Role of fibroblast-like synoviocytes in cartilage degradation during rheumatoid arthritis

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Introduction

Introduction

The joint

Joints are the hinges in the skeleton and they facilitate movement of the body. They are indispensable for all kinds of movements, like walking, grabbing things and jumping. A joint is composed of two opposing bones connected by a capsule (See figure 1). The inner layer of the capsule forms the synovial membrane. Sometimes a joint also contains ligaments, menisci or additional bones like the patella. The rest of this introduction will discuss the structure and function of the synovium and its cells.

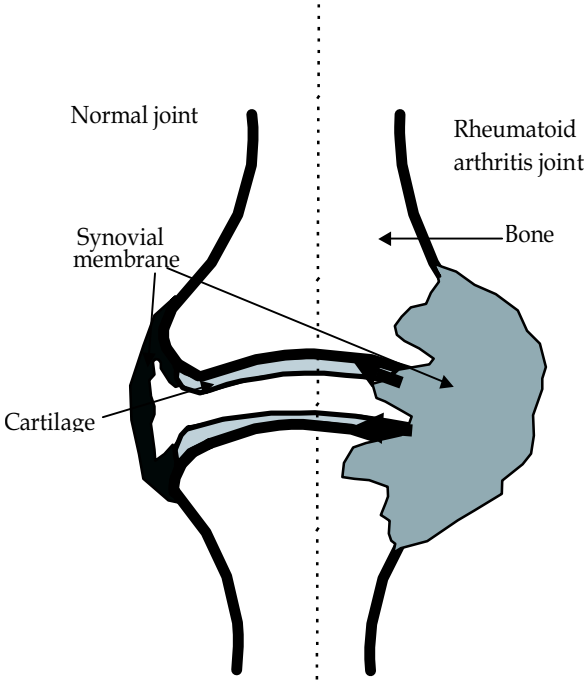

Figure 1 Schematic representation of a normal healthy joint (left) and a joint from a patient with rheumatoid arthritis (right).

Synovium and fibroblast-like synoviocytes

Synovium is very variable, being composed of several layers, any of which may be present or absent at a given site (see figure 2). The anatomical and histological boundaries of the tissue are often hard to identify (1). The function of the synovium is to facilitate skeletal movement by the maintenance of a fluid-filled space around cartilage or tendon surfaces.
The presence of fluid is dependent on 3 factors:
1) the subatmospheric pressure within the cavity at rest, which encourages the entry of plasma dialysate.
2) the addition of hyaluronan (HA) to this dialysate.
3) the presence of a compact uninterrupted superficial synovial tissue layer or membrane (1).
The synovial intima consists of a layer of overlapping cells, rarely more than 3 cells thick, without a basal lamina or tight junctions (2).
Intimal cells are a mixture of macrophages and fibroblast-like cells (1;3) and synovial intimal cells vary in shape from polygonal cells with round or oval nuclei to elongated cells with spindle shaped nuclei (2). The differences are based on electron microscopy. The different cells are called type A and type B synoviocytes. Type A synoviocytes contain prominent Golgi complex and many vesicles and vacuoles, while type B synoviocytes contain a lot of rough endoplasmic reticulum and only sparse Golgi complex and vacuoles. Another feature of type A cells are greater amounts of filopodia, mitochondria, intracytoplasmic filaments and lysosomes than type B cells (2).
During the 1980s the type A synoviocytes were considered to be tissue macrophages and type B synoviocytes the fibroblast-like synoviocytes (FLS) or fibroblasts. The work presented in this thesis focuses on FLS and their role in joint destruction during rheumatoid arthritis.
FLS form together with macrophages in the lining layer the border between the stroma of the synovium and the synovial joint filled with synovial fluid. Synovial fluid is an ultrafiltrate from the blood in which several molecules are added by FLS. This synovial fluid functions as a lubricant in the joint and facilitates movement, but it also contains nutrients for the chondrocytes in the cartilage, relevant because cartilage is hardly vascularized. Cultured FLS show a characteristic fibroblast spindle shaped morphology and express fibroblast markers, like vimentin and collagens (4).

Development of synovium and differentiation of FLS

Synovial joint development in the foetus can be divided into 2 stages:

1. Formation of a primitive skeletal core or anlage. This is composed of cartilaginous elements and is destined to form individual bones. These "prebones" are separated by transverse bands of relatively flattened cells, known as interzones. The periphery of each interzone is destined to become synovium and becomes vascularised, while the centre remains avascular.
2. Cavitation of the interzone. During this process the cartilaginous and synovial elements separate and take on their final form (5).
How this separation is initiated and elongates is not exactly known, however it is thought that hyaluronan (HA) plays an important role. The cells involved in the cavitation of synovial joints are CD44 positive, which is a receptor for HA (6).
HA is a nonsulphated, high molecular weight unbranched polysaccharide composed of repeating disacchariride units of glucuronate linked to N-acetyl glucosamine. The substrate precursors of HA are (uridine diphospho-) UDP-glucuronate and UDP-N-acetylglucosamine. Hyaluronan synthase transfers these two precursors alternately to nascent HA. Uridine diphosphoglucose dehydrogenase (UDPGD) is the enzyme responsible for the conversion of UDP-glucose to UDP-glucuronate and this conversion is thought to be the rate limiting step in the synthesis of HA (7). Cells immediately bordering the line of joint separation have high UDPGD activity just like synoviocytes of human adult synovium (4;5).
After synovial tissue has been clearly separated from adjacent cartilage at 12 weeks after gestation or later, significant staining for β-integrin was seen in synovium. At this stage, staining is relatively prominent on the surface layer of cells. These data are the only indication that a distinct intimal synoviocyte phenotype starts to develop, because these cells were still negative for VCAM-1 as opposed to adult intimal layer synoviocytes (5). In normal adult synovium fibroblasts two phenotypes are observed: intimal (present in the lining) and subintimal (present in the stroma of the synovium). The intimal fibroblasts express UDPGD, HA, VCAM-1 and CD55. The intimal fibroblasts also express fibronectin, type IV collagen, laminin and chondroitin-6-sulphate bearing proteoglycans, which are associated with basal laminae. Other molecules that are expressed by intimal fibroblasts are two types of microfibril based on fibrillin-1 and type VI collagen, Lubricin, phospholipids and unusual carbohydrate entities (1;4;6).

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, inflammatory disease of the joints with an unknown etiology. Both genetic and environmental factors contribute to disease susceptibility and the prevalence of RA is greater in women than in

