Review article

Cytokine networks in the pathogenesis of rheumatoid arthritis

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Abstract
Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic systemic inflammation causing progressive joint damage that can lead to lifelong disability. The pathogenesis of RA involves a complex network of various cytokines and cells that trigger synovial cell proliferation and cause damage to both cartilage and bone. Involvement of the cytokines TNF-α and IL-6 is central to the pathogenesis of RA, but recent research has revealed that other cytokines such as IL-17, IL-23, GM-CSF, IL-33, and IL-2 also play a role. Clarification of RA pathology has led to the development of therapeutic agents such as biological disease-modifying anti-rheumatic drugs (DMARDs) and Janus kinase (JAK) inhibitors, and further details of the immunological background to RA are emerging. This review covers existing knowledge regarding the roles of cytokines, related immune cells and the immune system in RA, manipulation of which may offer the potential for even safer and more effective treatments in the future.

Key words: rheumatoid arthritis, TNF-α, IL-6, IL-17, IL-23, GM-CSF, IL-33, IL-2

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease causing progressive joint damage that can lead to lifelong disability [1]. Although RA itself is not life-threatening, it may cause secondary amyloidosis, which carries a risk of organ failure and death [2]. This systemic disease is characterized by synovial inflammatory cell infiltration, synovial hyperplasia, angiogenesis and cartilage damage, which in turn can lead to bone destruction [3]. Various autoantibodies such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibody (ACPA) are important serological markers for not only RA diagnosis but also prediction of treatment responsiveness and progression of bone destruction [4]. The level of ACPA is elevated with high specificity in RA, and clinically it is widely used for diagnosis [5]. The production of ACPA is thought to be correlated with certain genetic background factors such as HLA-DR [6], other existing conditions such as periodontal disease [7], and smoking [8]. ACPA and RF form immune complexes with citrullinated proteins and activate macrophages, triggering the release of inflammatory cytokines such as TNF-α and IL-6 [9]. This cytokine-mediated pathway is central to the pathogenesis of RA. At the affected joint, infiltrating immune cells are associated with the release of a variety of cytokines, which are important mediators of cell differentiation, inflammation, immune pathology, and immune response [10]. As further details of RA pathogenesis have emerged, biological disease-modifying anti-rheumatic drugs (DMARDs) and Janus kinase (JAK) inhibitors have been developed as therapeutic agents, and the underlying immunological conditions are being further clarified. It has become evident that numerous cytokines are associated with the pathophysiology of RA, and that some of them might become targets for future therapeutics. Here we review several of these cytokines, including TNF-α, IL-6, IL-17, IL-23, IL-33, GM-CSF, and IL-2, which are known to be active from the acute to the chronic stage of RA, and may have potential for therapeutic targeting.

1. TNF and RA

Based on the rationale that TNF-α plays a central role in the regulation of RA-related molecules, anti-TNF drugs were the first biological agents to be introduced for treatment of RA, starting with infliximab, a chimeric anti-TNF-α monoclonal antibody (mAB), in 1999 [11]. Since then, it has become clear that TNF regulates the production of IL-6, IL-8, MCP-1, and VEGF, recruitment of immune and inflammatory cells into joints, angiogenesis, and the blood levels of matrix metalloproteinases (MMP) 1 and 3 [12]. TNF was originally named by Carswell et al. on the basis of their observation that an endotoxin caused host macrophages to release a substance that induced necrosis of tumor cells [13].

Human TNF-α is a 17-kDa secreted protein comprising 157 amino acids and is produced mainly by macrophages [14]. Primarily, the TNF-α precursor molecule is produced as a transmembrane protein (memTNF), which is then cleaved by a metalloproteinase, such as TNF-α converting enzyme (TACE), leading to the release of soluble TNF (sTNF). sTNF then forms a homotrimer and binds to its receptor. There are two known receptor isoforms – TNF receptors 1 (TNFR1) and TNFR2 – both of which form a homotrimer. TNFR1 possesses the intracellular DEATH domain, which regulates apoptosis through activation of caspases, whereas TNRF2 does not.

MemTNF activates both TNFR1 and TNFR2, whereas sTNF activates mainly TNFR1 [15]. TNFR2 can only be activated properly by memTNF, because activation of TNFR2-associated signaling pathways requires subsequent stabilization of TNF-TNFR2 homotrimer complexes. This subsequent stabilization is achieved only by memTNF [16], perhaps because of the memTNF costrain physical distance of the inter-TNF-TNFR2 complex. TNFR1 recruits the adaptor protein, TNF receptor-associated death domain (TRADD), through its DEATH domain. Usually NF-κB binds IκBα and is localized in the cytosol. Activated TNFR1 recruits TRADD, TNF receptor-associated factor 2 (TRAF2), receptor-interacting protein kinase 1 (RIPK1), and cell inhibitor of

apoptosis protein-1/2 (cIAP1/2) to form Complex I. A precisely controlled multistep ubiquitination process activates the I kappa B kinase (IKK) complex through the NF-κB essential modulator (NEMO). The IKK phosphorylates IκBα, and then IκBα is K48-ubiquitinated, leading to its degradation. Finally, NF-κB becomes detached from IκBα and translocates to the nucleus. TNFR1 signaling also activates c-Jun N-terminal kinase (JNK) and p38MAPK through TGF-β-activated kinase 1 (TAK1) and caspase-8, via FADD. Although TNFR2 signaling also activates NF-κB and p38MAPK through a TRAF2-dependent mechanism, this process is dependent on NF-κB inducing kinase (NIK), and independent of NEMO. In addition, recruitment of TRAF2 by TNFR2 leads to depletion of cytosolic TRAF2 and perturbation of TNFR1-mediated signaling [14].

Although TNF inhibitors have advanced the management of RA and led to dramatic improvements in disease status [1,17], the cellular source of TNF and its relationship to the complex pathogenesis of RA have remained unclear. Kruglov et al.performed a series of studies using mice with conditional TNF knockout [18]. These revealed that memTNF has a protective function against arthritis, and suppresses autoreactive T cells. It was also shown that TNF produced by myeloid cells controls arthritis onset by regulating the activation of synovial fibroblasts, that B cell-derived TNF regulates the severity of arthritis through induction of autoantibodies, and that T cell-derived TNF exerts a protective action by regulating the development of autoreactive T cells.

TNF also regulates the function and differentiation of T regulatory (Treg) cells, which are essential for maintenance of immune homeostasis and prevention of autoimmunity, and whose differentiation is regulated by FoxP3 [19]. While signaling via TNFR1 promotes the pathogenesis of arthritis, TNFR2 signaling exerts protective functions [20, 21, 22]. Several lines of evidence suggest that interaction of TNF with TNFR2 promotes Treg function [23]. For example, the TNF-TNFR2 axis expands the number and function of $mCD4^{+}CD25^{+}$ Tregs [24]. Human peripheral blood $CD4^{+}CD25^{+}TNFR2^{+}$ cells have been shown to markedly inhibit proliferation of, and cytokine production by, co-cultured T-responder cells, whereas $CD4^{+}CD25^{+}TNFR2^{-}$ cells do not [25]. In a study using TNFR2-knockout mice, FoxP3 gene methylation in Treg cells was shown to be greater than in wild-type mice, and Treg cells differentiated into pro-inflammatory Th17-like cells. Thus, TNFR2 signaling appears to block methylation of the promoter region of FoxP3, thus maintaining FoxP3 transcription and preventing pathogenic conversion of Tregs to Th17-like cells [26]. Furthermore, transfer of TNFR2-expressing Treg cells has been shown to ameliorate inflammation in an experimental arthritis model [27]. On the other hand, TNFR1 deficiency increases Treg activity, suggesting that TNFR1 signaling mediates the disease exacerbation attributable to Tregs. TNF-TNFR1 signaling activates NF-κB, leading to transcription of protein phosphatase 1 (PP1), which dephosphorylates Ser418 of FoxP3. Because dephosphorylated FoxP3 is unable to bind the target sequence, Treg differentiation is blocked [28].

During the course of RA, from the very early preclinical stage to the established disease stage, the DNA methylation status of RA synovial fibroblasts changes dynamically [29]. TNF controls gene expression by regulating methylation and acetylation. It has been shown that hypomethylation is associated with increased gene expression, and that many genes involved in RA pathogenesis, including signal transducer and activator of transcription 3 (STAT3) and TRAF2, are hypomethylated in synovial fibroblasts derived from RA patients [30]. Cytokines such as TNF and IL-1β can inhibit the expression of DNA methyltransferase (DNMT), leading to reduced DNA methylation and increased pathogenic gene expression [31]. Acetylation is another mechanism by which TNF can regulate gene expression. Histone deacetylases (HDAC) are a class of enzymes that remove acetyl groups from lysine on histone, allowing histones to wrap DNA more tightly. DNA expression is controlled through a balance between acetylation and deacetylation. HDAC5 is known to exert an anti-inflammatory role. TNF and IL1β suppress HDAC5 and upregulate the production of inflammatory cytokines and chemokines [32]. Loh et

al. investigated genome-wide changes in gene expression and chromatin remodeling induced by TNF in synovial fibroblasts and macrophages. They stimulated synovial fibroblasts from RA patients and human $CD14^+$ monocyte-derived macrophages with TNF *ex vivo*, and identified 280 TNF-inducible arthritogenic genes, including IL-6, CXCL8, CXCL10, and MMP-19. These genes were expressed transiently in macrophages, but their expression was sustained in synovial fibroblasts. They further identified 80 of these genes that lost their reactivity with TNF when stimulated repeatedly in macrophages, but which retained their reactivity in synovial fibroblasts. Use of Assay for Transposase-Accessible Chromatin using SEQuencing (ATAC-seq), which can identify open chromatin, and chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) for Histone 3 lysine 27 acetylation (H3K27ac) of these genes in macrophages and synovial fibroblasts demonstrated persistent TNF regulation of H3K27 acetylation and increased chromatin accessibility in the regulatory elements of arthritogenic genes in TNF-stimulated synovial fibroblasts [33]. Not only TNF, but also other cytokines contribute to epigenetic modification. When stimulated with a mixture of 8 cytokines, including IL-1β, IFN-α, and IFN-γ, the genomic structure of synovial fibroblasts changes dramatically, with formation of chromatin loops that can be detected by high-throughput chromosome conformation capture (Hi-C) analysis. The transcription factors metal-regulatory transcription factor-1 (MTF1) and runt-related transcription factor-1 (RUNX1) could be key regulators of chromatin remodeling for expression of pathogenic molecules in fibroblasts [34]. Table 1 summarizes the RA-related cytokines, signaling pathways, and clinical assessments described in this article.

Tabel 1. Representative cytokines, signal transduction, and their pathogenic roles in rheumatoid arthritis. Abbreviations,
TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; mem TNF, membrane TNF; JAK, janus kinase; Tyk2, tyrosine kinase 2; STAT, signal transducer and activator of transcription; PI3K, phosphatidylinositol-3 kinase; SHP-2, Src homology region 2 domain-containing phosphatase 2; ERK, extracellular signal regulated kinase; Th17, T helper ; Treg, T regulatory; IL-33, interleukin-33; IL1-RacP, IL-1 receptor accessory protein; MyD88, *myeloid differentiation factor* 88; IRAKs, interleukin-1 receptor associated kinases; TRAF6, tumor necrosis factor receptor-associated factor 6; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor-kappa B; AP1, activator protein 1; Th2, type 2 helper T cell; ILC2. group 2 innate lymphoid cells; IL-23, interleukin-23; IL12R, IL-12 receptor; IL-23R, interleukin-23 receptor; Th17, T helper 17; NKT, natural killer T cell ; IL-17, interleukin-17; IL-17R, interleukin-17 receptor; ACT1, nuclear factor activator 1; GM-CSF, granulocyte macrophage colony-stimulating Factor; GM-CSFR, Granulocyte Macrophage colony-stimulating factor receptor; SHC-1, Src homology domain-containing transforming protein 1; IFN-γ, interferon-gamma.

