studies are necessary to elucidate whether and how JAK inhibition regulates structural damage in vivo.

Emerging therapeutic targets

To reinstate the joint structure, it is necessary to determine how to enhance the osteoblastic bone formation under arthritic conditions[137]. Blockade of DKK1 and sclerostin, both of which inhibit Wnt signalling, have been shown to exert considerable effects on bone formation in arthritis[67,113,138,139]. Treatment with an anti-DKK1 antibody prevents bone damage and leads to bone formation in TNF-Tg arthritis[113]. An anti-sclerostin antibody blocks periarticular and systemic bone loss in TNF-Tg arthritis and CIA, although it does not affect joint inflammation[138,139]. Thus, blockade with Wnt inhibitors could serve as a treatment for reinstating the joint structure in RA. However, TNF-Tg arthritis is exacerbated in sclerostin-deficient mice, consistent with a role for sclerostin in attenuating TNF signalling[67]. Therefore, treatment with a sclerostin inhibitor needs to be carefully conducted with much attention given to potential adverse effects.

There are other candidate molecules that can increase bone formation under arthritic conditions. Sema3A exerts bone anabolic effects by increasing osteoblastic formation and inhibiting osteoclastogenesis[77]. Sema3A has also been identified as an immunosuppressive factor, and the administration of Sema3A not only ameliorated inflammation and bone erosion but also increased bone formation in a serum transfer-induced model of arthritis[140]. In addition, Sema4D is known to be an osteoimmune molecule that promotes inflammation and inhibits osteoblastic bone formation[78,79]. Administration of an anti-Sema4D antibody inhibits inflammation and bone erosion in CIA[79]. Moreover, Notch signalling is important for the polarization of inflammatory synovial fibroblasts as well as the inhibition of osteoblastic bone formation, and studies in mice have shown that Notch inhibition increases bone volume by enhancing osteoblastic bone formation[141]. Furthermore, the CX_3CR1–CX_3CL1 axis and the CXCL10–CXCR3 axis promote not only the migration of T cells and macrophages, but also the activation of synovial fibroblasts[99,100,142,143]. Blockade of CX_3CL1 and CXCL10 as well as blockade of GM-CSF and M-CSF have been shown to inhibit inflammation and bone erosion in mouse models of arthritis and are now being investigated in a clinical trial[100,144–148]. Thus, therapeutic strategies targeting molecules involved in the immune cell–fibroblast–bone triad will be beneficial for both inhibition of inflammation and restoration of the joint structure in RA (TABLE 1).

Current therapies are not universally effective in all patients because RA pathogenesis is heterogeneous. The lack of predictors of treatment success presents a problem in relation to the choice of the best therapy for each individual patient. It is thus important to establish therapeutic strategies that are based on patient subpopulations. It remains to be seen whether the analysis of cells and transcriptomes in synovial tissue samples can appropriately delineate disease subsets and provide better targets for therapeutics. Alternatively, targeting the pathogenic synovial fibroblasts common to all patients with RA is an attractive therapeutic strategy. In terms of targeting surface molecules expressed on synovial fibroblasts, administration of antibodies directed against cadherin-11 and depletion of $FAP\alpha^+$ synovial fibroblasts have been shown to be effective in mouse models of RA[4,28]. An anti-cadherin-11 antibody has been shown to be ineffective in clinical studies, while therapies targeting FAPα are still under clinical investigation[149,150]. At present, there are no therapies targeting synovial fibroblasts that inhibit both bone erosion and cartilage degradation. Further identification of the surface or intracellular proteins specifically expressed by tissue-destructive synovial fibroblasts will contribute to the development of agents designed to treat structural damage.

