The histopathology of early synovitis

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List of abbreviations
RA: rheumatoid arthritis
OA: osteoarthritis
PsA: psoriatic arthritis
SpA: spondyloarthropathy
AS: ankylosing spondylititis
UA: undifferentiated arthritis
SLE: systemic lupus erythematosus
PMN: Polymorphonuclear cell
MIP1: macrophage inflammatory protein 1
NIH: National Institutes of Health
VEGF: vascular endothelial growth factor
ICAM1: intercellular adhesion molecule 1
VCAM1: vascular cell adhesion molecule 1
PECAM1: platelet-endothelial cell adhesion molecule 1
VLA: very-late antigen
MCP1: monocyte chemoattractant protein
TH1, TH2: T helper cell type 1; T helper cell type 2
TNF: tumor necrosis factor
INF: interferon
IL: interleukin
MMP: matrix metalloproteinase
TIMP: tissue inhibitor of matrix metallo proteinase
TUNEL: Terminal deoxynucleotidyl trans ferase mediated dUTP nick end labeling
FLICE: FADD-like interleukin 1β converting enzyme
FADD: Fas-associated death domain protein
FLIP: FLICE-like inhibitory protein
NFκβ: nuclear factor kappa beta
RANK: receptor activator of NFκβ
RANKL: receptor activator of NFκβ ligand
OPG: osteoprotegerin

ABSTRACT
Early diagnosis and therapeutic inter - vention are needed to prevent the mor - bidity related to erosive arthropathies such as rheumatoid arthritis (RA), yet it is difficult to distinguish various forms of synovitis early in the disease course. The availability of synovial tis - sue biopsy techniques has facilitated the analysis of synovial tissue from patients with early disease. Compari - son of the histopathologic features of synovial tissue in early RA, established RA, and in non-RA synovitis has shown subtle, but potentially important differ - ences in histologic features, cytokine and protease expression patterns, and apoptosis. Ultimately, it remains to be shown definitively that analysis of the histopathological features of synovial tissue early in disease is of independent value in identifying patients destined to have persistent synovitis or erosive dis - ease, and in turn, in allocating patients to specific therapeutic strategies.

Introduction

Distinguishing rheumatoid arthritis (RA) from other forms of synovitis is difficult early in the course of disease, yet it is now well established that early aggressive treatment is beneficial in preventing the long-term morbidity related to erosive arthropathies. In order to understand the pathological processes that initiate and contribute to the ongoing synovitis occurring in conditions such as RA, attention is being directed towards identifying events occurring early in the course of the disease. This article will address the histopathology of early inflammatory arthritis, focussing on comparisons with late or established disease and between different clinical variants. Studies of histological features, cytokine expression patterns, protease expression patterns, and additional features such as apoptosis will be highlighted.

How early is early synovitis?

The definition of "early" disease varies between different studies. Arbitrarily, symptom duration of less than 12 months is generally considered to be "early" disease in most studies although some have included patients with 18 or more months of symptoms. Even at 12 months, the initial features may have evolved and a number of groups have looked at "very early" disease with symptoms of 6 weeks or less. As all clinicians know, defining the onset of disease is often difficult, as joint symptoms can develop insidiously and increase gradually. Patients may have arthralgias for varying periods prior to the development of true synovitis as defined by joint swelling. Presumably arthralgias represent pre-clinical synovitis in some patients; however, clearly not all patients with arthralgia develop true inflammatory arthritis. Newer imaging approaches such as MRI (1-3), ultrasound (4-6), and infrared spectroscopy (7) may be of value in addressing this important question. Biopsy studies of clinically unaffected joints in patients with established RA demonstrate evidence of subclinical histologic changes, implying that the early synovial events that ultimately lead to persistence and destructiveness may be entirely asymptomatic for an unspecified period of time (8, 9).

The challenge of studying pathological features in early synovitis

The primary challenge involved with studies of histological features in early inflammatory arthritis relates to the difficulty in obtaining representative tissue samples from patients with early disease. Establishing an organized referral base and dedicated early arthritis clinics facilitates the identification of appropriate patients, as well as providing the necessary long-term follow-up required for outcomes research. Tissue acquisition is also a challenge. Many of the early studies in established RA used samples obtained at the time of joint arthroplasty or autopsy, which allows directed sampling of specific

joint areas and usually produces good amounts of tissue. In contrast, tissue from patients with early disease is primarily obtained through closed needle biopsies or arthroscopic guided biopsies. Each of these techniques has advantages and disadvantages. Both allow sampling of both small and large joints. The major advantage of arthroscopic biopsy is that it allows accurate, visually directed sampling of the cartilage-pannus junction. Samples obtained by closed needle biopsy generally show comparable findings to arthroscopic samples, but it has been shown that they may underestimate the degree of inflammatory activity at the cartilage-pannus junction, this being the most important and active area to study in erosive diseases (10, 11). Since synovial inflammation is clearly not uniform throughout the joint, regardless of the method used to obtain tissue, multiple tissue samples from different locations in the joint are required to provide a reliable assessment of the histologic variation within the joint (12).

