

Molecular Mechanisms of Rheumatoid Arthritis Revealed by Categorizing Subtypes of Fibroblast-Like Synoviocytes

Katsuhiko Ishihara and Hideya Igarashi
Kawasaki Medical School
Japan

1. Introduction

The immune system is a highly organized defense system, which recognizes invading microorganisms and aims to exclude them. In order to do this effectively and safely, the immune system must distinguish between self- and non-self-antigens, and be tolerant of self-antigens. Autoimmune diseases develop through the breakdown of self-tolerance, as a result of immune deregulation. This is caused by the combined influence of genetic and environmental factors, including infectious microorganisms. Rheumatoid arthritis (RA) is a systemic autoimmune disease, characterized by synovial hyperplasia leading to the destruction of bones and joints. This severely impairs the life of patients. RA is a relatively common autoimmune disease, occurring in approximately 1% of the population. However, its etiology and pathophysiology are not completely understood. The incidence of RA is correlated with certain human leukocyte antigen (HLA)-DR haplotypes, and the production of autoantibodies such as rheumatoid factor and anticitrullinated protein autoantibody. Thus, the involvement of the deregulated immune system is strongly implicated. Various molecules, including type II collagen, gp39, citrullinated peptides, and glucose-6-phosphoisomerase, have been reported as potential pathogenic autoantigens. However, their involvement explains only a proportion of RA cases. Autoantigens are abundant in the body and, theoretically, the immune response to them continues indefinitely. Thus, systemic autoimmune diseases exhibit the characteristics of chronic inflammation.

In the pathological condition of RA, the joints are infiltrated with T cells, B cells, macrophages, and plasma cells, all of which are characteristic chronic inflammation cells driven by the immune system. Recently, Th17, a novel helper T-cell subset producing interleukin (IL)-17, has been recognized as a pivotal player in the local inflammation driven by acquired immunity. In addition to immune-competent cells, there is accumulating evidence for abnormalities in non-hematopoietic cells, especially fibroblast-like synoviocytes (FLSs) (Bartok & Firestein, 2010; Firestein, 2009; Mor *et al.*, 2005; Pap & Gay, 2009). The cartilage and bone are destroyed by the invasion of pannus, which is formed from proliferating FLSs and multi-nucleated osteoclasts. Osteoclasts are specialized to resorb bone, and play a major role in bone destruction in RA. However, there is strong evidence that FLSs themselves are aggressive enough to destroy bone. When cultured FLSs derived from RA or osteoarthritis (OA) were co-implanted with human cartilage under the renal

capsule of a severe combined immunodeficiency (SCID) mouse, the FLSs derived from RA, but not from OA, destroyed the cartilage (Muller-Ladner *et al.*, 1996; Pierer *et al.*, 2003). In RA, cytokines produced by surrounding cells in the inflamed joints, such as basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF)- β , tumor necrotizing factor (TNF)- α , and IL-1 β , are thought to be responsible for the hyperplasia of FLSs. On the other hand, activated FLSs produce TNF- α , IL-1 β , IL-6, chemokines, and matrix metalloproteinases (MMPs), thereby establishing the chronic and destructive inflammatory circuit driven by cellular interaction. Thus, it appears that some passively activated FLSs may be changed to be in a distinctly activated state, autonomously destroying bone and joints.

The critical roles of inflammatory cytokines are evidenced by the effectiveness of cytokine-blockade therapies for RA, using anti-TNF- α or anti-IL-6 receptor antibodies (Brennan & McInnes, 2008; Nishimoto & Kishimoto, 2006). In spite of the promising effects shown by these anti-cytokine therapies, several problems remain, such as suppression of the normal immunity and substantial numbers of resistant cases (Firestein, 2007). To overcome these difficulties, increased knowledge of the molecular mechanisms involved in the complex and multi-factorial pathophysiology of RA is required. In this context, our research on the disease-associated genes of RA is based on the theory that FLSs are heterogeneous in physiological, and also in pathological situations. In this chapter, we briefly overview the current understanding of FLSs in RA, and introduce the pathophysiological natures of FLSs as revealed by our subtyping studies.

2. Fibroblast-like synoviocytes

2.1 Fibroblast-like synoviocytes in the normal synovium

The synovium is a membranous structure that extends from the margins of articular cartilage and lines the capsule of diarthrodial joints. The synovium supports the joint structure, provides nutrition to the cartilage and lubricates the surface. The synovial membrane has 2 compartments: the intimal lining layer and the sublining layer. The intimal lining layer is the superficial layer that faces the intra-articular cavity, and produces synovial fluid as lubricant. This lining layer is normally 2 to 3 cells thick and consists of 2 types of synovial cells: macrophage-like synoviocytes (Type A synoviocytes), and fibroblast-like synoviocytes (Type B synoviocytes). Type A synoviocytes are hematopoietic in origin, bone marrow-derived, and terminally differentiated, as are other tissue-resident macrophages. Type B synoviocytes are mesenchymal cells with vimentin in the cytoskeleton, and Thy-1 (CD90) on their surface. Type B synoviocytes display many characteristics of fibroblasts, such as the production of extracellular matrix, and collagen type IV and V. Specific characteristics for FLSs in the intimal lining layer include expression of cadherin-11 for homotypic aggregation (Lee *et al.*, 2007) and uridine diphosphoglucose dehydrogenase for synthesis of hyaluronic acid, an essential joint lubricant. Expression of decay accelerating factor, CD55, and adhesion molecules (VCAM-1 and ICAM-1) is also characteristic.

2.2 Fibroblast-like synoviocytes in the synovium of RA

In the synovium of RA, the histopathological characteristics are hyperplasia of FLSs, and infiltration with inflammatory cells. The pathophysiological reactions are joint destruction and perpetuation of inflammation.

2.2.1 Hyperplasia

Hyperplasia of FLSs exhibits features of stable activation—the so-called tumor-like transformation. Features of tumor-like transformation include anchorage-independent growth, adhesion to the extracellular matrix of cartilage, resistance to apoptotic signaling, and invasiveness to cartilage and bone. Tumor-like transformation may be cell-autonomous or non-cell-autonomous. The non-cell-autonomous pathway is indirectly driven by factors produced by autoimmune-competent cells in the microenvironment. These include cytokines, growth factors, lipid mediators, and reactive oxygen species. By contrast, the cell-autonomous pathway results from the cell-intrinsic changes of FLSs themselves.

Reflecting cell-intrinsic changes, FLSs in RA have a characteristic morphology, i.e., an abundant cytoplasm; a dense, rough endoplasmic reticulum; and large, pale nuclei with several prominent nucleoli (Pap & Gay, 2009). One of the important molecular characteristics of FLSs in RA is the expression of proto-oncogenes (Bartok & Firestein, 2009), including *c-fos*, (Aikawa *et al.*, 2008), *ras*, *raf*, *sis*, *myb*, and *myc* (Roivainen *et al.*, 1999). Interestingly, proto-oncogenes are predominantly expressed by FLSs attached to cartilage and bone (Muller-Ladner *et al.*, 2000). Furthermore, some of these proto-oncogenes regulate gene expression of MMPs or cathepsin L. Thus, in the SCID mouse, inhibition of c-Raf-1 or c-Myc significantly reduced the expression of *MMP-1* and *MMP-3*, resulting in decreased invasiveness of FLSs to the cartilage (Pap *et al.*, 2004).

Among the various cells in the inflamed synovium, macrophages and T cells are thought to be most responsible for producing various stimuli for stable activation of FLSs. Various combinations of PDGF, TGF- β , TNF- α , IL-1, and the arachidonic acid metabolites induce the proliferation of FLSs (Konttinen *et al.*, 1999). On the other hand, FLSs in RA have been shown to exhibit defective apoptosis, rather than enhanced proliferation (Jacob *et al.*, 1995; Korb *et al.*, 2009). Apoptosis was rapidly induced in RA-derived FLSs by retroviral transduction of a combination of dominant-negative c-Raf-1 and dominant-negative c-Myc (Pap *et al.*, 2004), indicating that some proto-oncogenes are involved. Death receptor Fas is expressed and is functional in FLSs *in vitro*. However, apoptosis induced by anti-Fas antibody was prevented by TNF- α , IL-1 β , and IL-6, suggesting that FLSs in the inflamed joints are resistant to apoptosis (Ohshima *et al.*, 2000; Wakisaka *et al.*, 1998). The anti-apoptotic function of nuclear factor (NF)- κ B activated by TNF- α signaling, and the induction of the anti-apoptotic molecule Bcl-xL by IL-1 β are involved (Jeong *et al.*, 2004). In addition to the effects of cytokines, the adhesion molecule VLA-5 (integrin α 5 β 1), upon ligation with fibronectin, is involved in this resistance to Fas-mediated apoptosis (Kitagawa *et al.*, 2006). Under conditions of genotoxic stress, the tumor-suppressor p53 induces cell-cycle arrest, followed by either DNA repair or apoptosis, depending on the degree of DNA damage (Gudkov & Komarova, 2010). A main effector of p53-dependent apoptosis, PUMA (p53 up-regulated modulator of apoptosis) is present in very low concentrations in the synovium. Adenovirus-mediated transfer of the *p53* gene into FLSs induced production of the p53 protein, leading to p21 expression; however, PUMA expression was not enhanced and apoptosis was not induced (Cha *et al.*, 2006). This suggests that, under conditions of genotoxic stress, the FLSs in RA tend to undergo cell-cycle arrest rather than apoptosis.

2.2.2 Infiltration with inflammatory cells

Infiltration with inflammatory cells mainly involves chemokines, cytokines, lipids of chemical mediators, and adhesion molecules. It comprises the mutual activation of interacting cells of distinct lineages, leading to the perpetuation of inflammation.