Conclusions

Synovial fibroblasts play an important part in exacerbating inflammation and joint damage in RA by enhancing osteoclastogenic bone erosion and cartilage destruction as well as inhibiting osteoblastic bone formation. Structural remission will be achieved by completely inhibiting inflammation in addition to inhibiting the specific pathways related to joint damage. To this end, it will be important to further elucidate the mechanisms of immune cell–fibroblast–bone interplay and their effects on joint destruction and the generation of pathogenic synovial fibroblasts. T_H17 cells has been considered to play a key role in autoimmune inflammation and bone destruction[3,12]. The activation of immune cells, including induction of a T_{reg} cell–T_H17 cell imbalance, is important for the arthritogenic effects of synovial fibroblasts. T_{reg} cells not only inhibit inflammation, but also inhibit osteoclastogenic bone resorption and promote osteoblastic bone formation. It would be interesting to investigate whether T_{reg} cells modulate joint damage by regulating the function or polarization of synovial fibroblasts. Thus, in future studies more attention will need to paid to T_{reg} cells and synovial fibroblasts as well as cells that promote bone formation.

Table 1 | **The effect of molecules on immune cells, synovial fibroblasts and bone**

Molecule	Main source	Effect on immune cells	Effect on synovial fibroblasts	Effect on bone
Established therapeutic targets for treatment of RA				
TNF	Macrophages, T cells, B cells	Activation	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑
IL-6	Synovial fibroblasts	Activation, T_{reg} cell–T_H17 cell imbalance	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑
IL-1β	Macrophages	Activation	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑
CTLA4	T_{reg} cells	Inhibition of T cell priming and DCs	ND	Osteoclasts ↓, osteoblasts ↑[a]
IL-17	T_H17 cells	Activation, accumulation	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑[b]
JAKs	Various cell types	Activation	Activation, ↑ RANKL expression	Osteoblasts ↓, osteoclasts ↑[b]
Autoantibodies	B cells	Activation	ND	Osteoclasts ↑
Selected candidate therapeutic targets for treatment of RA				
Sema3A	Osteoblasts, synovial fibroblasts	Inhibition	Activation	Osteoblasts ↑, osteoclasts ↓
Sema4D	T cells	Activation	ND	Osteoblasts ↓
NOTCH3	Synovial fibroblasts	Activation	Polarization of pro-inflammatory synovial fibroblasts	Osteoblasts ↓
Cadherin-11	Synovial fibroblasts	ND	Activation, cell–cell adhesion for the maintenance of synovial architecture	ND
DKK1	Synovial fibroblasts	ND	ND	Osteoblasts ↓
Sclerostin	Osteocytes, synovial fibroblasts	ND	ND	Osteoblasts ↓
CXCL10	Synovial fibroblasts	Recruitment of T cells	Activation	ND
Fractalkine (CX_3CL1)	Synovial fibroblasts, endothelial cells	Recruitment of T cells and monocytes	Activation	Osteoclasts ↑
GM-CSF	Synovial fibroblasts, T cells, ILCs	Activation of DCs and macrophages	ND	Osteoclasts ↓
M-CSF	Mesenchymal cells	Activation of macrophages	ND	Osteoclasts ↑

DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; ILC, innate lymphoid cell; JAK, Janus kinase; M-CSF, macrophage colony-stimulating factor; ND, not determined; RANKL, receptor activator of NF-κB ligand; T_H17 cell, T helper 17 cell; T_{reg} cell, regulatory T cell. [a]CTLA4 indirectly promotes osteoblastic bone formation by inducing T cell anergy and production of T cell derived Wnt10b. [b]IL-17 and JAK indirectly promote osteoclastic bone absorption by inducing RANKL expression on synovial fibroblasts.

Technological advances in the past several years have revealed the heterogeneity of cell subsets and enabled the identification of pathogenic and protective cell populations. From a therapeutic point of view, it is important to restore the joint structure by increasing osteoblastic bone formation and by targeting the pathogenic immune cell–fibroblast axis. Clarification of the interaction between immune cells and fibroblasts at the single-cell level will provide new insights into the pathogenesis of RA. In order to prove the pathological relevance of the findings obtained by scRNA-seq analysis, it will be necessary to perform loss-of-function analysis in vivo, such as cell-type-specific gene deletion. Clarifying how skeletal stem cells or the nervous system contribute to joint damage in RA will also be important. Integration of *in silico* and in vivo studies will provide a complete atlas of the immune cell–fibroblast–bone triad in RA, providing a molecular basis for the development of future therapeutic strategies aimed at providing protection against structural damage as well as restoration of damaged joints.

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