Quantification of histological or immunohistological parameters also varies in different studies. Several methods have been applied to interpret the degree of synovial inflammation and to quantify various features. These range from visual estimates using semi-quantitative scales to labor intensive counts of individual positively stained cells or structures. As with the biopsy sampling, multiple tissue areas must be scored, given heterogeneity of the synovial lesions. Computer image assisted analysis may provide an objective means of quantifying immunohistochemical staining (13-16).

Histopathologic findings in early RA compared to established RA

The classical features of established RA including lining layer hypertrophy, cellular infiltration with lymphocytes, macrophages, and plasma cells, and the presence of secondary lymphoid organs or follicles/aggregates have been well described (17, 18). There is also evidence of exuberant angiogenesis and the increased expression of inflammatory cytokines and chemokines, collagen degrading enzymes such as cathepsin and metalloproteinases, and variable degrees of apoptosis (19-23). Established seronegative arthritis has similar features to established RA, although potentially to a lower degree. Psoriatic arthritis (PsA) is distinguished from RA by characteristic changes in the synovial vasculature both macroscopically and microscopically (24, 25).

Several studies have compared early RA to established RA. Synovial tissue obtained from patients with a disease duration as early as 6 weeks from symptom onset shows evidence of lining layer hypertrophy, cellular infiltration with perivascular aggregates, and increased vascularity, although the inflammatory features are patchy (26, 27). The majority of cellular subtypes typical for established RA including monocytes, lymphocytes, macrophages, and even plasma cells are found in early disease. Surface and villus fibrin have also been described, though they were not found to be specific for RA (27, 28). The degree of lymphocytic, plasma cell and PMN infiltration is similar between early RA (disease duration less than 12 months and as early as 2 weeks) and late RA (29). Importantly, in one study this infiltration did not correlate with disease duration (30). Macrophage numbers and their chemokine products, macrophage inflammatory protein1 (MIP1), are increased in the lining and sublining layers of late RA compared to early RA (31). Cytotoxic cells and natural killer cells expressing granzyme B are present in RA and osteoarthritis (OA) and are increased in early RA (32, 33).

As mentioned, synovial inflammation is present even in the asymptomatic joints of patients with established RA (8, 9) and early synovitis (34). The asymptomatic joints of patients with established RA had evidence of macrophage infiltration in the synovial sublining, though less than in the clinically involved joints (8). CD3 T cells, plasma cells, and fibroblast-like cells were present, but not increased in asymptomatic joints compared to control tissues. In a separate study, clinically uninvolved joints of RA patients had lining layer hyperplasia and perivascular mononuclear cell infiltration was comprised of primarily helper T cells (9). Of patients with histologically apparent, but clinically inapparent synovitis, only 2 developed clinical synovitis after 36 months of follow-up. In a biopsy study of asymptomatic joints in early synovitis patients, Pando *et al.* found definite evidence of synovitis in 11 of 20 patients with increased microvasculature, lining layer hypertrophy and an inflammatory cell infiltrate consisting predominantly of monocytes/macrophages and T lymphocytes (34).

In summary, the histological features that appear to be more prominent in

Table I. Comparison of biopsy techniques for sampling synovium.

	Closed needle	Needle arthroscopy	Surgical arthrotomy
Joints	Small and large joints	Small and large joints	Small and large joints
Invasiveness	Bedside/outpatient procedure Minimally invasive Local anesthetic	Outpatient procedure Modestly invasive Local anesthetic/mild sedation	In-patient procedure requiring surgical expertise General/spinal anesthetic
Tissue sampling	Blind	Visually directed	Visually directed
Amount of tissue	Small	Small	Large
Main advantage	Minimally invasive	Visually directed samples	Visually directed and large amounts of tissue
Main disadvantage	Blind sampling	Small tissue samples	Surgical procedure

Table II. Summary of published studies.