Cytokines-Receptor-major signaling molecules	Proposed roles in rheumatoid arthritis	Clinical application
TNF - TNFR 1/2-NFkB, MAPKs, PI3K	Osteoclastogenesis, Pro-inflammatory cytokine production TNFR1; Treg inhibition TNFR2; Treg activation Epigenomic modification (Acetylation /Methylation) memTNF; Protective for arthritis	Widely used
IL-6 - IL-6R-gp130, JAK1/ 2, Tyk2, STAT1/3, PI3K, SHP-2, ERK	Osteoclastogenesis Pro-inflammatory cytokine production Auto-antibody production Th17 differentiation, Treg inhibition	Widely used(anti-receptor antibody)
IL-33 - ST2-IL1-RacP, MyD88, IRAKs, TRAF6, MAPKs, NFkB, AP1	Pro-inflammatory cytokine production Activation of mast cell, Treg, Th2, and ILC2	
IL-23 – IL-12Rβ1/IL-23R-	Activation of Th17, NKT, and ILC3 cells	

Tyk2, JAK2, STAT3	Cytokine production (IL17, TNF- α , GM-CSF)	
IL-17 - IL17R- ACT1, TRAF6, NFkB, MAPKs	Pro-inflammatory cytokine production Osteoclastogenesis	Did not meet primary endpoint
GM-CSF - GM-CSF-R- JAK2, STAT3/5	Macrophage activation Pro-inflammatory cytokine production	Phase II a
IL-2 - IL2R- JAK1/3, STAT3/5, SHC-1, ERK	Late phase: arthritogenic Activation of ILC2, NK cell, Th17 cell, IL-33 production, Early phase: Anti- arthritogenic via IFN-γ	

2. IL-6 and RA

IL-6, another key regulator of RA, is a 26-kDa secreted protein that was originally identified in 1986 as a secreted factor that induced immunoglobulin production [35]. IL-6 is involved in a wide range of physiological processes, such as the immune response, inflammation, and bone metabolism, and has also been implicated in the pathogenesis of RA. Anti-IL-6 receptor antibody was first approved for RA in 2008, and is now used for treatment of various rheumatic diseases [36].

IL-6 activates a signal cascade via three modes of receptor-ligand interaction, the first of which is classical signaling whereby IL-6 binds to its receptor, IL-6Rα, which is expressed on cells of lymphoid or myeloid lineage, thus activating an intracellular signal transduction pathway via gp130 dimerization. The second mode is trans-signaling. IL-6R also exists as a secreted form (sIL-6Rα), unlike the membrane-bound form, IL-6Rα, which is cleaved by proteases such as TACE. sIL-6Rα binds to IL-6, and the resulting complex binds to gp130 on endothelial cells and synovial fibroblast, which usually do not express IL6R. In the third mode, so-called trans presentation, circulating IL-6 binds to IL-6Rα expressed on dendritic cells (DC), and then the IL-6/IL-6Rα complex binds to gp130 expressed on $CD4^+$ T-cells. This mode of interaction is required for priming of Th17 cells [37].

The IL-6/IL-6Rα complex binds gp130 to form a gp130 homodimer, thus activating Janus kinase (JAK). JAK activates the STAT family transcription factors, mainly STAT3, and src homology region 2 domain-containing phosphatase 2 (SHP-2). JAK phosphorylates STAT3 to form a homodimer, which then translocates to the nucleus where it functions as a transcription factor. JAK also activates the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway through SHP-2. STAT3 induces suppressor of cytokine signaling1 (SOCS1) and SOCS3; SOCS1 inhibits JAK directly, and SOCS3 inhibits gp130 [36].

Sustained production of IL-6 after TNF-α stimulation in synovial fibroblasts is one of the features of RA [38]. F759 mice, which carry the Y759F mutation in gp130 and lack the negative feedback loop mediated by SOCS3, develop arthritis spontaneously [39]. Genetic experiments have shown that F759 mice develop arthritis only if mutated gp130 functions in non-immune cells. Detailed experiments have shown that accumulation of IL-6 secreted from synovial fibroblasts leads to proliferation of $CD4^+$ T-cells, differentiation of Th17 cells, and subsequent development of arthritis [40, 41]. This suggests that the pathogenesis of RA requires coordinated interaction of TNF, IL17, and IL-6. In fibroblasts, TNF induces various types of cytokines and chemokines, whereas IL-17A alone does not induce cytokines and/or chemokines significantly. Slowikowski et al. reported that TNF induced the expression of 370 genes, but that the expression of these genes was unaffected by addition of IL-17A. They also identified 26 genes whose expression was induced only upon co-stimulation with TNF and IL17-A, and 25 genes whose expression was induced by TNF and dose-dependently amplified by IL-17A; these included C-X-C motif chemokine ligand 1 (CXCL1), CXCL2, CXCL3, IL-6, IL-8, and MMP-3. This synergistic control

is regulated by atypical IκB factor IκBζ, which is induced in proportion to IL17A concentration and functions in a dose-dependent manner. Synergistic induction of IL-6 is canceled when IκBζ is repressed by siRNA [42].

IL-6 also regulates the differentiation of Treg, Th17, and Tfh cells. The IL-6-STAT3 pathway is required for Th17 cell development, and enhancement of the IL6-STAT3 signaling axis causes IL17A-dependent autoimmune arthritis in mice [43]. The importance of STAT3 in the differentiation of Th17 cells has also been demonstrated in humans [44]. Furthermore, IL-6 downregulates Foxp3 expression through STAT3 and induces the genetic reprogramming of Treg cells to Th17-like cells [45]. Thus, IL-6 regulates the Treg vs Th17 cell balance. Conversion of Treg cells to Th17 cells is induced by IL-6 derived from synovial fibroblasts, and the converted Th17 cells are more osteoclastogenic than conventional Th17 cells [46].

T follicular helper (Tfh) cells have been identified as $CXCR5^+$ $PD\text{-}1^+$ $CD4^+$ T cells, which regulate B cell differentiation into plasma cells and memory B cells by mediating class switching and affinity maturation of antibodies in germinal centers [47]. In mice, $Bcl6^+CXCR5^+$ Tfh differentiation is severely impaired in the absence of IL-6, suggesting that IL-6 is an essential factor for the development of murine Tfh cells [48]. In humans, however, Tfh differentiation might be less affected by IL-6, and TGF-β together with IL-12 or IL-23 induces various Tfh markers on $CD4^+$ T cells [49].

IL-6 signaling regulates osteoclastogenesis in combination with TNF. Extensive analysis of osteoclast differentiation has identified various osteoclast types. Osteoclasts regulate bone absorption and bone mineral density (BMD), and cause bone erosion in both RA patients and model mice [50]. Differentiation of osteoclasts depends on TNF and receptor activator of nuclear factor-κB ligand (RANKL), IL-6, and IL-17.

3. Synovial structure, inflammation, and pathogenesis of RA

The synovium of humans is composed of a lining and a sublining layer [51]. The synovium is the primary site of inflammation in RA, and undergoes marked hyperplasia, becoming inflamed and invasive, thus destroying the cartilage and bone. Fibroblasts are the major component of the synovium, and those in the sublining layer undergo major expansion in RA. Synovial tissue is also composed of lymphocytes, macrophages, and dendritic cells. Recently, the molecular characteristics of these cells, as well as their mechanism of differentiation and contribution to RA development have been revealed at high resolution using modern techniques such as single-cell (sc) RNA sequencing, bulk RNA sequencing, and mass cytometry.

Fibroblasts are key players in synovial inflammation in RA. Stephenson et al. have identified $Thy1^+$ fibroblasts in the sublining, and $CD55^+$ fibroblasts in the lining. $Thy1^+$ fibroblasts are rich in modules associated with metallopeptidase activity, while $CD55^+$ fibroblasts express functional modules associated with endothelial cell proliferation [52]. Mizoguchi et al. have classified RA-related synovial fibroblasts into at least three subpopulations: a $CD34^-Thy1^+$ population in the sublining, especially the perivascular area, a $CD34^-Thy1^-$ population in the lining area, and a $CD34^+$ population. The $CD34^-Thy1^+$ population expresses a higher level of RANKL, and is expanded three-fold in RA patients relative to patients with osteoarthritis (OA), whereas the $CD34^-Thy1^-$ population expresses a higher level of MMP-3 [53]. Zhang et al. have identified $Thy1^+HLA\text{-}DR^+$ synovial fibroblasts in the sublining layer as the origin of IL-6 [54]. Croft et al. have identified $FAPα^+Thy1^-$ lining fibroblasts as driving joint injury, and $FAPα^+Thy1^+$ sublining fibroblasts as driving inflammation [55]. Wei et al. have shown that differentiation of $Thy1^-$ $lubricin^+$ lining fibroblasts and $Thy1^+$ sublining fibroblasts is regulated by the vascular endothelium expressing Jagged1 through fibroblasts expressing its receptor, Notch3. Blocking of the Jagged1-Notch3 pathway inhibits Thy1-positive sublining fibroblasts and blocks joint destruction [56]. Orange et al. have reported that a $CD45^-CD31^-PDPN^+$ population emerges in the blood one week before relapse of arthritis, and have named this population preinflammatory mesenchymal (PRIME) cells. These cells show an expression pattern similar to that of

Thy1^{+}HLA-DR^{+} synovial fibroblasts, and are considered to be one of the origins of sublining inflammatory fibroblasts [57]. Although several researchers have reported a variety of fibroblast subsets to date, those subsets and the functional molecules they express as well as their proposed cellular functions have been divergent depending on the experimental design used.

Macrophages also play a crucial role in the pathogenesis of RA. Traditionally, macrophages were identified as "phagocytes", and were believed to be involved only in the clearance of debris or pathogens. However, recent studies have revealed diverse biological functions of macrophages in various tissues. Macrophages not only scavenge tissue debris or pathogens, but also regulate the immune system, functioning as drivers of tissue inflammation or key regulators of tissue repair. Furthermore, in some instances they help to maintain tissue homeostatic balance. For example, osteoclasts, which are bone-related macrophages, destroy old bone tissue and promote its reconstruction [50, 58].

Macrophages that migrate via peripheral blood are often pathogenic. Hasegawa et al. have identified CX3CR1^{hi}, Ly6C^{int}, F4/80^{hi}, MHCII^{+} macrophages in the joints of CIA model mice. In *ex vivo* culture, these macrophages differentiate into osteoclasts. This population has been referred to as arthritis-associated osteoclastogenic macrophages (AtoM), and express molecular markers of osteoclasts, as well as the transcription factor FoxM1. Inhibition of FoxM1 blocks the differentiation of AtoM cells into osteoclasts [59]. Another macrophage type, tissue-intrinsic macrophages, colonize sites during the embryonic period and exert a tissue protective function. Culemann et al. have found that CX3CR1^{+} lining macrophages form a barrier on the synovium and protect the joint by maintaining tight junctions. Molecules such as TREM2, MERTK, AXL and claudin function to maintain these tight junctions, and silencing of these molecules causes severe joint inflammation [60].

MerTK^{+}CD206^{+} synovial macrophages have also been recently identified as having a protective function [61]. They are abundant in the synovial lining layer of patients with OA or RA in remission. On the other hand, MerTK^{-}CD206^{+} synovial macrophages are abundant in the synovial sublining layer in patients with active RA. MerTK^{+}CD206^{+} synovial macrophages express pro-resolving mediators such as CD163, which functions as a scavenger receptor, resolvin D1 and IL10. These molecules play a role in suppressing inflammation [62,63].

