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Cell-cell Interactions in Rheumatoid Arthritis Synovium

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Synopsis

Understanding the pathogenesis of joint inflammation and destruction in rheumatoid arthritis involves dissection of the cellular and molecular interactions that occur in synovial tissue. Development of effective targeted therapies has been based on progress in achieving such insights. Safer and more specific approaches to treatment could flow from discovery of cell-cell interaction pathways that are relatively specific for inflammation of the joint, and less important in defense against systemic infection. This chapter highlights selected cell-cell interactions in rheumatoid arthritis synovium that may be worthy of evaluation as future therapeutic targets.

Keywords

synovial fibroblasts; T lymphocytes; B lymphocytes; antigen presenting cells; endothelial cells; cytokines

Introduction

Although the cause of rheumatoid arthritis (RA) remains unknown, insights into the pathogenesis of RA have been achieved by careful study of inflammatory, immune and tissue-destructive processes that take place in synovial tissue. Successful approaches have included *ex vivo* analysis of RA synovium, experiments in animal model systems, and use of cultured cell lines, especially fibroblast-like synoviocytes, derived from patients' synovial tissue. These

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insights have led to remarkable advances in the treatment of RA and other diseases, such as the use of TNF-blocking biologics.

None of the molecular targets of medications currently used in the treatment of RA are expressed uniquely in synovial tissue; instead, all of them are of great importance in host defense as well. Nevertheless, recent research is revealing important molecules and pathways pertinent to joint inflammation and damage that may be less central to hosts defenses compared to currently-targeted molecules. A conceptual framework for such investigations is the realization that although RA synovium can display some features of an immune organ and is justifiably regarded as a tertiary lymphoid structure, it also contains cells that are quite distinct from those found in primary or secondary lymphoid tissue – namely, the intrinsic structural cells of the joint such as fibroblast-like synoviocytes.

It is clear that no one cell type explains the pathologic behavior of RA synovial tissue. Rather it is the interactions between these cells that define the disease. The three most abundant cell populations in RA synovium are the monocyte/macrophage (type A) synoviocytes, the fibroblast-like (type B) synoviocytes, and T lymphocytes (which are strikingly heterogeneous). Other critically important cells of the RA synovium include B lymphocytes, plasma cells, dendritic cells, mast cells, endothelial cells, osteoclasts and adjacent chondrocytes. These various cell types can interact in two general ways: first through secreted mediators, notably inflammatory cytokines such as TNF, IL-6, IL-17 and many others; and second through direct cell-cell contact that is mediated by cell surface receptors and ligands, including some membrane-anchored cytokines.

This chapter will focus on selected cell-cell interactions that may be important in the pathogenesis of RA. It is hoped that the relative molecular specificity of some of these interactions for events in the joint compared to the systemic immune response will ultimately provide more specific targets (Table 1) for a new generation of biologic and non-biologic therapeutics.

Homotypic and autocrine interactions of fibroblast-like synoviocytes (FLS)

Cadherin-11

During the course of RA the cells of the synovial lining undergo extensive hyperplasia to form the synovial pannus which invades and destroys cartilage and bone. Recent discoveries have highlighted a critical role for cadherin-11 in these events. Cadherin-11 has been identified as a strongly expressed intercellular adhesion molecule on both human and mouse FLS^{1,2}. Transfection of L cells with cadherin-11 led to formation of sheet-like structures with an organization similar to that seen in synovial lining¹; furthermore, cadherin-11 localized to cell-to-cell junctions between FLS². Joints in cadherin-11 null mice had an underdeveloped synovial lining and decreased extracellular matrix². This data indicates that cadherin-11 plays a vital role in formation of the synovial lining layer by mediating FLS/FLS connections.

Cadherin-11 staining correlated strongly with cellular infiltration of macrophages and T lymphocytes in RA synovium³. Cadherin-11 staining also correlated with erythrocyte sedimentation rate and C-reactive protein level although not as strongly³. Cadherin-11 expression in synovium is not specific to RA, as cadherin-11 staining on synovial biopsies was similar in inflamed joints from RA, osteoarthritis (OA), and psoriatic arthritis (PsA)³. There was also a positive correlation between cadherin-11 staining of lung tissue from patients with RA-associated interstitial pneumonitis (IP) and CD4+ T cell infiltration of the lung³. Cadherin-11 null mice showed an average of 50% reduction in clinical arthritis activity in the K/BxN serum transfer model². Cadherin-11 has been explored as a possible therapeutic target using the same mouse serum transfer model. Both cadherin-11-Fc and an anti-cadherin-11

mAb ameliorated clinical arthritis when administered with arthritogenic serum². More significant to potential treatment of human disease, anti-cadherin-11 mAb ameliorated established arthritis in a modified K/BxN serum transfer model².

Fractalkine and its receptor

Fractalkine (FKN) is a potent chemoattractant and adhesion molecule that is found in increased levels in RA synovium. RA FLS secrete FKN and express its receptor, CX₃CR1⁴. Soluble FKN induced proliferation of FLS which was blocked by addition of anti-CX₃CR1⁵. Even in the absence of sFKN the antibody was able to decrease FLS proliferation, thus revealing an autocrine growth loop⁵. FKN also induced migration of RA FLS and caused significant reorganization of F-actin within FLS⁴. It is likely that FKN could act in an autocrine fashion to aid in pannus invasion of the bone and cartilage through FLS growth and migration.

