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REVIEW

Synovial cellular and molecular markers in rheumatoid arthritis

M. Asif Amin[1] · **David A. Fox**[1] · **Jeffrey H. Ruth**[1]

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Abstract The profound alterations in the structure, cellular composition, and function of synovial tissue in rheumatoid arthritis (RA) are the basis for the persistent inflammation and cumulative joint destruction that are hallmarks of this disease. In RA, the synovium develops characteristics of a tertiary lymphoid organ, with extensive infiltration of lymphocytes and myeloid cells. Concurrently, the fibroblast-like synoviocytes undergo massive hyperplasia and acquire a tissue-invasive phenotype. In this review, we summarize key components of these processes, focusing on recently-described roles of selected molecular markers of these cellular components of RA synovitis.

Cellular composition of synovial tissue

Normal synovium is comprised primarily of two cell populations, termed type A and type B synoviocytes [1]. Type A synoviocytes are cells of the monocyte/macrophage lineage, while type B synoviocytes are of mesenchymal origin and are referred to as fibroblast-like synoviocytes (FLS). These two cell populations form the normal synovial lining, which is approximately two cell layers in thickness, lacks a true basement membrane, and is positioned above a poorly-demarcated connective tissue matrix that contains a modest vascular network, a few mast cells and few, if any, lymphocytes.

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✉ David A. Fox
dfox@med.umich.edu

[1] Division of Rheumatology and the Clinical Autoimmunity Center of Excellence, University of Michigan, Ann Arbor, MI, USA

In RA, a massive increase in synovial cellularity occurs that represents a combination of cellular influx, hyperplasia of synovial cells (FLS), and activation/proliferation/differentiation of infiltrating immune system cells. Early disease in RA is associated with synovial vascular inflammation, followed by a robust infiltration of leukocytes into the synovium resulting in hyperplasia of the synovial lining, formation of lymph follicles, and development of the mature pannus. The three most abundant cell types in RA synovium are FLS, monocyte-lineage cells, and T lymphocytes (Table 1), but other cell populations, including B lymphocytes, plasma cells, dendritic cells, vascular endothelial cells, mast cells, and osteoclasts, are all very important in the pathogenesis of RA. Granulocytes (polymorphonuclear cells) are rare in RA synovium but are often the most abundant cellular component of RA synovial fluid. Growing appreciation of the molecular markers expressed in RA synovium not only provides insight into pathogenesis, but also offers targets for molecular imaging of RA synovitis [2].

FLS in RA

FLS are cells of mesenchymal origin that display many characteristics in common with fibroblasts, such as the expression of several types of collagens and the protein vimentin, a component of cytoskeletal filaments. FLS also secrete unique proteins that are normally absent in other fibroblast lineages, such as lubricin, a protein crucial for joint lubrication. Furthermore, these cells express a number of molecules important for the mediation of cell adhesion such as vascular cell adhesion molecule-1 (VCAM-1), various integrins and their receptors, and cadherin-11 [1]. Cadherins are particularly important for intercellular adhesion and coordinate morphogenesis during tissue development. They also support tissue integrity and architecture [3]. FLS express cadherin-11 in vivo, and this

Table 1 Cellular components of the rheumatoid synovium

Abundant cell populations
• T lymphocytes
• Machrophage-like (type A) synoviocytes
• Fibroblastic (type B) synoviocytes
Other cell populations
• Dendritic cells
• B lymphocytes
• Plasma cells
• Mast cells
• Osteoclasts
• Vascular endothelial cells

molecule plays a key role in homotypic adhesion of FLS and in synovial tissue organization (Table 2) [3]. Cadherin-11 can also generate activation signals in FLS, and its function may be regulated by controlled shedding of its N-terminal domain from the FLS surface [3, 4].

