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Factors underlying chronic inflammation in rheumatoid arthritis

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Summary

Rheumatoid arthritis (RA) is a debilitating chronic inflammatory disease whose characteristic pathology includes swollen, painful, and deformed joints. In recent decades, both clinical and basic scientific research have tried to determine the factors involved in the pathogenesis of this common disease. Although the cause of RA is still unknown, several factors that contribute to RA have been identified. Among these are the discoveries of: susceptibility genes, disease-causing immune cells, and cytokine and signal transduction networks involved in promoting persistence of inflammation. Various therapeutic strategies, including anti-tumor necrosis factor α therapy, have been developed to target one or more of these factors. Although none of these therapeutic strategies can actually cure the disease, some of these novel agents have proven to be more effective than others. This implies that the success of a therapy is very much dependent on the therapeutic targets chosen. Therefore, improved understanding of the cellular and molecular events occurring in the rheumatoid joint during the pathogenesis of the disease is particularly important if we are to better combined therapeutic strategies. In this article we summarize current understanding of the factors that contribute to disease pathogenesis in RA and identify cellular and molecular events that could drive the development of the disease and represent potential new therapeutic targets.

Key words: inflammation • fibroblasts • cytokines • apoptosis • chemokines

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INTRODUCTION

Rheumatoid arthritis (RA) is a common inflammatory arthritis affecting about 0.5–1% of the population worldwide. The disease is primarily associated with inflammation within synovial joints. Synovial joints are composed of bone, hyaline cartilage, synovial membrane, ligaments, and tendons (Fig. 1a). The synovial membrane is made up of a cellular inner layer, the synovial lining (synovium), mainly composed of macrophage-like and fibroblast-like synoviocytes. Virtually all peripheral joints can be affected by RA, although the most commonly affected joints are those of the hands, feet, and knees⁸³. In addition to inflammation of the synovium, expansion of the synoviocytes leads to thickening of the joint lining and formation of the so-called pannus tissue, which invades and destroys local articular structures and bone (Fig. 1b). The ultimate hallmark of RA is joint erosion.

Although the precise cause of RA is unknown, it has been proposed that the initiating event in RA involves an infectious agent or other environmental insult. This induces a local inflammatory response by activating the cells in the synovial lining and recruiting leukocytes into the joint. This leads to the earliest symptom of arthritis, called synovitis. In most people such arthritic episodes resolve and the inflammation subsides, but if this inflammatory response persists in the joint, then progress to chronic inflammation and RA occurs. The joint does not normally contain immune cells, but in RA substantial numbers of various leukocytes are seen²³. These include B cells, T cells, macrophages, and neutrophils. As the disease progresses there is also hyperplasia of the synovial lining resulting from a marked increase in infiltrating macrophages and synoviocytes. Various pro-inflammatory cytokines as well as degradative enzymes have also been detected in both the synovial tissues and the synovial fluid of RA patients. Several therapeutic agents have been developed, including an anti-tumor necrosis factor (TNF)- α antibody and an IL-1 receptor antagonist (IL-1Ra), to target these various factors therapeutically. These drug therapies, although reasonably successful at ameliorating the symptoms of RA, have not led to elimination of the disease. Thus it is important to evaluate the factors that have been suggested to be important in RA pathogenesis in order to improve current therapeutic strategy. The genetic and environmental basis of RA and the interaction of cellular and molecular events in the RA joint is extremely complex, but it is only by taking all these factors together that we can start to establish a wider and deeper perspective on the understanding of the pathogenesis of RA.