Inflammatory mediators produced by FLSs include IL-15 (Miranda-Carus *et al.*, 2004), IL-16 (Pritchard *et al.*, 2004), IL-18 (Gracie *et al.*, 1999), TNF- α , TGF- β (Pohlers *et al.*, 2007), NO, and prostagrandin E2 (Kojima *et al.*, 2003). Various chemokines are reported to be produced by FLSs in RA (Iwamoto *et al.*, 2008). The production of IL-8/CXCL8 and GRO α /CXCL1, which recruit neutrophils, is induced by stimulation of FLSs with IL-1 α , IL-1 β , TNF- α , or IL-17 (Hosaka *et al.*, 1994; Kehlen *et al.*, 2002; Koch *et al.*, 1991, 1995). Neutrophils are abundant in the synovial fluid of RA, but rare in the synovial tissue. The levels of lymphotactin/XCL1 are elevated in the synovial fluid and tissues of RA. Moreover, infiltrating mononuclear cells and FLSs in the tissues of RA express XCR1, a receptor for lymphotactin/XCL1 (Wang *et al.*, 2004). The levels of macrophage inflammatory protein (MIP)-1 α /CCL3 (a ligand of CCR1 and CCR5) are higher in the synovial fluid of RA. Furthermore, upon stimulation with lipopolysaccharide and TNF- α , isolated FLSs produce MIP-1 α /CCL3 (Koch *et al.*, 1994). The migration of CD4⁺ memory T cells to the synovium of RA, and the inhibition of activation-induced apoptosis of T cells, are induced by stromal cell-derived factor (SDF)-1/CXCL12. Thus, the accumulation of CD4⁺ memory T cells in the synovium plays an important role in RA (Nanki *et al.*, 2000). The production of RANTES (Regulated upon Activation, Normal T cell Expressed and Secreted)/CCL5 is histologically detected in the synovial lining and sublining layers of affected rheumatoid joints (Robinson *et al.*, 1995). All these suggest that, in microenvironments rich with TNF- α and IL-1 β , FLSs themselves recruit monocytes, neutrophils, Th1 cells, eosinophils, and basophils (Rathanaswami *et al.*, 1993). In addition to the regulation of migration, stimulation with MCP-1/CCL2, SDF-1/CXCL12, IP-10/CXCL10, Mig/CXCL9, and MCP-4/CCL13 enhances the proliferation of FLSs, leading to synovial hyperplasia (Garcia-Vicuna *et al.*, 2004; Iwamoto *et al.*, 2007). Furthermore, continuous infusion of human IL-8/CXCL8 into the knee joints of rabbits for 14 days led to severe arthritis, characterized by erythema, joint pain, infiltration of leucocytes and mononuclear cells in the synovial tissue, and hypervascularization in the synovial lining layer (Endo *et al.*, 1994). Thus, the angiogenic properties of chemokines, such as IL-8/CXCL8, GRO α /CXCL1, MCP-1/CCL2, SDF-1/CXCL12, and fractalkine/CX3CL1 (Koch *et al.*, 1992; Salcedo *et al.*, 1999, 2000; Volin *et al.*, 2001) may play an important role in the development of RA. Angiogenic factors, including FGF (Thomas *et al.*, 2000), vascular endothelial growth factor (VEGF) (Cho *et al.*, 2002), IL-18, and angiopoietin (Scott *et al.*, 2002), are also produced by FLSs. This suggests that FLSs are involved in neovascularization, and may cause critical pathological changes to sustain pannus formation in RA (Szekanecz & Koch, 2007).

2.2.3 Joint destruction

Proteinases, such as MMPs and cathepsins, are produced by FLSs attached to cartilage and bone, and play an important role in joint destruction. The expression of *MMP-1/interstitial collagenase* and *MMP-3/stromelysin* correlates with the invasive growth of FLSs in RA (Tolboom *et al.*, 2002). MMP-1 is found in the synovial membranes of all RA patients. Moreover, the levels of MMP-1 in the synovial fluid, but not in the sera, correlate with the degree of synovial inflammation (Konttinen *et al.*, 1999; Maeda *et al.*, 1995; Sorsa *et al.*, 1992). MMP-3 plays a key role in joint destruction, not only by degrading matrix molecules, but also by activating other pro-MMPs into their active forms (Okada, 2009). The major source of MMP-3 is FLSs in the lining layer (Tetlow *et al.*, 1993). High concentrations of MMP-3 have been detected in the synovial fluid and sera of RA patients (Beekman *et al.*, 1997; Taylor *et al.*, 1994). Moreover,

elevated serum levels of MMP-3 are correlated with systemic inflammation at the clinical and also the serologic level (Manicourt *et al.*, 1995; Yoshihara *et al.*, 1995). Although expression of MMP-13/collagenase-3 correlates with elevated levels of systemic inflammatory markers, this is not specific to RA (Lindy *et al.*, 1997; Westhoff *et al.*, 1999). MT1-MMP/MMP-14 degrades the extracellular matrix, and activates MMP-2/gelatinase A and MMP-13 (Pap *et al.*, 2000a).

The expression of MMPs in synovial cells is regulated by several extracellular signals, including inflammatory cytokines, growth factors, and molecules of the extracellular matrix, such as collagen and fibronectin (Pap & Gay, 2009). Among these, IL-1 is the most potent inducer of MMPs, including MMP-1, MMP-3, MMP-8, MMP-13, and MMP-14. FGF and PDGF also act as potent inducers for MMPs, by enhancing the effects of IL-1. TNF- α and TGF- β induce MMP-1 and MMP-13, respectively, while IL-17 induces MMP-1 and MMP-9.

Another group of proteinases involved in joint destruction is the cathepsins, which cleave cartilage types II, IX, and XI, and proteoglycan. The expression of the cysteine proteases, cathepsins B and L, was increased in the synovium of RA, especially at the sites of cartilage invasion (Keyszer *et al.*, 1995, 1998). Similarly to MMPs, the production of cathepsins is induced by proto-oncogene, IL-1, and TNF- α (Joseph *et al.*, 1987; Huet *et al.*, 1993; Lemaire *et al.*, 1997). Cathepsin K, which plays an important role in bone resorption by osteoclasts, is also expressed by FLSs and macrophages at the site of synovial invasion into the articular bone (Min *et al.*, 2004).

2.2.4 Perpetuation of inflammation

When cells producing soluble factors or expressing ligands on their surfaces are located close to cells that receive signals through the specific receptor, a circuit of chronic inflammation may be generated through an exchange of cell roles. For example, activated Th1 cells produce IFN γ , which activates macrophages. The activated macrophages produce IL-1 and TNF- α , which in turn activate T cells. In cases of RA, IL-1 and TNF- α from macrophages can activate FLSs, creating another circuit with a distinct cellular combination. Although one can easily imagine the operation of such a circuit at a certain time point of autoimmune diseases, it is difficult to demonstrate the mechanism by a suitable model system. Recently, Ogura *et al.* (2008) proposed that IL-17 secreted from Th17 cells induces fibroblasts to produce more IL-6, in a manner dependent on the transcription factor NF- κ B, and the signal transducer and activator of transcription (STAT) 3. The mechanism, designated as "IL-17A-triggered positive-feedback loop of IL-6 signaling", is thought to amplify the inflammatory responses mediated by interactive cytokines. Enhancement of this loop was shown to be involved in the development of RA-like arthritis or experimental autoimmune encephalomyelitis in knock-in mice gp130F759, which are defective in the negative regulation of signaling through a common receptor subunit of IL-6 family cytokines, gp130. The identification of such a powerful circuit, specific to each autoimmune disease, will facilitate the development of a critical target point for effective therapy.

3. Progress in the study of RA by the molecular genetic approach

3.1 Genome-wide screening for disease-related genes

The risk of developing RA and the severity of the disease are significantly affected by genetics. It has long been recognized that certain HLA alleles, especially HLA-DR4, are

associated with increased risk of onset and severity of RA (Weyand *et al.*, 1992). A shared epitope on certain HLA haplotypes is thought to affect the binding of peptides derived from self-antigens, leading to autoimmune responses by T cells (Wordsworth *et al.*, 1989). To identify non-HLA genes that regulate the development and severity of RA, human genome-wide studies have been performed. Some of these studies have used a combined approach, with factors such as microsatellites (Tamiya *et al.*, 2005), or disease subsets; serum autoantibody alone (Stahl *et al.*, 2010) or combined with a shared epitope (Sugino *et al.*, 2010); race, or nation (Freudenberg *et al.*, 2011; Martin *et al.*, 2010); correlation with other autoimmune diseases (Cui *et al.*, 2009; Zhernakova *et al.*, 2011); or responsiveness to therapies targeting specific cytokines (Liu *et al.*, 2008; Plant *et al.*, 2011). Single nucleotide polymorphisms that may be involved in the development of RA include protein tyrosine phosphatase, nonreceptor-type 22 (*PTPN22*), cytotoxic T-lymphocyte antigen 4 (*CTLA4*), *STAT4*, and peptidylarginine deiminase type 4 (*PADI4*). Among these, *PADI4* has been identified by genome-wide screening (Suzuki *et al.*, 2003) as being able to modify self-antigens by citrullination. Moreover, the presence of anti-cyclic citrullinated antibody in the serum is highly specific to RA and has a high diagnostic value. The role of *PADI4* in the pathogenesis of RA, especially with respect to “autoimmunity” to modified self-antigens, will be intriguing to clarify.

Large-scale, genome-wide association studies, based firmly on statistics, have provided valuable information on the candidate genes for RA. Nevertheless, to understand the complex pathophysiology of RA, data from studies on additional aspects must be integrated. Such studies should include molecular and cell-biological analyses of clinical materials from individual RA cases, and functional analyses of candidate genes *in vitro* and *in vivo*, including experimental system using engineered mutant mice.

3.2 Transcription profiling reveals disease-specific genes and heterogeneity in RA tissues

Gene expression profiling of FLSs, comparing RA and OA, has revealed disease-specific genes. The genes highly and exclusively expressed in RA were *HOXD10*, *HOXD11*, *HOXD13*, *CCL8*, and *LIM homeobox 2*. Further analysis of the relationships between gene expression on RA-FLSs and clinical disease parameters revealed specific and unique correlations as follows; *HLA-DQA2* with Health Assessment Questionnaire (HAQ) score; *Clec12A* with rheumatoid factor; *MAB21L2*, *SIAT7E*, *HAPLN1*, and *BAIAP2L1* with C-reactive protein level; and *RGMB* and *OSAP* with erythrocyte sedimentation rate (Galligan *et al.*, 2007). The data indicated the heterogeneity of gene expression in patients with the same disease. These RA-specific or clinical state-related genes differ from those identified by genome-wide screening, indicating that the complete pathophysiology of RA, as a multifactorial disease, involves genomic and also epi-genomic regulation of genes. The functional roles of these genes remain to be determined.

Evidence for the heterogeneity of gene expression in synovial tissues from erosive RA cases has been demonstrated by large-scale profiling studies. Systemic characterization of the differentially expressed genes highlighted the existence of at least 2 molecularly distinct forms of RA tissues (van der Pouw Kraan *et al.*, 2003). The first is RA tissue with high-grade inflammation (RA^{high}), which exhibits abundant expression of gene clusters indicative of adaptive immune responses, such as genes expressed by T cells, B cell, and antigen-presenting cell (APC). The second form of RA tissue is a low-grade inflammatory gene

expression signature (RA^{low}), common to the tissues from patients with OA, and characterized by increased expression of genes involved with tissue remodeling activity. Importantly, the cluster of RA^{high} showed an increased expression of STAT1-pathway related genes; STAT1-inducing receptors (*IL-2R γ* , *CCR5*), and STAT1 target genes (*MMP-1*, *MMP-3*, *caspase-1*, *TAP-1*, and *IRF-1*), suggesting a prominent role for this pathway. Furthermore, patients with the high-grade inflammation tissue type had higher Disease Activity Scores in 28 joints, higher C-reactive protein levels, higher erythrocyte sedimentation rates, increased numbers of platelets, and shorter disease durations (van Baarsen *et al.*, 2010).

Several trials have profiled gene expression in the synovial tissues of RA patients undergoing molecular-targeting therapy (Lindberg *et al.*, 2006; Wijbrandts *et al.*, 2008). Further analysis is expected to yield valuable data on cytokine activity in the human body, facilitating the development of effective therapy with a clear target point.