Rheumatoid Arthritis Fibroblast-like Synoviocytes (RA FLS) are important mediators of joint destruction, since they are able to invade adjacent collagenous structures, including articular cartilage. Using a severe combined immunodeficient (SCID) mouse chimera model in which human synovium is engrafted into mice, it was shown that inhibition of endogenous p53 leads to increased invasiveness and cellularity of FLS, highlighting the possibility that somatic mutations in the *p53* tumor suppressor gene may contribute to synovial hyperplasia and cartilage damage in RA [5]. Using a similar SCID chimera model, it was shown that RA FLS display active recruitment to naïve cartilage via the neovasculature, independent of the site of application of the RA fibroblasts in vivo, leading to profound destruction of the target cartilage. These experimental findings support the idea that destructive arthritis can spread between joints, which can be partly explained by the transmigration of activated RA FLS [6].

In RA, FLS also secrete factors that promote inflammation, neovascularization, and cartilage degradation [7]. For example, RA FLS express high levels of cyclooxygenase-2 (COX-2) protein and synthesize prostaglandin E2 (PGE2) in vitro, and in rodent models of arthritis [8, 9]. Moreover, these same cells secrete pro-inflammatory and angiogenic chemokines and cytokines [10]. In response to cytokines produced by macrophages such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), RA FLS secrete matrix-degrading enzymes, such as matrix metalloproteinases (MMPs), aggrecanases, and cathepsins [7]. MMPs released from RA FLS can modulate activity of cytokines and chemokines, release pro-apoptotic ligands from cell surfaces, and promote FLS invasion of the cartilage [8]. MMP-2, -3, and -1 aid in tissue invasion and the breakdown of proteoglycans and collagen types I and II [8]. MMPs also activate cathepsins that are potent collagen-degrading proteases produced in the synovium [11]. Moreover, MMPs from RA FLS invade more aggressively in a Matrigel matrix than FLS from osteoarthritis (OA) or avascular necrosis (AVN) synovium [12]. Despite these properties of secreted MMPs, recent work has identified a more crucial role for the membrane-anchored MMP-14 in invasion of types I and II collagen matrices and articular cartilage [13]. Moreover, MMP-14 is also required for the angiogenic response to FLS invasion through articular cartilage [13], and is highly expressed in RA synovium [14].

Chemokines, largely derived from activated fibroblasts, are responsible for recruitment of neutrophils (PMNs) as well as lymphocytes and monocytes. There is evidence for roles of IL-8/CXCL8 [15, 16], epithelial neutrophil-activating protein-78 (ENA-78/CXCL5) [17], monocyte chemoattractant protein-1 (MCP-1/CCL2) [18], macrophage inflammatory protein 1-alpha (MIP-1α/CCL3) [19], CX_3CL1 (fractalkine) [20], MIP-3α [21], CXCL16 [22] and regulated on activation, and normal T cell expressed and secreted (RANTES/CCL5) [23, 24] in RA pathogenesis. Roles for some of these cytokines in RA initiation are possible, as MCP-1/CCL2 and MIP-1α/CCL3 are both increased in RA serum prior to disease

Table 2 Functions of selected surface markers on synovial cell subsets

Cadherin 11	Mediates homotypic adhesion of FLS and important in the organization of normal and diseased synovial tissue
CD13	A surface ectoenzyme expressed by FLS and synovial monocytes. When shed by FLS can function as a chemoattractant
B7H3	The principal B7 family member expressed by FLS-involved in T cell/FLS interactions
MMP-14	A membrane-anchored matrix metalloproteinase that is the enzyme principally involved in FLS invasion of collagenous structures, including articular cartilage
PD-1	A surface marker for a novel subset of CD4+ T cells that is expanded in RA synovium and that supports B cell differentiation

FLS fibroblast-like synoviocytes (also known as type B synoviocytes), *PD-1* programmed cell death protein-1 (also known as CD279)