Study	Sample studied	Question	Sampling method	Findings
Schumacher 1972 (26)	Symptoms < 1 mo. RA 6 , JRA 3, SLE 2 , SS 1	Histology	Closed needle	No findings specific for RA – all had lymphocytic infiltrate, no follicles, rare plasma cells Vascular changes present in all: Prominent vascular changes included congestion, RBC extravasation, and venular lumen occlusion
Bayliss 1975 (27)	RA Early RA 14 (< 1 yr) Early non-RA 35 (< 1yr) Late RA 18 (> 1 yr) Late non-RA 18 (> 1 yr)	Histology	Closed needle	Increased vascularity, synovial hyperplasia, perivascular or aggregate mononuclear cell infiltration, fibroblast and mononuclear cuffing of small vessels and surface fibrin, predicted but were not specific for RA diagnosis
Konttinen 1985 (28)	RA Acute 4 (symptoms and disease duration < 3 mo) Subacute 3 (symptoms < 3 mo. disease duration < 3 yr) Chronic 5 (symptoms and disease duration > 3 yrs)	Histology	Not stated	Three patterns of histology with sequential changes: Acute: sublining mononuclear phagocytes, patchy infiltrates of granulocytes, fibrin layer, sparse T cell infiltrate Subacute: lymphocytes in perivascular aggregates, mononuclear phagocytes, some plasma cells Chronic:dense infiltrate of plasma cells. T cells main inflammatory cells, fewer mononuclear phagocytes
Konttinen 1986 (39)	JRA Early JRA 8 (< 5 mo.) Late JRA or other 4	Histology	Arthroscopy Arthrotomy	Early JRA had non-specific synovitis, fibrin exudation, LL hyperplasia, few lymphocyte aggregates, vascular congestion. Late JRA had increased plasma cells and T lymphocyte infiltrates.
Schumacher 1988 (41)	Reiters syndrome n =15 DxDur: < 1mo – 24 yr and rebiopsy 9 DxDur < 1 yr 10 SxDur < 4 wks	Histology	Closed needle Arthrotomy	Early symptoms: surface fibrin lining layer hyperplasia, vascular congestion, moderate infiltrate but few plasma cells Late symptoms: surface fibrin lining layer hyperplasia and vascular congestion, moderate lymphocyte and plasma cell infiltrate
Kraan 1999 (36)	Early arthritis Early RA 36 (<1 yr) Early non-RA 59 (< 1 yr) (UA 21, OA 17, ReA 10, PsA 2, AS 3, crystal 6) Control 5	Histology	Closed needle	Early RA has greater plasma cells, B cells, and macrophages compared to non-RA
Tak (32) 1994	RA Early RA 10 (< 1 yr) Late RA 10 (> 5 yrs) OA 10	Histology	Closed needle	Natural killer cells expressing granzyme B are present in RA and OA but increased in early RA
Tak 1997 (30)	RA Early RA 31 (< 1 yr) Late RA 36 (> 5 yr)	Histology Clinical	Closed needle	No histologic features to distinguish early from late RA,knee pain correlated positively with IL6, TNFα, CD68 and negatively with CD4 cells
Soden 1989 (9)	Unaffected joint in established RA Established RA 16 Post mortem control 15	Histology	Closed needle	Asymptomatic synovitis precedes clinical synovitis Five patients had synovitis with primarily helper T cell infiltrate
Kraan 1998 (8)	Unaffected joint in established RA Established RA 10 Control 8	Histology	Arthroscopy	Asymptomatic synovitis precedes clinical synovitis: Macrophage (CD68) counts, and IL1β, IL6 and TNFα expression were greater in unaffected joints than controls. CD68, CD3, CD38 cells and IL1β, TNFα expression was greater in clinically affected joints than unaffected joints
Pando 2000 (34)	Unaffected joint in early arthritis Early RA 6 Early non-RA 14	Histology	Closed needle	Asymptomatic synovitis precedes clinical synovitis 11 asymptomatic joints had definite synovitis (increased vasculature, lining layer hypertrophy, and inflammatory infiltrates) but less severe than symptomatic joints RA and UA more likely to have synovitis of asymptomatic joint than ReA
Fearon 2003 (35)	Early arthritis Early PsA 12 (< 1 yr) Early RA 14 (< 1 yr) Late RA and PsA 21 (> 1.5 yr) OA 12 (> 2 yr)	Histology Vascularity	Arthroscopy	Early RA had increased CD3 T cells and lining layer thickness than PsA. Early PsA had greater vascularity with "tortuous" vessels, increased perivascular VEGF and Ang2 expression than early RA.