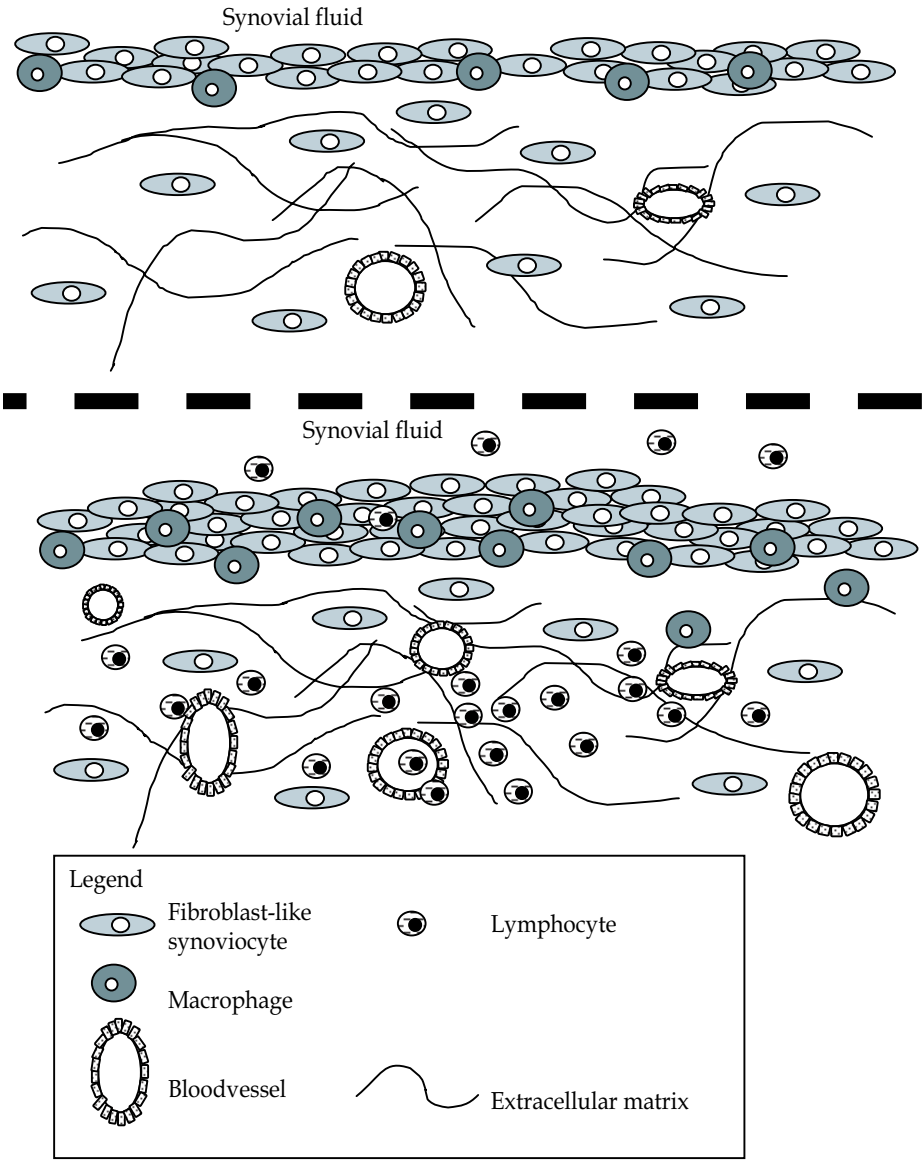

Figure 2 Structure of the synovium in healthy persons (upper panel) and in patients with rheumatoid arthritis (lower panel).

men. One of the genetic factors associated with RA is the shared epitope. This implicates in increased presence of certain HLA alleles in patients in RA. RA is believed to be an autoimmune disease characterized by infiltration of immune and inflammatory cells into the synovium, hyperplasia of the synovial lining, formation of lymph follicles and development of pannus. There is progressive cartilage destruction and finally erosion of the underlying bone. Fibroblast-like synoviocytes (FLS) are thought to be one of the principle cells involved in pannus formation as demonstrated by histology and the intimate physical relation of the cartilage pannus junction suggests a prominentrole in cartilage degradation. In this thesis the role of FLS in the pathology of RA is investigated with emphasis on the invasive properties of FLS.

Characteristics of FLS in RA as compared to FLS in healthy human adult synovium

Morphology and behaviour

Characteristic lesions found in RA synovium are hypertrophy, proliferation of synovial connective tissue (pannus formation) and subintimal infiltration with mononuclear cells (see also figure 2) The pannus tissue is found on the border between synovium and cartilage and in the pannus tissue cells were identified that showed characteristics of both FLS and chondrocytes. They expressed on-cogenes, mRNA for matrix degrading enzymes, vimentin and collagen type II (8;9). These data are compatible with a possible contribution to the destruction of cartilage by pannocytes in RA. Because FLS can undergo chondrogenesis when cultured in the presence of TGF-β1, this suggests that these pannocytes have a synovial origin (10).

Invasiveness

The first study to show that FLS from patients with RA are invasive into cartilage comes from the severe combined immunodeficient (SCID) mouse co-implantation model of RA (11). In this model, isolated RA FLS are implanted together with normal human cartilage under the renal capsule of SCID mice (11). Because these mice lack a functional immune system, they do not reject the implants and this allows for the study of the interaction between RA FLS and normal human cartilage.
In table 1 cytokines and other proteins are listed that are known to affect FLS

migration and invasion. In joints of patients with RA fibrin deposits adhering to the synovial surface are typical of the disease. Aggregation of fibrin to the synovial surface in rabbits with antigen-induced arthritis can activate the FLS in the lining layer to invade into the aggregates (12).

Table 1 Effector molecules with an effect on migration and invasion of FLS

Factor	Cellular or tissue source	Effect
TNF-α	Macrophage, activated monocyte, B cell, T cell, fibroblast	+
PDGF	Platelet, macrophage, endothelial cell, skeletal muscle cell, glial cell, type I astrocyte, myoblast, kidney, epithelial cell, mesangial cell	+
TGF-β	Platelet, macrophage, T cell, skeletal muscle cell, fibroblast	+
bFGF	Brain, retina, bone matrix, endothelial cell, macrophage	+
EGF	Granulocyte, ectodermal cell, kidney, duodenal gland, platelet	+
IGF-I (SmC)	Fibroblast, skeletal muscle cell, liver, endothelial cell, T cell	+
Collagen	ECM	+
Fibronectin	ECM	+
Fibrin	Platelets	+
Interferon	T lymphocytes, NK cell (IFN-γ), all cells (IFN-α)	-

Recently, an inverse correlation between invasion and proliferation was shown (13), suggesting a dissociation between proliferation and invasion. This has also been shown for several tumours, were an inverse correlation has been found between rate of proliferation and metastasis of a tumour. This is indirectly confirmed by Kasperkovitz et al (14), who found that two groups of FLS exist in RA patients. One group expressed genes involved in proliferation, like growth factors and the other group expressed genes involved in cartilage degradation (see also below). Besides adhesion CD44 is also involved in invasion of RA FLS. FLS expressing the splice variants v3 and v6 were significantly more invasive than FLS not expressing these variants and antibodies against these variants inhibited the invasiveness. Furthermore, the invasive cells showed reduced expression of CD44v7/v8 (15), which confirms the observations that invasive cells show reduced proliferation compared to non-invasive cells, because CD44v7/v8 is associated with increased proliferation of FLS (16).

Adhesion molecules

Integrins
One of the most striking features of RA FLS is their ability to grow anchorage independent in soft agarose for mutiple passages and escape contact inhibition, which could be inhibited by TGF-β or retinoids (17). Furthermore, RA FLS up-regulate different adhesion molecules that allow them to attach to cartilage. One family of adhesion molecules that is up-regulated on RA FLS are the integrins (18). Integrins consist of two non-covalently bound subunits, α and β. The α subunit family consists of α1 to α6 or αv and the β subunit has three isoforms β1, β3 and β4. The expression of β1 integrins contributes to increased binding to the cartilage (18;19) and antibodies against β1 integrin inhibited, at least in part, the binding of RA FLS to the ECM (18). Several integrins function as fibronectin receptors and therefore it has been suggested that the fibronectin-rich environment of the RA cartilage surface facilitates adhesion of FLS to the articular cartilage. Furthermore, adhesion to fibronectin via αv integrin receptors downregulated the expression of MMP-1 and induced proliferation in response to PDGF (20). In contrast, loss of cell adhesion was associated with loss of cytoskeletal structure and change in cell shape and increased MMP-1 expression (20). Crosslinking of β1 integrin or binding of fibronectin or collagen type I induces the expression of ICAM-1 and Fas on FLS from patients with RA (21). Normal FLS constitutively express the integrins αv and β3, however expression of β3 integrin is less in RA FLS and expression of these integrins was further downregulated by the pro-inflammatory cytokines IL-1β and TNF-α (22). IL-1β induced invasion of RA FLS into cartilage slices and this invasion was dependent on the integrins β1, α4, α5 and αv (23).
The expression of the oncogenes c-fos and c-myc is also regulated by integrins. This demonstrates that integrins not only play a role as receptor molecules involved in cell adhesion, but they can also interact with several signaling pathways involved in the pathogenesis of RA (24). These pathways mainly involve oncogenes and can confer independency of adherence to cells.