FLS/T cell Interactions

In RA, FLS as well as other cells produce chemokines that attract T cells to the joint. FLS and T cells then interact in the synovium through both secreted factors and direct cell-to-cell interactions, resulting in activation of both cell types. FLS proliferate when cocultured with CD4⁺ T cells, especially when RA T cells are used⁵.

Synovial T cells in RA patients include an expanded population of CD4⁺CD28⁻ cells and this subset of cells in particular greatly enhances FLS proliferation. CD4⁺CD28⁻ cells, which are sometimes considered to be senescent, aberrantly express CX₃CR1, and anti-CX₃CR1 decreases the FLS growth-promoting activity of these cells without a significant effect on CD4⁺CD28⁺ T cells⁵. Stimulation of CD4⁺CD28⁻ T cells by FKN through CX₃CR1 increases TNF α production, and TNF α can then act on FLS to increase growth, FKN secretion, and CX₃CR1 expression^{4,5}. This data suggests an important relationship between TNF α and FKN/CX₃CR1 in FLS/T cell interactions in the RA joint, in which production and action of these molecules occurs in linked paracrine and autocrine loops involving T cells and FLS.

IL-15

IL-15 is constitutively expressed on FLS and is a potent T cell growth factor that can cause activation/proliferation of both effector T cells (T_{eff}) and regulatory T cells (T_{reg}) in T cell/FLS cocultures⁶. Moreover, IL-15 can decrease apoptosis of various cell types including FLS and T cells^{7,8}. The IL-15 receptor is a trimer - IL-15R α , β , γ_c - and subunits of IL-15R are expressed by various cell types, including FLS and T cells⁷. IL-15 can function either as a secreted or membrane bound cytokine, with signaling similar to other cytokines through the full trimeric IL-15R or through dimeric IL-15R β γ_c receptor (*cis*-presentation)⁷. IL-15 can also signal through a unique *trans* signaling form, in which distinct subunits of IL-15R are expressed on the surface of interacting cells. IL-15 can even be recycled by the cell and presented on the cell surface by IL-15R α , a pathway that allows for persistence of an IL-15 signal even when soluble IL-15 is no longer available⁷. In cultures containing both T_{eff} and T_{reg} co-cultured with RA FLS, proliferation and function of both T cell subsets was stimulated, with a net pro-inflammatory effect. These effects were not observed when OA FLS or dermal fibroblasts were used and were dependent upon cell-to-cell contact. Neutralizing IL-15 during T cell/FLS cocultures significantly attenuated the proliferation of T_{eff} and T_{reg}, T_{eff} production of TNF α and IFN γ , and T_{reg} inhibition of T_{eff}⁶. The overall pro-inflammatory effect of IL-15 makes it a potential target for RA therapy. A proof-of-concept study has been conducted using a human IgG1 anti-IL-15 monoclonal antibody in RA. This antibody suppressed effects of IL-15 *in vitro*, and showed meaningful efficacy in phase I-II trials⁸.

FLS as APCs

FLS can also act as antigen presenting cells (APCs) in the initiation of T lymphocyte responses. For example, FLS induced secretion of IL-2 by class II MHC-restricted CD4⁺ T cell hybridomas specific for arthritogenic autoantigens, specifically human cartilage gp-39 (HC gp-39) and human type II collagen (CII)⁹. The T cell hybridomas in these experiments were developed from HLA-DR4 transgenic mice, and therefore respond to peptide antigens that are loaded onto and presented by HLA-DR4. Activation of the hybridomas required re-expression of class II on the FLS, which occurs *in vivo* in RA and which is re-induced *in vitro* by IFN γ . The T cell response to peptide antigen presented by FLS was exquisitely MHC-restricted, identical to experiments in which professional APC were used⁹, and blocking antibodies to either human class II MHC or murine CD4 prevented IL-2 production. Since T cell hybridomas do not require a second signal in order to respond to peptide antigen, this system was not useful for defining potential co-stimulatory ligands on FLS. Of note, FLS do not express the classic APC co-stimulatory molecules CD80 or CD86 (B7-1, B7-2) at functionally relevant levels¹⁰.

B7-H3

Of the molecules that belong to the B7 family, B7-H3 is expressed strongly and constitutively on FLS *in vitro*¹⁰. Moreover, immunostaining of RA synovium showed broad B7-H3 expression nearly identical in distribution to the FLS marker cadherin-11. Dual color immunohistochemical analysis showed CD3⁺ T cells in close proximity to FLS expressing B7-H3¹⁰. Furthermore, in co-culture experiments B7-H3 localized to the contact point between FLS and cytokine activated T cells (Tck), or during FLS presentation of superantigen to T cells¹⁰. Prior studies had indicated that B7-H3 can have either stimulatory or inhibitory effects on T cells, and results with FLS/T cell co-cultures were consistent with a dual role for B7-H3. RNAi knockdown of B7-H3 in FLS decreased production of TNF α , IFN γ , and IL-2 by co-cultured Tck but increased production of these cytokines by resting T cells¹⁰. The T cell ligand or ligands for B7-H3 have not yet been defined. B7-H3, in contrast to B7-1 and B7-2, is expressed on human solid tumors¹¹, in part controlled by the microRNA miR-29¹². In early-phase human clinical trials, the B7-H3-specific mAb 8H9 was reported to prolong survival in patients with solid tumors and central nervous system metastasis¹². Further trials of anti-B7-H3 in cancer could yield safety information pertinent to consideration of therapeutic trials of this antibody in RA.