Reece 1999 (24)	Early arthritis RA 18 (< 1 yr) PsA 14 (< 1 yr) ReA 12 (< 1 yr)	Vascularity	Arthroscopy	PsA and ReA had mainly "tortuous, bushy" blood vessels RA patients had mainly straight blood vessels
Baeten 2000 (25)	Early arthritis Early RA 16 (< 1 yr) Early SpA 23 (< 1 yr) Late RA (> 1 yr) OA 12	Histology Vascularity Clinical	Arthroscopy	Early & late RA had similar cellularity, vascularity and integrin expression Histology features increased in effused joints in RA and SpA and correlated with systemic disease activity in RA SpA tissue had increased vascularity with "tortuous vessels" and αvβ3 expression. RA tissue had more cellularity and αvβ5 expression
Tak 1995 (44)	RA Early RA 11 (<1 yr; 8 < 6 months) Late RA14 OA 15	Histology Adhesion molecules	Closed needle	RA greater inflammatory infiltrate and increased expression of adhesion molecules E selectin, ICAM1,VCAM1,PECAM1, VLA-4 & Mac1 than OA Early and late RA had similar infiltrates of lymphocytes, plasma cells, and PMN, LL hypertrophy, vascularity, and adhesion molecule expression
Fraser 2001 (38)	Early arthritis Early RA 12 (< 1 yr) Early PsA12 (< 1 yr) OA 10	Vascularity Protease Apoptosis	Arthroscopy	Synovial fluid (SF) MMP9 higher in early PsA than early RA and correlated with blood vessel morphology and SF VEGF levels. Early RA had higher endothelial cell apoptosis than early PsA.
Katrib 2001 (31)	RA Early RA 22 (<1 yr) Late RA 22 (> 5 yr)	Histology Chemokine Protease	Closed needle	Late RA had greater macrophage (CD68) expression and this correlated with MIP1α. Early and late RA had similar MMP expression
Smeets 1998 (51)	RA vs ReA Early RA 10 (< 1 yr) Late RA 10 (> 1 yr) Early ReA 6 (< 1 yr) Late ReA 5 (> 1 yr)	Histology Cytokine	Closed needle	RA had greater infiltration of lymphocytes and plasma cells than ReA but no differences early vs late RA or ReA RA higher INFγ than ReA independent of disease duration (similar expression of IL2, IL4, IL10 between RA and ReA.) Early RA had higher IL2, IL4, IL10 than late RA(opposite in ReA but not significant)
Smeets 1998 (29)	RA Early RA 12 (<1 yr) Late RA 12 (>5 yr) Control tissue (tonsil) 5	Histology Cytokine	Closed needle	Similar T cell and IFNγ expression in early and late RA. RA T cell activation and proliferation reduced, suggesting hyporesponsiveness compared to tonsil T cells
Ulfgren 2000 (48)	RA Early RA 15 (< 1.5 yr) Late RA 5	Cytokine	Arthroplasty Arthroscopy	Early RA had greater IL-1α and IL-1β expression than TNFα expression. TNFα expression was greater at the cartilage pannus junction than at the villous in early RA. Early RA had greater TNFα, IL-1α, and IL-1β expression than late RA.
Kotake 1997 (50)	Early arthritis RA 13 (< 1 yr) ReA 11 (< 1 yr) UA 28 (< 1 yr)	Cytokine	Closed needle	Nested PCR of synovial tissue IL2 and IFNγ higher in ReA than RA
Kotake 1999 (49)	ReA Chlamydia 6 (<1 yr) UA 29 (<1 yr)	Cytokine	Closed needle	IFN gamma and IL10 mRNA higher in chlamydia arthritis vs UO or normal volunteers
Goldbach-Mansky 2000 (55)	Early arthritis Early RA (< 12 mo.) Early non-RA 7 (< 12 mo.) Normal 4	Protease	Closed needle	MMP expression higher in patients than controls Active MMP2 associated with erosive disease
Cunnane 1999 (53)	Inflammatory arthritis Early RA 16 (< 18 mo.) Early PsA 9 (<18 mo.) Early AS 1 (<18 mo.) Gout 1 (<18 mo.) UA 1 (< 18 mo.) Late erosive RA 2 Normal 3	Protease	Closed needle	MMP1, and cathepsins B and L expression greater in patients than controls – No differences between diseases or in disease duration – MMP1 seen in primarily in lining layer and also in perivascular areas and endothelium – Cathepsin B seen in sublining more than lining layer but not in aggregates
Cunnane 2001 (54)	Early arthritis RA 12 (<18 mo.) PsA 6 (<18 mo.) Other 2 (< 6 mo.)	Protease Clinical	Arthroscopy	MMP1 expression correlated with development of erosions