Cadherins
Cadherins are transmembrane glycoproteins expressed in restricted patterns. They mediate homophilic adhesion between cells. Several cadherins exist with a specific tissue localization, like E-cadherin in epithelia and N-cadherin in the nervous system. Together with intracellular catenins they bind to the actin cytoskeleton and can activate intracellular signaling pathways, influence cytoskeletal organization and orchestrate multicellular arrangements (25;26). Changes in cadherin expression are associated with cell transformation and tumor metastasis.

Cadherin-11 and not E-cadherin was found to be expressed in FLS from healthy individuals and patients with RA and OA and cadherin-11 mediated adhesion of FLS on Cadherin-11/ Fc fusion protein coated plates, which facilitate cadherin-cadherin interactions (27). Cadherin-11 staining was seen at sites of cell-cell contact in ladder-like series of lines. This corresponded with the filopodial processes on FLS (27). Cadherin-11 is associated with invasive tumour behaviour in breast and prostate carcinomas (28;29). Therefore, it will be of interest to study whether this cadherin is important in the locally invasive behaviour of the rheumatoid synovium. Although it is unknown whether synoviocytes are epithelial cells, some reports show that they express E-cadherin (30;31), in contrast to other reports (27) that do not find E-cadherin expression in FLS. Triggering of LTβR on FLS increases the expression of the adhesion molecules VCAM-1 and ICAM-1, indicating increased adhesive properties of FLS, which can help with the formation of ectopic germinal centers (GC) in RA synovium (32) (see also below in "Interactions with other cell types"). ICAM-1 has been demonstrated to distinguish between proliferative RA FLS and apoptotic/ growth arrested FLS, because cells positive for ICAM-1 represent growth arrest with up-regulation of Fas and p53, whereas ICAM-1 negative cells were proliferative (33).

CD44

CD44 has previously been identified as a lymphocyte homing factor. However, nowadays many functions for CD44 are known, most of which are concerned with cell adhesion. It is the major cell surface receptor for hyaluronan (34). The gene of CD44 consists of various exons that can be spliced out of the protein and different splicing combinations give the protein different functions and some splice variants are associated with increased metastasis of tumours. FLS from patients with RA show a wide variety of splicing combinations at high levels (35). Expression of CD44 exons 7 and 8 (CD44v7/8) is associated with increased proliferation of FLS and antibodies against this epitope, but not other exons, inhibited proliferation (16). This implicates a role for CD44 in the transformation of RA FLS. Another function of CD44 recently discovered is the up-regulation of Fas on the membrane of FLS and this amplified the Fas-mediated apoptotic change (36).

Matrix degradation

A hallmark of the inflammatory synovitis seen in RA patients is the subsequent erosion of articular cartilage and bone presumably caused by pannus tissue, which is not seen in osteoarthritis (OA). Addition of FLS and monocytes to cultured and radiolabeled cartilage slices resulted in cartilage degradation and

this was augmented by addition of TNF-α, IL-1β and IL-6 (23;37). Degradation only occurred by direct contact with the cartilage via CD44 and was dependent on β1, α4, α5 and αV integrin expression.

ADAMs and MMPs

In RA several enzymes that can degrade the extracellular matrix (ECM) are overexpressed. Families of these enzymes are the matrix metalloproteinases (MMPs), a disintegrin and metalloproteinase family (ADAMs) and the cathepsins. ADAM15 is overexpressed in RA synovial tissue. It is expressed by FLS, macrophages and plasma cells (38). This implicates a role for this metalloprotease in the pathogenesis of cartilage destruction in RA. In the synovium, several MMPs play an important role in the degradation of ECM. MMP-1, MMP-3, MMP-9, MMP13, MMP-14 and MMP-15 (39-45) are the most important MMPs that are up-regulated in RA FLS compared to OA or normal healthy FLS.
In contrast, Reboul et al. demonstrated that MMP-13 is not expressed in FLS, but only in chondrocytes and that it probably play a role in OA and not in RA (46). Expression of MMP-3 was found mainly in the lining FLS, but also in the sublining layer (47-49) (50;51) and MMP-3 activity was crucial for aggrecan and collagen type II breakdown during adjuvant induced arthritis in mice (52). Expression of MMP-8 in FLS is rather controversially, because Konttinen et al found no expression in synovium from patients with RA or trauma, but previously from the same group expression of this MMP has been demonstrated in FLS from patients with RA (53). The membrane type (MT-) MMP MT1-MMP (MMP-14), MMP-2 and MMP-13 showed strong co-expression in RA FLS (54). MT1- and MT3-MMP are abundantly expressed in RA synovial lining cells (55;56) and this expression of MT-MMPs is important because MT-MMPs not only degrade ECM, but they also play an important role in the activation of other MMPs, such as MMP-2 and MMP-13 (57). MT1-MMP deficient mice develop spontaneous arthritis among other diseases due to inadequate collagen turnover (58). In contrast, MMP-9 knockout mice display milder arthritis after immunization with antibody, while MMP-2 knockout mice also show more severe diasease (59).
MMPs are also found in the synovial fluid of patients with RA (42), with very high expression of MMP-1 and –3 (60;61). Expression of MMP-1 and -3 and tissue inhibitors of metalloproteinases (TIMP)-1 is also found in the serum of patients with RA and the levels of MMP-1 and -3 correlate with disease activity and predict clinical response (62-66). Serum levels of MMP-3 have been shown to be higher in patients who carry the shared epitope, especially early in the disease course in RA patients. This may partly reflect the association between shared epitope and more erosive disease (64). Anti-TNF-α therapy down-regulates serum levels of MMP-1, –3 and MMP-TIMP ratio (67).

Expression of MMP-13 is of importance for RA, because it is the main protease to cleave type II collagenase and it can cleave aggrecan, a large cartilage proteoglycan (68). Its mRNA was induced in FLS when they were co-cultured with normal human cartilage or when FLS were cultured within 3 dimensional collagen gels (69) and expression of MMP-13 correlates with levels of systemic inflammation markers (43).
The activity of MMPs is counterbalanced by TIMPs, which are also produced by FLS in the lining of the synovium (49). Expression of TIMPs is enhanced by IL-6, IL-11 and TGF-β via expression of the transcription factor Egr-1 (70). Over expression of TIMP-2 has been shown to ameliorate collagen induced arthritis in mice (71) and overexpression of TIMP-1 in TNF-α transgenic mice reduces joint damage (72). Expression of MMPs is tightly regulated and oncogenes and cytokines play an important role in this regulation. Ras can induce expression of MMP-1, -3, -9 and –13 via c-Raf-1 (73). When FLS were transduced with dominant negative c-Raf-1 (a downstream messenger of Ras) or dominant negative c-Myc, invasiveness in this model was significantly inhibited as compared to mock transduced cells (73). The inhibition of invasiveness was not complete, however due to rapid apoptosis induction after transduction with both vectors, this could not be used in the SCID mouse model. Egr-1 and fos are known to induce expression of MMP-1 in fibroblasts (74). IL-1β induces the expression of MMP-1, -3 and –9 in fibroblasts (75) and IL-1β induced expression of MMP-1 is dependent on Bcl-3 (76).
It is known that many MMPs contain AP-1 binding sites in their promotors, indicating that the AP-1 transcription factor is involved in the tissue specific expression of MMPs (77;78). However, although AP-1 is necessary for transcription of MMPs, it is not sufficient. It cooperates with other transcription factors to regulate MMP expression (77). Like AP-1, NF-κB induces the expression of MMPs (79), but it is unclear which regulatory pathway contributes most significantly to the altered expression of MMPs in RA (80). Inhibition of NF-κB activation inhibits the expression of MMP-1 (81) and –3 but leaves the expression of TIMP-1 unaffected (79) shifting the balance towards repair. Transforming Growth Factor (TGF)-β has been shown to induce expression of MMP-1 (82) and inhibit the expression of MMP-3 probably via the expression of Fos which can bind in a multimeric protein complex to a DNA sequence identified as the TGF-β1 inhibitory element (83). This complex is probably different from AP-1, because AP-1 induces expression of MMPs, including MMP-3. Furthermore, TGF-β induces the expression of tissue inhibitor of MMPs (TIMP) shifting the balance proteinase/inhibitor towards a net neosynthesis of ECM (84) via which TGF-β is also involved in the formation of fibrosis, another characteristic of RA in some patients.
Extracellular matrix metalloproteinase inducer (EMMPRIN) can induce local

production of at least MMPs-1, -2 and –3 and its expression is up-regulated in the rheumatoid synovial membrane compared to OA. This supports a role for EMMPRIN in joint destruction in RA (85;86).
Recently, it was shown that expression of MMP-1, -3, and –9 is increased by enhanced activity of the MAPK ERK (87). Beside a role in apoptosis, the tumor suppressor p53 has also been demonstrated to play a role in the regulation of MMP expression. Wild-type p53 and retinoblastoma (88), also a tumour suppressor, downregulate the expression of MMP-1 and –13, but in cells with mutant p53 this downregulation is inhibited and in one mutant, expression is even enhanced 2-4 fold (89).