T cells and APCs in the RA synovium

The following paragraphs will highlight examples of selected cytokine-mediated and cognate cell-cell contact driven interactions between T cells and synovial APCs that are current or potential therapeutic targets for the treatment of RA. Interruption of T cell co-stimulation between T cells and antigen presenting cells (APC)s in the synovium is a worthwhile approach given the clinical success of blocking T cell co-stimulation with CTLA-4Ig in rheumatoid arthritis (RA). Resting T cells require at least two signals to differentiate into effector T cells. The first signal through engagement of the T cell antigen receptor by the antigen-MHC complex on the APC, and the second signal by engagement of co-stimulatory molecules such as CD28, on T cells by ligands such as CD80/86 on APCs. Effector T cell differentiation leads to the expression of additional surface molecules. These inducible structures may have stimulatory (ICOS, OX40) or inhibitory (CTLA-4) potential.

CTLA-4 and IDO

CD28 is the prototypic TCR for co-stimulatory signals. The ligation of CD28 by CD80/86 (B7.1 and B7.2) sends activating signals into the T cell and the APC. CTLA-4 (CD152) is up-regulated on activated T cells and binds to the CD28 ligands CD80/86. The ligation of CD80/86 by CTLA-4, sends inhibitory signals directly into the T cell. The ligation of CD80/86 by

CTLA-4 can also deliver regulatory signals to the APC. The interaction of CD80/86 with CTLA-4 leads to the induction of indoleamine dioxygenase (IDO) in APCs¹³. IDO is thought to be critical in inducing anergy in T cells, as IDO depletes tryptophan which is necessary for T cell activation. Blocking the activity of IDO in a mouse model of arthritis led to increased severity of arthritis and accumulation of Th1 and Th17 cells in the inflamed joints^{14,15}. Conversely, administration of L-kynurenine, a metabolite of L-tryptophan, resulted in amelioration of arthritis. These findings suggest that manipulation of tryptophan degradation as a therapeutic target in RA¹⁵.

OX40/OX40L

OX40 (CD134) is predominantly expressed on activated CD4 and CD8 T cells following stimulation via TCR and CD28. Pro-inflammatory cytokines IL-1, IL-2 and TNF- α can further augment the expression of OX40. The ligand of OX40, OX40L (CD252), is expressed on APCs including dendritic cells, B cells and macrophages.¹⁶ OX40L is induced on APCs after stimulation via CD40¹⁷. There is bidirectional activation of T cells and APCs via the OX40/OX40L pathway. Stimulation of OX40 on T cells induces proliferation and cytokine secretion and signaling via OX40L into APCs induces the secretion of pro-inflammatory cytokines by the APCs¹⁷.

In RA, there is increased expression of OX40 on peripheral blood CD4 T cells with a trend towards a positive correlation with serum CRP levels¹⁸. OX40 is expressed on both CD4 and CD8 positive synovial T cells, more so on CD4 positive T cells, and levels of expression correlate with disease activity^{19–21}. OX40 as well as OX40L expressing cells are also present in the RA synovium¹⁹. Although there is increased expression of OX40 and OX40L in the RA synovium, the role of OX40/OX40L in mediating immune events in RA is unclear. However, preclinical studies in animal models blocking the interaction of OX40/OX40L have shown promise¹⁹.

CCL-20

Chemokines mediate inflammatory responses by stimulating the recruitment of leucocytes. The chemokine CCL20 (MIP-3 α) and its receptor CCR6 play a role in the migration of different cell types to the RA synovium. CCL20 is a CC-chemokine expressed on macrophages, dendritic cells and lymphocytes. CCR6, the receptor for CCL20, is expressed on Th17 cells, immature dendritic cells and B cells. CCL20 is chemotactic for Th17 cells and dendritic cells which express CCR6. Th17 cells secrete CCL20 and recruit other CCR6-expressing Th17 cells to the site of Th17 cell-mediated damage²². Increased levels of CCL20 have been observed in the synovial fluid of RA patients compared to osteoarthritis patients and protein concentrations of CCL20 are elevated in the peripheral blood of patients with RA^{23,24}. Expression of CCL20 is induced in FLS by the synergistic interaction of pro-inflammatory cytokines, including TNF- α , IL-1 β and IL-17, and cytokine-stimulated FLS can recruit mononuclear cells in a CCL20/CCR6 dependent manner^{25,26}. Taken together, the results suggest CCL20 produced by FLS recruits monocytes and Th17 T cells to the synovium and is an important chemokine in the pathogenesis of RA. Moreover, blockade of CCL20 binding to CCR6 with a neutralizing antibody was effective at treating arthritis in a T cell transfer model in mice²². Further supporting the role of CCL20 as an important chemokine in RA, a recent study demonstrated that treatment with infliximab, etanercept or tocilizumab reduced serum levels of CCL20 in patients with RA²⁷.

