[Frontiers in Bioscience 9, 2323-2334, September 1, 2004]

SYNOVIAL ACTIVATION IN RHEUMATOID ARTHRITIS

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1. ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease with progressive articular damage. Activated cells of the synovium produce pro-inflammatory and matrix-degrading effector molecules, which maintain the inflammation and lead to the destruction of the involved joints. In addition to macrophages and T- and B-cells, fibroblast-like synoviocytes must be considered key cells in driving the pathological processes. They can be distinguished by their transformed-appearing phenotype and their invasion into adjacent cartilage and bone.

Synovial activation is driven by pro-inflammatory cytokines as well as cytokine independent pathways including endogenous retroviral elements and Toll-like receptors (TLR).

These pathways are connected by a complex network of autocrine and paracrine acting factors. Another feature of RA synovium is hyperplasia of the lining layer, which results from increased proliferation and decreased apoptosis of synovial fibroblasts. Thanks to new techniques in basic research, novel insights into the cellular and molecular mechanisms of the pathogenesis of RA were gained and led to the development of new, specific therapeutic strategies.

2. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease with progressive articular damage caused by an invasive infiltrate composed of inflammatory cells and synoviocytes. The etiology and pathogenesis of RA are not yet fully understood. It is believed that the exposure of a genetically susceptible individual to an environmental factor, possibly an infectious agent, leads to an immune response, which results in the activation of the synovium. After activation, matrix-degrading enzymes, e.g. metalloproteinases, are expressed by the synovium and destroy the surrounding articular structures.

Cells that play an active role in RA are macrophages that are located in the intimal lining and CD4+ T-cells, B-cells, dendritic cells and mast cells, which infiltrate the sublining. They contribute significantly to various aspects of the disease either through cell-cell interactions or through the production of cytokines and other mediators.

In recent years it has become clear that the fibroblast-like synovial cells play an important role in the pathogenesis of RA. They contribute to the synovial cell hyperplasia in RA and mediate autonomously cartilage destruction after their activation.

A better understanding of the complex cellular and molecular mechanisms in RA led to the development of promising, new therapies. One of these important advancements is the application of biological therapeutics ('biologicals'). Compounds that inhibit the pro-inflammatory cytokine TNF-alpha like the soluble receptor Etanercept and the monoclonal antibodies Infliximab and Adalimumab, are highly successful. However, the pathophysiological effects of TNF-alpha are not yet fully understood. The rather high number of patients unresponsive to anti-TNF therapy needs further investigation (1). Furthermore, TNF-alpha inhibition has been associated with an increased risk of tuberculosis and other opportunistic infections (2). The wide use of TNF inhibitors demands further evaluation of the safety of these biological agents. The lack of sustained benefit after termination of anti-TNF-alpha therapy shows that even though TNF-alpha overproduction is an important mechanism in RA, it is certainly not the only pathogenic pathway implicated in maintaining disease chronicity. Rituximab, an anti CD20 antibody eliminates B-cells and thus blocks their antigen presenting, T-cell activating function. Even though the ACR 20 and ACR 50 scores of these treatments are impressive (Rituximab: ACR 20 65%; ACR 50 33% (3)), an ACR 70 response over 45% has not been achieved. This is an indication for pathogenic pathways that are independent of the classical inflammatory response and appear to be mediated by activated RA fibroblast-like synoviocytes (FLS). Therefore, the present review will specifically focus on the role of synovial fibroblasts in the activation of synovial cells in RA.

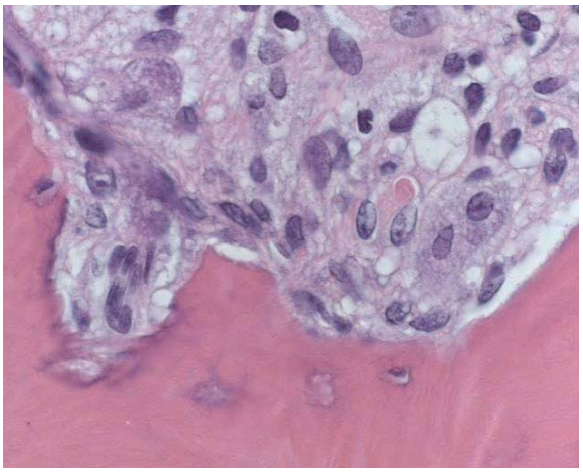

Figure 1. Synovium invading bone in rheumatoid arthritis. Note the activated phenotype of RA FLS with large nucleus and nucleoli.