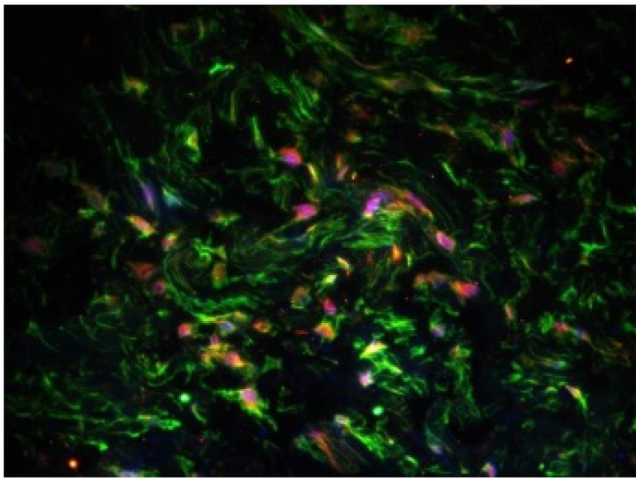

Fig. 1 Strong expression of CD13 in RA synovium. CD13 (*green*) in RA synovium, co-localized (*yellow*) with cadherin-11 (*red*), a marker of FLS. CD13 is also extensively expressed on monocyte-macrophage lineage cells in RA synovial tissue, which do not express cadherin-11

onset [25]. Specific chemokines, including MCP-1, RANTES, growth-regulated protein alpha (Gro-α/CXCL1), and IL-8 are produced by RA FLS via distinct signaling pathways that could become targets for new therapeutic approaches [26].

Other chemoattractant proteins, distinct from conventional chemokines, also may have important roles in RA. For example, aminopeptidase N/CD13, an ecto-peptidase expressed by myeloid cells and FLS, has recently been examined in RA (Fig. 1). CD13 is released into synovial fibroblast culture supernatants by MMP-14 mediated shedding of CD13 from the FLS membrane [27, 28] and is found in high concentrations in RA synovial fluid. Using an as yet unidentified G-protein-coupled receptor and independent of its enzymatic activity, CD13 induces chemotaxis of cytokine-activated T cells, a T cell population similar to that found in RA synovium, at a concentration that is similar to the gradient between the CD13 concentration in RA serum versus RA synovial fluid, suggesting that CD13 could play an important role as a T cell chemoattractant in a positive feedback loop that contributes to RA synovitis [27].

Angiogenesis is also considered to be an invasive process that requires proteolysis of the extracellular matrix, proliferation and migration of endothelial cells, as well as synthesis of new matrix components. Angiogenesis is a key event in the expansion of the synovial lining of joints. CXC chemokines as well as some CC chemokines, that are potent inducers of angiogenesis, are primarily produced in the RA joint by FLS [16, 20, 22, 29]. Typically, CXC chemokines are pro-angiogenic if they possess the ELR motif, but anti-angiogenic if they do not [29].

Citrullination of various proteins in RA, mediated by various isoforms of peptidyl arginine deiminase (PAD), is increasingly recognized as not only a mechanism for generation of RA-specific autoantigens, but also as a pathway for altering the function of various inflammatory mediators and receptors. PAD4 is expressed by FLS, supporting strong evidence for citrullination of FLS proteins being integral to inflammation and the pathogenesis of RA [30–33]. For example, citrullination of ENA-78 converts the primary target of this chemokine from the neutrophil to the monocyte and changes its pattern of chemokine receptor engagement [33]. A second example is the citrullination of calreticulin which enhances the ability of this molecule to function as a novel cell-activating receptor for the shared epitope MHC allele that is associated with susceptibility to and severity of RA [34].