Cathepsins
Cathepsins B, D, K and L are cysteine proteases that are expressed in RA synovium (90-96). Expression levels of cathepsin K in RA FLS correlated with disease severity (94) and enzymatic activity of cathepsins in AIA rats was positively correlated with joint destruction and inflammation (97). Ras oncogene can induce expression of cathepsin L and B in several transformed cell lines and in RA synovium combined overexpression of Ras and cathepsin L are found mainly at the site of invasion (98). Expression of cathepsins can be up-regulated by cytokines including PDGF, TNF-α (cathepsin B, L and K), IL-1β (cathepsin K), bFGF (cathepsin L), IL-1β (cathepsin B and L) and IFN-γ (cathepsin B and L) (92;96).

Serine proteases
A third family of proteases involved in the degradation of extracellular matrix are the serine proteases. This family include plasmin and plasminogen activators which are important in RA because of their fibronolytic function and their capacity to degrade a wide range of ECM molecules and activation of MMP-1 and –3 (99). Furthermore, the plasminogen activation system and their inhibitors are expressed at significantly higher levels in RA synovium as compared to OA or normal synovium. Inhibition of the urokinase-type plasminogen activator resulted in a significant reduction of cartilage matrix degradation in vitro and in cartilage invasion in vivo (99).

Aggrecanases
A recently discovered family of matrix degrading enzymes, aggrecanase-1 and –2, are expressed in RA FLS and expression of aggrecanase-1 is induced by TGF-β1, indicating a role for these enzymes in joint destruction of RA (100).

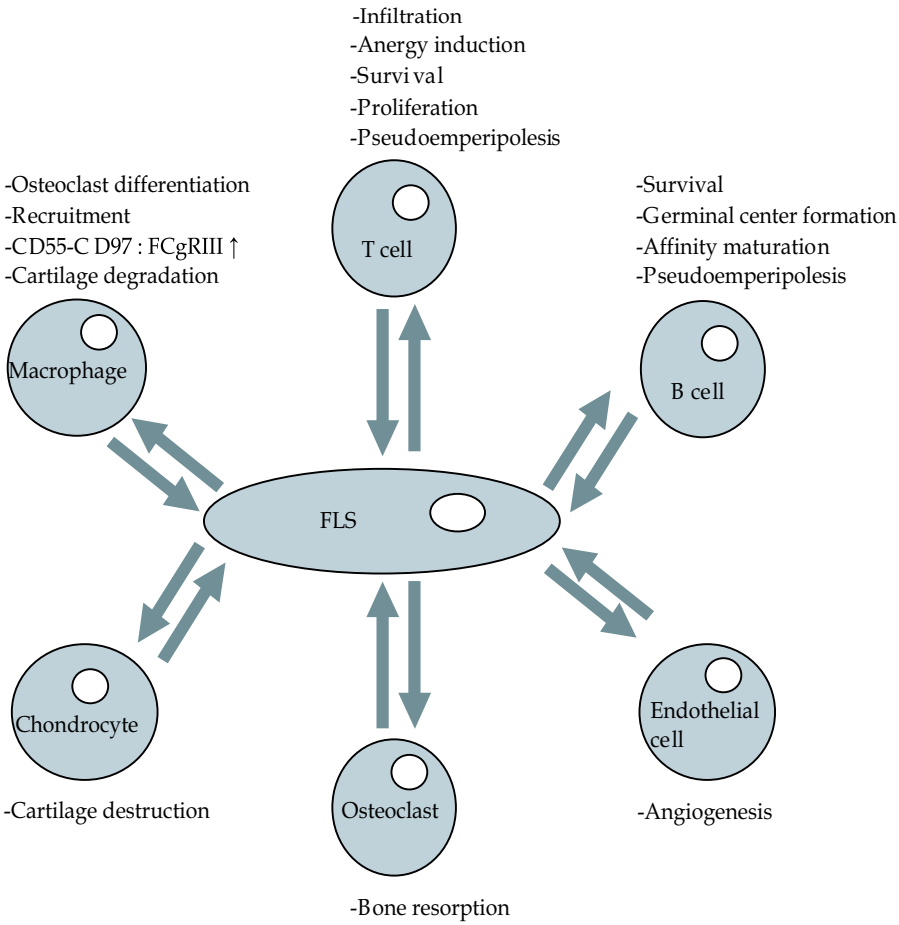

Figure 3 Interaction between FLS and other cell types play an important role in the modulation of synovitis and joint destruction

Interaction with other cell types

In the rheumatoid synovium the FLS also interact with various cell types to modulate synovitis and joint destruction (see figure 3).

Macrophages and osteoclasts
RA FLS play an important role in the activation of macrophages in the lining and their differentiation into multinucleated, osteoclast-like bone resorbing cells (101). RA FLS express large amounts of the osteoclast-differentiation factor (ODF), which correlates with the ability of these cells to induce osteoclastogenesis in peripheral blood monocytes in vitro (102) and in synovial cells (103)FLS express functional IL-2 receptor β- and γ-chains (CD122 and CD132) and are able to recruit macrophages into the rheumatoid synovium by secreting macrophage chemoattractant protein-1 (MCP-1) in response to IL-2 derived from T cells (104). MCP-1 has also been shown to stimulate expression of MMP-1 in human fibroblasts (105). RA FLS in the lining can also interact with macrophages via CD55-CD97 interaction (106), which may be involved in induction of FcγRIIIa expression on macrophages (6). Coculture of macrophages and FLS induced cartilage degradation and this could be inhibited by antibodies against IL-1β, IL-6, CD44 and TNF-α (37;107).
FLS also play a role in bone catabolism by secreting RANKL and macrophage-colony stimulating factor (M-CSF) which are necessary for osteoclast development (108). As a counteraction of bone catabolism, FLS also secrete bone morphogenic protein-2 (BMP-2) and -6 after stimulation with IL-1β and TNF-α (109). BMP-2 participates in the compensatory response by stimulating new bone formation (110). As an additional function, BMP-2 induces apoptosis in FLS, while BMP-6 protected against apoptosis induced by nitric oxide (109).

T and B cells
RA FLS in the lining layer of the synovium are an important source of IL-16 (111), which has been demonstrated as a chemoattractant for CD4+ T cells and appears at least in part responsible for the anergic state of T cells in the synovial state (112). Furthermore, direct contact between T cells and FLS caused up-regulation of CD69 and CD25 on T cells, negligible T cell proliferation and HLA-DR on FLS (113). IL-1 exists as a soluble form, but also as a membrane bound isoform (MA-IL-1). MA-IL-1 is expressed on RA FLS and macrophages and induced proliferation of T cells, but also of FLS in an autocrine manner. Furthermore, it promoted proteoglycan release from cartilage, a measure for cartilage destruction (114).
FLS fom patients with RA have similar properties as follicular dendritic cells in the sense that they can bind B cells and are able to inhibit T and B cells from

apoptosis (115;116). Cell-cell contact between B cells and RA FLS via VLA-4 and V-CAM-1 up-regulates expression of BCL-XL in B cells which inhibits apoptosis of these B cells (117;118) and together with CXCL12-CXCR4 interaction support migration of B and T cells below a monolayer of FLS (pseudoemperipolesis) (119;120). The pseudoemperipolesis of B cells is dependent on VLA-4, but not VCAM-1 and binding of B cells to the FLS induces expression of IL-6 and IL-8. However this is dependent on VLA-4 independent cell-cell contact (121).