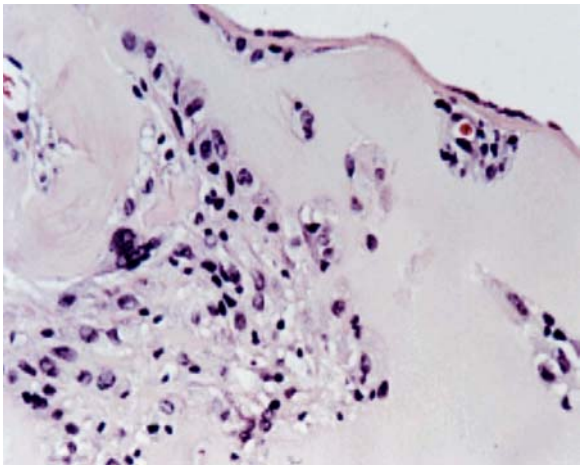

Figure 2. RA FLS invading co-implanted human cartilage in the SCID mouse.

The cellular activation in the RA synovium is driven and maintained by a complex network of paracrine and autocrine acting cytokines. In addition, there are cytokine-independent pathways of activation including endogenous retroviral elements and Toll-like receptors (TLR).

The use of new techniques gives us novel insights into cellular and molecular mechanisms of the pathogenesis of RA and lead to the development of more specific therapeutic strategies. Although we are in a position to control the symptoms of the disease, we are still far from curing this complicated and disabling illness.

3. CHARACTERISTICS OF ACTIVATED SYNOVIAL FIBROBLASTS

RA FLS must be considered key cells in mediating cartilage degradation. They differ both in their morphology and in their biological features from normal synoviocytes. They are mainly found in the synovial lining layer which is thickened and hyperplastic in RA. However, also fibroblast-like "floating" cells which can be isolated from the synovial fluid of RA patients, show an activated phenotype and invasive behavior (4).

Morphologically, RA FLS can be distinguished by their more roundish shape and their large pale nucleus with prominent nucleoli (5). They grow anchorage-independent and lack contact inhibition (6). The most striking indication for their activation, however, is their invasive growth into adjacent tissues (Figure 1). Co-implantation experiments with isolated RA FLS and human cartilage into SCID mice showed that their destructive behavior is maintained at least for 60 days independently of B- and T-cells (7). This suggests that the activation is intrinsic and persistent (Figure 2).

Matrix-degrading enzymes, such as matrix metalloproteinases (MMP's), are responsible for the destruction of articular structures in RA (8). In addition to their ability to directly degrade cartilage and bone, they are capable of activating other proteases and thus start a cascade of further matrix degrading factors. Besides MMP's, RA FLS abundantly produce cysteine proteases and components of the plasminogen activation system.

MMP-1 and MMP-3 are elevated both in the synovial fluid and in the serum of RA patients and are mainly produced by RA FLS (9-11). Moreover, MMP-13 expression showed an exclusive association with RA and could also be localized in FLS (12, 13). Additionally, RA FLS express MMP-14 and MMP-16 which belong to the family of membrane-type MMP (MT-MMP) and are known to activate MMP-2 and MMP-13 (14, 15).

The other important group of matrix degrading enzymes are cysteine proteases, which include cathepsins. Cathepsin K has not only been implicated in cartilage but also in bone degradation. Cathepsin K-positive FLS are regularly present at sites of cartilage and bone degradation, but also cathepsins B and L are known to be produced by FLS (16, 17).

Gravallese *et al.* and Shigeyama *et al.* could show that FLS and activated T-cells express a receptor activator of nuclear factor kappaB ligand (RANKL) (18, 19). RANKL, its receptor RANK and its soluble decoy receptor osteoprotegerin regulate the sensitive balance between bone loss and growth (20). Over-expression of RANKL leads to the differentiation of osteoblasts into osteoclasts and the activation of mature osteoclasts through the stimulation of RANK. Thus, RA FLS contribute directly to osteoclast formation and activation at sites of bone erosion.

A further aspect of RA, apart from articular damage, is the severe inflammation associated with rheumatoid synovitis. A critical mediator of this inflammation is prostaglandin E_2 (PGE_2). Enzymes that catalyze PGE_2 production are cyclooxygenases (COX), which are targeted by non-steroidal anti-inflammatory drugs (NSAID) and membrane-associated prostaglandin E synthases (mPGES), which lie downstream of COX in PGE_2 synthesis. Both are up-regulated in RA FLS and

cause abundant PGE_2 production at sites of inflammation in the rheumatoid synovium (21).