IL-7

IL-7 is a member of the IL-2 family. IL-7 is associated with endothelial cells, FLS and macrophages in the RA synovium and co-localizes with deposits of extracellular matrix collagen IV²⁸. IL-7R is composed of IL-7R α and IL-2R γ and is expressed on CD4+ and CD8

+ T cells, NK-T cells and monocytes. IL-7 levels are increased in RA compared to OA synovial fluid. Serum IL-7 levels correlate with disease activity in RA²⁹. In RA patients who are poor responders to anti-TNF therapy, persistently elevated serum levels of IL-7 are seen²⁹. Moreover, reduced levels of serum IL-7 were observed in patients with early RA treated with methotrexate, and the reduction correlated with disease suppression²⁹.

Within RA synovial biopsies, samples with lymphoid follicles demonstrate consistent IL-7 staining and gene expression analysis of RA synovial samples revealed increased expression of genes involved in IL-7 signal transduction²⁸. IL-7 may be responsible for generation of tertiary lymphoid follicles observed in RA synovium, as IL7 is known to be crucial for the development of lymphoid tissue. Enhanced expression of IL-7R α and IL-7 in RA patients may contribute to joint inflammation by activating T cells, B cells and macrophages as treatment with soluble human IL-7R α inhibited IL-7R mediated immune activation *in vitro*³⁰. Taken together, these research findings suggest IL-7 and its receptor as potential targets for immune modulation in RA therapy.

B cell interactions with synovial T cells

Recent successes of therapeutic interventions using B cell depleting reagents have highlighted the importance of B cells to the pathogenesis of rheumatoid arthritis³¹. These current therapies, which target CD20 positive B cells, and several others that are in the pipeline that target other B cell markers, are designed to eliminate or disrupt the activation of a large percentage of the entire population of B cells. Although effective as treatment for RA in the short term, each of these reagents has the potential to have long-term deleterious effects on the immune response to common infectious microorganisms. Therefore, it is desirable to continue pursuing B cell-directed treatments that are more specific to the pathogenic subset of B cells that mediate joint inflammation. The following is a summary of some recently recognized cell-cell interactions involving synovial B cells, that point to emerging potential targets of interest.

Germinal centers in RA synovium

It has been established that structures resembling lymph node germinal centers can be found in the inflamed synovium of a subset of RA patients. The recently described expression of activation-induced cytidine deaminase (AID) within these ectopic germinal centers supports the hypothesis that maturation of the antibody response through somatic hypermutation and class-switch recombination could occur within the RA synovium³². This study went on to demonstrate that germinal centers remained functionally active following implantation of RA synovial tissue in SCID mice³². Analysis of a panel of cytokines and chemokines that are thought to contribute to germinal center formation showed correlations between the presence of germinal centers and TNF α , lymphotoxin- β , APRIL and B-lymphocyte chemoattractant (BLC, CXCL13). Interestingly, another study by the same researchers demonstrated that mononuclear cells from RA synovium had 400 fold higher expression of CXCL13 than cells isolated from the peripheral blood of the same patients³³. The majority of synovial CXCL13 was produced by CD45RO+/CD4+/CD3+ T lymphocytes, suggesting that this T cell subset was of an activated phenotype. Further analysis of surface markers on these T cells suggested a phenotype that was distinct from T follicular helper cells found in other lymphoid structures. Although systemic blockade of CXCL13 or its receptor CXCR5 may be expected to interrupt T cell-B cell interactions that are important to host defenses, the findings concerning CXCL13 expression within the RA synovium open the possibility of intra-articular immune therapy directed at CXCL13/CXCR5 or other contributors to germinal center formation. Localized neutralization of other cytokines or cytokine receptors involved in germinal center formation including lymphotoxin, IL-21, APRIL and BAFF may also provide therapeutic benefit in some patients.

ICOS and its ligand

As noted above, B cells constitute an important APC population for T lymphocyte activation. Therapies directed at OX40/OX40L, CD80 and CD86 are expected to act on B cell antigen presentation as well as on dendritic cells and macrophages. Another notable interaction between CD4⁺ T helper cells and B cells that has gained interest recently is that of T cell expressed inducible co-stimulator (ICOS, CD278) with its ligand on B cells, B7RP-1 (ICOS-L, CD275). These molecules are also involved in the formation of germinal centers and are critical to the initiation and further development of antigen-specific antibody responses. Disruption of interactions between ICOS and its ligand have been demonstrated to diminish arthritis incidence and severity in two independent studies^{34,35}. In the latter study, treatment with a blocking antibody decreased the number of T follicular helper cells and germinal center B cells in the draining lymph nodes and spleens of mice in the collagen-induced arthritis model. This led to an overall reduction in T cell cytokine production and titers of anti-collagen antibodies in the serum. ICOS/ICOS-L blockade was also effective in the NZB/NZW F₁ model of lupus nephritis. As was noted above for CXCL13, systemic blockade of ICOS/ICOS-L interactions may not prove to be desirable due to adverse side effects, but local inhibition of ICOS/ICOS-L in the synovium may be a viable treatment option.

Regulatory B cells and NKT cells

It is important to understand that not all interactions between B cells and T cells will result in increased disease pathogenesis in arthritis. Regulatory B cells that express IL-10 have been studied for many years, but have recently gained prominence through the demonstration that they have unique surface markers (CD1d^{hi}CD5⁺) and may represent a subset of B cells distinct from those that are pathogenic in autoimmune diseases³⁶. Interestingly, the high expression of CD1d by these regulatory B cells suggests that they may present antigens to invariant NKT cells. The role of NKT cells in arthritis has been controversial with almost equal numbers of papers suggesting a pathogenic or suppressive phenotype³⁷. The conflicting roles of NKT cells in arthritis might be explainable by differences in the B cell populations that are presenting antigen to them. A recent study has demonstrated the increased presence of invariant NKT cells with regulatory phenotype following depletion of B cells with anti-CD20 therapy³⁸. This study suggests a novel mechanism contributing to the success of B cell depletion therapy, and demonstrates the potential efficacy of targeting the interaction of B cells with iNKT cells.