Lymphoid structures
FLS from patients with RA express the lymphotoxin β receptor (LTβR) (32), which is indispensible in the formation lymphoid tissue. Tertiary lymphoid tissue is formed in several patients with RA (122) and these germinal center (GC) like structures are capable of supporting B cell affinity maturation. Stimulation of LTβR on RA FLS increased the expression of IL-1α and –β, IL-6, granulocyte and monocyte colony stimulating factor (GM-CSF), TNF-α, MMP-1 and –3 (32). These molecules all play an important role in the pathogenesis of RA. Stimulation of RA FLS with LTα1β2 increased the adhesiveness for T cells probably via the increased expression of VCAM-1 and ICAM-1 (32). Triggering of LTβR on RA FLS also increased the secrestion of the pro-inflammatory chemokines CCL2, CCL5 and CXCL8 and this stimulated the migration of T cells in a tran - swell migration experiment (32). TNF-α, IL-1β and TGF-β1 regulate the expression of CCL2, CCL5 and CXCL12 by RA FLS and these cells also express the corresponding receptors CCR2, CCR5 and CXCR4 (123;124). It has also been shown that the number of CXCR4 expressing CD4+ T cells was elevated in RA synovium and that CXCL12-CXCR4 interactions inhibited activation-induced apoptosis of these cells (124). This implicates that chemokines not only play a role in inflammatory cell migration, but are also involved in the activation of FLS in RA synovium. Expression of IL-6, IL-8, CXCL1 and CXCL2, COX-2 and PGE2 (125) in FLS are up-regulated by IL-17/IL-17R interaction, which is a cytokine that is produced by T cells and is abundantly expressed in RA syn - ovium. Its receptor is expressed on several cell types including FLS (126;127). Treatment of mice after the onset of collagen induced arthritis (CIA) with a neutralizing anti-murine IL-17 antibody reduced joint damage, systemic levels of IL-6 and the severity of CIA (128).

Angiogenesis
RA FLS also express abundant angiopoietin-1 (Ang-1) and Ang-2 which are important regulators of angiogenesis (133). Expression of Ang-1 is up-regulated by TNF-α (134). The receptors of Ang-1 and –2 are Tie1 and Tie2 and are also expressed on FLS. Signaling via Ang/Tie2 is not only important for the up-regulated angiogenesis in RA, but also for synoviocyte behaviour by regulating

chemotactic cell movement (133).
Cell-cell contact between FLS and stimulated T cells can markedly increased the production of MMP-1 and –3, VEGF, PGE2, IL-15, TNF-α and IL-18 (129-131) in FLS. Up-regulated production of MMP-1 and –3 is at least partially mediated by cell-bound IL-1α and TNF-α and integrins play an important role in VEGF production, because antibodies against integrins inhibited the expression of VEGF (130). Cell-cell contact between FLS and T cells also works the other way around via CD47 and its ligand on FLS, thrombospondin, which induced proliferation and survival of T cells (132).

Cartilage
Invasiveness of FLS from patients with RA is significantly decreased when FLS are co-implanted with cartilage stored for 24 hours as compared to fresh cartilage. Furthermore, the cartilage breakdown by FLS is decreased when chondrocyte protein biosynthesis is blocked by cycloheximide. The cartilage breakdown was restored by addition of IL-1β (135). This implicates a role for chondrocytes in cartilage breakdown by RA FLS even when the chondrocytes come from normal healthy human cartilage.

Table 2 Effector molecules released by FLS

Signal function	Effector molecules
Angiogenesis	IL-8, TGF-β, PDGF, GM-CSF, G-CSF, FGF, VEGF, EGF
Chemoattractant	IL-8, IL-16, MCP-1, MIP-1α
Pro-inflammatory	IL-1, IL-6, IL-7, IL-8, IL-11, IL-15, LIF, PDGF, MIF, GM-CSF, TRX
Anti-inflammatory	p55 TNFR, p75 TNFR, IL-10
Matrix degradation	PGE2, MMP-1, MMP-2, MMP-3, cathepsins B, L and K
Inhibit matrix degradation	TIMP, TGF-β, IL-11
Osteoclastogenesis	RANKL, VEGF
Bone formation	TGF-β, BMP-2

Soluble mediators

Many cytokines, chemokines and growth factors are secreted by FLS in patients with RA and table 2 shows a short summary of these mediators and their effects.
Myofibroblasts are activated or transformed fibroblasts exhibiting increased ex

pression of cytokines and chemokines like stromal cell derived factor-1 (SDF-1) (4), IL-6 and IL-8. These cytokines are abundantly expressed in RA FLS (127) and this implicates another role for myofibroblasts in RA as mentioned above (morphology and behaviour) (14). These cytokines play an important role in the influx of inflammatory cells in the synovium.

Angiogenic factors
Platelet derived growth factor (PDGF) is not only expressed in platelets, but also in endothelial cells, macrophages and synovial cells (136). PDGF stimulates proliferation and anchorage independent growth in RA FLS (17). Synergisti - cally with IL-1, PDGF can induce PGE2 (137), however IL-1 antagonizes the proliferative effect of PDGF on FLS and PDGF did not increase the steady-state levels of MMP-1 as IL-1 does (137). PDGF is overexpressed in the lining layer of RA synovium (138) and the intensity of PDGF expression correlates with the expression of heparin-binding growth factor (HBGF), which is a precursor of acidic FGF (136). HBGF is abundantly expressed in rheumatoid synovium and is a potent mitogen for FLS. FLS from patients with RA spontaneously express acidic FGF and expression is increased compared to OA and normal FLS, which is increased by TGF-β (139).
New blood vessel formation or angiogenesis is a characteristic feature of RA and it is now appreciated that RA FLS play an important role in the forma - tion of new blood vessels. They secrete important pro-angiogenic cytokines and growth factors including TGF-β, IL-8, PDGF, GM-CSF, EGF, FGF and VEGF. VEGF is one of the most potent angiogenic factors and is constitutively ex - pressed by RA FLS (140). Expression of VEGF is further increased by IL-1. Due to the inflammation in RA, hypoxic conditions are likely to occur and this also leads to enhanced expression of VEGF.

Monocyte chemoattractants
Beside angiogenesis, RA FLS have the potential to induce synovitis by releasing mediators that attract leukocytes and monocytes into the joint. After cytokine stimulation, FLS can secrete macrophage chemotactic protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α, that are responsible for attracting monocytes (82;141;142). FLS are stimulated to express IL-1β, IL-6, IL-8, COX-2, PGE2 by MIF (143;144)and MIF induced phosphorylation of p38 and ERK and p38 activation was responsible for the induced expression of COX-2 and IL-6 (144).

Inflammation modulators
RA FLS also play a role in the modulation of the inflammatory response. TGF-β1 induces the expression of IL-1β, TNF-α, IL-8 and MIP-1α by RA FLS thereby

contributing to the progression of inflammation (82). RA FLS in the lining layer express more Complement 2 (C2) and HLA-DR compared to OA FLS (40). They can also trigger the acute phase response by releasing the IL-6-type cytokines IL-6, IL-11 and Leukemia inhibitory factor (LIF) (145), furthermore, FLS promote T-cell activation and expansion by IL-15 and IL-7 (146) and IL-15 is also involved in the activation of FLS via an autocrine feedback loop. FLS express the IL-15 receptor and triggering via exogenous IL-15 up-regulated expression of BCL-xL and enhanced proliferation (147). Expression of IL-15 by FLS is inhibited by PGE2 via NF-κB (148). Oxidative stress in RA FLS and monocytes induces thioredoxin and this augments the secretion of TNF-α and IL-1 and also blocks apoptosis (149). Thioredoxin is important for the maintenance of an appropriate intracellular redox balance, but secreted thioredoxin functions as a cytokine-like molecule.
In an attempt to downregulate the inflammation, FLS also express IL-10 (150) and this expression is up-regulated by PGE2 (148), IL-1β and TNF-α (150).
FLS from patients with RA are a source of nitric oxide (NO) in the synovial fluid. NO may mediate pathology of RA through the induction of TNF-α production (151).
IL-18 plays a role in the activation of T cells and macrophages in the synovium, however not on FLS, because FLS do not express functional IL-18 receptor (152). FLS do express the IL-18 antagonist (IL-18 binding protein (IL-18BP)) after stimulation with IFN-γ, which may limit IL-18 biological activity in arthritis (153).