4. Mouse models for RA

The generation of RA-like joint diseases in engineered mutant mice appears to reflect the heterogeneous and complicated mechanisms of human arthritis, diagnosed simply as rheumatoid arthritis. In contrast to previous years, when animal models for human diseases rarely emerged by point mutations in nature, current research on autoimmune diseases such as RA benefits from the existence of various engineered mutant mice models. For example, mechanisms for RA-like disease revealed by murine models include, the abnormal T-cell receptor (TCR) signaling by a natural mutant ZAP70 in SKG mouse (Sakaguchi *et al.*, 2003), an autoantibody to glucose-6-phosphoisomerase in K/BxN TCR transgenic mouse (Korganow *et al.*, 1999), defective autoantigen clearance in *DNaseII^{-/-}* mouse (Kawane *et al.*, 2006), overexpression of the viral gene in HTLV-1 *pX* transgenic mouse (Iwakura *et al.*, 1991), and excessive amounts or activity of cytokines. RA-like disease developed in TNF- α transgenic mice (Keffer *et al.*, 1991) and IL-1 α transgenic mice (Niki *et al.*, 2001) with overproduction of inflammatory cytokines and in TNF AU-rich elements-deficient (Δ ARE) mice (Kontoyiannis *et al.*, 1999) with increased stability of cytokine messenger RNA. Excessive activities of arthritogenic cytokines were evoked in IL-1 receptor antagonist knock-out mouse (Horai *et al.*, 2000) lacking a physiological negative feedback molecule, and in gp130F759 with a defective, intracellular negative-regulatory signaling pathway (Atsumi *et al.*, 2002; Ohtani *et al.*, 2000).

These wide variety of murine arthritis models with a defined genetic defect will be useful for analyzing the mechanisms for the synergistic action of genetic and environmental factors in RA development (Ishihara *et al.*, 2004), and also the mechanisms for initiation or perpetuation of joint inflammation (Murakami *et al.*, 2011; Ogura *et al.*, 2008). Furthermore, bone marrow transplantation experiment revealed a unique feature of gp130F759 that non-hematopoietic cells with a point mutation Y759F in gp130 are sufficient to induce passive but arthritogenic activation of wild type CD4⁺T cells (Sawa *et al.*, 2006). In human TNF- α transgenic mouse, arthritogenic FLSs showed increased expression of *MMP-1* and *MMP-9*, and also diminished adhesion to extracellular matrix components. These changes could induce increased proliferation and migration, which are critical for the spread of hyperplasia in the joints (Aidinis *et al.*, 2003). Dispensable roles for RAG in arthritis have been observed in TNF^{ARE} mouse (Kontoyiannis *et al.*, 1999) and *DNaseII^{-/-}* mouse (Kawane *et al.*, 2010), indicating that synovial hyperplasia may develop independently of acquired immunity.

5. Fibroblast-like synoviocytes and mesenchymal stem cells

FLSs are characterized mainly by *in vitro* analyses. The synovial membranes are easily obtained by joint surgery. The cells liberated from the synovial tissues by treatment with collagenase can be cultured under the appropriate conditions. Although primary FLSs are useful, experiments must be carefully designed, because the composition of the cells in the culture changes after 4 passages, when contaminated hematopoietic cells disappear (Zimmermann *et al.*, 2001).

The source of pathogenic FLSs proliferating in the synovium of RA is an intriguing issue, and several possibilities can be considered. Growth of FLSs can be stimulated by adjacent hematopoietic cells in the microenvironment, or initiated by the acquisition of cell-intrinsic properties for unregulated growth. Alternatively, growing FLSs can be derived from resident FLSs in the normal synovial membrane, or migrated from other organs. The latter possibility was demonstrated by experiments to inject FLSs from human TNF- α transgenic mice into the knee joint, and to transplant human synovial fibroblasts into SCID mice (Aidinis *et al.*, 2003; Lefevre *et al.*, 2009).

Fibroblast-like cells that initiate growth during the very early stages of RA can originate from mature FLSs or from other mesenchymal cells at the primitive stage, such as mesenchymal stem cells (MSCs). The presence of MSCs in the synovium has been reported by several researchers. MSCs with the potential to differentiate into 3 lineages, osteogenic, adipogenic, and chondrogenic cells, were obtained from the synovial membrane following digestion with collagenase and more than 3 passages (De Bari *et al.*, 2001). Fibroblast-like MSCs expressing fibroblast marker D7-FIB, but not CD45, were detected in the synovial fluid (Jones *et al.*, 2004). The number of these MSCs was lower in the synovial fluid of RA than in that of OA. In addition to the synovium, MSCs have been derived from blood (Zvaifler *et al.*, 2000), adipose tissue (Zuk *et al.*, 2002), and the periosteal region (De Bari *et al.*, 2006). Although not genuine MSCs, circulating CD14⁺ monocytes may contain progenitors with the potential to differentiate into mesenchymal cells (Kuwana *et al.*, 2003).

In terms of the underlying mechanism for the transformation-like phenotype of FLSs, and the involvement of MSCs, Li & Makarov (2006) reported intriguing data from animal models of RA. Arthritic FLSs contained a substantial fraction of bone marrow-derived precursors, with the ability to differentiate *in vitro* into various mesenchymal cell types. However, inflammation prevented multilineage differentiation. The transcription factor NF- κ B played a key role in repressing osteogenic and adipogenic differentiation of arthritic FLSs. On the other hand, specific activation of NF- κ B profoundly enhanced proliferation, motility, and matrix-degrading activity of FLSs by the induction of MMPs. These data suggest an intriguing mechanism, namely, that arthritic FLSs are bone marrow-derived MSCs, which are arrested during the early stages of differentiation, by the activation of NF- κ B induced by inflammatory cytokines (Li & Makarov, 2006).

6. Search for RA-related genes through the classification of fibroblast-like synoviocytes

6.1 Subtypes of fibroblast-like synoviocytes in RA

Kasperkovitz *et al.* (2005) reported that subtypes of FLSs in RA differ in their gene expression. Complementary DNA microarrays of the synovial tissues and cultured FLSs obtained from RA patients revealed that the gene expression profiles of high- and low-grade

inflammation synovial tissues were characterized by high and low expression of genes of immune-competent cells (T cells, B cells, and APCs), respectively. Furthermore, hierarchical clustering identified 2 groups of FLSs, characterized by distinctive gene expression profiles and correlation with the inflammatory profiles of the synovial tissues. The first group correlated with the high-grade inflammation tissue, and exhibited increased expression of a TGF- β /activin A-inducible gene profile, which is characteristic of myofibroblasts, a cell type involved in wound healing. The second group correlated with the low-grade inflammation tissue, and showed increased expression of the genes involved in autocrine growth regulation, cell transformation, complement activation, and oxidative stress. Reflecting the gene expression profile, an increased proportion of myofibroblast-like cells in the heterogeneous population of FLSs were immunohistochemically detected in the high-grade inflammation tissue. These data suggest that the inflammatory state of the synovium is determined by the composition of heterogeneous FLSs.

6.2 Transformed fibroblast-like synoviocyte lines reveal heterogeneity irrespective of arthritis types

The data of Kasperkovitz *et al.* (2005), Galligan *et al.* (2007) and others indicate that combining gene expression profiling with other parameters, such as clinical data or characteristics of FLS lines, constitutes a powerful tool for identifying novel disease-related genes. To identify the cell-intrinsic abnormalities of RA-FLSs, we established transformed cell lines from the synovium of RA or OA cases, by immortalization with SV40 large T Ag (unpublished data of Ishihara *et al.*). Characterization of FLSs from 2 types of arthritis revealed no significant differences in surface molecules, growth rates, patterns of tyrosine-phosphorylated proteins, or expression of the genes related to inflammation (*IL-1 β* , *IL-6*, *MMP-1*, *MMP-3*, etc.). Since the expression levels of these genes vary (ranges exceeding 1,000-fold) among FLS lines from each type of arthritis, we tentatively categorized them into 2 subtypes reflecting resting (r) and active (a) stages, based on the expression levels of *IL-1 β* and *MMP-1*. Next, we performed a micro DNA array to obtain the gene expression profiles for 4 representative cell lines, r-OA-FLS, a-OA-FLS, r-RA-FLS, and a-RA-FLS, and obtained 10 gene clusters. Although no disease-specific clusters were obtained, 2 reciprocal, stage-specific clusters were detected, suggesting the validity of our hypothesis for the presence of subtypes in FLSs. Using these data we are presently searching for 2 types of candidate genes; master genes that determine the states of FLSs, and genes that could play a role in the pathophysiology of RA by inference based on our current understanding of FLSs. In the following sections, we will review the potential roles of activation-induced cytidine deaminase (*AID*) (Igarashi *et al.*, 2010) and the *A20/ABIN* family.

6.3 Ectopic expression of *AID* and acquisition of a tumor-like phenotype by fibroblast-like synoviocytes

6.3.1 *P53* mutation in fibroblast-like synoviocytes of RA and *AID* expression in inflammation

In addition to the properties described above, the expression of the tumor-suppressor gene *p53* with somatic mutations (Firestein *et al.*, 1997; Inazuka *et al.*, 2000; Kullmann *et al.*, 1999; Reme *et al.*, 1998; Yamanishi *et al.*, 2002), and the down-regulation of the tumor suppressor *PTEN*, a protein phosphatase gene, have been demonstrated in RA-FLSs (Pap *et al.*, 2000b).

In particular, the somatic mutation of the *p53* gene appears consistent, not only in terms of increased resistance to apoptosis, but also with respect to pro-inflammatory responses such as production of IL-6 and MMP-1 (Han *et al.*, 1999; Sun *et al.*, 2004; Yamanishi *et al.*, 2005). However, little is known about the mechanism by which the somatic mutations are introduced into the *p53* gene in RA-FLSs.

AID is a member of the APOBEC family, which is a cellular cytidine deaminase involved in protection from retroviral infection or regulation of cholesterol metabolism (Goila-Gaur & Strebel, 2008). AID was originally identified as an indispensable molecule for somatic hypermutation at the immunoglobulin variable region, and also for class-switch recombination in germinal center B lymphocytes (Di Noia & Neuberger, 2007; Honjo *et al.*, 2004). Recently, several investigators have demonstrated up-regulation of AID in non-lymphoid tumor cells such as breast cancer, cholangiocarcinoma, hepatoma, and colorectal cancer cells (Babbage *et al.*, 2006; Chan-On *et al.*, 2009; Endo *et al.*, 2007, 2008; Komori *et al.*, 2008; Kou *et al.*, 2007; Morisawa *et al.*, 2008). During the process of oncogenesis, NF- κ B activation in inflammation is thought to be important for aberrant expression of AID. For example, the infection of gastric mucosal cells with *Helicobacter pylori*, or of hepatocytes with hepatitis C virus, activates NF- κ B and successfully induces local production of pro-inflammatory cytokines such as TNF- α and IL-1 β . Together, these secreted cytokines also activate NF- κ B, and lead to the induction of AID. In fact, stimulation with TNF- α or IL-1 β induces AID expression even in non-tumor hepatocyte or colon epithelial cells. Moreover, the somatic mutations of *p53* found in these cancer cells appeared to be a direct target of AID (Endo *et al.*, 2008; Kou *et al.*, 2007; Takai *et al.*, 2009).