RA FLS also display an altered phenotype compared to cells present in normal synovium. This can be easily observed in culture in which RA FLS lose contact inhibition and dependency, thus contributing to the increase in the number of fibroblasts in the inflammatory tissue [10]. This is remarkably similar to the growth of progenitor like cells. Hyperproliferating synoviocytes in the RA joint have many features of progenitor cells and display proteins that are uniquely attributable to both cell phenotypes. For example, Inhibitor of DNA binding 1 (Id1) is a nuclear protein containing a basic helix-loop-helix (bHLH) domain that regulates cell growth and is expressed in endothelial progenitor cells [35]. Histologic analysis of synovial tissue reveals that Id1 is also highly expressed in the vasculature of RA synovial tissue [35], and by FLS [36], suggesting that Id1 displays pleiotropic properties in cells that exhibit hyperproliferative responses. RA synovial fluid also contains abundant amounts of Id1 with the primary source being from activated FLS [36]. Once released, Id1 acts as a potent inducer of angiogenesis [35]. Id1 is also packaged into FLS exosomes and released, setting the stage for the intriguing possibility that Id1 (and possibly other nuclear proteins) may be delivered to other cells within the RA synovium that do not themselves produce Id1 [36]. Similarly, alveolar macrophages secrete the STAT-induced STAT signaling inhibitors SOCS1 and SOCS3 in exosomes and microparticles for uptake by alveolar epithelial cells and subsequent inhibition of STAT activation in vitro and in vivo [37].

FLS may also amplify joint inflammation by cognate cell–cell interactions within the rheumatoid synovium. Such interactions may alter the phenotype of FLS, and also provide additional pathways for FLS to exert control over the behavior of the other cell types in RA synovium. Interactions between FLS and T lymphocytes are considered in more detail below. The multiple interactions between FLS and other key cell populations in RA, through secreted mediators, microparticles, and direct cell–cell contact, create numerous positive feedback loops that enhance the inflammatory environment within the RA synovium, attracting and activating more

immune cells and ultimately contributing to joint destruction [38–40].

Monocytic cells in RA

Monocyte (MN)/macrophage recruitment into and activation within the synovial membrane are critical steps in the pathogenesis of RA. An early hallmark of active RA is an increased number of synovial MNs/macrophages which correlates with the degree of joint erosion while their depletion from inflamed synovium results in therapeutic benefit [41, 42]. Upon recruitment and activation in the synovial tissue (ST), these MNs/macrophages secrete pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 which contribute to RA pathogenesis [43]. Biologic therapies targeting these mediators highlight their importance in RA. In this section, we will discuss the heterogeneity of human and murine MNs/macrophages and their characteristics and functions in RA.

Macrophages are co-localized with FLS in the normal ST membrane where the predominant cells are FLS. In RA, a greater fraction of the cells in ST are MNs/macrophages [44]. Resident tissue macrophages are typically derived from circulating MNs, which originate from $CD34^+$ bone marrow progenitors. Recent lineage-tracing studies indicate that some tissue-resident macrophages originate from embryonic precursors instead of $CD34^+$ bone marrow progenitors [45] and maintain themselves by self-renewal [46]. For example, intestinal lamina propria and dermal macrophages originate from $CD34^+$ bone marrow progenitors and are continually replaced by MNs from the circulation [47, 48]. The sources of ST macrophages and their self-renewal capacity have not been characterized in humans.

Murine MN subset classification and functions have been well characterized and accepted [49]. However, classification of human MN subsets linked to their functional phenotypes remains only partially defined. Two major MN subsets have been identified in mice: "classical" ($Ly6C^{hi}$ $CX3CR1^{int}$ $CCR2^+$); and "non-classical" ($Ly6C^{low}$ $CX3CR1^{hi}$ $CCR2^-$) [50]. $Ly6C^+$ is further divided into $Ly6C^{hi}$ and $Ly6C^{int}$. The surface markers for $Ly6C^+$ subsets are $CD11b^+CD115^+$ and chemokine receptors are $CCR2^{hi}CX3CR1^{low}$. The surface markers and chemokine receptors for $Ly6C^-$ ($Ly6C^{low}$) MNs are $CD11b^+CD115^+$ and $CCR2^{low}CX3CR1^{hi}$ [51].