Catrina 2002 (57)	RA Early RA 8 (median dur. 5 mo) Late RA 11 (median dur. 21 yr)	Apoptosis	Arthroscopy Arthroplasty	Apoptosis increased in late RA CD68 cells, and FLIP expression (inhibits apoptosis) increased in early RA
Tak 1999(58)	RA Early Ra 13 (< 6 mo) Late RA 14 (> 5 yr) ReA 8 (mean15 mo) Inflammatory OA 10 Uninvolved RA knee 9	Apoptosis	Closed needle Arthroscopy	P53 expression in inflammatory arthritis only or in uninvolved joints of RA patient No difference between early and late RA P53 expression greater in RA than in ReA or OA
Crotti 2002 (60)	Active RA 21 (< 1 yr) Inactive RA 9 (7 > 1 yr) SpA 12 (10 > 2 yr)	RANKL/OPG	Arthroscopy	RANKL expression highest in active RA and in some SpA RANKL expression was seen mainly in T cell aggregates and some macrophages
Haynes 2003 (61)	Active RA 16 (< 1 yr) Inactive RA 6 (> 1.5 yr) SpA 12 OA 10 Normal 18	RANKL/OPG	Arthroscopy	OPG was expressed in inactive RA, SpA, OA and normals primarily by macrophage type lining layer cells and endothelial cells RANKL was predominantly expressed in active RA tissue

RA:rheumatoid arthritis; JRA:juvenile rheumatoid arthritis; SLE:systemic lupus erythematosus; SS:systemic sclerosis; ReA: reactive arthritis; PsA:psoriatic arthritis; SpA:spondyloarthropathy; UA:undifferentiated arthritis,RBC: red blood cell,LL: lining layer; dxdur:disease duration; sxdur:symptom duration; PCR: polymerase chain reaction; MMP: matrix metalloproteinase.

late compared to early RA are a tendency for greater lining layer hypertrophy, seen in some but not all studies, greater numbers of macrophages and the presence of lymphoid follicles.

Histopathologic findings in early RA compared to early non-RA synovitis

Distinguishing RA from other forms of synovitis is difficult early in the course of disease. Initial studies of the histopathology in early disease suggest the presence of increased vascularity, synovial hyperplasia, and mononuclear cell infiltration, and fibroblastic and mononuclear cuffing of blood vessels are more commonly seen in early RA than early non-RA synovial tissues (27). Several small studies of cellular subsets have suggested that early RA has more CD3 T cells (25, 35) compared to psoriatic arthritis (PsA) or the spondyloarthropathies (SpA), and higher CD4 and CD20 T cells than SpA (25). Another group examined a spectrum of early arthritis patients, and assigned a diagnosis after at least two years of follow-up. These investigators found that early RA patients had significantly higher numbers of plasma cells and B cells than non-RA arthritis patients (undifferentiated, psoriatic, reactive and spondyloarthropathy), and that increased macrophage numbers best distinguished RA, non-RA arthritis and non-inflammatory arthritis (36). Histopathologic analysis of synovial tissues from the National Institutes of Health (NIH) early synovitis cohort determined that the presence of lining layer hypertrophy, lymphocytic aggregates and lower vascularity scores were more commonly associated with early RA than early non-RA and that stromal proliferation was associated with early erosions (37).

Vascular changes in early synovitis

One of the striking differences between RA, SpA and PsA early in the disease appears to relate to blood vessel morphology (24, 25, 35, 38). Macroscopically, early PsA and SpA synovium is characterized by increased numbers of blood vessels with a "tortuous and bushy" appearance in contrast to the relatively straight blood vessels in RA synovium (24, 35, 38). This pattern is seen even in PsA patients whose joint counts and disease activity are similar to RA patients (35, 38). Microscopically, the number of blood vessels is increased in early SpA with joint effusions compared to RA (25), and in early PsA with similar CD4 and macrophage infiltration to early RA (35) . However, both early and established RA tissues were shown to have increased vascularity (25, 39).