Pro-inflammatory effector molecules like tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-8 sustain the inflammatory process in the rheumatoid joint. They lead to chemotaxis and expansion of macrophages and T-cells as well as to the activation of RA FLS. Of interest are the elevated levels of migration inhibitory factor (MIF) in the synovial fluid of RA patients. MIF is produced by macrophages, RA FLS and to a lesser extent T-cells and mediates joint destruction and pro-inflammatory responses through an increase of MMP production by RA FLS (22). It activates T-cells, increases COX-2 activity and is able to counter-regulate the immunosuppressive effects of glucocorticoids (23, 24).

The increased production of thioredoxin (TRX) is caused by the inflammation in RA joints. Radical oxygen intermediates (ROI), which are produced in the inflamed joint in response to oxidative stress, induce the expression of TRX in RA FLS(25). TRX in turn stimulates the production of pro-inflammatory cytokines (26).

IL-6 is one of the most pleiotropic cytokines with multiple effects (27). It induces the acute phase reaction in the liver and exhibits pro-inflammatory as well as anti-inflammatory properties. IL-6 and other members of the IL-6 family, like IL-11, leukemia inhibitory factor (LIF), and oncostatin M (OSM) are expressed by RA FLS at sites of invasion (28).

Angiogenesis is a characteristic feature of the inflamed synovium and a key event in the initiation and persistence of RA. Several pro-angiogenic cytokines are released by FLS including TNF-alpha, IL-8, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and transforming growth factor (TGF)-beta (29, 30).

RA FLS contribute to the massive migration and activation of CD4+ T-cells and macrophages in the synovium by the production of chemoattractants and stimulating factors. In contrast, IL-2, which was considered essential for T-cell proliferation and activation, was found to be expressed only in low concentrations in RA synovial fluid and synovial tissue (31). Instead it could be shown that IL-15, IL-16 and IL-7 secreted by RA FLS are mainly responsible for T-cell expansion and activation in RA synovium (32, 33). Macrophage chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1alpha and MIP-3alpha are important for the attraction of monocytes as well as T-cells.

Interestingly, TNF-alpha and IL-1beta stimulate the synthesis of many cytokines, chemokines and matrix-degrading factors (34-36).

4. CYTOKINE DEPENDENT PATHWAYS OF ACTIVATION

Among the many cytokines leading to synovial activation, TNF-alpha and IL-1 are probably the best characterized. The benefit of TNF-alpha inhibition in RA treatment emphasizes its key role in maintaining the inflammatory process, whereas the success of the IL-1 receptor antagonist Anakinra has proven to be only limited (37). Both cytokines mediate their effects through the activation of nuclear factor kappaB (NF-kappaB) and activator protein (AP)-1 (Figure 3).

NF-kappaB is a dimeric transcription factor that classically comprises p50 (NF-kappaB1) and p65 (RelA), but can also consist of other components such us p52 (NF-kappaB2) or RelB. Apart from TNF-alpha and IL-1, NF-kappaB can be activated by various other stimuli such as LPS and viruses, antigen stimulation of T- and B-cells, H_2O_2 and ultraviolet light (38). After proteolytic degradation of the inhibitor of nuclear factor kappaB (I kappaB), a nuclear localization signal (NLS) is exposed and enables NF-kappaB to translocate into the nucleus and initiate the transcription of its target genes. NF-kappaB is expressed in nearly all cells and controls a wide range of genes that are involved in inflammatory and immunological reactions as well as cell growth. In RA it plays a pivotal role in the expression of macrophage derived cytokines and the activation of RA FLS (39, 40). Pro-inflammatory cytokines, chemoattractants and matrix degrading enzymes are regulated by NF-kappaB (41-43). Furthermore it could be shown that NF-kappaB mediates synovial hyperplasia by suppressing apoptosis (44).

An additional way for TNF-alpha and IL-1 to activate cells is through AP-1. AP-1 can be found either as a Jun-Jun homodimer or as a Jun-Fos heterodimer. After stimulation of cells by TNF-alpha or IL-1, the signal is transferred by the so-called stress-activated protein kinases (SAPK) p38 and JNK, which belong, together with the extracellular signal-regulated kinase (ERK), to the family of mitogen-activated protein kinases (MAPK). All 3 MAPK pathways regulate the transcription of Fos and Jun family genes and therefore regulate AP-1 activity. Whereas DNA binding activity of AP-1 is high in RA synovium, no or little activity is detected in patients with osteoarthritis (OA) (45). Target genes of AP-1 which are implicated in the pathogenesis of RA are MMP's and various pro-inflammatory cytokines (46, 47).