B cell interactions with other synovial cell populations

Although B cell interactions with T cells in the RA synovium may present important and obvious targets for future therapy, it is critical to understand that signaling between B cells and other synovial cell populations may also contribute to joint pathology.

BAFF

A recent study has shown that the B cell-activating factor, BAFF, is expressed on the cell surface of RA-FLS but not on OA-FLS³⁹. This RA-FLS-associated BAFF acted as a first signal for the transcription of recombination-activating genes (RAG) in B cells, which are involved in maturation of the antibody response. RAG expression in B cells co-cultured with RA-FLS was blocked by interference with BAFF gene translation or by neutralization of IL-6. Forced expression of membrane-associated BAFF in OA-FLS did not result in RAG gene transcription unless IL-6 was also present in the co-culture. These results highlight that RA-FLS can have a direct effect on B cell activation through the combined expression of cell surface BAFF and IL-6. Evidence for the importance of local synovial BAFF expression to pathogenesis of arthritis comes from a study in which intra-articular injection of a lentiviral vector containing a BAFF silencing reagent provided long-term protection from joint inflammation in the collagen-induced arthritis model⁴⁰. This protective effect of BAFF gene silencing within the

joint was accompanied by decreases in production of anti-collagen antibodies and IL-17 by cells of the joint and draining lymph node, but not in the spleen of treated mice. This study demonstrates another potential approach to localized treatment of arthritic inflammation.

Osteopontin

Another notable interaction between RA-FLS and B cells that may contribute to joint inflammation was found to be associated with the expression of an RA-associated isoform of osteopontin (OPN)⁴¹. This larger form of osteopontin was preferentially detected in RA-FLS on Western blot analysis, and was found to form a unique macrocomplex with fibrinogen on the cell surface of RA-FLS. Co-cultures of purified B cells with OPN-fibrinogen complex-positive FLS led to increased expression of IL-6, an effect that was blocked by specific inhibition of OPN production. Immunohistochemistry of RA synovial tissue demonstrated the co-localization of OPN, IL-6 and B cells to highly inflamed areas of the joint.

RANKL on B cells

Provocative new data suggests that B cells can express the receptor activator of nuclear factor- κ B ligand (RANK-L)⁴². The expression of RANK-L in the synovium is known to drive differentiation of osteoclasts that mediate the erosion of bone in RA. The study by Han et al demonstrated that RANK-L expression was stimulated on rat splenic B cells by culture with the inactivated bacterium, *Aggregatibacter actinomycetemcomitans*, a pathogenic microbe associated with human periodontal disease⁴². Purified splenic B cells from A. *actinomycetemcomitans* infected rats stimulated a reporter cell line to differentiate toward osteoclasts⁴². Thus, oral infection has systemic effects on RANK-L expression by B cells and may be a partial explanation of the association between RA and periodontal disease. Although the expression of RANK-L by synovial B cells has not yet been reported, it will be interesting to determine whether B cells contribute to RA tissue damage through this mechanism, and what local or systemic signals might be involved in B cell expression of RANK-L.

Leucocyte-endothelial interactions in RA synovium

Cell migration into inflammatory lesions is a tightly-regulated and complex process that involves adhesion receptors on both leucocytes and vascular endothelial cells, multiple chemokines, and retention signals within target tissues. Space does not permit a comprehensive description of the extensive knowledge concerning these processes in RA synovium, which have been recently reviewed elsewhere⁴³. Relevant adhesion molecules include selectins, integrins and members of the immunoglobulin gene superfamily⁴³. These molecules are also used in various combinations in leucocyte efflux into non-synovial tissues, but it appears likely that in some respects both the synovial vasculature and the cell homing mechanisms to synovium are unique.

Neutrophil recruitment

Intravital microscopy has emerged as an elegant technique for assessing leucocyte ingress into synovium, at least in animal model systems⁴⁴. Such studies have revealed an unexpected prominence of neutrophil versus lymphocyte recruitment that, in RA, could be reflected by the cell composition of synovial fluid more than synovial tissue. In the mouse model of proteoglycan-induced arthritis neutrophil expression of CD44 and CD62L (L-selectin) is crucial for entry into the joint⁴⁵. In human RA, *in vitro* studies suggest that FLS control neutrophil recruitment indirectly, through their influence on endothelial cells⁴⁶. Co-culture of endothelial cells with RA-FLS but not RA dermal fibroblasts markedly augmented adherence of neutrophils to the endothelial cells, a process that depended on IL-6 production by the FLS. A role for FLS IL-6 was also identified in a similar co-culture system that measured lymphocyte recruitment by endothelial cells⁴⁷. In these experiments OA-FLS and RA-FLS behaved

similarly, but FLS from injured but otherwise normal joints or dermal fibroblasts inhibited lymphocyte-endothelial adhesion. Although limited by the use of umbilical vein rather than synovial microvascular endothelial cells, these interesting studies provide striking evidence for a role of FLS in the control of endothelial cell function.