Tu et al. have also identified two types of synovial macrophage in mouse: one is an intrinsic macrophage present in the joint synovium since their birth, and the other is an extrinsic macrophage derived from bone marrow. Intrinsic macrophages express anti-inflammatory cytokines such as IL-4 and IL-10, whereas extrinsic macrophages express pro-inflammatory cytokines such as IL1-β and TNF-α. They also identified similar cells in patients with RA [64].

In lymph nodes, CXCL13 recruits CXCR5^{+} Tfh cells and Tfh cells regulate lymphoid follicle formation. Tfh cells function to assist B cell maturation, and have a PD-1^{hi}CXCR5^{+} CD4^{+} T cell phenotype [47]. The synovium in RA is rich in lymphoid follicles. However, RA synovial tissue contains few PD-1^{hi}CXCR5^{+}Tfh cells, and almost 85% of PD-1^{hi}CD4^{+}cells in synovial tissue lack CXCR5 expression [65]. PD-1^{hi}CXCR5^{-}CD4^{+} cells express functional molecules such as IL-21, CXCL13, ICOS, and MAF, which are known to be expressed in Tfh cells, but do not express CXCR5 and BCL-6, which are important for Tfh function in the germinal centers of lymph nodes. In terms of function, these cells assist the maturation of B cells, as do Tfh cells, and have therefore been named T peripheral helper (Tph) cells. Because CXCR5 is required for migration into the germinal center, Tph cells cannot do so. Instead, they express chemokine receptors, such as CCR2, CX3CR1, and CCR5, which allow Tph cells to migrate into sites of inflammation. Because Tph cells in peripheral blood do not express CXCL13, whereas those in joints affected by RA express CXCL13 strongly, it has been proposed that the TGF-β-abundant, IL-2-limiting inflammatory milieu in RA joints induces the transcriptional factor Sox4 in Tph cells, which migrate from the peripheral blood, and that Sox4 then upregulates the expression of CXCL13. The CXCL13-expressing Tph cells then attract B cells, leading to the formation of ectopic lymphoid-like structures [66]. These findings indicate that tissue-localized T-B interactions might play an

important role in the development of RA.

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In lymph nodes, CXCL13 recruits CXCR5^{+} Tfh cells and Tfh cells regulate lymphoid follicle formation. Tfh cells function to assist B cell maturation, and have a PD-1^{hi}CXCR5^{+} CD4^{+} T cell phenotype [47]. The synovium in RA is rich in lymphoid follicles. However, RA synovial tissue contains few PD-1^{hi}CXCR5^{+}Tfh cells, and almost 85% of PD-1^{hi}CD4^{+}cells in synovial tissue lack CXCR5 expression [65]. PD-1^{hi}CXCR5^{-}CD4^{+} cells express functional molecules such as IL-21, CXCL13, ICOS, and MAF, which are known to be expressed in Tfh cells, but do not express CXCR5 and BCL-6, which are important for Tfh function in the germinal centers of lymph nodes. In terms of function, these cells assist the maturation of B cells, as do Tfh cells, and have therefore been named T peripheral helper (Tph) cells. Because CXCR5 is required for migration into the germinal center, Tph cells cannot do so. Instead, they express chemokine receptors, such as CCR2, CX3CR1, and CCR5, which allow Tph cells to migrate into sites of inflammation. Because Tph cells in peripheral blood do not express CXCL13, whereas those in joints affected by RA express CXCL13 strongly, it has been proposed that the TGF-β-abundant, IL-2-limiting inflammatory milieu in RA joints induces the transcriptional factor Sox4 in Tph cells, which migrate from the peripheral blood, and that Sox4 then upregulates the expression of CXCL13. The CXCL13-expressing Tph cells then attract B cells, leading to the formation of ectopic lymphoid-like structures [66]. These findings indicate that tissue-localized T-B interactions might play an important role in the development of RA.

5. IL-17 and RANKL elicit bone resorption by driving the function of osteoclasts

Several T-cell subsets and their complex interactions likely contribute to RA pathology. It is largely accepted that regulatory T cells accumulate in RA synovial fluid and that the equilibrium between them and effector cells is a key factor in controlling the inflammatory processes involved in RA [67]. Th17 cells are known to comprise a third T-cell subset since Th1/Th2 cells, and are induced by the cytokines IL-6, IL-1β, IL-21, TGF-β, and IL-23. They are present in synovial joints [68, 69] and secrete IL-17, which is a pro-inflammatory cytokine contributing to osteoclastogenesis along with TNF and IL-6.

Bone erosion by osteoclasts is one of the most important pathologic features of RA [70, 71]. Receptor activator of nuclear factor kappa-B ligand (RANKL) is a cytokine belonging to the TNF superfamily and mainly stimulates osteoclast differentiation. It binds to receptor activator of nuclear factor kappa-B (RANK) on osteoclasts and osteoclast precursors and promotes osteoclast differentiation and activation. Osteoprotegerin (OPG) is a soluble decoy receptor of RANKL, and upon binding to RANKL inhibits the activation of RANK. The inflamed synovium and pannus in RA produce significantly higher levels of RANKL and lower levels of OPG in comparison to healthy synovium [72-74]. The cells responsible for the increased expression of RANKL in the inflamed synovial membrane are fibroblast-like synoviocytes (FLS) and T lymphocytes. The increased RANKL/OPG ratio results in increased osteoclast differentiation and activation at the synovium-bone interface and the development of bone erosions in RA [74, 75].

RANKL on T cells or fibroblasts, which are activated by a combination of IL-6, IL-17, and

TNF, regulates the differentiation of osteoclasts. IL-6 signaling induces RANKL expression in RA-FLS through expression of NFATc1 and TRAP5b mRNA in co-cultures of RA-FLS and osteoclast precursor cells [76].

IL-17 increases RANKL expression in adjuvant-induced arthritis-derived synovial fibroblasts, leading to increased osteoclastogenesis *in vitro*. RANKL expression and osteoclastogenesis are dependent on IL-17R and STAT-3, as both are reduced by blocking these molecules [77]. Furthermore, IL-17 modulates osteoclast precursor cells. Raw264.7 cell culture experiments have shown that a low level of IL-17A promotes the RANKL-RANK system by mediating the JNK signaling pathway and activating autophagy and osteoclastogenesis in induced osteoclast precursor cells. However, a high level of IL-17A inhibits osteoclastogenesis [78].

A RANKL-independent osteoclast differentiation pathway has also been reported. TNF induces differentiation of osteoclasts from mouse bone marrow myeloid cells (mBMM) and human peripheral blood monocytes (PBMC) independently of RANKL function [79]. Tartrate-resistant acid phosphatase (TRAP)-positive cells induced by TNF lack bone absorption ability, but gain it upon treatment with a combination of IL-6 and TNF. Thus IL-6 is required for the bone-resorbing activity of TRAP-positive cells induced by TNF. Interestingly, whereas bone absorption activity cannot be abolished in mice with STAT3 conditional knockout, treatment with JAK inhibitor or MEK inhibitor achieves this [80]. Furthermore, the number of osteoclasts induced *ex vivo* from RA patient PBMC with TNF and IL-6 is positively correlated with the host patient modified Total Sharp score [81].

In RA patients, reactive oxygen species (ROS) are highly expressed in neutrophils and synovium [82]. Oxidative stress is one of the main mechanisms responsible for destructive proliferative synovitis [83], and ROS activate osteoclasts and drive bone resorption. RANKL itself induces nitric oxide synthase and N-acetyl cysteine (NAC). NOS and NAC inhibit RANKL-induced ROS production and inhibit the differentiation of osteoclasts from bone marrow monocyte-macrophage lineage cells [84].

In RA synovial fibroblasts, NAC dose-dependently attenuates the induction of RANKL mRNA by IL-17 [85]. NAC inhibits both ROS and MMP-3 mRNA by interfering with the JNK signaling pathway [86]. Thus, JNK has potential as a target of intervention for RA.

AtoM cells are another type of osteoclast that develop when macrophages derived from peripheral blood are stimulated with TNF and RANKL [54].

6. Biological DMARDs, JAK/STAT inhibitors and osteoclastogenesis

A previous study using synovial samples from 18 RA patients has evaluated the expression of OPG and RANKL protein by immunohistochemistry. After 8 weeks of treatment, infliximab and etanercept were found to increase the expression of osteoprotegerin (OPG) in synovial tissue, but neither influenced the expression of RANKL. In TNF inhibitor-treated groups of patients, the RANKL:OPG ratio decreased following therapy, suggesting that TNF inhibitors in RA modulate the OPG/RANKL system, possibly explaining the retardation of radiographically evident damage following anti-TNF therapy [87].

In another study, BMD in the spine, hip, and hand, as well as serum RANKL and RANKL/OPG, were monitored for 12 months in 102 RA patients receiving infliximab. BMD in the spine and hip showed no significant change, but BMD in the hand showed a significant decrease. The serum RANKL and RANKL/OPG ratio were significantly decreased by infliximab treatment [88]. On the other hand, infliximab directly promotes the differentiation of osteoclast precursor cells in PBMC and lacunar resorption induced by RANKL. Addition of infliximab has been shown to markedly increase the number of TRAP-positive multinucleated cells ($TRAP^+$ MNCs) and the extent of lacunar resorption in comparison with control cultures [89].

IL-6 and soluble IL-6 receptor (sIL-6R) induce RANKL expression in RA-FLS. Although IL-17 and TNF-alpha stimulate cell growth and IL-6 production in RA-FLS, they do not induce RANKL expression. IL-6 and sIL-6R induce the expression of NFATc1 and TRAP5b mRNA in cocultured

RA-FLS and osteoclast precursor cells [76].
In mouse calvarial osteoblasts, IL-6 and sIL-6R induce bone resorption, and this is decreased by osteoclast inhibitors. Thus, IL-6 signaling influences osteoclastogenesis [90]. Tocilizumab significantly ameliorates bone erosion in metacarpal bones of patients with RA when injected subcutaneously weekly for 52 weeks, whereas adalimumab and methotrexate do not ameliorate bone erosion to any significant degree [91]. Tocilizumab suppresses the number of histologically evident osteoclasts and the degree of RANKL-induced bone erosion in the metacarpal bones of model monkeys with collagen-induced arthritis, indicating that IL-6/IL-6R is involved in subchondral bone and bone marrow change in RA patients [92].
Abatacept, a chimeric molecule consisting of the extracellular domain of the co-inhibitory molecule CTLA-4 fused to the Fc portion of a human IgG1 antibody, neutralizes binding of the CTLA-4 part to either CD80 or CD86 on the surface of activated antigen-presenting cells [93,94]. Abatacept dose-dependently inhibits RANKL-mediated osteoclast formation in monocytes, exerting an anti-bone resorbing effect [95].
Recent studies have clarified the action of abatacept on osteoclastogenesis in more detail. Bozec et al. have described a mechanism for control of bone resorption by the adaptive immune system and showed that CD80/86 negatively regulated the generation of bone-resorbing osteoclasts. In CD80/86-deficient mice with osteopenia due to increased osteoclast differentiation, inhibition of CD80/86 by administration of CTLA-4 activated the enzyme indoleamine 2,3-dioxygenase (IDO) in osteoclast precursors, causing degradation of tryptophan and promotion of apoptosis [96]. CTLA4-Ig inhibits osteoclast differentiation and reduces the expression of nuclear factor of activated T cells1 (NFATc1) in bone marrow macrophages. It also suppresses calcium oscillations dependently on FCgammaR [97].