A number of investigators have demonstrated that microvascular changes are commonly seen even very early in the course of disease (26,40,41). In a small series of patients biopsied within 6 weeks of symptoms, vascular congestion and obliteration were prominent findings, with several tissues also showing evidence of PMN and mononuclear cells infiltrating the walls of venules and erythrocyte extravasation. These changes were not limited to RA, as similar findings were seen in non-RA tissues, including those from patients with reactive arthritis and connective tissue diseases such as SLE and scleroderma (26). Electron microscopy revealed that many vessels were occluded by platelets, and had fibrin deposition in the vessel walls and disrupted endothelial cell contacts or multi-laminated basement membranes. A detailed analysis of the microvascular morphology was done in the NIH early synovitis cohort with symptoms of less than 1 year (42). No differences were seen between early RA and early non-RA tissues in the numbers of high endothelial blood venules, angiomatoid vessels described as grape-like clusters of small vessels, or obliterated or vasculitic blood vessels. However, non-RA patients expressed higher amounts of an

angiogenesis related Lewis 6/H-5-2 glycoconjugate on synovial microvasculature, suggesting that non-RA synovium has greater angiogenic potential (42). In addition, there is greater expression of angiogenic proteins (VEGF, ang2) and their mRNA in the perivascular tissue of early PsA synovium compared to early RA synovium (35). Selective upregulation of corticotrophin-releasing hormone signaling by both vasculature and perivascular aggregates in early arthritis has been proposed as a contributor to the vascular changes seen in early disease (43). These findings suggest that subtle differences in angiogenic or other growth factors may contribute to the observed macroscopic vascular phenotypes in RA and non-RA tissue.

Table III. Histologic features of early rheumatoid and non-rheumatoid synovitis.

	Early RA	Early non-RA arthritis
Lining layer hypertrophy	±± (35, 62)	±
Cellularity		
T lymphocytes (CD3)	±± (25, 35)	±
T lymphocytes (CD4)	± (36)	±
Macrophage	± (36)	±
B lymphocytes	±± (36)	±
Plasma cells	±± (36)	±
Vascularity	± (62)	±±

Adhesion molecule expression

The vascular endothelium plays a key role in the recruitment and retention of inflammatory cells and mediators to sites of tissue damage and inflammation. Endothelial expression of adhesion molecules such as E-selectin, integrin intercellular adhesion molecule1 (ICAM1) and vascular cell adhesion molecule1 (VCAM1) facilitate leukocyte adherence to the vessel walls, and platelet-endothelial cell adhesion molecule (PECAM) expression facilitates subsequent migration into the tissue. Inflammatory cytokines upregulate adhesion molecules, and adhesion molecules are commonly expressed on high endothelial venules that histologically appear as venules of varying size with tall endothelial cells.

Adhesion molecule expression in the synovium of early RA has been compared to established RA and OA. E selectin, P-selectin and very-late-antigen1 (VLA1) were found primarily on endothelial cells, VCAM1 on lining layer cells, PECAM1 on the lining layer, sublining layer and infiltrating cells but primarily on endothelial cells, VLA4 and Mac1 on lining and sublining cells, and ICAM was expressed throughout the synovium (44). Integrins αvβ3 and αvβ5 were expressed by lining layer and endothelial cells, and αvβ5 was also expressed by sublining cells (25). Adhesion molecule expression in early RA was similar to that seen in established RA but greater than in the OA tissues (44). In the NIH cohort, P selectin and E selectin expression by blood vessels was greater in synovium from early non-RA compared to RA (42).

Thus evidence to date suggests that endothelial activation is similar in both early and established RA; however, subtle differences may exist between RA and non-RA synovitis at least in the initial stages of disease. In addition, other factors important in promoting local tissue inflammation, MIP1, and monocyte chemoattractant protein (MCP1), do not differ between early and late disease (31). Thus, the subtle differences in the inflammatory cell infiltration patterns seen between early and late RA and between early RA and early non-RA cannot be explained by adhesion molecule expression alone and are likely the result of multiple other factors.

Cytokine and chemokine patterns in early synovial tissue

It has been shown that rheumatoid synovitis is characterized by having a predominance of TH1 type cytokines that are involved in cell-mediated immunity including TNF-α, IL1β, and INFγ. Similar patterns are also seen in psoriatic synovium (45, 46). In contrast, reactive arthritis (ReA) has been proposed to be a TH2 predominant, or TH1 deficient, disease. TH1 cytokine deficiency, in combination with genetic defects, could result in defective pathogen elimination and persistence of the organisms could contribute to persistent synovitis.