$Ly6C^{hi}$ MNs, referred as inflammatory MNs, require CCR2 expression for egress from the bone marrow [52, 53]. Granulocyte-macrophage colony-stimulating factor (GM-CSF), a pro-inflammatory cytokine involved in myeloid cell differentiation, plays an important role in many autoimmune diseases, including RA, by regulating the inflammatory signature and progeny of $Ly6C^{hi}$ MNs [54]. $Ly6C^{hi}$ MNs contribute to the initiation of tissue inflammation [55], secrete TNF-α, nitric oxide, and IL-1β upon bacterial infection [56] and type 1 interferon (IFN) in response to viruses [57]. The interaction between CCR2-CCL2 in $Ly6C^{hi}$ MNs alters the conformation of very late antigen-4 (VLA-4), which results in increased MN transmigration into inflamed tissue [58]. $Ly6C^{hi}$ MNs also differentiate into M1 macrophages, which secrete pro-inflammatory cytokines such as TNF-α and IL-6 and contribute to tissue degradation and T cell activation.

CD14 is highly expressed on the surface of human MNs and macrophages and is a pattern recognition receptor. Three subsets of MNs have been defined in humans: classical ($CD14^{++}CD16^-$), intermediate ($CD14^{++}CD16^+$), and non-classical ($CD14^+CD16^{++}$) [59]. The classical and non-classical human MNs are homologous to their classical and non-classical counterparts in mice. Functionally, classical ($CD14^{++}CD16^-$) and intermediate ($CD14^{++}CD16^+$) MNs possess more phagocytic and pro-inflammatory properties than non-classical MNs [60].

Intermediate ($CD14^{++}CD16^+$) MNs express CCR2 and selectively CCR5, which bind, respectively, MIP-1α, which is chemotactic for macrophages, and CCL5/RANTES. Intermediate MNs produce significantly more TNF-α and IL-1β compared to other populations in response to lipopolysaccharide (LPS); however, production of IL-6, IL-8, and IL-10 are not different in intermediate and classical monocytes when compared to non-classical monocytes [60, 61]. Human $CD14^{++}CD16^+$ intermediate monocytes display pro-inflammatory functions and are involved in antigen presentation and T cell activation, as indicated by gene signature studies [61]. These MNs produce TNF-α and IL-1β in high concentration in response to LPS but have low peroxidase activity [60]. During inflammation, classical and intermediate monocytes adhere to and invade tissue by interaction of CCR2/CCL2 (MCP-1/CCL2) and/or CCR5/CCL5 in a very late antigen 1 (VLA1)/VCAM1 dependent manner. The frequency of intermediate MNs is increased in peripheral blood of patients with chronic RA compared to sex- and age-matched healthy donors, while the frequency of non-classical $CD14^+CD16^{++}$ MNs does not differ between patients and controls [62, 63]. However, another study found that absolute cell counts of both intermediate and non-classical MN populations are increased in RA patient blood [64].

Human $CD14^+CD16^{++}$ non-classical MNs patrol the vessel wall and invade tissues when triggered by interaction of CX3CR1 with CCL3 in a lymphocyte functional antigen-1 (LFA-1)/intercellular adhesion molecule-1 (ICAM-1)-dependent manner. This subset releases IL-1β and TNF-α in response to ligation of toll-like receptor 7 (TLR7) or TLR8, suggesting a pathological role in autoimmune disease [60]. Classical MNs represent ~90% of blood MNs while the number of CD16 expressing cells is very small in healthy individuals but the number of CD16 expressing cells increases in infection and inflammatory conditions [59]. Classical ($CD14^{++}CD16^-$) and intermediate $CD14^{++}CD16^+$ MNs resemble mouse $Ly6C^+$ inflammatory MNs, while non-

classical ($CD14^+CD16^{++}$) MNs act like $Ly6C^-$ anti-inflammatory MNs and patrol vascular endothelium [50]. However, some studies suggest a pro-inflammatory role of $CD14^+CD16^{++}$ cells because of the production of inflammatory cytokines.