The JAK/STAT signaling pathway appears to be related to bone homeostasis. JAK protein associated with the receptor after formation of the ligand-receptor complex is activated by transphosphorylation. JAK activation induces the phosphorylation of tyrosine on a cytoplasmic tail subunit of the receptor at docking sites for STAT proteins. The STAT proteins then undergo phosphorylation and dimerization, and the dimer translocates into the nucleus, where it binds to DNA and activates the transcription of targeted genes that affect cell behavior. Suppressors of cytokine signaling (SOCS) provide negative feedback to the receptor and prevent continuous signaling. JAK1 and STAT3 signaling is mediated by IL-6 family cytokines, which bind to the glycoprotein 130 (gp130) IL-6 receptor subunit and are indispensable for normal skeletal development in mice and humans [98]. IL-6 family cytokines such as IL-6, IL-11, oncostatin M, cardiotrophin 1, leukemia inhibitory factor, and ciliary neurotrophic factor are needed for normal levels of bone formation but also induce pathological bone formation such as heterotopic ossification. They also stimulate osteoclast formation by acting indirectly on osteoblasts through STAT3 signaling [99]. The JAK1/STAT3/SOC3 pathway is also activated by G-CSF, thus inhibiting bone formation and promoting osteoclast formation [100-102]. Tofacitinib (a JAK1/JAK3 inhibitor) dose-dependently reduces RANKL expression in cultured T cells [103]. In a rat adjuvant-induced arthritis model, tofacitinib has been reported to increase bone cortical and trabecular hardness, but it does not reverse the effects of arthritis on cortical and trabecular bone structure [104].

Stattic, a STAT3-specific inhibitor, suppresses STAT3 and NF-kB, resulting in inhibition of RANKL-mediated osteoclastogenesis in RANKL-induced Raw264.7 cells. Stattic also inhibits bone loss caused by ovariectomy [105]. Baricitinib (a Jak1/Jak2 inhibitor) exerts an inhibitory effect on osteoclastogenesis. It suppresses RANKL expression in murine calvaria-derived osteoblasts. Furthermore, shRNA-mediated knockdown of Jak1 or Jak2 suppresses RANKL expression in osteoblasts and inhibits osteoclastogenesis. Therefore it has been suggested that Jak1 and Jak2 represent novel therapeutic targets for osteoporosis as well as RA [106].
In RA patients, ROS are highly expressed in neutrophils and synovium [82]. Oxidative stress is one prominent mechanism causing destructive proliferative synovitis [83]. RANKL itself induces

nitric oxide synthase, and N-acetyl cysteine (NAC) inhibits RANKL-induced ROS production and differentiation of osteoclasts in bone marrow monocyte-macrophage lineage cells [84]. Osteoclasts are activated by ROS to drive bone resorption [107]. In RA synovial fibroblasts, NAC attenuates the expression of RANKL mRNA and production of soluble RANKL in an IL-17 dose-dependent manner. IL-17 enhances the phosphorylation of mammalian target of rapamycin (mTOR), c-Jun N-terminal kinase (JNK), and inhibitor of kappaB alpha (IkN-α) [85]. NAC inhibits both ROS and MMP-3 mRNA by interfering with the JNK signaling pathway [86]. Thus, JNK may have potential as a target for intervention in RA patients.

5. GM-CSF and the pathogenesis of RA

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a well-known hemopoietic growth factor produced mainly by T-cells and stromal cells. It is also essential for regulating the functions of mature myeloid cells such as macrophages. As well as its stimulating effects on mature granulocytes, it induces the expression of HLA class II-antigen on synovial tissue-cells in patients with RA [108]. GM-CSF levels are increased in the sera, synovial fluid and bone marrow of patients with RA, especially at the chronic stage [109, 110]. IL-2, IL-7, and IL-33 are known to regulate the function of type 2 innate lymphoid cells [111]. These cytokines are involved in the production of GM-CSF by innate lymphoid cells of the synovium. In addition, the production of IL-33 is significantly increased in inflamed joints relative to healthy controls, and IL-2 is supplied from activated Th17 cells [112]. When the innate lymphoid cells of the synovium are stimulated with IL-2, IL-7, or IL-33 alone, GM-CSF is produced. Additionally, the production is synergistically increased when IL-33 and IL-2 are applied in combination [112].

Myeloid cells, T and B cells, and tissue resident cells can secrete GM-CSF, which in turn is capable of polarizing the function of macrophages into M1-like production of inflammatory cytokines. These cytokines induce the recruitment of inflammatory cells and activation of tissue resident cells. GM-CSF induces the production of IL-6 and IL-23 by antigen-presenting cells. IL6 and IL-23 cause activation of T cells and differentiation to Th17 cells, which in turn secrete GM-CSF and IL-17, thus maintaining the cycle. GM-CSF produced by Th17 cells also induces inflammation by activating the monocyte-macrophage system and neutrophils [113]. Macrophage populations in synovial tissue are associated with articular damage [114]. GM-CSF receptor activation leads to downstream involvement of Janus kinase-signal transducer and activator of transcription-suppressor of cytokine signaling (JAK-STAT-SOCS) as well as other pathways involving mitogen-activated protein kinases (MAPK), phosphatidylinositol 3 kinase (PI3K), and NF-kB [115 ,116].

Clinical trials of agents targeting the GM-CSF pathway in RA have been reported. Mavrilimumab is a human monoclonal antibody that inhibits the human GM-CSF receptor [117]. A phase 2a trial of mavrilimumab at doses of up to 100 mg found that 55.7% of the subjects met the primary endpoint of a ≥1.2 decrease from baseline disease activity scores at week 12 (vs. only 34.7% of subjects given a placebo) [118]. Theoretically, the signal of GM-CSF could be controlled by blocking the signal transduction pathway, which involves JAK-STAT and MAPK. Jak2 inhibits the GM-CSF signaling pathway by acting on JAK-STAT. For the treatment of RA, peficitinib is an oral JAK inhibitor that targets JAK family enzymes (JAK1, JAK2, JAK3, TYK2) and blocks the signal transduction of various cytokines including GM-CSF [119]. Baricitinib is another inhibitor exerting similar effects [120]. Since these two drugs also inhibit Jak1 and block the IL-6 signaling pathway, any inhibitory effect on GM-CSF alone remains unclear.

6. IL-33 and the pathogenesis of RA

IL 33 is a member of the IL 1 family and recognized as a ligand for the ST2 receptor [121]. IL-33 binds to a specific heterodimeric cell surface receptor, which is a dimer of IL1Receptor associated protein (IL-1RacP) and ST2. ST2 exists in two forms as splice variants: a soluble form (sST2), which acts as a decoy receptor, sequesters free IL-33, and does not signal, and a

membrane-bound form (ST2), which activates the NF-κB and MAP kinase signaling pathway to enhance the functions of mast cells, Th2 regulatory T cells (Tregs), and innate type 2 lymphoid cells [122]. The IL-33 protein is expressed mainly in epithelial and endothelial cells, particularly in high endothelial venules in human synovial tissue, and in cultured human RA fibroblasts [123]. IL-33 expression is strongly induced by IL-1 and/or TNF-α. Furthermore, IL-33 is highly expressed and its mRNA also detected in the joints of mice with collagen-induced arthritis (CIA) and increased during the early phase of the disease. In mice with CIA, and in arthritic mouse joints, inhibition of IL-33 signaling attenuates the severity of experimental arthritis. Administration of a blocking anti-ST2 antibody at the onset of disease has been reported to attenuate the severity of CIA and reduced joint destruction. In addition, anti-ST2 antibody treatment is associated with a marked decrease in interferon production as well as with a more limited reduction of IL-17 production by *ex vivo*-stimulated draining LN cells, and levels of RANKL mRNA in the joint are reduced [123]. IL-33 levels are increased in the sera and synovial fluid of patients with RA [124,125]. Increased levels of IL-33 in sera and synovial fluid are associated with disease activity [126] and bone erosion [127] in RA patients. Since it has been suggested that IL-33 is strongly associated with the pathogenesis of RA, several attempts to neutralize IL-33 have been conducted in model CIA mice. One experiment demonstrated that anti-IL-33 treatment also significantly decreased the serum levels of IFN-γ, IL-6, IL-12, IL-33, and TNF-α [128]. It is widely known that suppression of IL-6 and TNF-α directly prevents joint destruction. Despite promising findings from animal models, no clinical trials have yet been conducted in humans. The network of the IL-33 signaling pathway was recently revealed, and the findings suggest that it may affect a wide variety of diseases, although it is complex [129]. The activity of IL-33 is mediated by binding to the IL-33 receptor complex (IL-33R) and activation of NF-κB signaling via the classical MyD88/IRAK/TRAF6 module. IL-33 also induces phosphorylation and activation of the ERK1/2, JNK, p38 and PI3K/AKT signaling modules, resulting in the production and release of proinflammatory cytokines [129]. Theoretically, the IL-33 signal should be controllable by blocking the transduction pathway. In reality, however, inhibition of pathways other than the Jak-Stat system is not considered to be easy due to the problem of crosstalk in which signals enter from other pathways. Nevertheless, intervention with anti-IL-33 antibody and T2 / IL-33 signaling might be an effective therapeutic option for RA.

7. IL-2 and the pathogenesis of RA

IL-2 is a Th1 lymphocyte-derived cytokine, and the principal autocrine growth factor that promotes T-cell activation and proliferation [130].

Clinical studies have shown that the serum IL-2 level is correlated with disease activity in RA [131, 132]. We have also shown that the levels of IL-2 in serum and bone marrow are correlated with disease activity in mutilans-type RA [110]. The effects of IL-2 are mediated by cell surface receptors (IL-2 R) expressed on activated T cells. The serum sIL-2 R level in RA reflects disease activation, and a rising level may also predict exacerbation of disease activity [133]. On the other hand, most studies of cytokines in RA have failed to detect IL-2 protein in RA synovial fluid [134]. In the CIA animal model, IL-2 was reported to exert two opposite effects, namely a direct stimulatory effect and an indirect suppressive effect. It was demonstrated that administration of recombinant human IL-2 (rhIL-2) at or just before disease onset exacerbated the disease (days 21–28 after the first immunization), whereas rhIL-2 administered before disease onset (days 14–21 after the first immunization) inhibited the CIA. It was concluded that the indirect suppressive effect was mediated by IFN-γ because in mice treated with an anti-IFN-γ Ab, both early and late IL-2 administration exacerbated the CIA [135]. In addition, IL-2/anti-IL-2 monoclonal antibody immune complexes have been reported to inhibit murine CIA. Histopathological examination of joints revealed inhibition of synovial cell proliferation and lower levels of IL-17, IL-6, and TNF-α [136]. Although these data may reflect the usage of different types of IL-2, they may also reflect

a change in the cytokine cascade in the mouse CIA model between the onset stage and the later stage. IL-2, IL-7, and IL-33 are known to regulate the function of type 2 innate lymphoid cells [137]. Stimulation with IL-33 caused GM-CSF production, and stimulation with a combination of IL-2 and IL-33 increased it. GM-CSF activates monocyte-macrophages and leads to joint damage. In RA patients, serum IL-2 levels are not only correlated with disease activity and autoantibody levels, but also impact their Th17/Treg immune imbalance. Also, in patients with active RA, levels of NK cells are abnormally elevated, possibly due to high serum levels of IL-2 [132]. IL-2-stimulated NK cells, which produce GM-CSF, induce monocyte-macrophage activation leading to joint damage. As mentioned in the section on GM-CSF, the network in which IL-2 is produced from Th-17 lymphocytes acts on NK cells and macrophages via GM-CSF. These networks characterize RA in the chronic phase.

Conclusions

Recent advances in molecular biology have greatly expanded our understanding of the pathophysiology of RA. We have overviewed the cytokines and molecules involved, starting from RF and ACPA, which represent the cornerstone autoantibodies operating in RA, and examined cytokines such as TNF-α, IL-6, IL-17, IL-23, IL-33, GM-CSF, and IL-2, which also play diverse roles. TNF and IL-6 inhibitors have already been used in many patients with remarkable clinical effects. GM-CSF inhibitors are also likely to be used as therapeutic agents. Jak inhibitors can suppress multiple molecules at the same time, but for RA, the Jak-Stat pathway is currently suitable for inhibition. Other possibilities for signaling inhibitors should be considered. Other cytokines may also have potential in future treatment avenues.