RA is characterized by an environment rich in pro-inflammatory cytokines and the existence of mutations in the *p53* gene. Thus, under chronic inflammatory circumstances, it is possible that aberrant expression of AID could introduce mutations into the *p53* gene of FLSs.

6.3.2 Aberrant expression of AID in RA-FLSs

First, we assessed the expression of the AID gene in the transformed FLS cell lines described in 6.2, by real-time reverse transcription polymerase chain reaction (RT-PCR). AID was transcribed in more than half of the RA-FLS cell lines (5 out of 9) and in none of the OA-FLS cell lines. Quantitative assay by RT-PCR showed 7- to 18-fold higher AID transcription in the RA-FLS lines compared to the OA-FLS lines that expressed a low but detectable level of AID transcription. The possibility of contaminated signals from AID-expressing B cells was excluded by the absence of pan B cell marker transcription. The translation of AID was further confirmed by the detection of protein in the cell lysate from RA-FLSs, with western blot analysis.

Patients who provided AID-expressing FLSs showed a tendency toward higher levels (approximately 2.7 times) of CRP in the serum. Regarding gender, the number of female patients with AID⁺ FLSs was approximately 1.9 times higher than the number of male patients. Although our data are not statistically significant because of the small sample numbers used, it appears that AID expression in FLSs is facilitated under conditions of inflammation in female patients. Indeed, we observed that estrogen, a representative female hormone, or TNF- α , a representative pro-inflammatory cytokine, augmented the transcription of AID in AID⁺ RA-FLSs to more than 20-fold higher levels compared with the basal levels in OA-FLSs. These results are similar to those previously reported for other cells

(Endo *et al.*, 2007, 2008; Pauklin *et al.*, 2009). The transcription levels of *TNF- α* , or the pro-inflammatory cytokines *IL-6* and *IL-1 β* , did not correlate with that of *AID*, suggesting that *AID* transcription is not induced by autocrine cytokines. No clear relationship was observed between aberrant expression of *AID* and other clinical parameters, such as age, serum MMP-3 levels, or medication.

6.3.3 Accumulation of *p53* gene mutations in *AID*-expressing RA-FLSs

The mutations of the *p53* tumor-suppressor gene frequently found in RA-FLSs could contribute to the tumor-like, and also the pro-inflammatory properties of RA-FLSs, such as aggressive growth, invasion, and destruction of cartilage and bone (Firestein *et al.*, 1997; Inazuka *et al.*, 2000; Kullmann *et al.*, 1999; Reme *et al.*, 1998; Sun *et al.*, 2004; Yamanishi *et al.*, 2002). Although genotoxic and oxidative stresses have been speculated to be causative candidates for the somatic mutation in the *p53* gene in RA-FLSs, the molecular mechanism has not yet been elucidated. As mentioned in 6.3.1, a clear relationship between *AID* expression and the frequency of *p53* somatic mutations has been demonstrated in some non-B lymphocytes, such as hepatocytes and colon epithelial cells (Chan-On *et al.*, 2009; Endo *et al.*, 2008; Komori *et al.*, 2008; Kou *et al.*, 2007; Morisawa *et al.*, 2008). Thus, we speculated that aberrant expression of *AID* might be involved in the introduction of the *p53* gene mutation. We amplified the coding region of *p53* from 3 *AID*⁺ RA-FLS cell lines with high-fidelity polymerase. We then determined the nucleotide sequence corresponding to that region and compared it with the intact *p53* gene sequence.

AID⁺ RA-FLSs harbored approximately 2- to 3.5-fold more mutations than the control RA-FLS subsets, which expressed *AID* at a lower level. In addition, the frequency of non-silent mutations was 3 times more than that of silent mutations. Notably, the base substitution pattern in *p53* was biased toward the transition type, which is typical for AID-mediated mutations at the variable region of the immunoglobulin gene (Di Noia & Neuberger, 2007). The mutations were distributed intensively at the DNA-binding domain of the *p53* gene, where the hotspot of somatic mutations is found in some malignant tumors. The Arg²⁴⁸ mutation, one of the cancer hotspot mutations (Ko & Prives, 1996), was found in *p53* from our *AID*⁺ RA-FLSs. In addition, among the amino acid mutations that we identified, 17% were identical to those previously reported. A further 33% were distinct amino acid mutations; however, the positions of base change were located in the same codons as previously reported. The apparent correlation between ectopic expression of *AID* and increased frequency of somatic mutations of *p53* strongly suggests that AID may be involved in the introduction of mutations to *p53*. Such mutations could lead to reductions or increases in the function of p53, which in turn may result in the tumor-like or anti-apoptotic phenotypes of FLSs in RA.

6.3.4 *AID* is produced by non-transformed RA-FLSs and in the RA synovium outside the B-cell follicles

The aberrant expression of *AID* in some RA-FLS transformed cell lines is not caused by the effects of transformation with SV40 large T Ag. Indeed, 3 to 8 times higher transcription levels of *AID* were observed, even in non-transformed primary FLS cell lines (4 out of 11 RA-FLSs, but none of the 6 OA-FLSs). In addition, cyto-staining with anti-AID antibody revealed a positive signal in *AID*-expressing primary RA-FLSs. Furthermore, dual-color immunohistostaining of the synovial sections from *AID*⁺ RA patients clearly demonstrated

the production of AID by FLSs in the RA synovial tissues (Figure 1), providing definitive evidence for the occurrence of ectopic and aberrant expression of *AID* in RA.

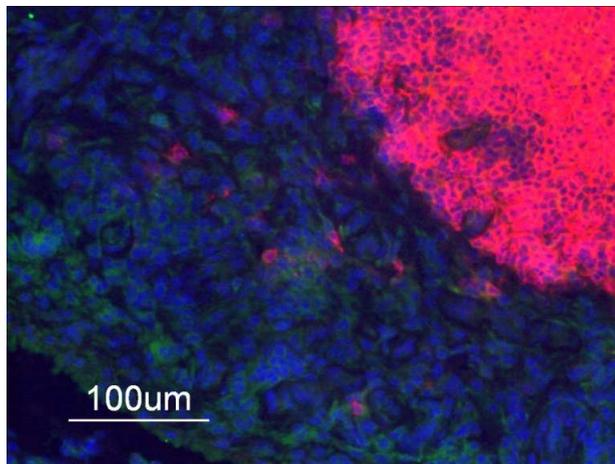


Fig. 1. Immunofluorescence staining of AID on synovial tissue sections from a representative RA patient. Sections were stained simultaneously with rat mAb for AID and anti-CD20 (B-cell marker) mAb. AID was visualized with alexa 488 fluoro-dye conjugated anti-rat secondary Ab (green); CD20 was visualized with alexa 594 fluoro-dye conjugated anti-mouse secondary Ab (red). The nucleus was stained with 4',6-diamino-2-phenylindole (blue). Scale bar is 100 μ m.

We concluded that AID is selectively expressed by a proportion of RA-FLSs and that its expression is associated with an increased frequency of somatic mutations in *p53* (Igarashi *et al.*, 2010). Thus, it is possible that the aberrant expression of AID within certain RA-FLSs induces somatic mutations in *p53*, leading to the acquisition of pro-inflammatory or tumor-like phenotypes.

6.4 Heterogeneous responsiveness of fibroblast-like synoviocytes to TNF- α

6.4.1 RA-FLS cell lines differentially respond to TNF- α

The chronic inflammation circuit in the joints of RA is initiated by the production of inflammatory cytokines by FLSs, following stimulation with TNF- α secreted from the surrounding inflammatory cells. In this context, the TNF- α /NF- κ B pathway plays an essential role in the transcription of pro-inflammatory cytokines. However, the regulation of NF- κ B activity downstream of TNF- α in FLSs is not fully understood. To investigate the heterogeneous responsiveness of RA-FLS cell lines to TNF- α stimulation, we examined the panels of primary RA-FLS cell lines for their induction levels of pro-inflammatory cytokines following TNF- α stimulation. Interestingly, RA-FLS cell lines can be clearly categorized into 2 types based on the responsiveness to TNF- α , namely, whether the transcription levels of pro-inflammatory cytokine gene are high (designated as the high-responder group) or not (designated as the low-responder group). This facilitated production of pro-inflammatory cytokines can be explained by the significant elevation of NF- κ B activity in the high-responder FLS lines compared with that in the low-responder lines.

6.4.2 Possible positive effect of A20/ABINs on pro-inflammatory cytokine induction

A20, also termed TNFAIP3 (TNF α -induced protein 3), was originally identified as an inducible zinc finger protein in human umbilical vein endothelial cell lines following stimulation with TNF- α . A20 has dual enzymatic activities, namely, ubiquitination and deubiquitination (Dixit *et al.*, 1990). The induction of A20 upon stimulation with TNF- α is NF- κ B dependent; moreover, induced A20 reversely suppresses the activation of NF- κ B through the regulation of ubiquitin-mediated degradation of NF- κ B activator (Vereecke *et al.*, 2009). This negative feedback loop is thought to be necessary to terminate inflammation and protect tissues from unnecessary damage. Recently, it was reported that the expression level of A20 in RA-FLSs was lower than that in OA-FLSs (Elsby *et al.*, 2010). Although the difference was not significant, this finding could provide *in vitro* evidence of altered A20 transcription by 6q23 intergenic SNPs associated with RA (Dieguez-Gonzalez *et al.*, 2009; Orozco *et al.*, 2009). Thus, we speculated that the down-regulation of NF- κ B inhibitors might be a possible mechanism for enhanced activation of NF- κ B in high-responder FLSs. Contrary to our speculation, the high-responder group with abundant mRNA levels of pro-inflammatory cytokines also exhibited marked induction of A20 following stimulation with TNF- α . Furthermore, the transcription of the NF- κ B inhibitory molecules ABIN (A20-binding inhibitor of NF- κ B activation, also called TNIP, TNFAIP3 interacting protein)-1 and ABIN-3, but not of ABIN-2, was increased (Igarashi *et al.*, in press).

These observations indicate that there is heterogeneity of RA-FLSs in the responsiveness to TNF- α stimulation and suggest that these “inhibitors” might not play negative regulatory roles in RA-FLS. The precise mechanism, cell-lineage specificity, disease specificity, and significance in cell biology of this unexpected possible positive role for A20/ABINs are currently under investigation.