In RA MNs/macrophages produce pro-inflammatory cytokines, including TNF-α, IL-6 and IL-1, and MMPs, leading to endothelial cell activation and cartilage damage (49.50). These cells also secrete various chemokines which help to recruit more leukocytes into the inflamed joint. $CD14^+CD68^+$ monocytes have an activated phenotype with increased expression of human leukocyte antigen (HLA-DR; involved in antigen presentation to $CD4^+$ T cells), costimulatory molecules (CD80, CD86, and CD40), adhesion molecules such as ICAM-1/CD54, and some chemokine receptors [65–69]. Depletion of activated MNs by repeated leukapheresis in patients with active RA leads to improvements in disease activity that persist for 10–12 weeks [70, 71].

CD68 and CD163 are two commonly used markers for identification of synovial macrophages in RA synovium. CD68 is a scavenger receptor that binds to oxidized low-density lipoprotein and is involved in cell–cell interactions [72]. The number of synovial $CD68^+$ macrophages correlates with disease activity, indicating the importance of these cells in RA pathogenesis [41, 73, 74]. CD68 is present on both cell surface and lysosomal membranes [75]. CD68 is a sensitive biomarker to predict the possible efficacy of new anti-rheumatic treatments, as the changes in the number of synovial sublining CD68+ macrophages correlate with clinical improvement [76]. The synovial sublining CD68+ macrophage count is correlated significantly with radiologic outcome and radiologic progression in RA patients [41]. CD163 is a type I transmembrane protein that belongs to the group B scavenger receptor cysteine-rich superfamily [77]. CD163 may be a better macrophage marker as compared with CD68 in RA synovium, because it discriminates between synovial macrophages and synovial intimal fibroblasts, some of which also stain positive for CD68 in diseased tissue [78]. Additionally, soluble CD163 in sera is a promising diagnostic marker for untreated new-onset systemic juvenile idiopathic arthritis and macrophage activation syndrome [79].

Polarized macrophages are referred to as M1 or M2 cells. Classically activated M1 macrophages are induced by IFN-γ, LPS, GM-CSF, and TNF-α, whereas alternatively activated M2 macrophages are induced by IL-4, IL-13, monocyte colony stimulating factor (M-CSF), immune complexes, IL-10, and glucocorticoids [80]. Transcription factors involved in M1 macrophage polarization include NF-κB, STAT1, and interferon regulatory factor 5 (IRF5), while IRF4, STAT6, c-Myc, PPARγ, and Kruppel-like factor 4 are involved in M2 polarization [81]. M1 macrophages express a high level of TNF-α, IL-1, IL-6, IL-23, IL-12, type I IFN, reactive nitrogen intermediate (RNI), reactive oxygen intermediate (ROI), and CXCL9, 10, and 11, while M2 macrophages express a high level of IL-4, IL-10, CD163, CD206, and CCL16, 17, 18, 22, and 24 [80, 82]. M1 polarized macrophages are generally involved in resistance to intracellular pathogens and to tumors in the context of Th1-driven responses, whereas M2 polarized macrophages are effectors of resistance to parasites, have immunoregulatory properties, promote tumor growth and invasiveness and orchestrate tissue repair and remodeling (including fibrosis) [83, 84]. The M1/M2 model is a useful scheme, but it may not fully reflect the flexibility and the diversity of the monocyte/macrophage activation process in various organs involved in immune-mediated diseases, in which many intermediate and diverse phenotypes have been demonstrated [85, 86]. The polarization state of synovial macrophages in RA is not yet well understood.

Expression of GM-CSF and M-CSF is increased in synovial fluid from RA patients. TNF-α and IL-1 induce production of GM-CSF and M-CSF by synovial fibroblasts and chondrocytes, suggesting that GM-CSF and M-CSF play a pathogenic role in arthritis. Both GM-CSF-deficient and Csf1op/op mice are resistant to collagen-induced arthritis [87]. Consistently, blockade of GM-CSF or M-CSF inhibits the development of arthritis [87, 88]. Phase 1 or phase 2 clinical trials and preclinical studies for inflammatory arthritis have employed specific antibodies against GM-CSF, M-CSF or their receptors [54, 89].