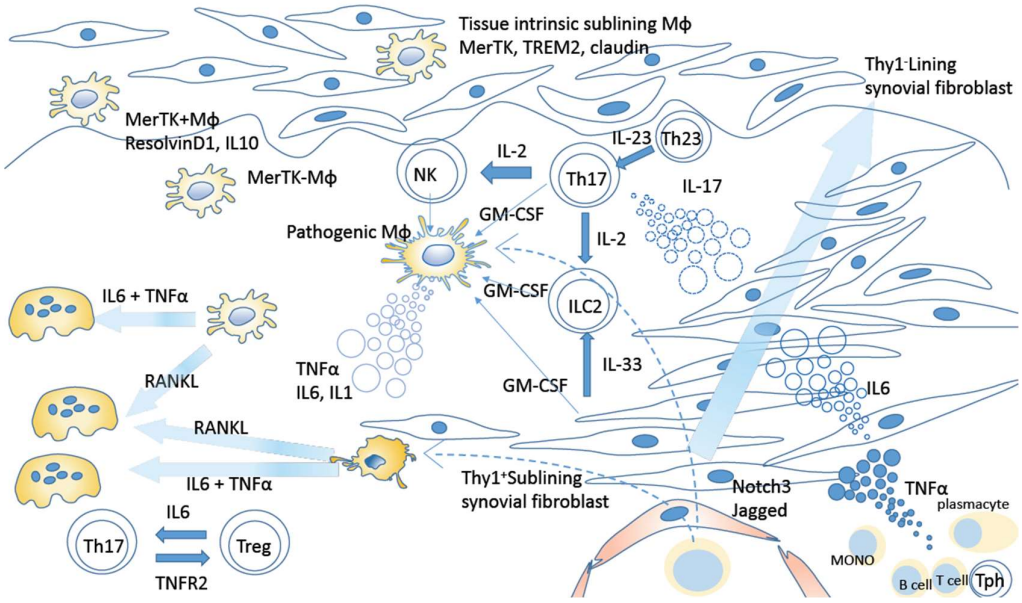

Figure 1. Possible mechanism of joint inflammation and destruction.

Interleukin (IL)-23 secreted by T helper (Th) 23 cells activate Th17, which in turn secretes IL-17 that activates group 2 innate lymphoid cells (ILC2) and natural killer (NK) cells. IL-2 and IL-33 released by Th17 cells, as well as NK, ILC2, and synovial fibroblast activates macrophages via the granulocyte-macrophage colony-stimulating factor (GM-CSF). Lastly, activated macrophages will secrete proinflammatory cytokines.

Cytokines, such as IL-6, tumor necrosis factor (TNF), and IL-17, activate synovial fibroblasts. Recent single-cell transcriptomics and mass cytometry analysis suggested the existence of various types of synovial fibroblasts, among which thymocyte differentiation antigen 1 (Thy1)-positive and -negative synovial fibroblasts in the lining and sublining layers, respectively, are the major subsets.

Cell identity between Thy1^{+} and Thy1^{-} is controlled by Jagged1 on the vascular endothelium and by Notch3 on synovial fibroblasts.

Macrophages can migrate from extra-synovial tissues and function as pathogenic cells by secreting proinflammatory cytokines. In contrast, tissue-intrinsic macrophages function as anti-arthritogenic, expressing c-mer tyrosine kinase (MerTK), claudin, resolvin D1, and IL-10.

Osteoclasts differentiate from extrinsic or intrinsic macrophages, through a process regulated by the receptor activator of nuclear factor-kappa B ligand (RANKL), TNF, and IL-6. In turn, RANKL expression in synovial fibroblasts is regulated by TNF-α, IL-6, and IL-17.

TNF receptor (TNFR) 2 and IL-6 signals regulate the balance between regulatory T-cells (Treg) and Th17, whereas peripheral Th cells (Tph) contribute to lymphoid follicle formation.

Conflict of Interest

The authors declare that they have no conflicts of interest in relation to this work.

Author Contributions

N.K., T.K., and D.K. contributed to the design and theoretical framework, to analysis of the results, and to writing of the manuscript.

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References

1. Smolen, J. S.; Aletaha, D.; McInnes, I. B.; Rheumatoid arthritis. Lancet. 2016.388.2023-2038.
2. Kuroda, T.; Tanabe, N.; Kobayashi, D.; Sato, H.; Wada, Y.; Murakami, S.; Saeki, T.; Nakano, M.; Narita, I; Treatment with biologic agents improves the prognosis of patients with rheumatoid arthritis and amyloidosis. J Rheumatol 2012. 39.1348-1854.
3. McInnes, I.B.; Schett, G.; The pathogenesis of rheumatoid arthritis. N Engl J Med 2011. 365. 2205–2219.
4. Klareskog, L.: Malmström, V.; Lundberg, K.; Padyukov, L.; Alfredsson, L.; Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. Semin Immunol. 2011. 23. 92−98.
5. Schellekens, G.A.; Visser, H.; de Jong, B. A.; van den Hoogen, F. H.; Hazes, J. M.; Breedveld, F. C.; van Venrooijet, W. J.: The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000. 43. 155−163.
6. James, E.A.; Moustakas, A.K.; Papadopoulos, G.K.; Bondinas, G.; Buckner, J.H.; Kwok, W.K.; HLA–DR1001 presents “altered-self ” peptides derived from joint-associated proteins by accepting citrulline in three of its binding pockets. Arthritis Rheum. 2010. 62. 2909−2918.
7. Koziel, J.; Mydel, P.; Potempa, J.; The link between periodontal dis-ease and rheumatoid arthritis: an updated review. Curr Rheumatol Rep. 2014.16: 408
8. Murata, K.; Ito, H.; Hashimoto, M.; Murakami, K.; Watanabe, R.; Tanaka, M.; Yamamoto, W.; Matsuda, S.; Fluctuation in anti-cyclic citrullinated protein antibody level predicts relapse from remission in rheumatoid arthritis: KURAMA cohort. Arthritis Research & Therapy. 2020. 22. 268.
9. Clavel, C., et al.: Induction of macrophage secre-tion of tumor necrosis factor through Fc receptor IIa engagement by rheumatoid arthritis–specific autoantibodies to citrullinated proteins complexed with fibrinogen. Arthritis Rheum. 2018.58.678−688.

10. McInnes, I.B.; Buckley, C.D.; and J. D. Isaacs, J.D.; Cytokines in rheumatoid arthritis—shaping the immunological landscape. Nat Rev Rheumatol.2016.12. 63–68.
11. Takeuchi, T.; Miyasaka, Y.; Yano, T.; Yoshinari, T.; Abe, T.; Koike, T.; Inhibition of plasma IL-6 in addition to maintenance of an efficacious trough level of infliximab associated with clinical remission in patients with rheumatoid arthritis: analysis of the RISING Study. Ann Rheum Dis 2012. 71.1583-1585.
12. Brennan, F.M.; Browne, K.A.; Green, P.A.; Jaspar, J-M.; Maini, R.N.; Feldmann. M.; Reduction of serum matrix metalloproteinase 1and matrix metalloproteinase 3 in rheumatoid arthritis patients following anti-tumor necrosis factor- α(cA2) therapy. Br J Rheumatol, 1997. 36. 643-650.
13. Carswell, EA.; Old, LJ.; Kassel, RL.; Green, S.; Fiore, N.; Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A. 1975. 72. 3666-3670.
14. Kalliolias, G.D.; Ivashkiv, L.B.; TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nat Rev Rheumatol. 2016. 12. 49-62.
15. Grell, M.; Douni, E.; Wajant, H.; Löhden, M.; Clauss, M.; Maxeiner, B.; Georgopoulos, S.; Lesslauer, W.; Kollias, G.; Pfizenmaie,r K.; et al. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor Cell. 1995. 83. 793-802.
16. Rauert, H.; Wicovsky, A.; Müller, N.; Siegmund, D.; Spindler, V.; Waschke, J.; Kneitz, C.; Wajant, H. Membrane tumor necrosis factor (TNF) induces p100 processing via TNF receptor-2 (TNFR2). J Biol Chem. 2010. 285. 7394-404.
17. Kobayashi, D.; Ito, S.; Takai, C.; Hasegawa, E.; Nomura, Y.; Otani, H.; Abe, A.; Ishikawa, H.; Murasawa, A.; Narita, I.; et al. Efficacy and safety of infliximab: A comparison with other biological disease-modifying anti-rheumatic drugs. Mod Rheumatol. 2018. 28. 599-605.
18. Kruglov, A.; Drutskaya, M.; Schlienz, D.; Gorshkova, E.; Kurz, K.; Morawietz, L.; Nedospasov, S. Contrasting contributions of TNF from distinct cellular sources in arthritis. Ann Rheum Dis. 2020. 1453-1459.
19. Wing, J.B.; Sakaguchi, S. Foxp3^{+} T(reg) cells in humoral immunity. Int Immunol. 2014. 26. 61-69.
20. Armaka, M.; Apostolaki, M.; Jacques, P.; Kontoyiannis, D.L.; Elewaut, D.; Kollias, G. Mesenchymal cell targeting by TNF as a common pathogenic principle in chronic inflammatory joint and intestinal diseases. J Exp Med 2008. 205. 331-337.
21. Blüml, S.; Binder, N.B.; Niederreiter, B.; Polzer, K.; Hayer, S.; Tauber, S.; Schett, G.; Scheinecker, C.; Kollias, G.; Selzer, E.; et al. Antiinflammatory effects of tumor necrosis factor on hematopoietic cells in a murine model of erosive arthritis. Arthritis Rheum 2010. 62. 1608-1619.
22. McCann, F.E.; Perocheau, D.P.; Ruspi, G.; Blazek, K.; Davies, M.L.; Feldmann, M.; Dean, J.L.; Stoop, A.A.; Williams, R.O. Selective tumor necrosis factor receptor I blockade is antiinflammatory and reveals immunoregulatory role of tumor necrosis factor receptor II in collagen-induced arthritis. Arthritis Rheumatol. 2014. 66. 2728-2738.
23. Wajant, H.; Beilhack, A. Targeting Regulatory T Cells by Addressing Tumor Necrosis Factor and Its Receptors in Allogeneic Hematopoietic Cell Transplantation and Cancer. Front Immunol. 2019. 10:2040. doi: 10.3389/fimmu.2019.02040. eCollection 2019.
24. Chen, X.; Bäumel, M.; Männel, D.N.; Howard, O.M.; Oppenheim, J.J. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse CD4+CD25+ T regulatory cells. J Immunol. 2007. 179. 154-61.
25. Chen, X.; Subleski, J.J.; Hamano, R.; Howard, O.M.; Wiltrout, R.H.; Oppenheim, J.J. Co-expression of TNFR2 and CD25 identifies more of the functional CD4+FOXP3+ regulatory T cells in human peripheral blood. Eur J Immunol. 2010. 1099-106.