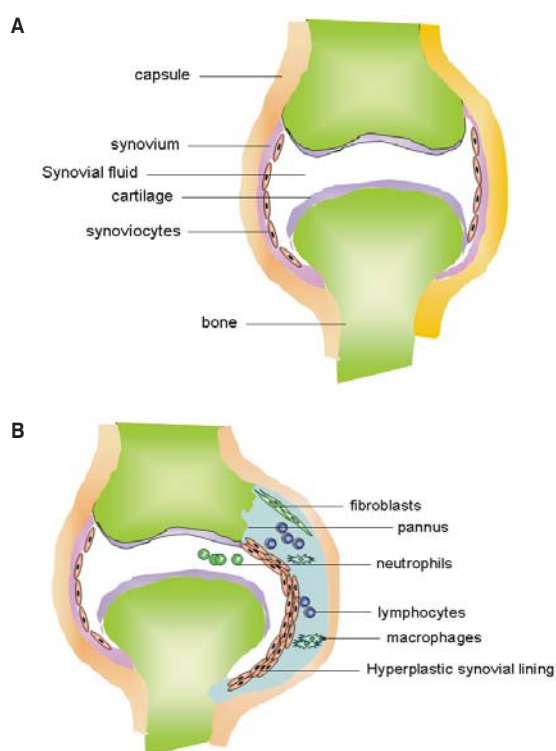


Figure 1. Key features of a normal joint and a rheumatoid joint. **A** – a synovial joint is composed of two bones, covered with articular cartilage at their ends, separated by a joint space filled with synovial fluid. The joint capsule is composed of the fibrous outer layer and the inner synovium, which lines the synovial cavity where articular cartilage is not present. The synovial lining consists of a thin layer of synoviocytes (fibroblast-like and macrophage-like). **B** – a rheumatoid joint has an inflammation of the synovial membrane (synovitis) which is caused by the influx of leukocytes, including lymphocytes, monocytes, and neutrophils. As the disease progresses, the synovium may grow considerably larger, eventually forming tissue called pannus. The ultimate hallmark of RA is bone erosion.

GENETIC FACTORS AND RA

The fast development of human genome research has provoked enthusiasm to identify susceptibility genes that can directly impact on molecular pathways involved in the pathogenesis of diseases such as RA. Epidemiology studies of RA have revealed that RA is exceptionally rare in much of sub-Saharan Africa^{1, 2}, and also has relatively low prevalence in South-East Asia, China, and Japan⁷⁸. In contrast, it is relatively common in certain American-Indian populations, such as the Pima (5%) and Chippewa Indians (7%)^{40, 45}. Relatives of people with RA also have an increased risk of developing the disease, and siblings of severely affected RA patients are at the highest risk. These observations suggest a role for specific genes in RA disease pathogenesis. Over the decades, strategies for identifying these susceptibility genes include linkage analysis, linkage disequilibrium mapping, and identi-

fication of candidate genes, which can be associated with disease by determination of functional polymorphisms within them. These efforts have led to the discovery of several susceptibility genes that influence RA disease development. Among these, major histocompatibility complex (MHC) class II, which is located on chromosome 6, has been shown to be consistently associated with RA⁶³. Human leukocyte antigens (HLA) DR, DP, and DQ are located in the MHC class II region, and people with the DRB1 allele, particularly with the subtypes DRB1*0401 (Dw4), DRB1*0404 and *0408 (Dw14), and DRB1*0405 (Dw15), have an increased susceptibility to development of RA.

Besides MHC molecules, genes for proteins that have relevant inflammatory functions have also been found to associate with RA. One of these is the TNF gene. TNF- α is an important pleiotropic mediator of inflammation, and excessive production of TNF- α and other pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, and granulocyte macrophage colony-stimulating factor (GM-CSF), are associated with pathology in RA²⁶. Much effort has focussed upon defining the polymorphisms in these genes that could lead to the excessive production of these inflammatory cytokines in RA patients. Nine single-nucleotide polymorphisms associated with the TNF- α gene have been identified so far. Some of these polymorphism lie within the 5'-regulatory region that regulates TNF- α gene transcription⁹³. However, whether the polymorphism in TNF- α contributes to RA independently or via linkage to some other primary factor has yet to be defined.

Although the genetic studies have provided invaluable information on factors that could contribute to pathogenesis, they have not been able to explain the primary cause of RA or allow identification of those people who will succumb to RA in later life. This is partly because RA, like many other diseases, has a polygenic basis. This makes the genetics approach to predicting the disease very complicated. There could also be many other genes that are associated with RA that have yet to be discovered. In addition, non-genetic environmental factors also have an impact on the development of RA, demonstrated by the relatively low concordance rate for monozygotic twins (<25%)^{79, 46}. This suggests that other factors should not be neglected when considering events that lead to the chronic inflammation so characteristic of RA.