T lymphocytes in RA

The T cell population in RA is polyclonal [90] and includes increased numbers of activated and memory cells compared to peripheral blood T cells (Fig. 2). Activated T cells in RA synovium express a combination of surface markers that characterize both early (CD69) and late (class II MHC) stages of T cell activation [91]. To what extent RA synovial T cells are activated elsewhere in the body, with subsequent migration to synovial tissue, versus stimulation by antigen and second signals from antigen-presenting cells locally, is not known, but both scenarios are likely. T cells can also be activated by cocktails of pro-inflammatory cytokines (including cytokines present in RA synovium), and such T cells display an array of surface structures similar to T cells isolated directly from the RA joint [91]. Moreover, these cytokine-activated T cells (Tck) are especially potent in inducing macrophages to secrete TNF [92], and interact closely with FLS in vitro, which results in activation of both cell types [93, 94]. Surface structures involved in these interactions include membrane-anchored TNF on the Tck [93] and B7-H3 on the FLS [94]. FLS lack robust expression of CD80 (B7.1) and CD86 (B7.2), along with most other members of the B7 family, but express B7-H3 constitutively, as do fibroblasts from other tissues [94]. The identity of the receptor(s) for B7-H3 on the T cell surface remains controversial. While many "professional" antigen-

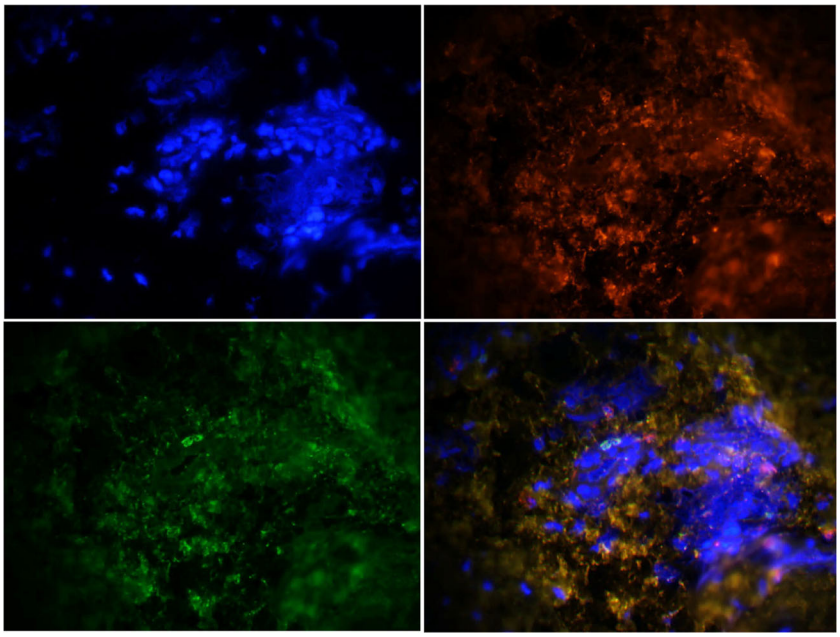

Fig. 2 A dense infiltrate of activated T cells in RA synovium. The CD30 molecule (*upper right panel*) is expressed on activated T and B lymphocytes, and the CD3 complex (*lower left panel*) is present on all T cells. CD30, which is found on a very small proportion of circulating lymphocytes, is expressed by >90% of the synovial T cells. The *lower right panel*, showing merged *red* and *green* fluorescence, which generates a *yellow* color, indicates that the great majority of the CD30+ cells in the RA synovium are T cells. The *blue* color is DAPI, which identifies nuclei

presenting cell populations are present in RA synovium, including dendritic cells, macrophages, and B lymphocytes, RA FLS also can present superantigen to naïve T cells [95], and arthritogenic, HLA-DR4-restricted peptide autoantigens to previously activated T cells [96]. Interactions between CD4 T cells and monocytes in RA synovium also have multiple important consequences, which can include induction of differentiation of pathogenic polarized T cell and macrophage subpopulations, differentiation of osteoclasts in synovial tissue, secretion of pro-inflammatory mediators, and induction of or protection from apoptosis [97].