FLS phenotype in RA?

In RA, transformed-appearing cells are discerned, characterized by large, pale nuclei, containing prominent nucleoli and an abundant cytoplasm. This transformed-appearing phenotype of synovial lining cells is accompanied by the production of matrix-degrading molecules and the upregulation and expression of signaling molecules such as growth factors, cytokines and adhesion molecules (154-156). These groups of molecules are discussed below. Using a general method for the identification of differences in patterns of gene expression, it was revealed that cultured RA FLS overexpress certain proinflammatory genes that are potentially relevant to lymphocyte and monocyte entry and interactions. They facilitate localization of immune reactions to the joint through leucocyte chemokinesis, cell-cell adhesion, and matrix specialization (157). The alterations of synovial components are thought to be due to activation of proto-oncogenes that are involved in the regulation of the cell cycle (138).
There are also indications that FLS in RA have lost their original differentiation state. This can be due to dedifferentiation or transdifferentiation or that there is an influx of non-differentiated cells showing characteristics of embryonal cells.

It has been shown that bone marrow CD34+ cells from patients with RA have abnormal capacities to respond to TNF-α and to differentiate into FLS producing MMP-1 and prolyl 4-hydroxylase (158). This was not seen with cells from controls (OA and healthy). And it has been shown that FLS can differentiate in a large variety of different cell types, like adipocytes, osteoclasts and chondrocytes (10;159), suggesting a un-differentiated cell type.
Further evidence for the hypothesis of dedifferentiation of FLS is the increased expression of wnt5a and fz5 (wingless and their receptors, frizzled families respectively) pointing to an undifferentiated state of FLS in RA, because the wnt pathway mainly plays a role during embryological development. Transfection of normal FLS with a wnt5a expression vector induces expression of IL-6, IL-8 and IL-15, which are overexpressed in RA (160). On the other hand, inhibition of either wnt5a or fz5 reduced expression of IL-6, IL-15 and RANKL in RA FLS (161). Another member of the Wnt family that is expressed in RA FLS is wnt-1. Expression of this member induces expression of fibronectin and pro-MMP-3 (162), compatible with RA.
FLS in patients with RA also express higher levels of H19 RNA, which is normally only expressed in embryonal tissues and adult skeletal muscle. H19 RNA is re-expressed in several tumours and chronic inflammation. It acts as a marker for chronic stress (163). Pleitrophin is another embryonic protein. It acts as a growth and differentiation factor which is normally not expressed in adult tissues. However, pleiotrophin is strongly up-regulated on RA FLS. Expression can be up-regulated by TNF-α and EGF and it stimulates proliferation of FLS (164).
Recently, two different phenotypes of FLS in patients with RA were detected. In one group of RA patients FLS expressed mainly markers for cell proliferation and differentiation like insulin-like growth factor 2 (IGF) and IGF binding protein 5. In the other group of RA patients, FLS expressed molecules associated with myofibroblasts. These include α-smooth muscle actin (SMA), activin A (TGF-β/activin pathway) and several types of collagens (14). The classification in different groups also correlates with a previous classification of synovial tissue into two groups, a high inflammatory group and a low/intermediate inflammatory group (165). In their analysis, the myofibroblast group of FLS correlates with the high inflammatory group of synovial tissue (14). Myofibroblasts play an important role in wound healing and pathologies with extensive fibrosis (166;167) and fibrosis is a feature which is often seen in RA synovium.

Signaling pathways

FLS isolated from RA exhibit a different behaviour compared to FLS from OA. Further evidence for this transformed state is the up-regulation of several sig -

naling pathways are in FLS (See figure 4). Many of these pathways have also been implicated in several cancers, which made researchers speculate about a comparison with tumor formation. However, several differences with tumor cells are observed. Cancers of the synovium are very rare. Synovial sarcomas are the only known cancers of the synovium, but its origin is still under debate because the cells of synovial sarcomas often show similarities with epithelial cells, while the synoviocytes do not. Furthermore synovial sarcomas can also form in tendon sheaths and other tissues in the joint (168).
In RA, FLS are not real tumor cells, but they certainly do show a transformed phenotype. They show up-regulation of oncogenes like c-myc, Ras etc.

AP-1 and c-fos
One of these oncogenes that is abundantly expressed in RA synovium is c-fos (74;169;170). It is involved in the activation of tissue degrading molecules, such as matrix metalloproteinases (MMPs) MMP-1 and -3 (171;172) and colocalizes with MMP-1 in FLS attached to bone erosions (74). Expression of c-fos is in - duced by various stimuli including platelet derived growth factor (PDGF), ba-

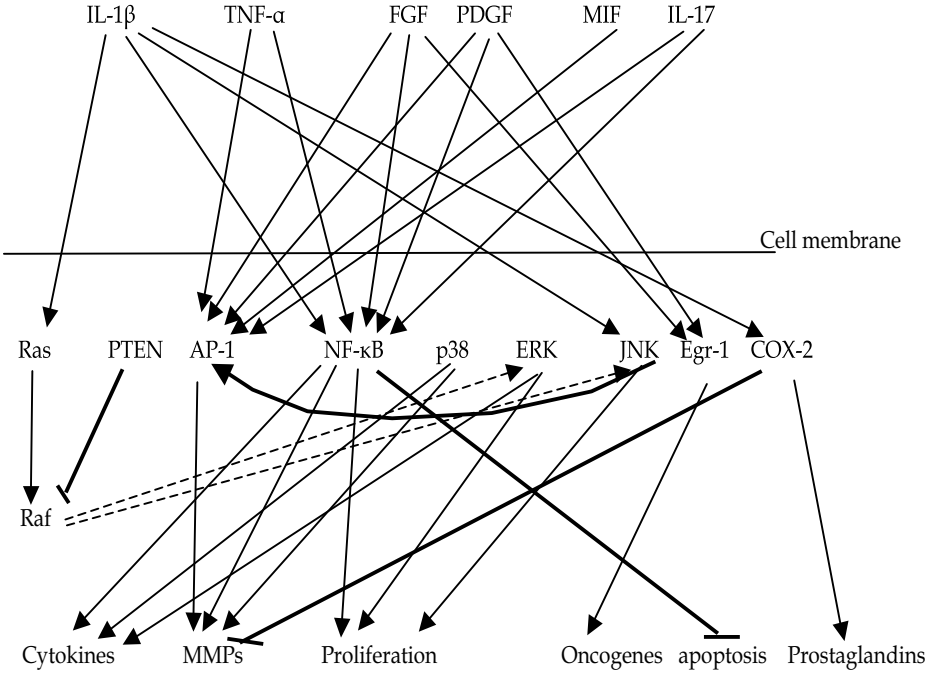

Figure 4 Example of the complexity of FLS signaling in RA. Note: This figure is far from complete, to keep it readable.

sic fibroblast growth factor (FGF), TNF-α and HOXD9 (173). HOXD9 is a transcription factor that enhances proliferation of RA FLS (173). Fos proteins dimerize with Jun proteins to form activator protein-1 (AP-1), a transcriptional activator. Various growth factors including TNF-α, IL-1 and macrophage migration inhibitory factor (MIF) (143), which are abundantly expressed in RA synovium, mediate the cellular production of AP-1. Combination of IL-1β, TNF-α and IL-17 synergistically activate AP-1 (174) and high nuclear binding activity of AP-1 in nuclear extracts from tissues of patients with RA but not in OA controls has been found (175). The family of Jun oncogenes not only promotes the growth of fibroblasts, but there are also members (like jun-d) that suppress fibroblast transformation and may antagonize ras. It was demonstrated that the proliferative c-jun and jun-b (176;177) are overexpressed in RA synovium and that the inhibitory jun-d is downregulated (176). This may contribute to synovial cell growth.