ROLE OF IMMUNE CELLS IN RA

Synovial B cells and T cells

The role of B lymphocytes in RA was proposed early on since the first factor found to correlate with the

pathogenesis of RA was the so-called "rheumatoid factor" (RF). RF is predominantly an IgM isotype antibody specific to the Fc portion of immunoglobulins. This observation led to the view that RA might be an autoimmune disease caused by auto-reactive B cells, which produce pathogenic auto-reactive antibodies⁸⁰. The primary pathogenic potential of RF in RA has been found to be the immune complexes formed by RFs which fix complement and release chemotactic factors such as C5a. As a result, inflammatory cells, such as neutrophils, are recruited to the rheumatoid joint where they are activated to engulf immune complexes and release proteolytic enzymes, causing tissue destruction. Although this hypothesis has been supported by many studies, it was later discovered that many normal individuals and patients with other chronic inflammatory diseases also produce RF⁶⁴, suggesting that the mere presence of the auto-antibody is insufficient to cause RA.

The prominent infiltration of T cells to the rheumatoid synovium has suggested a key role of these cells in RA. In the past, T cells have been the most frequently targeted cells in the biological therapy of RA due to the discovery that HLA-DR, a MHC class II which participates in antigen presentation, is associated with the disease³⁹. This led to the hypothesis that specific peptides that bind to the DR proteins in RA patients were able to promote inflammation and RA. However, the precise role of HLA-DR in RA could be more complex, since specific peptides that bind to the DR proteins in RA patients have not been identified. It could also be that the susceptibility epitope is closely linked to other genes in the MHC that are associated with RA. More recently, as described below, studies on the role of T cells in RA have focussed upon their function as drivers of inflammation via their cellular interactions and cytokine production. Brennan et al.⁸ have proposed that the interaction of T cells with macrophages is responsible for the initial production of TNF- α , which drives the initial stages of the disease. Indeed, as a result of their work it is TNF- α that is now being successfully targeted for the treatment of RA (see later).

Synovial macrophages

Macrophages are key players in many inflammatory responses. They are professional antigen-presenting cells that can activate T cells through their co-stimulatory molecules, such as CD80/86, CD40, and ICOS. Macrophages are numerous in the inflamed synovial membrane and at the cartilage-pannus junction⁶⁰. They show clear signs of activation, such as overexpression of MHC class II, production of pro-inflammatory cytokines (e.g. IL-1, TNF- α , GM-CSF, IL-6),

chemokines (e.g. IL-8, MIP-1 and MCP-1), metallo-proteinases, and neopterin⁴⁹. More importantly, macrophage numbers, as well as the levels of TNF- α and IL-1 β , strongly correlate with disease symptoms and with joint damage in RA^{27, 60, 88}.

The increased numbers of macrophages in RA have been suggested to result from a lack of apoptosis. Several mechanisms that prevent the apoptosis of these inflammatory cells have been identified. Among these, nuclear factor (NF)- κ B activation³⁵ and signaling through the PI-3K-AKT pathway⁶⁶ have been shown to inhibit apoptosis. In addition, FLIP (FADD-like IL-1 β -converting enzyme-inhibitory protein) is highly expressed in RA synovial macrophages, and this is sufficient to prevent Fas-mediated apoptosis⁶⁶. Therefore, therapeutic strategies targeted at inducing apoptosis in macrophages may be a novel way of treating the disease.

Neutrophils

Neutrophils are the most abundant leukocytes, comprising about 60% of total leukocytes in human peripheral blood. They play a very important role in acute inflammation, being one of the first cells to be recruited to the site of inflammation⁷. Once at the site of inflammation, they clear infectious agents mainly through phagocytosis, generation of reactive oxygen species, and secretion of antimicrobial substances, including elastase. The latter may cause serious, nonspecific tissue damage if not tightly regulated⁹⁶. In fact, neutrophils have been claimed to be the main “culprit” in the pathology of several inflammatory diseases, including adult respiratory distress syndrome¹⁹. However, over the years the possible role of neutrophils in rheumatoid arthritis has not been studied as extensively as those of other leukocytes, such as macrophages, T cells, and B cells²⁴. This is mainly because neutrophils are not usually found in pannus tissue. The pannus-cartilage interface is believed to be the site of cartilage destruction and it is usually heavily infiltrated with macrophage-like synoviocytes, plasma cells, and T cells.