RA synovium is a site of autoantibody production and some RA synovia contain structures that resemble germinal centers. A recent report describes a novel CD4+ T cell subset that is distinct from T follicular helper cells and that appears to be the principal T cell subset that drives B cell to plasma cell differentiation in RA synovium [98]. These cells express PD-1 and lack CXCR5, but express other chemokine receptors that direct migration to the joint. Expression of surface SLAMF5 and secretion of IL-21 are important in the interaction of these cells with B lymphocytes [98]. Another group recently demonstrated that a subset of CD4+ IL-21-producing T cells was expanded in RA synovial fluid, and that some of these cells also produced TNF-α and RANK-ligand [99]. Either IL-21 or co-culture with these T cells augmented FLS production of MMPs and IL-6 [99]. Interleukin 21 also promotes FLS migration and tissue invasion [100], suggesting that this novel T cell subset and/or its key mediators may become an important therapeutic target in RA.

Regulatory T cells (Tregs) are essential for prevention of autoimmunity, and Treg defects have been sought in many autoimmune diseases, including RA. CD4+ Tregs, which express CD25 and the transcription factor FoxP3, are present in RA, both systemically and in the joint. While not found to be numerically deficient in most studies, they appear to have impaired function, a defect that can be reversed in some patients following successful treatment of RA [101].

Heterogeneity of RA synovium and the potential for optimization of targeted therapies

The extent of lymphoid tissue organization is known to be heterogeneous among RA patients [102]. Recent work has extended our understanding of RA synovial tissue heterogeneity by global analysis of synovial tissue gene expression [103]. This analysis led to molecular definition of four RA synovial subsets, termed lymphoid, myeloid, fibroid, and low inflammatory. These subsets were also distinct when analyzed by immunohistochemistry and by flow cytometry. T cells were present in all subsets, but B cells were found only in the lymphoid and myeloid subsets and were abundant only in the lymphoid subset. Based on the patterns of gene expression in the lymphoid and myeloid subsets, two circulating biomarkers were identified: soluble ICAM1 (expressed most strongly in the myeloid-dominant RA synovial tissue) and CXCL13 (expressed most strongly in the lymphoid-dominant RA synovial tissue), that are elevated in RA sera but which do not correlate with each other with respect to their

serum concentrations. Baseline samples were then assayed for these two proteins, from an RA therapeutic trial that compared two biologic agents, adalimumab (anti-TNF) and tocilizumab (anti-IL-6R). Patients with high sICAM1 and low CXCL13 were more likely to achieve a robust clinical response ACR50) with adalimumab, while those with low sICAM1 and high CXCL13 were much more likely to achieve an ACR50 with tocilizumab [103]. If replicated, these findings point towards selection of optimal biologic therapeutics for individual RA patients, based on circulating biomarkers that reflect the molecular composition of synovial tissue subtypes.

Conclusion

RA synovium may be an environment that is sufficiently unique to promote patterns of gene expression, inflammatory mediator production, cell surface protein expression, and cell–cell interactions that are distinct from those essential to normal host defenses and immune system function. For example, the hypoxic conditions in RA synovium lead to the local production by FLS of hypoxia-inducible factor-1alpha, which alters FLS gene expression in multiple ways relevant to the pathogenesis of RA [104], including augmentation of production of pro-inflammatory cytokines and of interactions of FLS with both T and B cells. The pathogenesis of RA does not reflect the actions of any one cell lineage, but rather the complex interactions between all cell populations in RA synovium, mediated by both direct cell–cell contact and by molecules that are secreted or shed by the various types of synovial cells. This network of multiple interactions is perhaps surprisingly non-redundant, since neutralization of a single inflammatory mediator, (out of dozens or hundreds that are present in the RA synovium), can be clinically effective. Further advances in the efficacy and safety of the treatment of RA are likely to arise from definition of pathogenic pathways that are less relevant to host defenses against infection or neoplasm, and from identification of clinically meaningful heterogeneity in the molecular pathogenesis of RA that will guide selection of therapeutics.

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