Egr-1
Early growth response gene-1 (egr-1) regulates transcription of other oncogenes such as sis and ras and it can partially substitute for fos in the formation of AP-1 (178). Egr-1 is immediately transcribed in fibroblasts after stimulation with certain polypeptides like, PDGF and FGF (178). In RA FLS egr-1 is significantly up-regulated and this activated transcription of egr-1 persisted (74) (179). Because sis and ras expression are induced by egr-1 and their expression is also upregulated in RA synovium (98) egr-1 expression may be one of the initial steps in the pathogenesis of RA.

C-myc and ras
Another oncogene that is expressed in RA synovial lining is c-myc (47;169;180;181). It is expressed in approximately 30% of the cells (170) and these are mainly the proliferating cells (180). C-myc and myb, a related oncogene, protect cells from apoptosis when overexpressed and cooperation between myc and ras can result in transformation of cells (182).
Ras oncogene is also expressed in RA synovial tissue in the lining layer at sites of invasive growth and its expression level was similar as myc (98). Ras proteins transduce mitotic signals across the plasma membrane by regulating signaling enzymes such as tyrosine kinases and serine/threonine kinases. Ras can signal via c-Raf-1 to induce extracellular regulated kinases (ERK) and Janus kinase (JNK) phosphorylation, which are mitogen activated protein kinases (MAPKs) and lead to proliferation (73). Dominant negative Ras inhibits IL-1β induced ERK activation and expression of IL-6. Furthermore, injection of adenovirus encoding dominant negative Ras in joints of Rats with adjuvant-induced arthritis ameliorated inflammation and suppressed bone destruction (183).

MAPK
MAPK play an important role in proliferation of cells. Three families of MAPKs exist, ERK, JNK and p38 MAPK. P38 MAP kinase plays an important role in RA, because it is known to induce expression of IL-1, TNF-α, MMP-1 and –3, IL-6 and IL-8 (184). The upstream kinases of p38 MAPKK-3 and –6 are mainly expressed in the lining layer of synovium without differences between RA and OA, however they were more phosphorylated in RA synovium as compared to OA synovium (185). Both MAPKK-3 and –6 can form stable complexes with p38 and mediate downstream signaling in RA FLS.
The upstream kinases of JNK MAPKK-4 and –7 are also activated in RA FLS as compared to OA FLS. This increased activity is mediated by IL-1, which is abundantly present in RA synovium (186).

PTEN
Tumour suppressor genes encode for proteins that control the cell cycle and apoptosis in normal cells. In many tumours, these genes are mutated or other - wise inactivated, leading to disregulated cell cycle progression. Recently, it was demonstrated that aggressive FLS from patients with RA lack the expression of mRNA for PTEN (187). The protein product of this tumour suppressor exhibits tyrosine phosphatase activity. In situ hybridization on RA synovium revealed reduced expression of PTEN in the synovial lining layer but not in the sublining layer. Moreover, only 40% of the cultured FLS expressed PTEN and co-implantation experiments in the SCID mouse showed no staining for PTEN in those cells aggressively invading the cartilage (187). PTEN expression can regulate c-Raf-1, which is an important downstream messenger of Ras (73).

NF-κB
Another pathway expressed in RA FLS is the nuclear factor κB (NF-κB) path - way (188)(See table 3). NF-κB is a general name for dimeric transcription factors comprised of members of the Rel family that include RelA (p65), c-Rel, RelB, NF-κB1 (p50) and NF-κB2 (p52) and is mainly expressed on FLS adjacent to the cartilage-pannus junction (189). NF-κB is induced by several pro-inflammatory cytokines, like IL-1, TNF-α, bFGF and PDGF. TNF-α, IL-1β and IL-17 act synergistically to activate NF-κB (174). After activation it stimulates the tran - scription of disease-relevant genes such as those encoding adhesion molecules, MMPs and cytokines, including IL-1, TNF-α, IL-6, IL-8, VCAM-1 and ICAM-1 (79;81;191) (see table 3). NF-κB also renders FLS insensitive to apoptosis in - duced by TNF-α and FasL (188;192). Inhibition of NF-κB by decoys inhibited the development of arthritis in a rat model of streptococcal cell wall (SCW) induced arthritis (188) and the proliferation of FLS in vitro (81). As opposed to

many pro-inflammatory cytokines, anti-inflammatory cytokines IL-10 and IL-11 are independent of NF-κB. IL-11 is mainly produced by FLS (79).

Cyclo-oxygenases
Prostaglandins are pro-inflammatory lipids. Their production is generally high in cultured RA FLS. This is due to the expression of inducible cyclo-oxygenase (COX2) and can be suppressed by exogenous corticosteroids. IL-1β is an important inducer of COX-2 expression and is at least in part responsible for high prostaglandin levels in RA (193). Prostaglandin (PG)E1 raises intracellular cAMP levels in fibroblasts, which decreases gene expression of MMP-1 (194), but this was not seen by PGE2. Recently, it was shown that inhibiting COX2 enhanced expression of MMP-1 induced by IL-1β and TNF-α (87). This is probably due to a decrease in PGE1.
Due to the inflammation in RA synovium hypoxic conditions are likely to occur. The expression of hypoxia inducible factor-1α (HIF-1α) confirms this and hypoxia induces the expression of VEGF and CXCL12 in RA FLS (195), thus contributing to the persistence of synovitis. Hypoxia also increased the expression of COX-2, prostaglandins and MMPs in response to IL-1β (196).

Table 3 Targets of NF-κB (190)

Targets	**Effect**
TNF-α, IL-1, IL-6, IL-17	***Inflammation***
IL-8, MCP-1, ICAM-1, VCAM-1, GM-CSF	***Recruitment of inflammatory cells***
VEGF	***Neovascularization***
COX2, iNOS	***Prostaglandin and NO production***
MMP-1, MMP-3, MMP-9, MMP-13	***Tissue remodelling***
c-Myc, cyclin D	***Proliferation***
IEX-1L, TRAF 1/2, c-IAP 1/2, XIAP, A1/Bfl-1	***Anti-apoptosis***

Proliferation and apoptosis

One of the hallmarks of RA is hyperplasia of the synovium. Hyperplasia is formed by large numbers of FLS. Normally the lining layer of the synovium comprises one or two cell-layers, however in RA, the lining layer is thickened and can consist of ten layers or more. This excess of synoviocytes is caused