Although they are absent in pannus tissue, neutrophils have been found abundantly in synovial fluid from patients with active RA⁶⁸. Interestingly, they are in an activated state, expressing activation markers such as CD11b, CD43, and CD63⁵⁴. Their activated state could be explained by the large quantities of neutrophil chemoattractants, including leukotriene B₄, IL-8, and complement C5a, in the synovial fluid. Synovial fluid from RA patients also contains high levels of immune complexes and is a rich source of various pro-inflammatory cytokines. Most of these

cytokines, such as IL-1 β , TNF- α , tumor growth factor β , and IL-6, have been shown to play a critical role in the pathogenesis of RA⁷⁰. More importantly, neutrophils have been shown, at least *in vitro*, to be one of the potential sources of some of these cytokines, including IL- β and TNF- α ¹³, and so they themselves will potentiate inflammation in the joint.

The presence of immune complexes and pro-inflammatory cytokines may also contribute to the accumulation of neutrophils in synovial fluid. The half-life of neutrophils is short (8–12 h), but this is greatly extended by various pro-survival cytokines present in the synovial fluid. GM-CSF, IL-1 β , TNF, and interferon (IFN)- β present in the synovial fluid are capable of delaying spontaneous neutrophil apoptosis^{17, 18, 59, 70, 74}. In addition, the synovial environment is hypoxic, and low pO₂ is a powerful inhibitor of neutrophil apoptosis via the induction of HIF-1 α ⁵⁷. Once their apoptosis is delayed, neutrophils have a great potential to inflict tissue damage. They possess a range of proteinases and hydrolases within their subcellular granules, as well as the ability to generate a series of reactive oxygen intermediates (ROIs)²³. *In vitro* experiments have shown that released ROIs and granule enzymes can depolymerize long-chain hyaluronic acid (hyaluronan), a glycosaminoglycan responsible for the lubricative property of synovial fluid, into smaller molecules, leading indirectly to joint damage³³. Recent studies also showed that synovial fluid neutrophils transcribe and express MHC class II molecules in RA²¹. This suggests their possible role in the adaptive immune response in RA.

ROLE OF CYTOKINE NETWORKS IN RA

The role of cytokines in RA was not really studied until the late 1980's, when new molecular techniques were available to measure cytokines in RA synovial tissue and fluid. Through the considerable effort of many investigators, the cytokines that have a direct correlation with RA disease have been identified and these represent potential therapeutic targets. A broad array of cytokines, including IL-1, IL-6, IL-15, IL-18, IFN- β , TNF- α , and GM-CSF have been detected in the joints of RA patients. Among these, TNF- α and IL-1 have been proven to be the critical cytokines involved in synovitis in RA in a T cell-independent manner²⁹. The key evidence for the importance of TNF- α in RA is provided by the observation of the occurrence of severe arthritis in TNF- α -over-expressing mice, which could be prevented by inhibiting TNF- α ⁴⁸. Drugs that block the activity of TNF- α have now been shown to improve clinical symptoms in RA patients^{25, 53, 95}. Infliximab, adalimumab (both are antibodies against TNF- α), and etanercept

(a fusion protein of TNF receptor II) are all now in large-scale clinical trials, and 60–70% of patients appear to benefit from these treatments.

Another key pro-inflammatory cytokine, IL-1, has also been investigated in RA. IL-1 produces its effects by binding to receptors in the membrane of target cells. Such effects are regulated naturally by the IL-1Ra³. The higher the concentration of IL-1Ra, the fewer IL-1 molecules that can bind to the receptor. Conversely, the lower the concentration of IL-1Ra, the more IL-1 molecules that can bind to the receptor, thus causing a greater inflammatory effect. In patients with RA, the balance between IL-1 and IL-1Ra is disturbed. The level of IL-1 far exceeds that of IL-1Ra, causing a destructive response³. The importance of IL-1Ra in regulating the inflammatory response is suggested by the occurrence of a destructive arthritis in IL-1Ra-deficient mice⁴². The use of recombinant IL-1Ra (anakinra) has now been approved for the treatment of RA^{9, 16}. However, its effectiveness in treating the disease, again, is not absolute.

Besides TNF- α and IL-1, other cytokines, such as IL-6, IL-15, IL-12, IL-17, and IL-18, have also been suggested to be associated with RA^{22, 44}. The therapeutic efficacy of cytokine blockade may be limited by redundancy in their actions, as the role of one cytokine may be substituted by that of another. Acting on more than one cytokine may increase the percentage of responding RA patients as well as the degree of individual patient response. In reality it may be easier to target the signaling pathways that they employ rather than the cytokine or its receptor individually.