7. Conclusion

Anti-cytokine therapy for RA is a prominent achievement in the field of autoimmune diseases. Accumulated evidence from clinical and basic medical research indicates pivotal roles for FLS in the pathogenesis and pathophysiology of RA. Data from genome-wide screening, transcriptional profiling, and animal models indicate that RA consists with heterogeneous disease subsets. Together with several other researchers, we have presented evidence for heterogeneity in FLS. Based on this finding, we have successfully searched for disease-related genes by subtyping FLS. We have identified 2 groups of genes, AID and A20/ABINs. AID is involved in the irreversible transformation of FLS, whereas A20/ABINs participate in the reversible, but potentially harmful, responsiveness of them. Both groups of genes are constituent elements for distinct levels of heterogeneity in FLS, which may be involved in resistance to anti-cytokine therapies. Subtyping of FLS based on expression of AID did not coincide with that based on responsiveness to signal-utilizing NF- κ B, which is reasonable because RA is a multi-factorial disease. We believe that our approach to categorizing subsets of FLS based on differential gene expression, or on responsiveness to inflammatory stimuli, will facilitate a comprehensive understanding of the pathogenesis and pathophysiology of RA.

8. Acknowledgment

We are grateful to Dr. Jun Hashimoto, Dr. Tetsuya Tomita, and Professor Hideki Yoshikawa in Department of orthopaedics (Osaka University) for collaboration on our study of FLS in

RA. We thank Dr. Masaaki Murakami and colleagues in the Laboratory for Developmental Immunology (Osaka University) for co-operation in the study of gp130F759. We are indebted to Professor Toshio Hirano (Osaka University) for instruction, encouragement, and long-term collaboration on our research into RA. We also thank Ms. Reina Tanaka and Ms. Yuka Kenmotsu for technical assistance. This work was partly supported by Research Project Grants from Kawasaki Medical School, and Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture, and Technology of Japan.

9. References

- Aidinis, V., Plows, D., Haralambous, S., Armaka, M., Papadopoulos, P., Kanaki, M.Z., Koczan, D., Thiesen, H.J., & Kollias, G. (2003). Functional analysis of an arthritogenic synovial fibroblast. *Arthritis Res Ther* 5, R140-157.
- Aikawa, Y., Morimoto, K., Yamamoto, T., Chaki, H., Hashiramoto, A., Narita, H., Hirono, S., & Shiozawa, S. (2008). Treatment of arthritis with a selective inhibitor of c-Fos/activator protein-1. *Nat Biotechnol* 26, 817-823.
- Atsumi, T., Ishihara, K., Kamimura, D., Ikushima, H., Ohtani, T., Hirota, S., Kobayashi, H., Park, S.J., Saeki, Y., Kitamura, Y., *et al.* (2002). A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. *J Exp Med* 196, 979-990.
- Babbage, G., Ottensmeier, C.H., Blaydes, J., Stevenson, F.K., & Sahota, S.S. (2006). Immunoglobulin heavy chain locus events and expression of activation-induced cytidine deaminase in epithelial breast cancer cell lines. *Cancer Res* 66, 3996-4000.
- Bartok, B., & Firestein, G.S. (2010). Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev* 233, 233-255.
- Beekman, B., van El, B., Drijfhout, J.W., Runday, H.K., & TeKoppele, J.M. (1997). Highly increased levels of active stromelysin in rheumatoid synovial fluid determined by a selective fluorogenic assay. *FEBS Lett* 418, 305-309.
- Wordsworth, B.P., Lanchbury, J.S., Sakkas, L.I., Welsh, K.I., Panayi, G.S., & Bell, J.I. (1989). HLA-DR4 subtype frequencies in rheumatoid arthritis indicate that DRB1 is the major susceptibility locus within the HLA class II region. *Proc Natl Acad Sci U S A* 86, 10049-10053.
- Brennan, F.M., & McInnes, I.B. (2008). Evidence that cytokines play a role in rheumatoid arthritis. *J Clin Invest* 118, 3537-3545.
- Cha, H.S., Rosengren, S., Boyle, D.L., & Firestein, G.S. (2006). PUMA regulation and proapoptotic effects in fibroblast-like synoviocytes. *Arthritis Rheum* 54, 587-592.
- Chan-On, W., Kuwahara, K., Kobayashi, N., Ohta, K., Shimasaki, T., Sripa, B., Leelayuwat, C., & Sakaguchi, N. (2009). Cholangiocarcinomas associated with long-term inflammation express the activation-induced cytidine deaminase and germinal center-associated nuclear protein involved in immunoglobulin V-region diversification. *Int J Oncol* 35, 287-295.
- Cho, M.L., Cho, C.S., Min, S.Y., Kim, S.H., Lee, S.S., Kim, W.U., Min, D.J., Min, J.K., Youn, J., Hwang, S.Y., *et al.* (2002). Cyclosporine inhibition of vascular endothelial growth factor production in rheumatoid synovial fibroblasts. *Arthritis Rheum* 46, 1202-1209.
- Cui, J., Taylor, K.E., DeStefano, A.L., Criswell, L.A., Izmailova, E.S., Parker, A., Roubenoff, R., Plenge, R.M., Weinblatt, M.E., Shadick, N.A., *et al.* (2009). Genome-Wide

- Association Study of Determinants of Anti-Cyclic Citrullinated Peptide Antibody Titer in Adults with Rheumatoid Arthritis. *Molecular Medicine* 15, 136-143.
- De Bari, C., Dell'Accio, F., Tylzanowski, P., & Luyten, F.P. (2001). Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* 44, 1928-1942.
- De Bari, C., Dell'Accio, F., Vanlauwe, J., Eyckmans, J., Khan, I.M., Archer, C.W., Jones, E.A., McGonagle, D., Mitsiadis, T.A., Pitzalis, C., *et al.* (2006). Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis Rheum* 54, 1209-1221.
- Di Noia, J.M., & Neuberger, M.S. (2007). Molecular mechanisms of antibody somatic hypermutation. *Annu Rev Biochem* 76, 1-22.
- Dieguez-Gonzalez, R., Calaza, M., Perez-Pampin, E., Balsa, A., Blanco, F.J., Cañete, J.D., Caliz, R., Carreño, L., de la Serna, A.R., Fernandez-Gutierrez, B., *et al.* (2009). Analysis of TNFAIP3, a feedback inhibitor of nuclear factor-kappaB and the neighbor intergenic 6q23 region in rheumatoid arthritis susceptibility. *Arthritis Res Ther* 11, R42.
- Dixit, V.M., Green, S., Sarma, V., Holzman, L.B., Wolf, F.W., O'Rourke, K., Ward, P.A., Prochownik, E.V., & Marks, R.M. (1990). Tumor necrosis factor-alpha induction of novel gene products in human endothelial cells including a macrophage-specific chemotaxin. *J Biol Chem* 265, 2973-2978.
- Elsby, L. M., Orozco, G., Denton, J., Worthington, J., Ray, D. W., & Donn, R. P. (2010) Functional evaluation of TNFAIP3 (A20) in rheumatoid arthritis. *Clin Exp Rheumatol* 28, 708-14.
- Endo, H., Akahoshi, T., Nishimura, A., Tonegawa, M., Takagishi, K., Kashiwazaki, S., Matsushima, K., & Kondo, H. (1994). Experimental arthritis induced by continuous infusion of IL-8 into rabbit knee joints. *Clin Exp Immunol* 96, 31-35.
- Endo, Y., Marusawa, H., Kinoshita, K., Morisawa, T., Sakurai, T., Okazaki, I.M., Watashi, K., Shimotohno, K., Honjo, T., & Chiba, T. (2007). Expression of activation-induced cytidine deaminase in human hepatocytes via NF-kappaB signaling. *Oncogene* 26, 5587-5595.
- Endo, Y., Marusawa, H., Kou, T., Nakase, H., Fujii, S., Fujimori, T., Kinoshita, K., Honjo, T., & Chiba, T. (2008). Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. *Gastroenterology* 135, 889-898, 898 e881-883.
- Firestein, G.S. (2009). 65, In: *Kelly's Textbook of Rheumatology*, Firestein, G.S, Budd, R.C., Harris, Jr. E.D., McInnes, I.B., Ruddy, S., Sargent, J.S., 1035-1086, SAUNDERS ELSEVIER, 978-1-4160-3285-4, Philadelphia
- Firestein, G.S., Echeverri, F., Yeo, M., Zvaifler, N.J., & Green, D.R. (1997). Somatic mutations in the p53 tumor suppressor gene in rheumatoid arthritis synovium. *Proc Natl Acad Sci U S A* 94, 10895-10900.
- Firestein, G.S. (2007). Biomedicine. Every joint has a silver lining. *Science* 315, 952-953.
- Freudenberg, J., Lee, H.S., Han, B.G., Shin, H.D., Kang, Y.M., Sung, Y.K., Shim, S.C., Choi, C.B., Lee, A.T., Gregersen, P.K., *et al.* (2011). Genome-Wide Association Study of Rheumatoid Arthritis in Koreans. *Arthritis and Rheumatism* 63, 884-893.
- Galligan, C.L., Baig, E., Bykerk, V., Keystone, E.C., & Fish, E.N. (2007). Distinctive gene expression signatures in rheumatoid arthritis synovial tissue fibroblast cells: correlates with disease activity. *Genes Immun* 8, 480-491.

- Garcia-Vicuna, R., Gomez-Gaviro, M.V., Dominguez-Luis, M.J., Pec, M.K., Gonzalez-Alvaro, I., Alvaro-Gracia, J.M., & Diaz-Gonzalez, F. (2004). CC and CXC chemokine receptors mediate migration, proliferation, and matrix metalloproteinase production by fibroblast-like synoviocytes from rheumatoid arthritis patients. *Arthritis Rheum* 50, 3866-3877.
- Goila-Gaur, R., & Strebel, K. (2008). HIV-1 Vif, APOBEC, and intrinsic immunity. *Retrovirology* 5, 51.
- Gracie, J.A., Forsey, R.J., Chan, W.L., Gilmour, A., Leung, B.P., Greer, M.R., Kennedy, K., Carter, R., Wei, X.Q., Xu, D., *et al.* (1999). A proinflammatory role for IL-18 in rheumatoid arthritis. *J Clin Invest* 104, 1393-1401.
- Gudkov, A.V., & Komarova, E.A. (2010). Pathologies associated with the p53 response. *Cold Spring Harb Perspect Biol* 2:a001180
- Han, Z., Boyle, D.L., Shi, Y., Green, D.R., & Firestein, G.S. (1999). Dominant-negative p53 mutations in rheumatoid arthritis. *Arthritis Rheum* 42, 1088-1092.
- Honjo, T., Muramatsu, M., & Fagarasan, S. (2004). AID: how does it aid antibody diversity? *Immunity* 20, 659-668.
- Horai, R., Saijo, S., Tanioka, H., Nakae, S., Sudo, K., Okahara, A., Ikuse, T., Asano, M., & Iwakura, Y. (2000). Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* 191, 313-320.
- Hosaka, S., Akahoshi, T., Wada, C., & Kondo, H. (1994). Expression of the chemokine superfamily in rheumatoid arthritis. *Clin Exp Immunol* 97, 451-457.
- Huet, G., Flipo, R.M., Colin, C., Janin, A., Hemon, B., Collyn-d'Hooghe, M., Lafyatis, R., Duquesnoy, B., & Degand, P. (1993). Stimulation of the secretion of latent cysteine proteinase activity by tumor necrosis factor alpha and interleukin-1. *Arthritis Rheum* 36, 772-780.
- Igarashi, H., Yahagi, A., Saika, T., Hashimoto, J., Tomita, T., Yoshikawa, H., & Ishihara, K. (2011). A pro-inflammatory role for A20 and ABIN family proteins in human fibroblast-like synoviocytes in rheumatoid arthritis. *Immunol Lett.*, in press.
- Igarashi, H., Hashimoto, J., Tomita, T., Yoshikawa, H., & Ishihara, K. (2010) TP53 mutations coincide with the ectopic expression of activation-induced cytidine deaminase in the fibroblast-like synoviocytes derived from a fraction of patients with rheumatoid arthritis. *Clin Exp Immunol* 161, 71-80.
- Inazuka, M., Tahira, T., Horiuchi, T., Harashima, S., Sawabe, T., Kondo, M., Miyahara, H., & Hayashi, K. (2000). Analysis of p53 tumor suppressor gene somatic mutations in rheumatoid arthritis synovium. *Rheumatology* 39, 262-266.
- Ishihara, K., Sawa, S., Ikushima, H., Hirota, S., Atsumi, T., Kamimura, D., Park, S.J., Murakami, M., Kitamura, Y., Iwakura, Y., & Hirano, T. (2004) The point mutation of tyrosine 759 of the IL-6 family cytokine receptor gp130 synergizes with HTLV-1 pX in promoting rheumatoid arthritis-like arthritis. *Int Immunol* 16, 455-465.
- Iwakura, Y., Tosu, M., Yoshida, E., Takiguchi, M., Sato, K., Kitajima, I., Nishioka, K., Yamamoto, K., Takeda, T., Hatanaka, M., *et al.* (1991). Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. *Science* 253, 1026-1028.