by an imbalance between apoptosis and proliferation. At this moment it is not clear whether the cells show increased proliferation or decreased apoptosis or both. Some studies show an increase in proliferation of FLS in RA as compared to OA or normal controls (181), but other studies can find no difference in proliferating cells in both RA and normal FLS (179). In this section on proliferation and apoptosis, the following molecules will be discussed: c-myc, cyclin dependent kinase inhibitors (CDKI), Fas, Bcl-2, Sentrin, Flice inhibitory protein (FLIP), TNF-α, TGF-β, TRAIL, p53, synoviolin/Hrd1 and interferon-(IFN-)γ.
Hashiramoto et al. demonstrate a key role in the regulation of proliferation for c-myc. Inhibition of c-myc through gene transfer reduces growth of RA FLS and can also induce apoptosis (197). Induction of the p16INK4a senescence gene inhibited the growth of FLS from patients with RA in vitro and ameliorated the course of rat adjuvant arthritis in vivo (198). However in RA FLS P16INK4a is induced at sites of cartilage invasion in the SCID mouse coimplantation model (199). P16INK4a is member of the family of cyclin dependent kinase inhibitors (CDKI), which inhibit CDKs that are involved in cell cycle progression. Inhibition of CDK leads to senescence of cells. Another CDKI is p21. Expression of this protein is lower in RA FLS compared to OA FLS and expression of p21 is inversely correlated with median synovial lining thickness. P21 has an additional function by inhibiting IL-6 and MMP-3 expression mediated via inhibition of AP-1 activation (200).
Very little is known about the importance of apoptosis in RA. Using DNA labeling techniques, it has been shown that RA FLS undergo increased apoptosis (201), other data have demonstrated that apoptosis is present in RA synovium and not in OA or normal synovium (202;203) and that RA FLS are able to undergo apoptosis in response to Fas signaling (204). These cells were mainly found in the sublining layer (202). The lining layer cells in contrast did not undergo apoptosis or express Fas, but showed expression of the cell death suppressor gene product Bcl-2 (205). Expression of Bcl-2 is highly expressed in RA FLS (202) compared with OA FLS and this expression confers resistance to apoptosis (206). Dysregulation of apoptosis has been associated with established animal models of autoimmunity, including the MRL-lpr/lpr mouse (207), which have a disorder in the Fas antigen, and the MRL gld/gld mouse (208), which have a disorder in the Fas ligand. These mice get spontaneous arthritis (209).
It has been shown that RA FLS are susceptible to Fas induced apoptosis (210-212), with activation of caspase-3 and –8 and FADD (213). The majority of FLS are resistant to induction of apoptosis through the Fas pathway (212). In vivo it is also possible that apoptosis does not occur, because the expression of Fas ligand is deficient in RA synovium, leading to hyperplasia (214). Indication for this is the observation that gene transfer of FasL in mice ameliorates collagen induced arthrtitis and induced apoptosis in synovial cells (215). Inhibition of

Fas induced apoptosis can be conferred by pro-inflammatory cytokines (210), expression of antiapoptotic molecules or expression of soluble Fas. Expression of soluble Fas is increased in RA SF compared to OA SF and this can inhibit Fas-mediated apoptosis via capturing of FasL. RA FLS were one of the cell types that expressed soluble Fas (216). Nitric oxide (NO) levels are elevated in RA SF and it can inhibit Fas-induced apoptosis by directly inhibiting the activation of caspase-3 (217).
Sentrin is a ubiquitin-like protein that has been shown to interact with the signal-competent forms of Fas and TNF receptor type 1 and thereby protects cells from apoptosis mediated by Fas and TNF-α (218) and FLIP inhibits Fas-induced apoptosis by competition with caspase-8 and by activation of NF-κB and Erk (219). Sentrin and FLIP are both overexpressed in RA FLS mainly in the synovial lining layer (220;221). Down-regulation of FLIP by antisense oligonucleotide sensitizes RA FLS to Fas mediated apoptosis (222). Expression of FLIP can be induced by cytokines or growth factors like TNF-α or bFGF (223).
The serine-threonine kinase Akt is highly activated in RA FLS compared with OA FLS and plays a role in the inhibition of apoptosis by TNF-α and TGF-β (224-226). However, their mechanisms of inhibition differ. TGF-β decreases Fas and increases Bcl-2 (225;226) while TNF-α does not (227). TNF-α activates NF-κB in RA FLS leading to proliferation of these cells. This was accompanied by down-regulation of TNF receptor 1 (TNFR 1) and up-regulation of TNFR2 and TNFR associated factor (TRAF)1-6 (228). In contrast, in OA TNF-α renders the FLS sensitive to Fas-induced apoptosis by up-regulation of expression of caspase-3 and –8 (229). This may be an explanation for a putative beneficial effect of anti-TNF therapy on the hyperplasia of the synovium during RA. Possible mechanisms via which TNF-α desensitizes RA FLS for apoptosis is via up-regulation of FLIP (222) and/ or the activation of NF-κB, because inhibiting the activation of NF-κB induces apoptosis in FLS stimulated with TNF-α (230). Another TNF family member TNF-related apoptosis-inducing ligand (TRAIL) is also able to induce apoptosis and one of its receptors, death receptor 5, is overexpressed on RA FLS as compared to OA FLS (231). This can give therapeutic opportunities because an agonistic antibody against death receptor 5 induces apoptosis in vivo in tumours without severe toxicity to the organism (232) and in vitro in RA FLS (231). In addition, TRAIL inhibits the hyperproliferation of FLS in a mouse arthritis model (233).
Apoptosis can not only be induced via signals from outside the cell, but also via signals from inside the cell, like DNA damage. P53 is a tumour suppressor molecule activated by DNA damage that is mutated or inhibited in many tumours (234;235). In normal cells expression of p53 is hardly detectable due to its short halflife, while in tumour cells where it is inactivated large amounts of p53 can usually be seen. When the DNA in a cell is irreparably damaged the cell

activates p53 which can induce cell cycle arrest or apoptosis (236;237). Several studies have shown overexpression of p53 in RA synovium and FLS (202;238-245) and in rat adjuvant arthritis (246). Mutations have also been found in synovium from patients with RA but not in normal controls (247-251), however this is not the case in other patient populations (252). p53 can inhibit invasion of FLS in normal human cartilage when co-implanted (253) and in an in vitro system (239), but in contrast in a distinct population of FLS it is induced during the invasive process (254). Inhibition of p53 increased the rate of proliferation of FLS and decreased apoptosis (239) and it was shown that the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF), which is abundantly expressed in RA synovium, can downregulate the expression of p53 (255). Adjuvant induced arthritis in MIF knockout mice is less severe as compared to wildtype mice. This is probably due (at least in part) to lower rate of proliferation and higher rate of apoptosis of the FLS in these mice (255). Human FLS from patients with RA showed increased proliferation and decreased apoptosis when they were treated with pathophysiologically relevant concentrations of MIF (255;256).
A novel E3 ubiquitin ligase, Synoviolin/Hrd1 is overexpressed in RA FLS and confers resistance to apoptosis in these cells (257). Mice overexpressing Hrd1 show spontaneous athropathy, while decreased Hrd1 expression in mice (Hrd+/-) conferred resistance to arthritis induction. Hrd1 expression is up-regulated during endoplasmic reticulum (ER) stress in an attempt to degrade an excess of proteins in the ER which are responsible for ER stress (257).
Inhibition of IFN-γ signaling via blocking of signal transducer and activator of transcription (STAT)-3 inhibits growth of FLS from both RA and OA patients. Furthermore, these cells underwent apoptosis spontaneously and when triggered by EGF, which normally acts as a growth factor (258).

Aim

The data described above imply a role for FLS in the pathogenesis of RA. Although many characteristics of FLS in RA are investigated as descirbed above, the functional role of FLS in invasion of cartilage is not completely clear. Therefore, this thesis focuses on the invasive properties of FLS in the disease course of RA.

Outline of this thesis

· A description of the model of in vitro invasion used in most of the chapters is given in **chapter 2**.

· Researchers have assumed that FLS play an important role in the pathogenesis and clinical pathology of RA, however this has never been tested directly. In **chapter 3** an association between FLS invasiveness and clinical joint destruction is explored.
· To investigate the mechanism of invasion of RA FLS, the expression of MMPs in FLS from patients with RA or OA is tested and associated with a diagnosis of RA and with invasion is described in **chapter 4**.
· An association between expression of CD44 splice variants and invasion of RA FLS is explored in **chapter 5**.
· To investigate whether reduced apoptosis in RA is a cause of hyperplasia, the expression of FLIP in RA FLS is measured and compared with the expression in OA FLS and FLS from healthy individuals. This is described in **chapter 6**.
· The hypothesis whether FLS are epithelial cells that undergo a transition to mesenchymal cells during RA is investigated in **chapter 7**. This transition is a common feature in many fibrotic processes and is called Epithelial Mesenchymal transition (EMT).
· In **chapter 8** a possible therapy is investigated, which also gives evidence for transformation of FLS. The therapy is based on apoptin, a chicken virus protein that induces apoptosis in transformed cells, but not in normal cells.
· In **chapter 9** the findings in this thesis are summarized and discussed.

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