ROLE OF SIGNAL TRANSDUCTION NETWORKS IN RA

The involvement of multiple signal-transduction pathways has been investigated extensively in RA with the aim of finding a potential therapeutic intervention at the intracellular level. To date, several signaling molecules have been identified as potential therapeutic targets, including NF- κ B, a family of transcription factors involved in regulating genes central to the inflammatory response⁸⁶. NF- κ B molecules exist as homo- or heterodimeric complexes in the cytosol, complexed to their natural inhibitor, inhibitory κ B (I κ B). In response to various stimuli, such as lipopolysaccharide, TNF, and IL-1, I κ B is phosphorylated by I κ B kinases (IKKs), which leads to its degradation by the proteasome. NF- κ B can then rapidly translocate into the nucleus to regulate gene expression. In the RA synovium the constitutive activation of NF- κ B has been detected in the nuclei

of cells of the intimal lining and vascular endothelium^{37, 81}. NF- κ B activation has also been reported in cultured human synovial fibroblasts, and increased nuclear levels were observed after IL-1 β or TNF- α stimulation^{31, 71}. Transfection of cells from the synovium with an adenoviral vector encoding I κ B α was shown to inhibit selective cytokine expression, particularly of TNF and IL-6, and to downregulate the production of matrix metalloproteinases (MMPs)²⁶. In addition, transfection with dominant negative (dn)IKK β , but not (dn)IKK α , results in the blockade of TNF- α -induced NF- κ B activation and cytokine production by synovial fibroblasts⁴. *In vivo* studies using arthritis animal models further confirmed the important role of NF- κ B. These studies showed that NF- κ B activation appeared to precede the onset of disease, and the blockade of NF- κ B activation decreased mediator disease severity^{15, 37, 89}. Furthermore, intra-articular administration of adenoviral (dn)IKK β reduced ongoing inflammation in rat adjuvant-induced arthritis⁸⁷.

Besides NF- κ B, mitogen-activated protein (MAP) kinases represent another attractive target for RA, as they can regulate cell proliferation, apoptosis, cytokine expression, and MMP production. The three major family members of MAP kinase are c-Jun-N-terminal kinase (JNK), extracellular regulating kinase, and p38 kinase, and they have been detected in synovial tissue, in an active phosphorylated form, in RA⁷⁵. p38 MAP kinase has been shown to be important in macrophages for the regulation of cytokine production²⁸, and some reports have suggested that TNF production is regulated by this kinase⁵⁰. Animal models of arthritis have been used to test the efficacy of MAP kinase inhibitors and the data showed a potential for the use of p38 inhibitors in treating arthritis⁵. JNK is also a key MAP kinase involved in the induction of MMP genes in RA. The use of a JNK inhibitor has been shown to reduce bone destruction in the rat adjuvant arthritis model³⁶. The MAP kinases are regulated by upstream kinases known as MKKs. Both MKK4 and MKK7 can effectively activate JNK through phosphorylation on tyrosine and threonine residues³⁰. Two of these, MKK4 and MKK7, are increased in RA, especially in the synovial intimal lining and fibroblast-like synovio-cytes⁸⁵.

Another signaling molecule which has been shown to be up-regulated, both at the mRNA and the protein level, in the synovial tissue of patients with RA is signal transducer and activator of transcription-1 (STAT1)^{43, 91}. STAT1 is essential for IFN signaling. Interestingly, type I IFNs (IFN- α and - β) are also abundant in the RA synovium, mainly produced by

synovial fibroblasts⁶⁷. Type 1 IFNs are believed to have immunosuppressive functions, including antiangiogenic effects⁶. On the other hand, IFN- β produced by synovial fibroblasts can act as a survival factor for both T cells and neutrophils in the synovial joint through the STAT1 signaling pathway^{67, 74}. A recent study has also shown increased active STAT1 molecules in the fibroblast-like synoviocytes in the RA synovium, suggesting that the expansion of these cells in the pannus tissue may be mediated by extended survival via this signaling pathway⁴⁷. Our own studies have shown that IFN- β signals through PI-3K and protein kinase C (PKC)- δ to activate NF- κ B and induce anti-apoptotic signals in T cells and neutrophils⁹⁴. Therefore the PKC- δ signaling pathway is another potential therapeutic target in RA. The biological function of type I IFN in the pathogenesis of RA is not clear. Despite its detrimental role in prolonging the survival of inflammatory cells and stromal cells in the rheumatoid synovium, some have suggested the use of IFN- β in the treatment of RA due to its anti-inflammatory function⁹². It is possible that type 1 IFN given therapeutically would have quite different effects from those seen in a localized and inflamed site such as the synovium. The type I IFN signaling pathway thus remains as an unproven therapeutic target for the treatment of RA.