Permeability of the synovial vasculature

Ingress of leucocytes into synovium may also be facilitated by unique permeability properties of the synovial microvasculature that have been revealed by experiments in a murine immune-complex model of acute joint inflammation that is induced by serum transfer (as described earlier). Intravital microscopy was used to show that joints destined to become inflamed developed a unique degree of vascular leakage upon systemic administration of arthritogenic sera or even non-arthritogenic immune complexes, through a process dependent upon mast cells and vasoactive amines⁴⁸. Such studies may point to hitherto unappreciated physiological functions of normal synovium, and explain the high propensity for systemic inflammatory processes to target the joints. The relationship of this model system to chronic arthritis, particularly RA, is still difficult to define.

Blood vessel growth in RA synovium

Vasculogenesis and angiogenesis are distinct but related events that both occur in RA synovium⁴³. Vasculogenesis, the de novo formation of blood vessels from circulating endothelial cell precursors (EPCs), is thought to occur both in RA synovium and in corresponding animal models^{43,49}. Co-culture studies have implicated cell-cell contact between RA EPCs and RA FLS, mediated by binding of VCAM-1 (vascular cell adhesion molecule 1) to the integrin VLA-4 as critical to such interactions, which could retain EPCs in synovial tissue long enough to allow vasculogenesis to occur⁴⁹. New blood vessel formation in RA synovium also depends on TNF, and can be reversed by TNF blockade⁵⁰. TNF and other pro-inflammatory cytokines can induce molecules that are involved in angiogenesis, leucocyte-endothelial adhesion or both. One example is the group of molecules termed Le^y/H (identified by a carbohydrate-binding antibody termed 4A11) or its analog H-2g⁵¹. These molecules not only promote both angiogenesis and expression of adhesion ligands by endothelial cells, but also stimulate monocyte migration. This latter effect was observed *in vitro* and also *in vivo*, in assays of monocyte recruitment to human RA synovial tissues implanted into scid mice⁵¹.

Lymphocyte egress

Very little is known about the control of lymphocyte egress versus retention in RA synovium. It is possible that some current treatment approaches, such as TNF blockade, do stimulate cell egress, although this is difficult to prove. Some data exist to suggest that expression of chemokine receptors on T lymphocytes is altered upon entry into RA synovium, and that chemokine gradients in synovial lymphatics, endothelium and stromal cells are distorted so as to discourage lymphocyte egress⁵². Readjustment of these gradients so as to deplete synovial tissue of inflammatory cells would be a particularly compelling approach to resolving joint inflammation.

Conclusion

Analysis of cell-cell interactions in RA synovium is providing a vivid and detailed understanding of RA pathogenesis. Current biologic and non-biologic therapies are likely to be already targeting some of these interactions in ways that are as yet poorly understood. Several new possibilities for more targeted therapies have been identified, based upon interactions that are unique, or uniquely important, in the inflamed joint.

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References

1. Valencia X, Higgins JM, Kiener HP, et al. Cadherin-11 provides specific cellular adhesion between fibroblast-like synoviocytes. *J Exp Med* 2004;200:1673. [PubMed: 15611293]
2. Lee DM, Kiener HP, Agarwal SK, et al. Cadherin-11 in synovial lining formation and pathology in arthritis. *Science* 2007;315:1006. [PubMed: 17255475]
3. Vandooren B, Cantaert T, ter Borg M, et al. Tumor necrosis factor alpha drives cadherin 11 expression in rheumatoid inflammation. *Arthritis Rheum* 2008;58:3051. [PubMed: 18821672]
4. Volin MV, Huynh N, Klosowska K, et al. Fractalkine is a novel chemoattractant for rheumatoid arthritis fibroblast-like synoviocyte signaling through MAP kinases and Akt. *Arthritis Rheum* 2007;56:2512. [PubMed: 17665439]
5. Sawai H, Park YW, He X, et al. Fractalkine mediates T cell-dependent proliferation of synovial fibroblasts in rheumatoid arthritis. *Arthritis Rheum* 2007;56:3215. [PubMed: 17907166]
6. Benito-Miguel M, Garcia-Carmona Y, Balsa A, et al. A dual action of rheumatoid arthritis synovial fibroblast IL-15 expression on the equilibrium between CD4+CD25+ regulatory T cells and CD4+CD25- responder T cells. *J Immunol* 2009;183:8268. [PubMed: 20007590]
7. Dubois S, Mariner J, Waldmann TA, et al. IL-15Ralpha recycles and presents IL-15 In trans to neighboring cells. *Immunity* 2002;17:537. [PubMed: 12433361]
8. Baslund B, Tvede N, Danneskiold-Samsoe B, et al. Targeting interleukin-15 in patients with rheumatoid arthritis: a proof-of-concept study. *Arthritis Rheum* 2005;52:2686. [PubMed: 16142748]
9. Tran CN, Davis MJ, Tesmer LA, et al. Presentation of arthritogenic peptide to antigen-specific T cells by fibroblast-like synoviocytes. *Arthritis Rheum* 2007;56:1497. [PubMed: 17469112]
10. Tran CN, Thacker SG, Louie DM, et al. Interactions of T cells with fibroblast-like synoviocytes: role of the B7 family costimulatory ligand B7-H3. *J Immunol* 2008;180:2989. [PubMed: 18292521]
11. Modak S, Kramer K, Gultekin SH, et al. Monoclonal antibody 8H9 targets a novel cell surface antigen expressed by a wide spectrum of human solid tumors. *Cancer Res* 2001;61:4048. [PubMed: 11358824]
12. Xu H, Cheung IY, Guo HF, et al. MicroRNA miR-29 modulates expression of immunoinhibitory molecule B7-H3: potential implications for immune based therapy of human solid tumors. *Cancer Res* 2009;69:6275. [PubMed: 19584290]
13. Munn DH, Sharma MD, Mellor AL. Ligation of B7-1/B7-2 by human CD4+ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. *J Immunol* 2004;172:4100. [PubMed: 15034022]
14. Szanto S, Koreny T, Mikecz K, et al. Inhibition of indoleamine 2,3-dioxygenase-mediated tryptophan catabolism accelerates collagen-induced arthritis in mice. *Arthritis Res Ther* 2007;9:R50. [PubMed: 17511858]
15. Criado G, Simelyte E, Inglis JJ, et al. Indoleamine 2,3 dioxygenase-mediated tryptophan catabolism regulates accumulation of Th1/Th17 cells in the joint in collagen-induced arthritis. *Arthritis Rheum* 2009;60:1342. [PubMed: 19404944]
16. Zola, H., editor. *Leucocyte and stromal cell molecules: the CD markers*. Hoboken, N.J: Wiley-Liss; 2007.
17. Ohshima Y, Tanaka Y, Tozawa H, et al. Expression and function of OX40 ligand on human dendritic cells. *J Immunol* 1997;159:3838. [PubMed: 9378971]
18. Brugnoli D, Bettinardi A, Malacarne F, et al. CD134/OX40 expression by synovial fluid CD4+ T lymphocytes in chronic synovitis. *Br J Rheumatol* 1998;37:584. [PubMed: 9651095]
19. Yoshioka T, Nakajima A, Akiba H, et al. Contribution of OX40/OX40 ligand interaction to the pathogenesis of rheumatoid arthritis. *Eur J Immunol* 2000;30:2815. [PubMed: 11069062]