The T cell derived cytokine expression patterns appear to be similar in early and late RA. No differences were seen in T cells or INFγ expression between patients with disease of less than 12 months duration and established RA (greater than 5 years) (29) or in TNFα, IL1β, or IL6 expression between early and late RA (47). However, when the cytokine profiles of clinically uninvolved and involved synovia in patients with established RA were compared to control synovium, IL1β, TNFα and IL6 were found to be increased in uninvolved joints compared to OA controls, all of which were lower than in symptomatic joints (8). Ulfgren *et al.* studied cytokine expression at the cartilage-pannus junction (CPJ) in relatively early RA (disease duration of less than 18 months) and found that the CPJ of early RA had less TNFα expression than established RA; however, the expression of IL1α, and IL1β was greater than TNFα (48).

Synovial cytokine expression has been evaluated in the NIH early synovitis cohort using nested PCR. Even early in disease, there was a predominance of pro-inflammatory type 1 cytokines irrespective of the diagnosis, although the patients with early ReA tended to have higher synovial levels of IFNγ than patients with early RA (49, 50). In contrast to previous studies that suggested IL4 was the predominant anti-inflammatory cytokine in early RA, IL10 predominated in the NIH cohort. When the cytokine profile of Chlamydia related ReA was specifically compared to undifferentiated arthritis, INFγ

and IL-10 levels were higher in Chlamydia ReA patients. This is in contrast to a separate study in which IFNγ was higher in early and late RA compared to ReA, but no differences in IL4 or IL10 were seen. In this study, IL2, IL4, and IL10 tended to be higher in early RA than late RA, but lower in early ReA than late ReA (51). The discrepancies in these studies indicate that the TH1/TH2 paradigm is an oversimplification of cytokine profiles between different arthropathies.

Proteases in early disease

The development of joint damage due to erosions is a key distinguishing feature of inflammatory arthropathies such as RA and subsets of psoriatic arthritis. Mediators of joint damage including the cysteine proteases, cathepsin B and L, and several matrix metalloproteinases (MMP) have been studied in early disease. Serum and synovial fluid levels of MMPs, particularly MMP3 and MMP1, have correlated with erosive damage in early RA (52). A few studies have also attempted to correlate tissue protease presence and activity with joint damage.

Collagenase (MMP1), stromelysin (MMP3), the gelatinases (MMP2, MMP9) regulators of MMP function (MMP14, TIMP1, TIMP2), and cathepsins B and L have all been detected in synovial tissue from early RA (31, 53-55). MMP1 was strongly expressed in the synovial lining layer and perivascular tissue but spared lymphocytic aggregates (54). MMP3 and the cathepsins were also expressed in the lining layer. Comparisons of MMP1, MMP3 and cathepsin protein expression between early RA and established RA or between other early inflammatory arthritides such as PsA have shown similar expression patterns. MMP1 expression in the lining and sublining and cathepsin L mRNA expression in sublining synovial tissue was greater in early RA than non-RA (54). Importantly, in this study MMP1 expression was correlated with the development of new erosions after 1 year of follow-up (54).

The gelatinases (MMP2 and MMP9) are of particular interest in early inflammatory disease as they are critical for angiogenesis (56) and blood vessel morphology may be an early distinguishing feature between different types of arthritis. The gelatinases (MMP2, MMP9) and their regulators (MMP14, TIMP1 and TIMP2) were present in early RA synovium yet virtually undetectable in normal tissues (55). MMP2 was widely expressed, whereas MMP9 expression was more focal and present mainly in the sublining tissue. TIMP2, and MMP14 were expressed primarily in the lining layer. Early RA patients had higher levels of MMP2 expression than early non-RA patients and also had higher levels of MMP14, an activator of MMP2, and lower levels of TIMP2. Gelatinase activity was measured by a sensitive tissue-based zymographic technique in the NIH early synovitis samples. Early RA patients had higher levels of MMP2 activity than non-RA patients. Interestingly, MMP9 activity was higher, though not statistically significant in non-RA patients. In addition, MMP2 expression and activity was significantly correlated with radiographic erosions in the NIH early arthritis patients,suggesting it may be a marker for aggressive erosive disease. The Leeds early synovitis cohort also found greater MMP9 expression in PsA than in early RA patients (38). Importantly, MMP9 expression in synovial fluid of early PsA patients correlated with tissue vascularity and with synovial fluid VEGF. This suggests that increased gelatinase activity may influence the differences in vasculature found early in PsA and RA patients and may be a clinically useful predictor of erosive disease. More study is needed to confirm this.