IL-15 is a cytokine that is not only produced by activated macrophages and dendritic cells, but also by osteoclasts and FLS (48). It is found in the synovial fluid of RA patients and was mainly considered as a T-cell attractant factor (49). In addition to its T-cell activating properties, Kurowska *et al.* could show that IL-15 enhances proliferation of RA FLS and induces the expression of anti-apoptotic proteins of the Bcl-2 family (50).

Receptor mediated activation of T-cells also leads to the production of IL-17, which is considered to be an important factor for T-cells to initiate and maintain inflammation. It induces the expression of IL-6, IL-8 and PGE_2 in cultured RA FLS (51, 52). Furthermore IL-17 appears to contribute directly to matrix degradation in RA by inducing the production of MMP-1 (53). Levels of IL-17 are high in RA synovial fluid, but not detectable in OA synovial fluid (54).

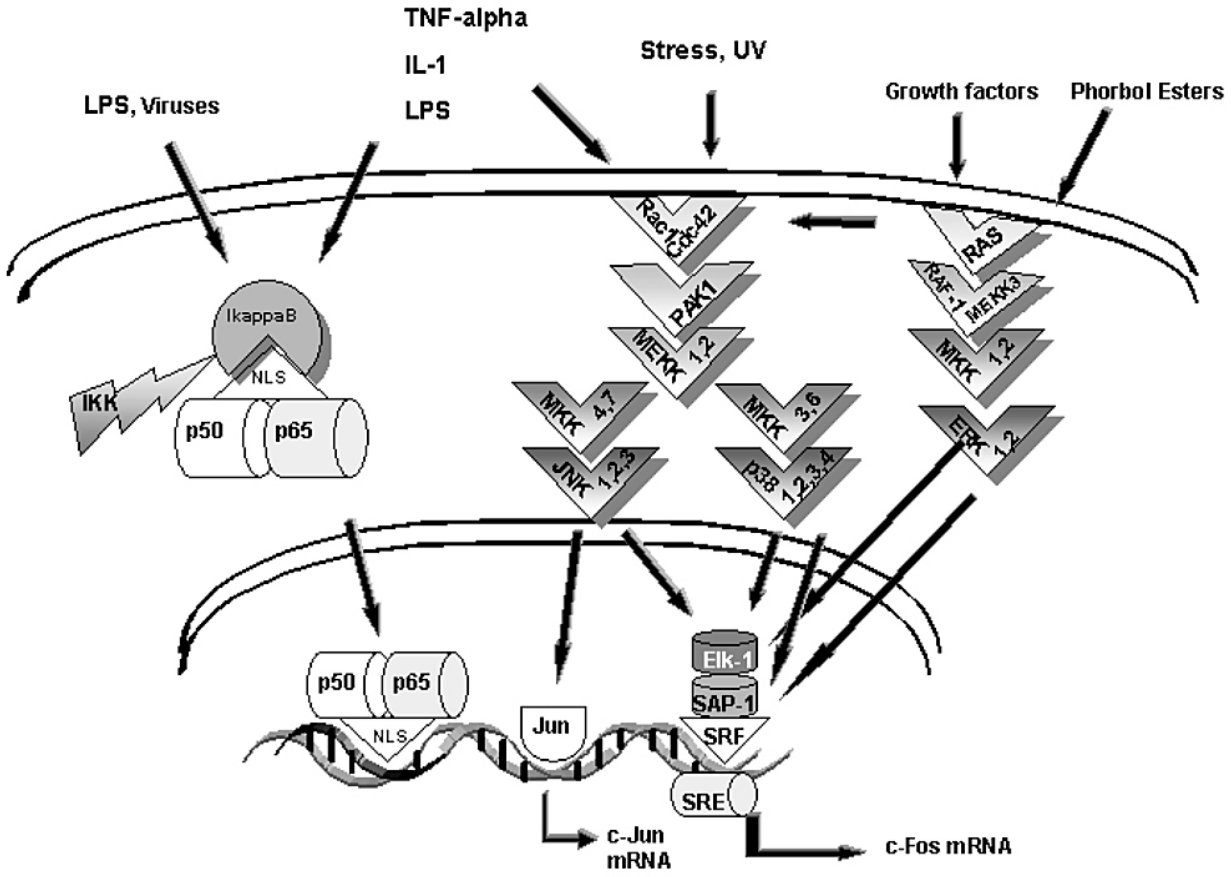

Figure 3. Important transcription factor pathways in RA. P50 and p65 form NF-kappaB, c-jun and c-fos together form the transcription factor AP-1. Cdc42: cell division cycle 42; ERK: extracellular signal-regulated kinase; IkappaB: Inhibitor of nuclear factor kappa B; IKK: IkappaB kinase; JNK: c-jun kinase; LPS: lipopolysaccharides; MEKK: MAP/ERK kinase kinase; MKK: MAP/ERK kinase; NLS: nuclear localization signal; PAK1: p21 activated kinase 1; SRE: serum response element; SRF: serum response factor.