- Iwamoto, T., Okamoto, H., Kobayashi, S., Ikari, K., Toyama, Y., Tomatsu, T., Kamatani, N., & Momohara, S. (2007). A role of monocyte chemoattractant protein-4 (MCP-4)/CCL13 from chondrocytes in rheumatoid arthritis. *FEBS J* 274, 4904-4912.
- Iwamoto, T., Okamoto, H., Toyama, Y., & Momohara, S. (2008). Molecular aspects of rheumatoid arthritis: chemokines in the joints of patients. *FEBS J* 275, 4448-4455.
- Jacob, R.A., Perrett, D., Axon, J.M., Herbert, K.E., Scott, D.L. (1995) Rheumatoid synovial cell proliferation, transformation and fibronectin secretion in culture. *Clin Exp Rheumatol* 13:717-723.
- Jeong, J.G., Kim, J.M., Cho, H., Hahn, W., Yu, S.S., Kim, S. (2004) Effects of IL-1beta on gene expression in human rheumatoid synovial fibroblasts. *Biochem Biophys Res Commun* 324:3-7
- Jones, E.A., English, A., Henshaw, K., Kinsey, S.E., Markham, A.F., Emery, P., & McGonagle, D. (2004). Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. *Arthritis Rheum* 50, 817-827.
- Joseph, L., Lapid, S., & Sukhatme, V. (1987). The major ras induced protein in NIH3T3 cells is cathepsin L. *Nucleic Acids Res* 15, 3186.
- Kasperkovitz, P.V., Timmer, T.C., Smeets, T.J., Verbeet, N.L., Tak, P.P., van Baarsen, L.G., Baltus, B., Huizinga, T.W., Pieterman, E., Fero, M., *et al.* (2005). Fibroblast-like synoviocytes derived from patients with rheumatoid arthritis show the imprint of synovial tissue heterogeneity: evidence of a link between an increased myofibroblast-like phenotype and high-inflammation synovitis. *Arthritis Rheum* 52, 430-441.
- Kawane, K., Ohtani, M., Miwa, K., Kizawa, T., Kanbara, Y., Yoshioka, Y., Yoshikawa, H., & Nagata, S. (2006). Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. *Nature* 443, 998-1002.
- Kawane, K., Tanaka, H., Kitahara, Y., Shimaoka, S., & Nagata, S. (2010). Cytokine-dependent but acquired immunity-independent arthritis caused by DNA escaped from degradation. *Proc Natl Acad Sci U S A* 107, 19432-19437.
- Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., & Kollias, G. (1991). Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J* 10, 4025-4031.
- Kehlen, A., Thiele, K., Riemann, D., & Langner, J. (2002). Expression, modulation and signalling of IL-17 receptor in fibroblast-like synoviocytes of patients with rheumatoid arthritis. *Clin Exp Immunol* 127, 539-546.
- Keyszer, G.M., Heer, A.H., Kriegsmann, J., Geiler, T., Trabandt, A., Keyszer, M., Gay, R.E., & Gay, S. (1995). Comparative analysis of cathepsin L, cathepsin D, and collagenase messenger RNA expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis, by in situ hybridization. *Arthritis Rheum* 38, 976-984.
- Keyszer, G., Redlich, A., Haupl, T., Zacher, J., Sparmann, M., Engethum, U., Gay, S., & Burmester, G.R. (1998). Differential expression of cathepsins B and L compared with matrix metalloproteinases and their respective inhibitors in rheumatoid arthritis and osteoarthritis: a parallel investigation by semiquantitative reverse transcriptase-polymerase chain reaction and immunohistochemistry. *Arthritis Rheum* 41, 1378-1387.

- Kitagawa, A., Miura, Y., Saura, R., Mitani, M., Ishikawa, H., Hashiramoto, A., Yoshiya, S., Shiozawa, S., & Kurosaka, M. (2006). Anchorage on fibronectin via VLA-5 (alpha5beta1 integrin) protects rheumatoid synovial cells from Fas-induced apoptosis. *Ann Rheum Dis* 65, 721-727.
- Ko, L. J., & Prives, C. (1996). p53: puzzle and paradigm. *Genes Dev.* 10, 1054-1072.
- Koch, A.E., Kunkel, S.L., Burrows, J.C., Evanoff, H.L., Haines, G.K., Pope, R.M., & Strieter, R.M. (1991). Synovial tissue macrophage as a source of the chemotactic cytokine IL-8. *J Immunol* 147, 2187-2195.
- Koch, A.E., Polverini, P.J., Kunkel, S.L., Harlow, L.A., DiPietro, L.A., Elner, V.M., Elner, S.G., & Strieter, R.M. (1992). Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science* 258, 1798-1801.
- Koch, A.E., Kunkel, S.L., Harlow, L.A., Mazarakis, D.D., Haines, G.K., Burdick, M.D., Pope, R.M., & Strieter, R.M. (1994). Macrophage inflammatory protein-1 alpha. A novel chemotactic cytokine for macrophages in rheumatoid arthritis. *J Clin Invest* 93, 921-928.
- Koch, A.E., Kunkel, S.L., Shah, M.R., Hosaka, S., Halloran, M.M., Haines, G.K., Burdick, M.D., Pope, R.M., & Strieter, R.M. (1995). Growth-related gene product alpha. A chemotactic cytokine for neutrophils in rheumatoid arthritis. *J Immunol* 155, 3660-3666.
- Kojima, F., Naraba, H., Sasaki, Y., Beppu, M., Aoki, H., & Kawai, S. (2003). Prostaglandin E2 is an enhancer of interleukin-1beta-induced expression of membrane-associated prostaglandin E synthase in rheumatoid synovial fibroblasts. *Arthritis Rheum* 48, 2819-2828.
- Komori, J., Marusawa, H., Machimoto, T., Endo, Y., Kinoshita, K., Kou, T., Haga, H., Ikai, I., Uemoto, S., & Chiba, T. (2008). Activation-induced cytidine deaminase links bile duct inflammation to human cholangiocarcinoma. *Hepatology* 47, 888-896.
- Kontoyiannis, D., Pasparakis, M., Pizarro, T.T., Cominelli, F., & Kollias, G. (1999). Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity* 10, 387-398.
- Konttinen, Y.T., Ainola, M., Valleala, H., Ma, J., Ida, H., Mandelin, J., Kinne, R.W., Santavirta, S., Sorsa, T., Lopez-Otin, C., *et al.* (1999). Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. *Ann Rheum Dis* 58, 691-697.
- Korb, A., Pavenstadt, H., & Pap, T. (2009). Cell death in rheumatoid arthritis. *Apoptosis* 14, 447-454
- Korganow, A.S., Ji, H., Mangialaio, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, C., Kikutani, H., Rajewsky, K., Pasquali, J.L., *et al.* (1999). From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity* 10, 451-461.
- Kou, T., Marusawa, H., Kinoshita, K., Endo, Y., Okazaki, I.M., Ueda, Y., Kodama, Y., Haga, H., Ikai, I., & Chiba, T. (2007). Expression of activation-induced cytidine deaminase in human hepatocytes during hepatocarcinogenesis. *Int J Cancer* 120, 469-476.
- Kullmann, F., Judex, M., Neudecker, I., Lechner, S., Justen, H.P., Green, D.R., Wessinghage, D., Firestein, G.S., Gay, S., Scholmerich, J., *et al.* (1999). Analysis of the p53 tumor