20. Giacomelli R, Passacantando A, Perricone R, et al. T lymphocytes in the synovial fluid of patients with active rheumatoid arthritis display CD134-OX40 surface antigen. *Clin Exp Rheumatol* 2001;19:317. [PubMed: 11407087]
21. Passacantando A, Parzanese I, Rascente M, et al. Synovial fluid OX40T lymphocytes of patients with rheumatoid arthritis display a Th2/Th0 polarization. *Int J Immunopathol Pharmacol* 2006;19:499. [PubMed: 17026834]
22. Hirota K, Yoshitomi H, Hashimoto M, et al. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J Exp Med* 2007;204:2803. [PubMed: 18025126]
23. Ruth JH, Shahrara S, Park CC, et al. Role of macrophage inflammatory protein-3alpha and its ligand CCR6 in rheumatoid arthritis. *Lab Invest* 2003;83:579. [PubMed: 12695561]
24. Kageyama Y, Ichikawa T, Nagafusa T, et al. Etanercept reduces the serum levels of interleukin-23 and macrophage inflammatory protein-3 alpha in patients with rheumatoid arthritis. *Rheumatol Int* 2007;28:137. [PubMed: 17619881]
25. Chabaud M, Page G, Miossec P. Enhancing effect of IL-1, IL-17, and TNF-alpha on macrophage inflammatory protein-3alpha production in rheumatoid arthritis: regulation by soluble receptors and Th2 cytokines. *J Immunol* 2001;167:6015. [PubMed: 11698482]
26. Tanida S, Yoshitomi H, Nishitani K, et al. CCL20 produced in the cytokine network of rheumatoid arthritis recruits CCR6+ mononuclear cells and enhances the production of IL-6. *Cytokine* 2009;47:112. [PubMed: 19535263]
27. Kawashiri SY, Kawakami A, Iwamoto N, et al. Proinflammatory cytokines synergistically enhance the production of chemokine ligand 20 (CCL20) from rheumatoid fibroblast-like synovial cells in vitro and serum CCL20 is reduced in vivo by biologic disease-modifying antirheumatic drugs. *J Rheumatol* 2009;36:2397. [PubMed: 19797510]
28. Timmer TC, Baltus B, Vondenhoff M, et al. Inflammation and ectopic lymphoid structures in rheumatoid arthritis synovial tissues dissected by genomics technology: identification of the interleukin-7 signaling pathway in tissues with lymphoid neogenesis. *Arthritis Rheum* 2007;56:2492. [PubMed: 17665400]
29. van Roon JA, Jacobs K, Verstappen S, et al. Reduction of serum interleukin 7 levels upon methotrexate therapy in early rheumatoid arthritis correlates with disease suppression. *Ann Rheum Dis* 2008;67:1054. [PubMed: 18556451]
30. Hartgring SA, van Roon JA, Wenting-van Wijk M, et al. Elevated expression of interleukin-7 receptor in inflamed joints mediates interleukin-7-induced immune activation in rheumatoid arthritis. *Arthritis Rheum* 2009;60:2595. [PubMed: 19714586]
31. Levesque MC. Translational Mini-Review Series on B Cell-Directed Therapies: Recent advances in B cell-directed biological therapies for autoimmune disorders. *Clin Exp Immunol* 2009;157:198. [PubMed: 19604259]
32. Humby F, Bombardieri M, Manzo A, et al. Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. *PLoS Med* 2009;6:e1. [PubMed: 19143467]
33. Manzo A, Vitolo B, Humby F, et al. Mature antigen-experienced T helper cells synthesize and secrete the B cell chemoattractant CXCL13 in the inflammatory environment of the rheumatoid joint. *Arthritis Rheum* 2008;58:3377. [PubMed: 18975336]
34. Iwai H, Kozono Y, Hirose S, et al. Amelioration of collagen-induced arthritis by blockade of inducible costimulator-B7 homologous protein costimulation. *J Immunol* 2002;169:4332. [PubMed: 12370365]
35. Hu YL, Metz DP, Chung J, et al. B7RP-1 blockade ameliorates autoimmunity through regulation of follicular helper T cells. *J Immunol* 2009;182:1421. [PubMed: 19155489]
36. Matsushita T, Yanaba K, Bouaziz JD, et al. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J Clin Invest* 2008;118:3420. [PubMed: 18802481]
37. Coppieters K, Dewint P, Van Beneden K, et al. NKT cells: manipulable managers of joint inflammation. *Rheumatology (Oxford)* 2007;46:565. [PubMed: 17307755]
38. Parietti V, Chiffot H, Sibilia J, et al. Rituximab treatment overcomes reduction of regulatory iNKT cells in patients with rheumatoid arthritis. *Clin Immunol*. 2009