Recent data suggest an essential role for IL-18 in the production of TNF-alpha in RA. IL-18 is expressed in RA, but not in OA tissue and is able to induce the production of TNF-alpha, interferon (IFN)-gamma and granulocyte/macrophage colony stimulating factor (GM-CSF) in monocytes derived from RA tissue and synovial fluid (55, 56). Furthermore, IL-18 functions as an angiogenic mediator in RA (57).

5. CYTOKINE INDEPENDENT PATHWAYS OF ACTIVATION

Whilst searching for a potential trigger of synovial activation in RA, retroviruses have been proposed as contributing factors. However, the isolation of an infectious retrovirus from RA synovial tissue never succeeded (58). Nevertheless, during this search, Neidhart *et al.* could detect L1 retrotransposable elements in synovial fluids as well as in cultured RA FLS and RA tissue (59). Retrotransposons are mobile genetic elements similar to retroviruses without the *env* gene and are widely distributed within our genome. It could be shown that functional L1 elements induce the expression of SAPK2-delta (p38delta). Although SAPK/p38 is known to be a critical signaling pathway for pro-inflammatory cytokines, it could be shown for the first time that a specific isoform of the p38 family, namely p38delta is induced through the expression of L1 in RA FLS in a cytokine-independent pathway (60). Subsequently, preliminary data showed that p38delta induces the production of MMP's. This cytokine-independent pathway of joint destruction might be responsible for the limitations of the currently used biologicals, which target cytokines.

Although neither retroviruses nor other infectious agents could ever been isolated from joints of RA patients, it is conceivable that transient exposure to an infectious agent triggers synovial activation, which might persist because of a pathological host response. The innate immune system is activated by infections through specialized pattern recognition receptors named Toll-like receptors (TLR). They recognize conserved microbial structures and initiate the accordant response. On the other hand, TLR are also able to bind endogenous ligands e.g. members of the heat-shock protein family (61, 62). Seibl *et al*. could show a strong expression of TLR2 in RA tissue and an increase in the expression of TLR2 mRNA after stimulation of cultured RA FLS with TNF-alpha and IL-1

(63). Most interestingly, several potent chemokines including granulocyte chemotactic protein (GCP)-2 and monocyte chemoattractant protein (MCP)-2 are up-regulated in RA FLS in response to the activation of TLR2 by its ligands (64). Moreover, it could be shown that incubation of cultured RA FLS with staphylococcal peptidoglycans (PG) induced the expression of MMP-1, MMP-3 and MMP-13 and increased the expression of IL-6 and IL-8 by TLR signaling (65).

As mentioned above RA FLS possess tumor-like features like anchorage independent growth and most importantly display an invasive growth into cartilage. In this respect it was interesting to examine molecules that are known to be involved in cancer growth and metastasis. One such molecule is PTEN, a tumor suppressor gene, which was found to be mutated in several human cancers (66). PTEN is a tyrosine phosphatase and shows homologies to cytoskeletal proteins. Even though no mutated PTEN was found in RA, RA FLS in culture, invasive RA FLS from the SCID mouse model and RA FLS in the lining layer of RA tissue showed only limited expression of PTEN (67). Since tyrosine kinase activity in RA is increased, it is feasible that the lack of PTEN leads to higher tyrosine kinase activity in RA FLS (68).

The tumor suppressor gene p53 is activated by DNA damage, leading to cell growth arrest and thereby giving the cell time for DNA repair. If the damage is too extensive, p53 leads to apoptosis (69). By inactivation of p53 in FLS, anchorage-independent growth, decrease of apoptosis and invasion into cartilage could be induced (70, 71). p53 appears to be higher expressed in synovial tissue of RA than of OA patients (72). It is unclear whether this up-regulated p53 is mutated. Some groups found p53 mutations in RA tissues, others could not find a specific mutation pattern (73, 74).