26. Tseng, W.Y.; Huang, Y.S.; Clanchy, F.; McNamee, K.; Perocheau, D.; Ogbechi, J.; Luo, S.F.; Feldmann, M.; McCann, F.E.; Williams, R.O. TNF receptor 2 signaling prevents DNA methylation at the Foxp3 promoter and prevents pathogenic conversion of regulatory T cells. Proc Natl Acad Sci U S A. 2019.116:21666-21672.
27. Santinon, F.; Batignes, M.; Mebrek, M.L.; Biton, J.; Clavel, G.; Hervé, R.; Lemeiter, D.; Breckler, M.; Busato, F.; Tost, J.; et al. Involvement of Tumor Necrosis Factor Receptor Type II in FoxP3 Stability and as a Marker of Treg Cells Specifically Expanded by Anti-Tumor Necrosis Factor Treatments in Rheumatoid Arthritis. Arthritis Rheumatol. 2020. 72. 576-587.
28. Nie, H.; Zheng, Y.; Li, R.; Guo, T.B.; He, D.; Fang, L.; Liu, X.; Xiao, L.; Chen, X.; Wan, B.; et al. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF-α in rheumatoid arthritis. Nat Med. 2013. 19. 322-328.
29. Karouzakis, E.; Raza, K.; Kolling, C.; Buckley, C.D.; Gay, S.; Filer, A.; Ospelt, C. Analysis of early changes in DNA methylation in synovial fibroblasts of RA patients before diagnosis. Sci Rep. 2018. 8. 7370.
30. Nakano, K.; Whitaker, J.W.; Boyle, D.L.; Wang, W.; Firestein, GS. DNA methylome signature in rheumatoid arthritis. Ann Rheum Dis. 2013. 72. 110-117.
31. Nakano, K.; Boyle, D.L.; Firestein, G.S. Regulation of DNA methylation in rheumatoid arthritis synoviocytes. J Immunol. 2013. 190. 1297-303. doi: 10.4049/jimmunol.1202572.
32. Angiolilli, C.; Grabiec, A.M.; Ferguson, B.S.; Ospelt, C.; Malvar-Fernandez, B.; van Es, I.E.; van Baarsen, L.G.; Gay, S.; McKinsey, T.A.; Tak, P.P.; et al. Inflammatory cytokines epigenetically regulate rheumatoid arthritis fibroblast-like synoviocyte activation by suppressing HDAC5 expression. Ann Rheum Dis. 2016. 75. 430-438.
33. Loh, C.; Park, S.H.; Lee, A.; Yuan, R.; Ivashkiv, L.B.; Kalliolias, G.D. TNF-induced inflammatory genes escape repression in fibroblast-like synoviocytes: transcriptomic and epigenomic analysis. Ann Rheum Dis. 2019. 78. 1205-1214.
34. Tsuchiya, H.; Ota, M.; Sumitomo, S.; Ishigaki, K.; Suzuki, A.; Sakata, T.; Tsuchida, Y.; Inui, H.; Hirose, J.; Kochi, Y.; et al. Parsing multiomics landscape of activated synovial fibroblasts highlights drug targets linked to genetic risk of rheumatoid arthritis. Ann Rheum Dis. 2020. annrheumdis-2020-218189. doi: 10.1136/annrheumdis-2020-218189. Online ahead of print.
35. Hirano, T.; Yasukawa, K.; Harada, H.; Taga, T.; Watanabe, Y.; Matsuda, T.; Kashiwamura, S.; Nakajima, K.; Koyama, K.; Iwamatsu, A.; et al. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature. 1986. 324. 73-76.
36. Hirano, T. IL-6 in inflammation, autoimmunity and cancer. Int Immunol. 2021. 33. 127-148.
37. Heink, S.; Yogev, N.; Garbers, C.; Herwerth, M.; Aly, L.; Gasperi, C.; Husterer, V.; Croxford, A.L.; Möller-Hackbarth, K.; Bartsch, H.S.; et al. Trans-presentation of IL-6 by dendritic cells is required for the priming of pathogenic T(H)17 cells. Nat Immunol. 2017. 18. 74-85.
38. Lee, A.; Qiao, Y.; Grigoriev, G.; Chen, J.; Park-Min, K.H.; Park, S.H.; Ivashkiv, L.B.; Kalliolias, G.D. Tumor necrosis factor alpha induces sustained signaling and a prolonged and unremitting inflammatory response in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum. 2013. 65. 928-38.
39. Atsumi, T.; Ishihara, K.; Kamimura, D.; Ikushima, H.; Ohtani, T.; Hirota, S.; Kobayashi, H.; Park, S.J.; Saeki, Y.; Kitamura, Y.; et al. A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. J Exp Med. 2002. 196. 979-90.

40. Ogura, H.; Murakami, M.; Okuyama, Y.; Tsuruoka, M.; Kitabayashi, C.; Kanamoto, M.; Nishihara, M.; Iwakura, Y.; Hirano, T. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. Immunity. 2008. 29. 628-636.
41. Sawa, S.; Kamimura, D.; Jin, GH.; Morikawa, H.; Kamon, H.; Nishihara, M.; Ishihara, K.; Murakami, M.; Hirano, T. Autoimmune arthritis associated with mutated interleukin (IL)-6 receptor gp130 is driven by STAT3/IL-7-dependent homeostatic proliferation of CD4+ T cells. J Exp Med. 2006. 203. 1459-1470.
42. Slowikowski, K.; Nguyen, H.N.; Noss, E.H.; Simmons, D.P.; Mizoguchi, F.; Watts, G.F.M.; Gurish, M.F.; Brenner, M.B.; Raychaudhuri, S. CUX1 and IkappaBzeta (NFKBIZ) mediate the synergistic inflammatory response to TNF and IL-17A in stromal fibroblasts. Proc Natl Acad Sci U S A. 2020. 117. 5532-5541.
43. Nishihara, M.; Ogura, H.; Ueda, N.; Tsuruoka, M.; Kitabayashi, C.; Tsuji, F.; Aono, H.; Ishihara, K.; Huseby, E.; Betz, UA.; et al. IL-6-gp130-STAT3 in T cells directs the development of IL-17+ Th with a minimum effect on that of Treg in the steady state. Int Immunol. 2007. 19. 695-702.
44. Tripathi, S.K.; Chen, Z.; Larjo, A.; Kanduri, K.; Nousiainen, K.; Äijo, T.; Ricaño-Ponce, I.; Hrdlickova, B.; Tuomela, S.; Laajala, E.; et al. Genome-wide Analysis of STAT3-Mediated Transcription during Early Human Th17 Cell Differentiation. Cell Rep. 2017. 19. 1888-1901.
45. Yang, X.O.; Nurieva, R.; Martinez, G.J.; Kang, H.S.; Chung, Y.; Pappu, B.P.; Shah, B.; Chang, S.H.; Schluns, K.S.; Watowich, S.S.; et al. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity. 2008. 29. 44-56.
46. Komatsu, N.; Okamoto, K.; Sawa, S.; Nakashima, T.; Oh-hora, M.; Kodama, T.; Tanaka, S.; Bluestone, J.A.; Takayanagi, H. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. Nat Med. 2014. 20. 62-68.
47. Crotty, S. T Follicular Helper Cell Biology: A Decade of Discovery and Diseases. Immunity. 2019. 50. 1132-1148.
48. Choi, Y.S.; Eto, D.; Yang, J.A.; Lao, C.; Crotty, S. Cutting edge: STAT1 is required for IL-6-mediated Bcl6 induction for early follicular helper cell differentiation. J Immunol. 2013. 190. 3049-3053.
49. Schmitt, N.; Liu, Y.; Bentebibel, S.E.; Munagala, I.; Bourdery, L.; Venuprasad, K.; Banchereau, J.; Ueno, H. The cytokine TGF-beta co-opts signaling via STAT3-STAT4 to promote the differentiation of human TFH cells. Nat Immunol. 2014. 15. 856-865.
50. Okamoto, K.; Takayanagi H. Osteoimmunology. Cold Spring Harb Perspect Med. 2019. 9. a031245.
51. Hitchon, C.A.; El-Gabalawy, H.S. The synovium in rheumatoid arthritis. Open Rheumatol J. 2011. 5. 107-114.
52. Stephenson ,W.; Donlin, L.T.; Butler, A.; Rozo, C.; Bracken, B.; Rashidfarrokhi, A.; Goodman, S.M.; Ivashkiv, L.B.; Bykerk, V.P.; Orange, D.E.; et al. Single-cell RNA-seq of rheumatoid arthritis synovial tissue using low-cost microfluidic instrumentation. Nat Commun. 2018. 9. 791.
53. Mizoguchi, F.; Slowikowski, K.; Wei, K.; Marshall, J.L.; Rao, D.A.; Chang, S.K.; Nguyen, H.N.; Noss, E.H.; Turner, J.D.; Earp, B.E.; et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. Nat Commun. 2018. 9. 789.
54. Zhang, F.; Wei, K.; Slowikowski, K.; Fonseka, C.Y.; Rao, D.A.; Kelly, S.; Goodman, S.M.; Tabechian, D.; Hughes, L.B.; Salomon-Escoto, K.; et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat Immunol. 2019. 20. 928-942.
55. Croft, A.P.; Campos, J.; Jansen, K.; Turner, J.D.; Marshall, J.; Attar, M.; Savary, L.; Wehmeyer, C.; Naylor, A.J.; Kemble, S.; et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. Nature. 2019. 570. 246-251.

56. Wei, K.; Korsunsky, I.; Marshall, J.L.; Gao, A.; Watts, G.F.M.; Major, T.; Croft, A.P.; Watts, J.; Blazar, P.E.; Lange, J.K.; et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. Nature. 2020. 582. 259-264.
57. Orange, D.E.; Yao, V.; Sawicka, K.; Fak, J.; Frank, M.O.; Parveen, S.; Blachere, N.E.; Hale, C.; Zhang, F.; Raychaudhuri, S.; et al. RNA Identification of PRIME Cells Predicting Rheumatoid Arthritis Flares. N Engl J Med. 2020. 383. 218-228.
58. Blériot C.; Chakarov S.; Ginhoux F. Determinants of Resident Tissue Macrophage Identity and Function. Immunity. 2020. 52. 957-970.
59. Hasegawa, T.; Kikuta ,J.; Sudo, T.; Matsuura, Y.; Matsui, T.; Simmons, S.; Ebina, K.; Hirao, M.; Okuzaki, D.; Yoshida, Y.; et al. Identification of a novel arthritis-associated osteoclast precursor macrophage regulated by FoxM1. Nat Immunol. 2019. 20. 1631-1643.
60. Culemann, S.; Grüneboom, A.; Nicolás-Ávila, J.Á.; Weidner, D.; Lämmle, K.F.; Rothe, T.; Quintana, J.A.; Kirchner, P.; Krljanac, B.; Eberhardt, M.; et al. Locally renewing resident synovial macrophages provide a protective barrier for the joint. Nature. 2019. 572. 670-675.
61. Alivernini, S.; MacDonald, L.; Elmesmari, A.; Finlay, S.; Tolusso, B.; Gigante, M.R.; Petricca, L.; Di Mario, C.; Bui, L.; Perniola, S.; et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. Nat Med. 2020. 26. 1295-1306.
62. Norling, L.V.; Headland, S.E.; Dalli, J.; Arnardottir, H.H.; Haworth, O.; Jones, H.R.; Irimia, D.; Serhan, C.N.; Perretti, M. Proresolving and cartilage-protective actions of resolvin D1 in inflammatory arthritis. JCI Insight. 2016. 1. e85922.
63. Chen, Z.; Bozec, A.; Ramming, A.; Schett, G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. Nat Rev Rheumatol. 2019. 15. 9-17.
64. Tu, J.; Hong, W.; Guo, Y.; Zhang, P.; Fang, Y.; Wang, X.; Chen, X.; Lu, S.; Wei, W. Ontogeny of Synovial Macrophages and the Roles of Synovial Macrophages From Different Origins in Arthritis. Front Immunol. 2019. 10. 1146.
65. Rao, D.A.; Gurish, M.F.; Marshall, J.L.; Slowikowski, K.; Fonseka, C.Y.; Liu, Y.; Donlin, L.T.; Henderson, L.A.; Wei, K.; Mizoguchi, F.; et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature. 2017. 542. 110-114.
66. Yoshitomi, H.; Kobayashi, S.; Miyagawa-Hayashino, A.; Okahata, A.; Doi, K.; Nishitani, K.; Murata, K.; Ito, H.; Tsuruyama, T.; Haga, H.; et al. Human Sox4 facilitates the development of CXCL13-producing helper T cells in inflammatory environments. Nat Commun. 2018. 9. 3762.
67. Chemin, K.; Gerstner, C.; Malmström, V. Effector functions of CD4+ T cells at the site of local autoimmune inflammation-lessons from rheumatoid arthritis. Front Immnol. 2019. 10. 353. doi: 10.3389/fimmu.2019.00353. eCollection 2019.
68. Gaffen, S.L.; Jain, R.; Garg, A.V.; Cua, D.J. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nat Rev Imunol. 2014. 14. 585-600. doi: 10.1038/nri3707.
69. Cascão, R; Moura, R.A.; Perpétuo, I.; Canhão, H.; Vieira-Sousa, E.; Mourão, A.F.; Rodrigues, A.M.; Polido-Pereira, J.; Queiroz, M.V.; Rosário, H.S.; et al. Identification of a cytokine network sustaining neutrophil and Th17 activation in untreated early rheumatoid arthritis. Arthritis Res Ther. 2010. 12. R196. doi: 10.1186/ar3168.
70. Udagawa, N.; Kotake, S.; Kamatani, N.; Takahashi, N.; Suda, T. The molecular mechanism of osteoclastogenesis in rheumatoid arthritis. Arthritis Res. 2002.4.281-289. doi: 10.1186/ar431.
71. Vandooren, B.; Cantaert, T.; Noordenbos, T.; Tak, P.P.; Baeten, D. The abundant synovial expression of the RANK/RANKL/Osteoprotegerin system in peripheral spondylarthritis is partially disconnected from inflammation. Arthritis Rheum. 2008. 58. 718-729. doi: 10.1002/art.23290.
72. Crotti, T.N.; Smith, M.D.; Weedon, H.; Ahern, M.J.; Findlay, D.M.; Kraan, M.; Tak, P.P.; Haynes, D.R. Receptor activator NF-kappa ligand (RANKL) expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathy, osteoarthritis, and from normal