Apoptosis in early disease

Accumulation of inflammatory cells and stromal proliferation can result from cellular proliferation, recruitment potentially through increased chemokine or adhesion molecule expression, or reduced apoptosis. The proportion of apoptotic cells in the synovium of patients with established RA, PsA and ReA inflammatory arthritis was greater than in control tissues and ReA tissue appeared to have greater apoptosis than RA and PsA (22). Catrina *et al*. compared the degree of apoptosis as measured by TUNEL staining in early and late RA (57). Early RA had limited apoptosis compared to late RA and the apoptosis in late RA was clustered in the lining and sublining layers. Interestingly, this group found that early RA had higher levels of FLICE-like inhibitory protein (FLIP) which is an inhibitor of apoptosis. They postulated that there is defective apoptosis early in disease and that apoptotic mechanisms are subsequently restored later in the disease. The p53 tumor suppressor gene also regulates apoptosis and p53 mutations have been associated with certain malignancies. In the setting of cellular damage, p53 can either arrest cell growth or induce apoptosis. The expression of p53 has been studied in early arthritis synovial tissue (58). p53 gene expression was detected in the lining layer, endothelium, lymphocytic aggregates where present, and diffuse leukocytic infiltrates of inflammatory synovium only. Importantly, p53 expression in diffuse leukocytic infiltrates was much higher in RA than in ReA or inflammatory OA synovium however, was similar in early and late RA synovium. Endothelial cell apoptosis was greater in early RA than early PsA (38) and this may account for some of the vascular patterns seen in early PsA. In this latter study apoptosis was also seen in perivascular and stromal tissue but minimal apoptosis was detected in the lining layer (38).

Histopathologic predictors of outcome

The primary concern with characterizing early arthritis relates to the need to identify patients destined to have persistent synovitis or aggressive erosive joint damage. Thus identifying pathologic features which can predict disease outcome – either in terms of progression of disease versus early remission or the development of joint damage and erosions – will be useful in clinical prognostication as well as provide further understanding of the pathobiology of the disease. A few studies have looked at pathologic features and the outcome of arthritis.

Protease activity is critical for joint

damage and serum levels of proteases have been shown to be predictive of erosive disease (52, 54). At the tissue level, collagenase (MMP1) mRNA and cathepsin L mRNA expression were highest in patients with erosive disease either at the time of biopsy or on followup. In addition, collagenase, (MMP1) mRNA in lining and sublining layers and cathepsin B mRNA in the sublining layer correlated with the number of new erosions that developed after 1 year of follow-up (54). In the NIH cohort, active MMP2 (gelatinase A) in the synovial tissue of early synovitis was associated with erosive disease (55). Synovial macrophage numbers early in disease have also correlated with the radiographic score after 5-6 years; however, CD3 T cells were not associated with the development of erosions (54).

Osteoclast activity is critical for the development of bone erosions. The formation and activation of osteoclasts is dependent on the interactions between the receptor activator of NFκβ (RANK) expressed by osteoclasts and osteoclast precursors, its ligand receptor activator of NFκβ ligand (RANKL) expressed by osteoblasts, stromal cells and fibroblasts, and a soluble decoy receptor osteoprotegerin (OPG) (59). RANKL and OPG have been detected in synovial tissue obtained from patients with rheumatoid arthritis and spondyloarthropathies (60,61). RANKL expression was greater in active RA synovium obtained from patients with less than 12 months of disease and spondyloarthropathy synovium obtained from patients with more established disease than in synovium from inactive RA, OA or normal controls (60). In addition, RANKL expression in active RA tended to be greater than in SpA. RANKL was expressed on CD3 T cells, primarily activated memory T cells, and some macrophages. In contrast, OPG expression was seen only in inactive RA, spondyloarthropathy, OA and normal synovium but not in active RA synovium and was primarily expressed by macrophage-like cells in the lining layer and endothelial cells (61). This suggests that a deficiency of OPG may lead to excessive osteoclast activity and predispose to the development of early erosions.

The role of synovial tissue biopsy in the early synovitis clinic

Analysis of synovial tissue biopsies obtained from patients early in the course of their disease can provide valuable insights into the pathogenic mechanisms occurring in the early stages of inflammatory arthritis. Specific features have not yet been identified which clearly distinguish clinical variants with a high degree of specificity, suggesting that similar mechanisms may be acting in different conditions. Importantly, clinically relevant measures of disease severity, the development of erosions and disease remission, can potentially be predicted using pathologic variables, although this remains to be clearly shown in large cohorts of patients. Thus,careful analysis of synovial tissue histopathology, in combination with known clinical features and novel RA specific autoantibodies has the potential to assist the clinician in identifying patients who need to be targeted for more aggressive therapy.

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