39. Rochas C, Hillion S, Saraux A, et al. Transmembrane BAFF from rheumatoid synoviocytes requires interleukin-6 to induce the expression of recombination-activating gene in B lymphocytes. *Arthritis Rheum* 2009;60:1261. [PubMed: 19404965]
40. Lai Kwan Lam Q, King Hung Ko O, Zheng BJ, et al. Local BAFF gene silencing suppresses Th17-cell generation and ameliorates autoimmune arthritis. *Proc Natl Acad Sci U S A* 2008;105:14993. [PubMed: 18820032]
41. Take Y, Nakata K, Hashimoto J, et al. Specifically modified osteopontin in rheumatoid arthritis fibroblast-like synoviocytes supports interaction with B cells and enhances production of interleukin-6. *Arthritis Rheum* 2009;60:3591. [PubMed: 19950274]
42. Han X, Lin X, Seliger AR, et al. Expression of receptor activator of nuclear factor-kappaB ligand by B cells in response to oral bacteria. *Oral Microbiol Immunol* 2009;24:190. [PubMed: 19416447]
43. Szekanecz Z, Koch AE. Vascular involvement in rheumatic diseases: 'vascular rheumatology'. *Arthritis Res Ther* 2008;10:224. [PubMed: 18947376]
44. Gal I, Bajnok E, Szanto S, et al. Visualization and in situ analysis of leukocyte trafficking into the ankle joint in a systemic murine model of rheumatoid arthritis. *Arthritis Rheum* 2005;52:3269. [PubMed: 16206283]
45. Sarraj B, Ludanyi K, Glant TT, et al. Expression of CD44 and L-selectin in the innate immune system is required for severe joint inflammation in the proteoglycan-induced murine model of rheumatoid arthritis. *J Immunol* 2006;177:1932. [PubMed: 16849507]
46. Lally F, Smith E, Filer A, et al. A novel mechanism of neutrophil recruitment in a coculture model of the rheumatoid synovium. *Arthritis Rheum* 2005;52:3460. [PubMed: 16255036]
47. McGettrick HM, Smith E, Filer A, et al. Fibroblasts from different sites may promote or inhibit recruitment of flowing lymphocytes by endothelial cells. *Eur J Immunol* 2009;39:113. [PubMed: 19130557]
48. Binstadt BA, Patel PR, Alencar H, et al. Particularities of the vasculature can promote the organ specificity of autoimmune attack. *Nat Immunol* 2006;7:284. [PubMed: 16444258]
49. Silverman MD, Haas CS, Rad AM, et al. The role of vascular cell adhesion molecule 1/ very late activation antigen 4 in endothelial progenitor cell recruitment to rheumatoid arthritis synovium. *Arthritis Rheum* 2007;56:1817. [PubMed: 17530710]
50. Izquierdo E, Canete JD, Celis R, et al. Immature blood vessels in Rheumatoid synovium are selectively depleted in response to anti-TNF therapy. *PLoS One* 2009;4:e8131. [PubMed: 19956574]
51. Amin MA, Ruth JH, Haas CS, et al. H-2g, a glucose analog of blood group H antigen, mediates mononuclear cell recruitment via Src and phosphatidylinositol 3-kinase pathways. *Arthritis Rheum* 2008;58:689. [PubMed: 18311817]
52. Burman A, Haworth O, Hardie DL, et al. A chemokine-dependent stromal induction mechanism for aberrant lymphocyte accumulation and compromised lymphatic return in rheumatoid arthritis. *J Immunol* 2005;174:1693. [PubMed: 15661933]

Table 1

Important Cell-Cell Interactions in RA synovium

<u>Cell types</u>	<u>Potential new molecular targets</u>
FLS-FLS	cadherin-11, fractalkine
FLS-T	B7-H3, IL-15
T-APC	OX40, CCL-20, IL-7
B-T	CXCL13, ICOS, OX40, BAFF
B-FLS	BAFF, osteopontin
Leucocyte-endothelial	Le ^x /H

[Please see text for explanation of abbreviations.]