patients: semiquantitative and quantitative analysis. Ann Rheum Dis. 2002. 61. 1047-1054. doi: 10.1136/ard.61.12.1047.

73. Haynes, D.R.; Barg, E.; Crotti, T.N.; Holding, C.; Weedon, H., Atkins, G.J.; Zannetino, A.; Ahern, M.J.; Coleman, M.; Robert-Thompson, P.J.; et al. Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. Rheumatology (Oxford, England). 2003. 42. 123-134. doi: 10.1093/rheumatology/keg047.
74. Takayanagi, H.; Iizuka, H.; Juji, T.; Nakagawa, T.; Yamamoto, A.; Miyazaki, T.; Koshihara, Y.; Oda, H.; Nakamura, K.; Tanaka, S. Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis and Rheum. 2000. 43. 259-269. DOI: 10.1002/1529-0131(200002)43:2<259::AID-ANR4>3.0.CO;2-W
75. Gravallese, E.M.; Manning, C.; Tsay, A.; Naito, A.; Pan, C.; Amento, E.; Goldring, S.R. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis and Rheum. 2000.43. 250-258. doi: 10.1002/1529-0131(200002)43:2<250::AID-ANR3>3.0.CO;2-P.
76. Hashizume, M.; Hayakawa, H.; Mihara, M. IL-6 trans-signaling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-alpha and IL-17. Rheumatology (Oxford). 2008. 47. 1635-1640. doi: 10.1093/rheumatology/ken363.
77. Ganesan, R.; Rasool, M. Interleukin 17 regulates SHP-2 and IL-17RA/STAT-3 dependent Cyr61, IL-23 and GM-CSF expression and RANKL mediated osteoclastogenesis by fibroblast-like synoviocytes in rheumatoid arthritis. Mol Immunol. 2017. 91.134-144. doi: 10.1016/j.molimm.2017.09.003.
78. Ke, D.; Fu, X.; Xue, Y.; Wu, H.; Zhang, Y; Chen, X.; Hou, J. IL-17A regulates the autophagy activity of osteoclast precursors through RANKL-JNK1signaling during osteoclastogenesis in vitro. Biochem Biophys Res Commun. 2018. 497. 890-896. doi: 10.1016/j.bbrc.2018.02.164.
79. O'Brien, W.; Fissel, B.M.; Maeda, Y.; Yan, J.; Ge, X.; Gravallese, E.M.; Aliprantis, A.O.; Charles, J.F. RANK-Independent Osteoclast Formation and Bone Erosion in Inflammatory Arthritis. Arthritis Rheumatol. 2016. 68. 2889-2900. doi: 10.1002/art.39837.
80. Yokota, K.; Sato, K.; Miyazaki, T.; Kitaura, H.; Kayama, H.; Miyoshi, F.; Araki, Y.; Akiyama, Y.; Takeda, K.; Mimura, T. Combination of tumor necrosis factor alpha and interleukin-6 induces mouse osteoclast-like cells with bone resorption activity both in vitro and in vivo. Arthritis Rheumatol. 2014. 66. 121-129. doi: 10.1002/art.38218.
81. Yokota, K.; Sato, K.; Miyazaki, T.; Aizaki, Y.; Tanaka, S.; Sekikawa, M.; Kozu, N.; Kadono, Y.; Oda, H.; Mimura, T. Characterization and Function of Tumor Necrosis Factor and Interleukin-6-Induced Osteoclasts in Rheumatoid Arthritis. Arthritis Rheumatol. 2021. 73. 1145-1154. doi: 10.1002/art.41666.
82. Wright, H.L.; Lyon, M.; Chapman, E.A.; Moots, R.J.; Edwards, S.W. Rheumatoid arthritis synovial fluid neutrophils drive inflammation through production of chemokines, reactive oxygen species, and neutrophil extracellular traps. Front Immunol. 2021.11.584116. doi: 10.3389/fimmu.2020.584116. eCollection 2020.
83. Lean, J.M.; Jagger, C.J.; Kirstein, B.; Fuller, K.; Chambers, T.J. Hydrogen peroxide is essential for estrogen-deficiency bone loss and osteoclast formation. Endocrinology. 2005. 146. 728-735. doi: 10.1210/en.2004-1021.
84. Lee, N,K,; Choi, Y.G.; Baik, J.Y.; Han, S.Y.; Jeong, D.W.; Bae, Y.S.; Kim, N.; Lee, S.Y. A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. Blood. 2005.106.852-859. doi: 10.1182/blood-2004-09-3662.
85. Kim, H.R.; Kim, K.W.; Kim, B.M.; Lee, K.A.; Lee, S.H. N-acetyl-l-cysteine controls osteoclastogenesis through regulating Th17 differentiation and RANKL in rheumatoid arthritis. Korean J Intern Med. 2019. 34. 210-219. doi: 10.3904/kjim.2016.329.
86. Kanai. T.; Kondo, N.; Okada, M.; Sano, H.; Okumura, G.; Kijima, Y.; Ogose, A.; Kawashima,

H.; Endo, N. The JNK pathway represents a novel target in the treatment of rheumatoid arthritis through the suppression of MMP-3. J Orthop Surg Res. 2020. 15. 87. doi: 10.1186/s13018-020-01595-9.

87. Catrina, A.I.; af Klint, E.; Ernestam, S.; Catrina, S.B.; Makrygiannakis, D.; Botusan, I.R.; Klareskog, L.; Ulfgren, A.K. Anti-tumor necrosis factor therapy increases synovial osteoprotegerin expression in rheumatoid arthritis. Arthritis Rheum. 2006. 54. 76-81. doi: 10.1002/art.21528.
88. Vis, M.; Havaardsholm, E.A.; Haugenberg, G.; Uhlig, T.; Voskuyl, A.E., van de Stadt, R.J.; Dijkmans, B.A.; Woolf, A.D.; Kvien, T.K.; Lems, W.F. Evaluation of bone mineral density, bone metabolism, osteoprotegerin and receptor activator of the NFkappaB ligand serum levels during treatment with infliximab in patients with rheumatoid arthritis. Ann Rheum Dis 2006. 65. 1495-1499. doi: 10.1136/ard.2005.044198.
89. Takita, C.; Fujikawa, Y.; Itonaga, I.; Taira, H.; Kawashima, M., Torisu, T. Infliximab acts directly on human osteoclast precursors and enhances osteoclast formation induced by receptor activator of nuclear factor kappaB ligand in vitro. Mod Rheumatol. 2005. 15. 97-103. doi: 10.1007/s10165-004-0373-7.
90. Palmqvist, P.; Persson, E.; Conaway, H.; Lemer, U.H. IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulates the expression of receptor activator of NF-kB ligand, osteoprotegerin, and receptor activator of NF-kB in mouse calvariae. J Immunol. 2002. 169. 3353-3362. doi: 10.4049/jimmunol.169.6.3353.
91. Finzel, S.; Kraus, S.; Figueiredo, C.P.; Regensburger, A.; Kocijian, R.; Rech, J.; Schett, G. Comparison of the effects of tocilizumab monotherapy and adalimumab in combination with methotrexate on bone erosion repair in rheumatoid arthritis. Ann Rheum Dis 2019. 78. 1186-1191. doi: 10.1136/annrheumdis-2018-214894.
92. Kato, A.; Matsuo, S.; Takai, H.; Uchiyama, Y.; Mihara, M.; Suzuki, M. Early effects of tocilizumab on bone and bone marrow lesions in a collagen-induced arthritis monkey model. Exp Mol Pathol. 2008. 84. 262-270. doi: 10.1016/j.yexmp.2008.03.003.
93. Bozec, A.; Luo, Y.; Engdahl, C.; Figueiredo, C.; Bang, H.; Schett, G. Abatacept blocks anti-citrullinated protein antibody and rheumatoid factor mediated cytokine production in human macrophages in IDO-dependent manner. Arthritis Res Ther. 2018. 20. 24. doi: 10.1186/s13075-018-1527-x.
94. Maxwell, L.J.; Singh, J. Abatacept for rheumatoid arthritis: A Cochrane systematic review. J Rheumatol. 2010. 37. 234-245. doi: 10.3899/jrheum.091066.
95. Axmann, R.; Herman, S.; Zaiss, M.; Franz, S.; Polzer, K.; Zwerina, J.; Hermann, M., Smolen, J., Schett, G. CTLA-4 directly inhibits osteoclast formation. Ann Rheum Dis. 2008. 67. 1603-1609. Doi: 10.1136/ard.2007.080713.
96. Bozec, A.; Zaiss, M.M.; Kagwiria, R.; Voll, R.; Rauh, M.; Chen, Z.; Mueller-Schmucker, S.; Kroczek, R.A.; Heinzerling, L.; Moser, M. et al. T cell costimulation molecules CD80/86 inhibit osteoclast differentiation by inducing the IDO/tryptophan pathway. Sci Transl Med. 2014. 6. 235ra60. doi: 10.1126/scitranslmed.3007764.
97. Okada, H.; Kajiya, H.; Omata, Y.; Matsumoto, T.; Sato, Y.; Kobayashi, T.; Nakamura, S.; Kaneko, Y.; Nakamura, S.; Koyama, T. et al. CTLA4-Ig directly inhibits osteoclastogenesis by interfering with intracellular calcium oscillations in bone marrow macrophages. J Bone Miner Res. 2019. 34. 1744-1752. doi: 10.1002/jbmr.3754.
98. Sims, NA. The JAK1/STAT3/SOCS3 axis in bone development, physiology, and pathology. Exp& Mol Med. 2020. 52. 1185-1197. doi: 10.1038/s12276-020-0445-6.
99. Tamura, T.; Udagawa, N.; Takahashi, N.; Miyaura, C.; Tanaka, S.; Yamada, Y.; Koishihara, Y.; Ousugi, Y.; Kumaki, K.; Taga, T. et al. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin-6. Proc Natl Acad Sci USA. 1993. 90. 11924-11928. doi: 10.1073/pnas.90.24.11924.
100. Morris, R.; Kershaw, N.J.; Babon, J.J. The molecular details of cytokine signaling via the

JAK/STAT pathway. Protein Sci. 2018. 27. 1984-2009. doi: 10.1002/pro.3519.