Another factor that was initially of interest because of its role in tumor growth is the transcription factor hypoxia-inducible factor (HIF)-1 (75). Various cytokines, growth factors and hypoxia are considered to activate HIF-1 (76, 77). Most interestingly, lack of PTEN in tumor cells contributes to tumor expansion through the activation of HIF-1-regulated gene expression (78). HIF-1 induces the expression of VEGF and therefore drives angiogenesis. Blocking of HIF-1 activity in tumor cells leads to reduced tumor growth by decreased vessel density (79). In RA, a hypoxic environment is established through vascular changes in combination with an increased metabolism and high pressure in the RA joint (25, 80). HIF-1 expression by macrophages is higher in RA synovium compared to OA synovium and is mainly localized in the lining layer (81). VEGF synthesis induced by HIF-1 mediates angiogenesis in RA, which is correlated with the presence of joint erosions in early RA (82). Furthermore, serum VEGF levels of RA patients correlate with the development of radiographic damage (83). Recently, it could be shown that inhibitor of differentiation (Id)-2, a basic helix-loop-helix transcription factor, is induced by hypoxia and pro-inflammatory cytokines in RA FLS in a HIF-1 independent way. Since Id-2 is known to contribute to the malignant transformation of tumor cells, its selective induction in RA FLS but not in skin fibroblasts is of particular interest (84, 85).

Members of the Wnt and Frizzled (Fz) family are known to be involved in tissue patterning and in proliferation, activation and transformation of mesenchymal cells (86-88). Whereas Wnt proteins are secreted glycoproteins, Fz proteins resemble G-coupled proteins and act as receptors for Wnt (89). At least one Wnt/Fz pair namely Wnt5a and Fz5 is expressed at higher levels in RA tissue compared to OA tissue. It could be shown that transfection of normal FLS with a Wnt5 expression vector induces the production of IL-6, IL-8 and IL-15, whereas inhibition of Wnt5a/Fz5 signaling in RA FLS reduces the production of IL-6 and IL-15 (90, 91).

In RA tissue the classical way of cellular activation is mediated by cell-cell interactions (92). T-cells as well as monocytes bind to stimulated FLS via intercellular adhesion molecule (ICAM)-1 (93). The production of pro-inflammatory cytokines, MMP-1 and PGE_2 by FLS and monocytes can be induced upon cellular contact with stimulated T-cells (94, 95). Together with the production of T-cell and monocyte activating cytokines by RA FLS, as mentioned above, this activation creates a positive feedback loop, which maintains synovial activation in RA. Remarkably, RA FLS but not normal FLS are able to bind B-cells from germinal centers and prevent their apoptosis (96).

6. SYNOVIAL HYPERPLASIA: APOPTOSIS VERSUS PROLIFERATION

Hyperplasia of the lining layer is a typical feature of the RA synovium. Whether this hyperplasia is due to the proliferation of RA FLS or due to an ability to evade apoptosis is a controversial topic.

An indication for rapid proliferation of RA FLS is provided by the increased expression of transcription factors and molecules regulating the cell cycle. In addition, several soluble factors present in the inflamed joint such as TNF-alpha, IL-1, MIF, bFGF and TGF-beta appear to enhance proliferation (44, 97-99). Products of oncogenes encompassing growth factors and its receptors, protein kinases and transcription factors are strongly involved in the regulation of the cell cycle, growth and intracellular signaling pathways (100). Proto-oncogenes like *myc*, *myb*, *ras* and their products can be found abundantly in RA FLS, predominately at sites of invasion into cartilage and bone (101, 102). Likewise it could be shown that activators of oncogene transcription like *egr-1* are up-regulated in RA FLS (103). *Egr-1* transcription can be enhanced by TNF-alpha and leads in the absence of its repressor p53 to the development of Wilms tumor (104, 105).

To clarify whether cartilage invasion of RA FLS is dependent on proliferation, Seemayer et al utilized SV40 transfected RA FLS and compared their behavior to non-transfected RA FLS (106). Whereas SV40 transformed RA FLS proliferate more rapidly than other RA FLS, they