The inhibition of specific signal-transduction pathways as a treatment for RA is attractive, but several difficulties need to be overcome before this could be realized. The signal-transduction network is extremely complicated, not only due to the number of molecules involved, but also the cross-talk between different signaling pathways. For example, although NF- κ B inhibition has great potential in treating RA, a major concern is that blockade of NF- κ B could potentially impair both innate and adaptive immunity due to its central role in immune responses. In addition, most molecules have a pleiotropic involvement in different signaling pathways. By inhibiting a molecule in a particular pathway this could affect other signaling pathways. Finally, it remains difficult to develop a pharmacological inhibitor which is specific to a particular signaling molecule, though the success of the c-abl inhibitor Gleevec in the treatment of chronic myeloid leukemia⁷² means that this is still an aim for many pharmaceutical companies and research groups.

ROLE OF SYNOVIAL FIBROBLASTS AND SYNOVIAL MICROENVIRONMENT IN RA

The previous sections have shown the complexity of RA as a disease and the vast number of potential therapeutic targets. In this section we add another

level of complexity: the contribution of fibroblast-like synoviocytes (FLS) to the pathogenesis of RA, independent of other immune-competent cells. Fibroblasts are ubiquitous cells that were previously considered to be only structural cells with little intrinsic heterogeneity⁹⁰. However, an emerging concept is that fibroblasts are not a homogeneous population, but rather consist of subsets that have organ-specific functions. Fibroblasts from different anatomical regions or even within a single tissue can display different characteristic phenotypes, which are stably maintained even with prolonged culture *in vitro*^{41, 55, 82}.

One of the characteristics of the onset of RA is the increased number of FLS synoviocytes and the redistribution of these cells throughout the synovial joint⁸⁸. FLS from RA patients are often characterized as aggressive or “transformed” cells due to their increased expression of several oncogenes^{61, 98}. Numerous studies also showed that these fibroblasts are clearly distinguishable from fibroblasts derived from non-diseased joints, from osteoarthritic joints, or from other tissues (skin or lung fibroblasts)³⁴. Several studies have demonstrated an increased percentage of these cells in S-phase in the rheumatoid joint, indicating their proliferation advantage^{14, 52, 69}. However, it remains unclear whether RA FLS cells in culture represent a single population of cells derived from the synovium that are capable of extensive phenotypic deviation, or whether RA FLS cells represent heterogeneous populations of cells, with the expansion of specific subpopulations depending on the micro-environment.

The studies of the characteristics and behavior of fibroblasts *in vitro* have generated much of the understanding of the phenotypic changes they undergo in RA. Cultured rheumatoid fibroblasts display a stellate morphology, enhanced growth, and altered migratory capacity, and they constitutively overexpress certain proinflammatory genes, oncogenes, metalloproteinase, and matrix proteins that facilitate the localization of immune cells in the joint^{10, 76}. They express a number of bone marrow stromal cell markers, such as VCAM-1 and CD157, and constitutively express cytokines, such as GM-CSF and IL-6^{58, 77}. Interestingly, FLS cells from some patients also show overexpression or mutation of the tumor-suppressor p53, and dominant negative mutations of p53 increased FLS invasive capacity, resistance to apoptosis, and the production and release of cytokines and MMP^{38, 84}. Another characteristic of the “transformed” FLS cells is the expression of CD44-splice variants, in different splicing combinations²⁰. It was shown that FLS-expressing variants containing exons

CD44v7/8 have a proliferative advantage over those that do not express them⁹⁷. Importantly, these “transformed” FLS cells have been shown, in mice, to invade and destroy cartilage even after isolation and co-implantation with human cartilage under the skin of SCID mice³². This elegant study showed that the transformed phenotype persists even under conditions where inflammatory-inducing agents are absent. Furthermore, mouse models of arthritis also revealed that fibroblast precursors in the peripheral blood can be preconditioned by the synovium prior to the development of lymphocyte aggregates and the inflammatory response, implying the critical and active role of fibroblasts in the early development of RA⁵⁶. These results suggest that the “transformation” of fibroblasts may have occurred in the early stage of the disease, which then defines the microenvironment that regulates RA pathogenesis.