- suppressor gene in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum* 42, 1594-1600.
- Kuwana, M., Okazaki, Y., Kodama, H., Izumi, K., Yasuoka, H., Ogawa, Y., Kawakami Y., & Ikeda, Y. (2003). Human circulating CD14+ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation. *Leukoc Biol* 74, 833-845.
- Lee, D.M., Kiener, H.P., Agarwal, S.K., Noss, E.H., Watts, G.F., Chisaka, O., Takeichi, M., Brenner, M.B. (2007). Cadherin-11 in synovial lining formation and pathology in arthritis. *Science* 315, 1006-1010.
- Lefevre, S., Knedla, A., Tennie, C., Kampmann, A., Wunrau, C., Dinser, R., Korb, A., Schnaker, E.M., Tarner, I.H., Robbins, P.D., *et al.* (2009). Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nat Med* 15, 1414-1420.
- Lemaire, R., Huet, G., Zerimech, F., Grard, G., Fontaine, C., Duquesnoy, B., & Flipo, R.M. (1997). Selective induction of the secretion of cathepsins B and L by cytokines in synovial fibroblast-like cells. *Br J Rheumatol* 36, 735-743.
- Li, X., Makarov, S. S. (2006). An essential role of NF-kappaB in the "tumor-like" phenotype of arthritic synoviocytes. *Proc Natl Acad Sci U S A* 103, 17432-17437.
- Lindberg, J., af Klint, E., Catrina, A. I., Nilsson, P., Klareskog, L., Ulfgren, A. K., & Lundeberg, J. (2006). Effect of infliximab on mRNA expression profiles in synovial tissue of rheumatoid arthritis patients. *Arthritis Res Ther* 8, R179.
- Lindy, O., Konttinen, Y.T., Sorsa, T., Ding, Y., Santavirta, S., Ceponis, A., & Lopez-Otin, C. (1997). Matrix metalloproteinase 13 (collagenase 3) in human rheumatoid synovium. *Arthritis Rheum* 40, 1391-1399.
- Liu, C.Y., Batliwalla, F., Li, W.T., Lee, A., Roubenoff, R., Beckman, E., Khalili, H., Damle, A., Kern, M., Furie, R., *et al.* (2008). Genome-wide association scan identifies candidate polymorphisms associated with differential response to anti-TNF treatment in rheumatoid arthritis. *Molecular Medicine* 14, 575-581.
- Maeda, S., Sawai, T., Uzuki, M., Takahashi, Y., Omoto, H., Seki, M., & Sakurai, M. (1995). Determination of interstitial collagenase (MMP-1) in patients with rheumatoid arthritis. *Ann Rheum Dis* 54, 970-975.
- Manicourt, D.H., Fujimoto, N., Obata, K., & Thonar, E.J. (1995). Levels of circulating collagenase, stromelysin-1, and tissue inhibitor of matrix metalloproteinases 1 in patients with rheumatoid arthritis. Relationship to serum levels of antigenic keratan sulfate and systemic parameters of inflammation. *Arthritis Rheum* 38, 1031-1039.
- Martin, J.E., Alizadeh, B.Z., Gonzalez-Gay, M.A., Balsa, A., Pascual-Salcedo, D., Fernandez-Gutierrez, B., Raya, E., Franke, L., van't Slot, R., Coenen, M.J.H., *et al.* (2010). Identification of the Oxidative Stress-Related Gene MSRA as a Rheumatoid Arthritis Susceptibility Locus by Genome-Wide Pathway Analysis. *Arthritis and Rheumatism* 62, 3183-3190.
- Min, D.J., Cho, M.L., Lee, S.H., Min, S.Y., Kim, W.U., Min, J.K., Park, S.H., Cho, C.S., & Kim, H.Y. (2004). Augmented production of chemokines by the interaction of type II collagen-reactive T cells with rheumatoid synovial fibroblasts. *Arthritis Rheum* 50, 1146-1155.
- Miranda-Carus, M.E., Balsa, A., Benito-Miguel, M., Perez de Ayala, C., & Martin-Mola, E. (2004). IL-15 and the initiation of cell contact-dependent synovial fibroblast-T

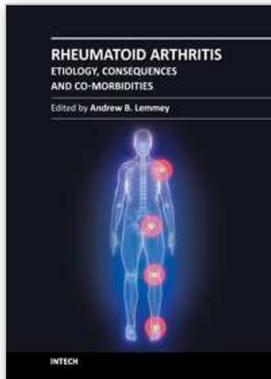
- lymphocyte cross-talk in rheumatoid arthritis: effect of methotrexate. *J Immunol* 173, 1463-1476.
- Mor, A., Abramson, S.B., & Pillinger, M.H. (2005). The fibroblast-like synovial cell in rheumatoid arthritis: a key player in inflammation and joint destruction. *Clin Immunol* 115, 118-128.
- Morisawa, T., Marusawa, H., Ueda, Y., Iwai, A., Okazaki, I.M., Honjo, T., & Chiba, T. (2008). Organ-specific profiles of genetic changes in cancers caused by activation-induced cytidine deaminase expression. *Int J Cancer* 123, 2735-2740.
- Muller-Ladner, U., Gay, R.E., & Gay, S. (2000). Activation of synoviocytes. *Curr Opin Rheumatol* 12, 186-194.
- Muller-Ladner, U., Kriegsmann, J., Franklin, B.N., Matsumoto, S., Geiler, T., Gay, R.E., & Gay, S. (1996). Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human cartilage when engrafted into SCID mice. *Am J Pathol* 149, 1607-1615.
- Murakami, M., Okuyama, Y., Ogura, H., Asano, S., Arima, Y., Tsuruoka, M., Harada, M., Kanamoto, M., Sawa, Y., Iwakura, Y., *et al.* (2011). Local microbleeding facilitates IL-6- and IL-17-dependent arthritis in the absence of tissue antigen recognition by activated T cells. *J Exp Med* 208, 103-114.
- Nanki, T., Hayashida, K., El-Gabalawy, H.S., Suson, S., Shi, K., Girschick, H.J., Yavuz, S., & Lipsky, P.E. (2000). Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. *J Immunol* 165, 6590-6598.
- Niki, Y., Yamada, H., Seki, S., Kikuchi, T., Takaishi, H., Toyama, Y., Fujikawa, K., & Tada, N. (2001). Macrophage- and neutrophil-dominant arthritis in human IL-1 alpha transgenic mice. *J Clin Invest* 107, 1127-1135.
- Nishimoto, N., & Kishimoto, T. (2006). Interleukin 6: from bench to bedside. *Nat Clin Pract Rheumatol* 2, 619-626.
- Ogura, H., Murakami, M., Okuyama, Y., Tsuruoka, M., Kitabayashi, C., Kanamoto, M., Nishihara, M., Iwakura, Y., & Hirano, T. (2008). Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity* 29, 628-636.
- Ohshima, S., Mima, T., Sasai, M., Nishioka, K., Shimizu, M., Murata, N., Yoshikawa, H., Nakanishi, K., Suemura, M., McCloskey, R.V., *et al.* (2000). Tumour necrosis factor alpha (TNF-alpha) interferes with Fas-mediated apoptotic cell death on rheumatoid arthritis (RA) synovial cells: a possible mechanism of rheumatoid synovial hyperplasia and a clinical benefit of anti-TNF-alpha therapy for RA. *Cytokine* 12, 281-288.
- Ohtani, T., Ishihara, K., Atsumi, T., Nishida, K., Kaneko, Y., Miyata, T., Itoh, S., Narimatsu, M., Maeda, H., Fukada, T., *et al.* (2000). Dissection of signaling cascades through gp130 in vivo: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. *Immunity* 12, 95-105.
- Okada, Y. (2009). 7. In: *Kelly's Textbook of Rheumatology*, Firestein, G.S, Budd, R.C., Harris, Jr. E.D., McInnes, I.B., Ruddy, S., Sergent, J.S., 115-134, SAUNDERS ELSEVIER, 978-1-4160-3285-4, Philadelphia.
- Orozco, G., Hinks, A., Eyre, S., Ke, X., Gibbons, L.J., Bowes, J., Flynn, E., Martin, P., Wilson, A.G., Bax, D.E., Morgan, A.W., Emery, P., Steer, S., Hocking, L., Reid, D.M., Wordsworth, P., Harrison, P., Thomson, W., Barton, A., Worthington, J. (2009).

- Combined effects of three independent SNPs greatly increase the risk estimate for RA at 6q23. *Hum Mol Genet* 18, 2693-2699.
- Pap, T., Shigeyama, Y., Kuchen, S., Fernihough, J.K., Simmen, B., Gay, R.E., Billingham, M., Gay, S. (2000a). Differential expression pattern of membrane-type matrix metalloproteinases in rheumatoid arthritis. *Arthritis Rheum* 43, 1226-1232.
- Pap, T., Franz, J.K., Hummel, K.M., Jeisy, E., Gay, R., & Gay, S. (2000b). Activation of synovial fibroblasts in rheumatoid arthritis: lack of Expression of the tumour suppressor PTEN at sites of invasive growth and destruction. *Arthritis Res* 2, 59-64.
- Pap, T., Nawrath, M., Heinrich, J., Bosse, M., Baier, A., Hummel, K.M., Petrow, P., Kuchen, S., Michel, B.A., Gay, R.E., *et al.* (2004). Cooperation of Ras- and c-Myc-dependent pathways in regulating the growth and invasiveness of synovial fibroblasts in rheumatoid arthritis. *Arthritis Rheum* 50, 2794-2802.
- Pap, T., & Gay, S. (2009). 11, In: *Kelly's Textbook of Rheumatology*, Firestein, G.S, Budd, R.C., Harris, Jr. E.D., McInnes, I.B., Ruddy, S., Sergent, J.S., 201-214, SAUNDERS ELSEVIER, 978-1-4160-3285-4, Philadelphia.
- Pauklin, S., Sernandez, I.V., Bachmann, G., Ramiro, A.R., & Petersen-Mahrt, S.K. (2009). Estrogen directly activates AID transcription and function. *J Exp Med* 206, 99-111.
- Pierer, M., Muller-Ladner, U., Pap, T., Neidhart, M., Gay, R.E., & Gay, S. (2003). The SCID mouse model: novel therapeutic targets - lessons from gene transfer. *Springer Semin Immunopathol* 25, 65-78.
- Plant, D., Bowes, J., Potter, C., Hyrich, K.L., Morgan, A.W., Wilson, A.G., Isaacs, J.D., Wellcome Trust Case Control Consortium; British Soc Rheumatology Biologics Register, & Barton, A. (2011). Genome-Wide Association Study of Genetic Predictors of Anti-Tumor Necrosis Factor Treatment Efficacy in Rheumatoid Arthritis Identifies Associations With Polymorphisms at Seven Loci. *Arthritis and Rheumatism* 63, 645-653.
- Pohlars, D., Beyer, A., Koczan, D., Wilhelm, T., Thiesen, H.J., & Kinne, R.W. (2007). Constitutive upregulation of the transforming growth factor- β pathway in rheumatoid arthritis synovial fibroblasts. *Arthritis Res Ther* 9, R59.
- Pritchard, J., Tsui, S., Horst, N., Cruikshank, W.W., & Smith, T.J. (2004). Synovial fibroblasts from patients with rheumatoid arthritis, like fibroblasts from Graves' disease, express high levels of IL-16 when treated with Igs against insulin-like growth factor-1 receptor. *J Immunol* 173, 3564-3569.
- Rathanaswami, P., Hachicha, M., Sadick, M., Schall, T.J., & McColl, S.R. (1993). Expression of the cytokine RANTES in human rheumatoid synovial fibroblasts. Differential regulation of RANTES and interleukin-8 genes by inflammatory cytokines. *J Biol Chem* 268, 5834-5839.
- Reme, T., Travaglio, A., Gueydon, E., Adla, L., Jorgensen, C., & Sany, J. (1998). Mutations of the p53 tumour suppressor gene in erosive rheumatoid synovial tissue. *Clin Exp Immunol* 111, 353-358.
- Robinson, E., Keystone, E.C., Schall, T.J., Gillett, N., & Fish, E.N. (1995). Chemokine expression in rheumatoid arthritis (RA): evidence of RANTES and macrophage inflammatory protein (MIP)-1 beta production by synovial T cells. *Clin Exp Immunol* 101, 398-407.