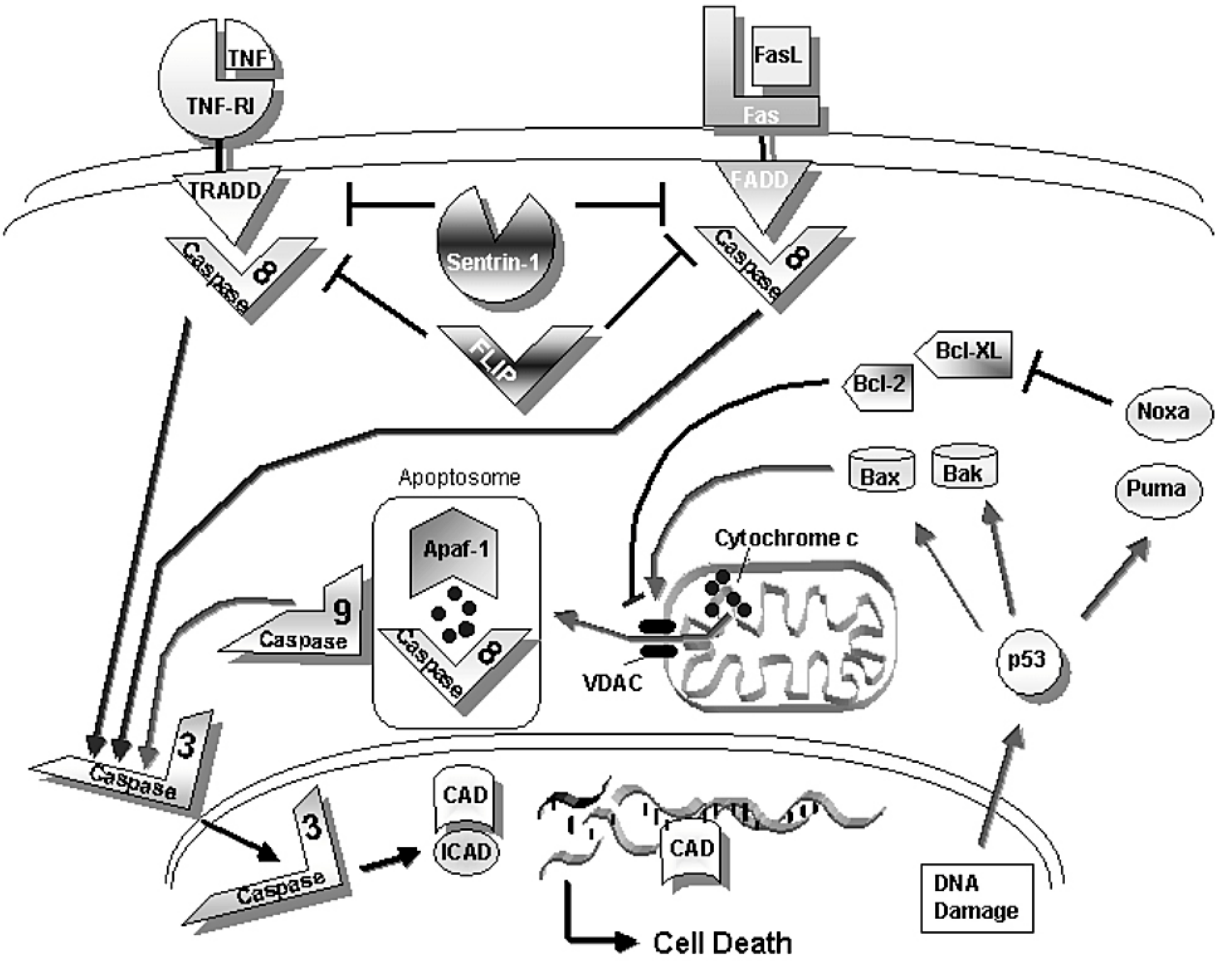

Figure 4. Apoptosis pathways and inhibitors in RA. Apaf-1: apoptotic protease activating factor-1; CAD: caspase activated deoxyribonuclease; FADD: Fas associated via death domain; FLIP: FLICE-inhibitory protein; ICAD: inhibitor of CAD; Puma: p53 upregulated modulator of apoptosis; TNF-RI: TNF- Receptor I; TRADD: TNF associated death domain; VDAC: voltage dependent anion channel.

produce lower levels of MMP-14 and cathepsin K and most importantly invade cartilage significantly less than other RA FLS. Thus, cartilage invasion and hyperplasia of the synovium have to be seen as two distinct processes in the pathogenesis of RA.

Another hypothesis regarding synovial hyperplasia suggests a decline of cells undergoing apoptosis (Figure 4). Even though expression of Fas on RA FLS appears to be high, they are rather resistant to Fas-ligand- (FasL) induced apoptosis (107). Soluble Fas has been detected in synovial fluid of RA patients, which could competitively inhibit Fas signaling (108). Furthermore, several studies examined the production of anti-apoptotic molecules in RA FLS. Sentrin-1/sumo-1 protects cells from TNF receptor-1- and Fas-induced apoptosis by interacting with their death domain (109). In RA synovium sentrin-1/sumo-1 expression can mainly be found in the invading synovium lining layer, whereas normal synovium shows no such production. In cell culture RA FLS showed a significantly higher production than OA FLS or normal FLS and maintained their high production of sentrin-1/sumo-1 for at least 60 days after implantation into the SCID mouse (110).