101. Winkler, I.G.; Sims, N.A.; Pettit, A.R.; Barbier, V.; Nowlan, B.; Helwani, F.; Poulton, I.J.; van Rooijen, N.; Alexander, K.A.; Raggatt, L.J. Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood. 2010.116. 4815-4828. doi: 10.1182/blood-2009-11-253534.
102. Semerad, C.L.; Christpher, M.J.; Liu, F.; Short, B.; Simmons, P.J.; Winkler, I.; Levesque, J.P.; Chappel, J.; Ross, F.P..; Link, D.C. G-CSF potently inhibits osteoblast activity and CXCL12 mRNA expression in the bone marrow. Blood. 2005. 106. 3020-3027. doi: 10.1182/blood-2004-01-0272.
103. LeBranche, T.P.; Jesson, M.I.; Radi, Z.A.; Storer, C.E.; Guzova, J.A.; Bonar, S.L.; Thompson, J.M.; Happa, F.A.; Stewart, Z.S.; Zhan, Y. et al. JAK inhibition with tofacitinib suppresses arthritic joint structural damage through decreased RANKL production. Arthritis Rheum. 2012. 64. 3531-3542. doi: 10.1002/art.34649.
104. Vidal, B.; Cascão, R.; Finnilä, M.A.J.; Lopes, I.P.; da Glória, V.G.; Saarakkala, S.; Zioupos, P.; Canhão, H.; Fonseca, J.E. Effects of tofacitinib in early arthritis-induced bone loss in an adjuvant-induced arthritis rat model. Rheumatology. 2018. 57. 1461-1471. doi: 10.1093/rheumatology/kex258.
105. Li, C.H.; Xu, L.L.; Jian, L.L.; Yu, R.H.; Zhao, J.X.; Sun, L.; Du, G.H.; Liu, X.Y. Stattic inhibits RANKL-mediated osteoclastogenesis by suppressing activation of STAT3 and NF-κB pathways. Int Immunopharmacol. 2018. 58. 136-144. doi: 10.1016/j.intimp.2018.03.021.
106. Murakami, K.; Kobayashi, Y.; Uehara, S.; Suzuki, T.; Koide, M.; Yamashita, T.; Nakamura, M.; Takahashi, N.; Kato, H.; Udagawa, N.; et al. A Jak1/2 inhibitor, baricitinib, inhibits osteoclastogenesis by suppressing RANKL expression in osteoblasts *in vitro*. PLoS ONE. 2017.12: e0181126. doi: 10.1371/journal.pone.0181126. eCollection 2017.
107. Garrett, J.R.; Boyce, B.F.; Oreffo. R.O.; Bonewald, L.; Poser, J.; Mundy, G.R. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. J Clin Invest. 1990.85. 632-639. doi: 10.1172/JCI114485.
108. Haworth, C.; Brennan F.M.; Chantry, D.; Turner, M.; Maini R. N.; Feldmann, M.; Expression of granulocyte-macrophage colony-stimulating factor in rheumatoid arthritis: regulation by tumor necrosis factor-alpha. Eur J Immunol 1991. 21.2575-2579.
109. Alvaro-Gracia, J. M.; Zvaifler, N.J.; Firestein, G.S.; Cytokines in chronic inflammatory arthritis. IV. Granulocyte/macrophage colony-stimulating factor-mediated induction of class II MHC antigen on human monocytes: a possible role in rheumatoid arthritis. J. Exp. Med. 1989. 170, 865-875.
110. Kuroda, T.; Tanabe, N.; Sakatsume, M.; Nozawa, S.; Mutsuka, T.; Ishikawa, H.; Tohyama, C.T., Nakazono, K.; Murasawa, A.; Nakano, M.; et al. Interleukin-2 levels are elevated in the bone marrow serum of patients with mutilans-type rheumatoid arthritis Clin Rheumatol 2002, 21.21-27.
111. Klose, C.S.; Artis, D.; Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. Nat. Immunol. 2016. 17,765-774.
112. Tanaka, M; Watanabe, H.; Kondoh, G.; Tanaka, A.; Yasuda, K.; Kopf, M.; Potocnik, A.J.; Stockinger, B.; Sakaguchi, N.; Sakaguch, S.; Autoimmune Th17 cells induced synovial stromal and innate lymphoid cell secretion of the cytokine GM-CSF to initiate and augment autoimmune arthritis. Immunity, 2018. 48, 1220-1232.e5.
113. Avci, A.B.; Feist, E.; Burmester, G.R.; Targeting GM-CSF in rheumatoid arthritis. Clin Exp Rheumatol. 2016. 34(4 Suppl 98). 39-44.
114. Mulherin, D.; Fitzgerld, O.; Breshinhan, B.; Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum 1996. 39. 115-124.
115. Hercus, T.R.; Thomas, D.; Guthridge, M.A.; Ekert, P.G.; King-Scott, J.; Parker, M.W.; Lopez, A.F. The granulocyte-macrophage colony-stimulating factor receptor: linking its structure to cell signaling and its role in disease. Blood. 2009. 114.1289–1298.

116. Sato, N.; Sakamaki, K.: Terada, N.; Arai, K.; Miyajima, A.: Signal transduction by the high-affinity GM-CSF receptor: two distinct cytoplasmic regions of the common beta subunit responsible for different signaling. EMBO J 1993; 12: 4181-9.
117. Burmester, G.R.: Feist, E.: Sleeman, M.A.: Wang, B.; White, B.; Magrini, F.: Mavrilimumab, a human monoclonal antibody targeting GM-CSF receptor-α, in subjects with rheumatoid arthritis: a randomised, double-blind, placebo-controlled, phase I, first-in-human study. Ann Rheum Dis. 2011. 70. 1542–1549.
118. Burmester, G.R.; Weinblatt, M.E.; McInnes, I.B.; Porter, D.; Barbarash, O.; Vatutin, M.; Szombati, I.; Esfandiari, E.; Sleeman, M.A. Kane, C.D.; et al. Efficacy and safety of mavrilimumab in subjects with rheumatoid arthritis. Ann. Rheum. Dis. 2013. 72. 1445–1452.
119. Takuchi, T.; Tanaka, Y.; Tanaka, S.; Kawakami, A; Iwasaki, M.; Katayama, K.; Rokuda, M.; Izutsu, H.; Ushijima, S.; Kaneko, Y.; et al. Efficacy and safety of peficitinib (ASP015K) in patients with rheumatoid arthritis and an inadequate response to methotrexate: results of a phase III randomised, double-blind, placebo-controlled trial (RAJ4) in Japan. Ann Rheum Dis. 2019. 78.1305-1319.
120. Keystone, E.C.; Taylor, P.C.; Drescher, E.; Schlichting, D.E.; Beattie, S.D.; Berclaz, P.Y.; Lee, C.H.; Fidelus-Gort, R.K.; Luchi, M.E.; Rooney, T.P.; et al. Safety and efficacy of baricitinib at 24 weeks in patients with rheumatoid arthritis who have had an inadequate response to methotrexate. Ann Rheum Dis 2015. 74. 333–340.
121. Schmitz, J.; Owyang, A.; Oldham, E.; Song Y.; Murphy E.; McClanahan, T.K.; Zurawski, G.; Moshrefi, M.; Qin, J.; Xiaoxia Li, X.: et al., IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity, 2005, 23, 479-490.
122. Griesenauer, B.; Paczesny, P.; IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Front Immunol 2017.24:475.
123. Palmer, G.; Talabot-Ayer, D; Lamacchia, C; Toy, D.; Seemayer C.A.; Sébastien Viatte. S.; Finckh. A.; Smith D.E.; Gabay. C.; Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis Arthritis Rheum 2009. 60; 738-249.
124. Talabot-Ayer, D.; McKee, T.; Gindre, P; Bas, S.; Baeten, D.L.; Gabay, C.; Palmer, G.; Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. Joint Bone Spine. 2012. 79. 32-37.
125. Hong, Y.S.; Moon, S.J; Joo, Y.B.; Jeon, C.H; Cho M.L;, Ju, J.H; Oh, H.J; Heo, Y.J.; Park, S.H.; Kim, H.Y.; et al. Measurement of interleukin-33 (IL-33) and IL-33 receptors (sST2 and ST2L) in patients with rheumatoid arthritis. J Korean Med Sci 2011.26.1132-1139.
126. Tang, S.; Huang, H.; Hu, F.: Zhou, W.; Guo, J,; HuJiang, H.: Mu, R.; Li, Z,; Increased IL-33 in synovial fluid and paired serum is associated with disease activity and autoantibodies in rheumatoid arthritis. Clin Dev Immunol, vol. 2013, Article ID 985301.
127. Xiangyang, Z.; Lutian, Y.; Lin, Z.; Liping, X.; Hui, S.; Jing, L.; Increased levels of interleukin-33 associated with bone erosion and interstitial lung diseases in patients with rheumatoid arthritis, Cytokine, 2012. 58. 6–9.
128. Li, Y.; Fu, Y.; Chen, H.; Liu. X.; Li. M.; Blocking Interleukin-33 Alleviates the Joint Inflammation and Inhibits the Development of Collagen-Induced Arthritis in Mice. J Immunol Res. Vol. 2020, Article ID 4297354.
129. Pinto, S.M: Subbannayya, Y.; Rex, D. A. B.; Raju, R.; Chatterjee, O.; Advani, J.; Radhakrishnan, A.; Prasad, T. S. K.; Wani, M.R.; Pandey, A.; A network map of IL-33 signaling pathway. J Cell Commun Signal. 2018. 12:615–624.
130. Norris, M.S.; McConnell, T.J.; Mannie, M.D.: Interleukin-2 promotes antigenic reactivity of rested T cells but prolongs the postactivational refractory phase of activated T cells. Cell Immunol 2001.211: 51 - 60.

131. Tebib, J. G.; T H Boughaba, T. H.; Letroublon, M. C.; Bienvenu, J.; Noel, E.; Armanet, P.; Colson, F.; Roullet, A.; Bouvier, M. Serum IL-2 level in rheumatoid arthritis: correlation with joint destruction and disease progression. Eur Cytokine Netw 1991. 2. 239-243.
132. Li. B.; Guo, Q.; Wang, Y.; Su. R,; Gao, C.; Zhao. J,: Li. X,; Wang. C,; Increased serum Interleukin-2 levels are associated with abnormal peripheral blood natural killer cell levels in patients with active rheumatoid arthritis. Mediators Inflamm. 2020. Article ID 6108342.
132. Wood, N.C.; Symons, J.A.; Duff, G.W.: Serum interleukin-2-receptor in rheumatoid arthritis: A prognostic indicator of disease activity? Journal of Autoimmunity. 1988, 1. 353-361
133. *Firestein, G. S;. Xu W.D.; Townsend, K.; Broide, D.; Alvaro-Gracia, J.; Glasebrook, A.; Zvaifler, N. J.; Cytokines in chronic inflammatory arthritis.* J. Exp. Med. 1988. 168*:* 1573-1586.
134. Thornton, S.; G. P. Boivin, G.P.; Kim K.N.; Finkelman, F.D.; Hirsch, R.; Heterogeneous effects of IL-2 on collagen-induced arthritis. J. Immunol. 2000. 165. 1557–1563.
135. Yokoyama, Y.; Iwasaki, T.; Kitano, S.; Satake, A.; Nomura, S.; Furukawa, T.; Matsui, K.; Sano, H.; IL-2-Anti-IL-2 Monoclonal antibody immune complexes inhibit collagen induced arthritis by augmenting regulatory T cell functions. J Immunol. 2018. 201.1899-1906.
136. Klose, C. S.; Artis, D.; Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. Nat. Immunol. 2016. 17. 765-774.