- Roivainen, A., Pirila, L., Yli-Jama, T., Laaksonen, H., & Toivanen, P. (1999). Expression of the myc-family proto-oncogenes and related genes *max* and *mad* in synovial tissue. *Scand J Rheumatol* 28, 314-318.
- Sakaguchi, N., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., Sakihama, T., Matsutani, T., Negishi, I., Nakatsuru, S., *et al.* (2003). Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature* 426, 454-460.
- Salcedo, R., Wasserman, K., Young, H.A., Grimm, M.C., Howard, O.M., Anver, M.R., Kleinman, H.K., Murphy, W.J., & Oppenheim, J.J. (1999). Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: In vivo neovascularization induced by stromal-derived factor-1 α . *Am J Pathol* 154, 1125-1135.
- Salcedo, R., Ponce, M.L., Young, H.A., Wasserman, K., Ward, J.M., Kleinman, H.K., Oppenheim, J.J., & Murphy, W.J. (2000). Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. *Blood* 96, 34-40.
- Sawa, S., Kamimura, D., Jin, G.H., Morikawa, H., Kamon, H., Nishihara, M., Ishihara, K., Murakami, M., & Hirano, T. (2006). Autoimmune arthritis associated with mutated interleukin (IL)-6 receptor gp130 is driven by STAT3/IL-7-dependent homeostatic proliferation of CD4+ T cells. *J Exp Med* 203, 1459-1470.
- Scott, B.B., Zarin, P.F., Colombo, A., Hansbury, M.J., Winkler, J.D., & Jackson, J.R. (2002). Constitutive expression of angiopoietin-1 and -2 and modulation of their expression by inflammatory cytokines in rheumatoid arthritis synovial fibroblasts. *J Rheumatol* 29, 230-239.
- Szekanecz, Z., & Koch, A.E. (2007). Mechanisms of Disease: angiogenesis in inflammatory diseases. *Nat Clin Pract Rheumatol* 3, 635-643.
- Sorsa, T., Kontinen, Y.T., Lindy, O., Ritchlin, C., Saari, H., Suomalainen, K., Eklund, K.K., & Santavirta, S. (1992). Collagenase in synovitis of rheumatoid arthritis. *Semin Arthritis Rheum* 22, 44-53.
- Stahl, E.A., Raychaudhuri, S., Remmers, E.F., Xie, G., Eyre, S., Thomson, B.P., Li, Y.H., Kurreeman, F.A.S., Zhernakova, A., Hinks, A., *et al.* (2010). Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nature Genetics* 42, 508-U556.
- Sugino, H., Lee, H.M., & Nishimoto, N. (2010). DNA microarray analysis of rheumatoid arthritis susceptibility genes identified by genome-wide association studies. *Arthritis Res Ther* 12, 401.
- Sun, Y., Zeng, X.R., Wenger, L., Firestein, G.S., & Cheung, H.S. (2004). P53 down-regulates matrix metalloproteinase-1 by targeting the communications between AP-1 and the basal transcription complex. *J Cell Biochem* 92, 258-269.
- Suzuki A, Yamada R, Chang X, Tokuhira S, Sawada T, Suzuki M, Nagasaki M, Nakayama-Hamada M, Kawaida R, Ono M, *et al.* (2003). Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 34, 395-402.
- Takai, A., Toyoshima, T., Uemura, M., Kitawaki, Y., Marusawa, H., Hiai, H., Yamada, S., Okazaki, I.M., Honjo, T., Chiba, T., *et al.* (2009). A novel mouse model of

- hepatocarcinogenesis triggered by AID causing deleterious p53 mutations. *Oncogene* 28, 469-478.
- Tamiya, G., Shinya, M., Imanishi, T., Ikuta, T., Makino, S., Okamoto, K., Furugaki, K., Matsumoto, T., Mano, S., Ando, S., *et al.* (2005). Whole genome association study of rheumatoid arthritis using 27 039 microsatellites. *Human Molecular Genetics* 14, 2305-2321.
- Taylor, D.J., Cheung, N.T., & Dawes, P.T. (1994). Increased serum proMMP-3 in inflammatory arthritides: a potential indicator of synovial inflammatory monokine activity. *Ann Rheum Dis* 53, 768-772.
- Tetlow, L.C., Lees, M., Ogata, Y., Nagase, H., and Woolley, D.E. (1993). Differential expression of gelatinase B (MMP-9) and stromelysin-1 (MMP-3) by rheumatoid synovial cells in vitro and in vivo. *Rheumatol Int* 13, 53-59.
- Thomas, J.W., Thieu, T.H., Byrd, V.M., & Miller, G.G. (2000). Acidic fibroblast growth factor in synovial cells. *Arthritis Rheum* 43, 2152-2159.
- Tolboom, T.C., Pieterman, E., van der Laan, W.H., Toes, R., Huidekoper, A., Nelissen, R., Breedveld, F., & Huizinga, T. (2002). Invasive properties of fibroblast-like synoviocytes: correlation with growth characteristics and expression of MMP-1, MMP-3, and MMP-10. *Ann Rheum Dis* 61:975-980.
- van Baarsen, L.G., Wijbrandts, C.A., Timmer, T.C., van der Pouw Kraan, T.C., Tak, P.P., & Verweij, C.L. (2010). Synovial tissue heterogeneity in rheumatoid arthritis in relation to disease activity and biomarkers in peripheral blood. *Arthritis Rheum* 62, 1602-1607.
- van der Pouw Kraan, T.C., van Gaalen, F.A., Kasperkovitz, P.V., Verbeet, N.L., Smeets, T.J., Kraan, M.C., Fero, M., Tak, P.P., Huizinga, T.W., Pieterman, E., Breedveld, F.C., Alizadeh, A.A., & Verweij, C.L. (2003). Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the STAT-1 pathway between rheumatoid tissues. *Arthritis Rheum* 48, 2132-2145.
- Vereecke, L., Beyaert, R., & van Loo, G. (2009). The ubiquitin-editing enzyme A20 (TNFAIP3) is a central regulator of immunopathology. *Trend Immunol* 30, 383-391.
- Volin, M.V., Woods, J.M., Amin, M.A., Connors, M.A., Harlow, L.A., & Koch, A.E. (2001). Fractalkine: a novel angiogenic chemokine in rheumatoid arthritis. *Am J Pathol* 159, 1521-1530.
- Wakisaka, S., Suzuki, N., Takeba, Y., Shimoyama, Y., Nagafuchi, H., Takeno, M., Saito, N., Yokoe, T., Kaneko, A., Asai, T., *et al.* (1998). Modulation by proinflammatory cytokines of Fas/Fas ligand-mediated apoptotic cell death of synovial cells in patients with rheumatoid arthritis (RA). *Clin Exp Immunol* 114, 119-128.
- Wang, C.R., Liu, M.F., Huang, Y.H., & Chen, H.C. (2004). Up-regulation of XCR1 expression in rheumatoid joints. *Rheumatology* 43, 569-573.
- Westhoff, C.S., Freudiger, D., Petrow, P., Seyfert, C., Zacher, J., Kriegsmann, J., Pap, T., Gay, S., Stiehl, P., Gromnica-Ihle, E., *et al.* (1999). Characterization of collagenase 3 (matrix metalloproteinase 13) messenger RNA expression in the synovial membrane and synovial fibroblasts of patients with rheumatoid arthritis. *Arthritis Rheum* 42, 1517-1527.
- Weyand, C.M., Hicok, K.C., Conn, D.L., Goronzy, J.J. (1992). The influence of HLA-DRB1 genes on disease severity in rheumatoid arthritis. *Ann Intern Med* 117,801-6.

- Wijbrandts, C.A., Dijkgraaf, M.G., Kraan, M.C., Vinkenoog, M., Smeets, T.J., Dinant, H., Vos, K., Lems, W.F., Wolbink, G.J., *et al.* (2008). The clinical response to infliximab in rheumatoid arthritis is in part dependent on pretreatment tumour necrosis factor alpha expression in the synovium. *Ann Rheum Dis* 67, 1139-1144.
- Yamanishi, Y., Boyle, D.L., Rosengren, S., Green, D.R., Zvaifler, N.J., & Firestein, G.S. (2002). Regional analysis of p53 mutations in rheumatoid arthritis synovium. *Proc Natl Acad Sci U S A* 99, 10025-10030.
- Yamanishi, Y., Boyle, D.L., Green, D.R., Keystone, E.C., Connor, A., Zollman, S., & Firestein, G.S. (2005). p53 tumor suppressor gene mutations in fibroblast-like synoviocytes from erosion synovium and non-erosion synovium in rheumatoid arthritis. *Arthritis Res Ther* 7, R12-18.
- Yoshihara, Y., Obata, K., Fujimoto, N., Yamashita, K., Hayakawa, T., & Shimmei, M. (1995). Increased levels of stromelysin-1 and tissue inhibitor of metalloproteinases-1 in sera from patients with rheumatoid arthritis. *Arthritis Rheum* 38, 969-975.
- Zhernakova, A., Stahl, E.A., Trynka, G., Raychaudhuri, S., Festen, E.A., Franke, L., Westra, H.J., Fehrmann, R.S.N., Kurreeman, F.A.S., Thomson, B., *et al.* (2011). Meta-Analysis of Genome-Wide Association Studies in Celiac Disease and Rheumatoid Arthritis Identifies Fourteen Non-HLA Shared Loci. *Plos Genetics* 7.
- Zimmermann, T., Kunisch, E., Pfeiffer, R., Hirth, A., Stahl, H. D., Sack, U., Laube, A., Liesaus, E., Roth, A., Palombo-Kinne, E., Emmrich, F., & Kinne, R. W. (2001). Isolation and characterization of rheumatoid arthritis synovial fibroblasts from primary culture--primary culture cells markedly differ from fourth-passage cells. *Arthritis Res* 3, 72-76.
- Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Alfonso, Z.C., Fraser, J.K., Benhaim, P., & Hedrick, M.H. (2002). Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 13, 4279-4295.
- Zvaifler, N. J., Marinova-Mutafchieva, L., Adams, G., Edwards, C. J., Moss, J., Burger, J. A., & Maini, R. N. (2000). Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res* 2, 477-488.



Rheumatoid Arthritis - Etiology, Consequences and Co-Morbidities

Edited by Dr. Andrew Lemmey

ISBN 978-953-307-847-2

Hard cover, 304 pages

Publisher InTech

Published online 11, January, 2012

Published in print edition January, 2012

The purpose of this book is to provide up-to-date, interesting, and thought-provoking perspectives on various aspects of research into current and potential treatments for rheumatoid arthritis (RA). This book features 16 chapters, with contributions from numerous countries (e.g. UK, USA, Japan, Sweden, Spain, Ireland, Poland, Norway), including chapters from internationally recognized leaders in rheumatology research. It is anticipated that Rheumatoid Arthritis - Etiology, Consequences and Co-Morbidities will provide both a useful reference and source of potential areas of investigation for research scientists working in the field of RA and other inflammatory arthropathies.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Katsuhiko Ishihara and Hideya Igarashi (2012). Molecular Mechanisms of Rheumatoid Arthritis Revealed by Categorizing Subtypes of Fibroblast-Like Synoviocytes, Rheumatoid Arthritis - Etiology, Consequences and Co-Morbidities, Dr. Andrew Lemmey (Ed.), ISBN: 978-953-307-847-2, InTech, Available from: <http://www.intechopen.com/books/rheumatoid-arthritis-etiology-consequences-and-co-morbidities/molecular-mechanisms-of-rheumatoid-arthritis-revealed-by-categorizing-subtypes-of-fibroblast-like-sy>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.