It is well known that a chronically inflamed joint can maintain a persistent infiltration of immune-competent cells. This reflects a distorted homeostatic balance in the synovial microenvironment between factors that enhance cellularity (cell recruitment, proliferation, and retention) and those that decrease cellularity (cell death and emigration). In fact, several studies have demonstrated that the synovial microenvironment prevents cell death or apoptosis^{65, 73} and promotes cell retention^{12, 62}. In joints of patients with active RA, few apoptotic cells are detected. Furthermore, the synovial fluid from the RA patients, but not from healthy controls, is capable of delaying neutrophil apoptosis *in vitro*⁶². Although synovial T cells in RA are highly differentiated (CD45RO⁺ CD45RB^{dull}) and should be highly susceptible to apoptosis, their death was actively inhibited. This inhibition of apoptosis has been mediated, at least partly, by type I interferons (IFN- β) produced by synovial fibroblasts⁶⁷. The same cytokine has also been shown to delay spontaneous neutrophil apoptosis *in vitro*⁷⁴. T cells in the rheumatoid joint have also been found to highly express chemokine receptor CXCR4. Interestingly, the ligand for CXCR4, stromal derived factor-1 or CXCL12, is also highly expressed on synovial endothelial cells. More importantly, Nanki's and Buckley's groups have, independently, also shown that factors produced within the synovial microenvironment were responsible for up-regulating CXCR4 on infiltrating synovial T cells and its ligand^{11, 62}.

These compelling data not only suggest the pivotal role of the synovial microenvironment, which is defined by fibroblasts, in modulating the behavior of the infiltrating cells that accumulate within the rheumatoid joint, but more importantly provide

another rationale for the development of a pharmacological intervention that targets the FLS. Kramer et al.⁵¹, in their recent review, have suggested possible ways to inhibit the FLS cell cycle with pharmacological inhibitors.

CONCLUSIONS AND PROSPECTS

Recent decades have seen great advances in the understanding of the pathogenesis of RA. Although the precise cause of RA still remains unknown, which is perhaps the primary reason why there still is no cure for this life-altering disease, many factors involved in this disease pathogenesis have been identified (Fig. 2). Several therapeutic strategies have been developed to intervene in these processes, notably anti-TNF therapies. However, even the best available therapy at present does not cure RA and can only not retard progression of the disease in the majority of the patients. This implies that RA pathogenesis is extremely complex and is not governed by one factor. Moreover, the various factors discussed here may all be involved, but may play their roles during different phases of the disease. Therefore, targeting therapy at the right factors at the right time (disease phase) could improve current therapeutic strategies, and we propose that synovial fibroblasts are likely to be a major focus of novel therapies in the coming decade.

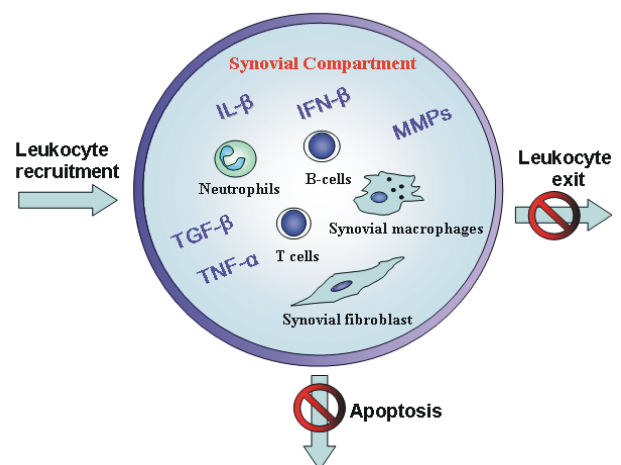


Figure 2. Schematic representation of factors involved in RA. In early arthritis, several leukocytes are recruited to the synovial compartment through a series of specific events involving chemokine-chemokine receptors. Once in the synovial compartment, these inflammatory cells will secrete several pro-inflammatory cytokines, including TNF- α and IL-1 β , which have a pivotal role in the pathogenesis of the disease. One of the hallmarks of RA is the persistence of these inflammatory cells in the synovial compartment. The apoptosis of these cells is inhibited by the microenvironment in the joint. Synovial fibroblasts, which define the microenvironment in the joint, may have a pivotal role in determining the outcome of the disease at its early phase.

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