FLICE-inhibitory protein (FLIP) displays its anti-apoptotic effect further downstream of the death receptor-mediated pathway. Due to its striking homology to caspase 8 it is able to inhibit binding of caspase 8 to the Fas-associating protein with death domain (FADD) and thus prevents apoptosis (111). Interestingly, expression of FLIP could be shown at sites of cartilage invasion and bone destruction in RA (112).

In addition to death receptor-dependent pathways of apoptosis, programmed cell death can be induced by mitochondrial-dependent pathways. Bcl-2 is known to be a strong inhibitor of these mitochondrial pathways.

The versatile transcription factor NF-kappaB is not only important in inflammation and activation of the innate and adaptive immune system, but is also able to inhibit apoptosis (113). It exerts its anti-apoptotic properties by inducting anti-apoptotic molecules including

FLIP and Bcl-2 family members (113, 114). Akt, a protein kinase increases the transactivation potential of NF-kappaB and thus enhances its anti-apoptotic potential (115). It is noteworthy that levels of phosphorylated Akt are higher in RA FLS than OA FLS and can be increased by TNF-alpha stimulation (116).

The association of NF-kappaB and TNF-alpha illustrates the complex correlation between inflammation, cell growth and cell death. Synovial cell hyperplasia in RA is not solely due to proliferation or decreased cell death, it must rather be seen as a result of various mitogen and anti-apoptotic stimuli during the course of inflammation.

7. PERSPECTIVES

The variety of factors involved in synovial activation gives an impression of the complexity of RA pathogenesis. At the same time it opens up new possibilities for therapeutic interventions. Blocking of pro-inflammatory effector molecules is successfully realized in the use of anti-TNF-alpha antibodies. An approach in the same direction is the treatment with MRA, an anti-IL-6-receptor antibody that inhibits the biological activity of IL-6. In a randomized, double-blind, placebo-controlled study MRA significantly reduced the mean activity score of the disease and brought the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) values to normal levels (117). Also humax-IL-15, a monoclonal antibody against IL-15, displayed encouraging results in a phase I/II trial with ACR 20 scores of 63%, ACR 50 scores of 38% and ACR 70 scores of 25% (118). Another potential therapeutic target is IL-18. Studies in murine collagen induced arthritis (CIA) showed that antibodies against IL-18 and the application of IL-18-binding protein significantly reduced clinical scores and protected against joint damage (119).

Non-cytokine targets like CD20 on B-cells blocked by Rituximab are other possible therapeutic approaches. CTLA4Ig is a fusion protein that is directed against CD80 and CD86 on antigen presenting cells. It blocks the binding of CD80/CD86 to CD28 on T-cells and thereby prevents T-cell activation. Patient treatment with a combination of CTLA4Ig and methotrexate significantly attenuated disease activity and improved health-related quality of life (120). Despite the major advancements in the therapy of RA, it needs to be stressed that no treatment with any of the new biologicals, even in combination with methotrexate, has resulted in an ACR 70 above 40%.

Several approaches have been made to use gene transfer to identify novel targets for the treatment of RA. Even if gene therapy in patients is far from realization, its benefit in elucidating pathogenic pathways of RA is of high value. An ideal model to study the effect of a gene on the invasive behavior of RA FLS is the co-implantation of the transfected cells with human cartilage into SCID mice. By these means van der Laan *et al.* showed that RA FLS transfected with specific tissue inhibitors of metalloproteinases (TIMP) were significantly less invasive than non- and mock-transfected cells (121). Although certain TIMP's are over-expressed in RA tissue, matrix degradation by MMP's occurs, which suggests an imbalance favoring MMP's. Raising the levels of TIMP-1 and/or TIMP-3 are therefore suggested as novel therapeutic strategies. Double gene transfer of IL-1 receptor antagonist and IL-10 showed similar results. Cartilage invasion and degradation by RA FLS could be significantly suppressed by transfection with these two joint protective genes, whereas single transfection with neither IL-10 nor IL-1 receptor antagonist could reduce both synovial invasion and pericellular chondrocyte degradation (122-124).

An innovative, new technique is the transfection of cells with small interfering RNA (siRNA) to suppress gene expression of a specific gene. Using this technique Masuda *et al.* reduced MMP-1 mRNA expression in RA FLS up to 90% and reached a MMP-1 protein reduction of 39% (125).

Thanks to these new techniques in basic research a better understanding of the pathogenesis of RA has been gained and will in future lead to further novel therapeutic strategies for the treatment of RA.

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Key Words: Rheumatoid arthritis, Synovial activation, Review

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