Commentary

Gene therapy in rheumatoid arthritis: how to target joint destruction?

Thomas Pap, Ulf Müller-Ladner, Renate Gay and Steffen Gay

Department of Rheumatology, University Hospital, Zurich, Switzerland

Received: 7 September 1999
Revisions requested: 20 September 1999
Revisions received: 23 September 1999
Accepted: 23 September 1999
Published: 26 October 1999

Important note about how to cite this article
This article is also available online in the *Arthritis Research* website. To avoid confusion, please ensure that only the online version of the article is cited in any reference, as follows:
Pap T, Müller-Ladner U, Gay R, Gay S: Gene therapy in rheumatoid arthritis: how to target joint destruction? [commentary]. http://arthritis-research.com/26oct99/ar0101c02

Rheumatoid arthritis is a chronic, inflammatory disorder that affects primarily the joints and causes significant disability to affected individuals. Although the recent years have brought exciting new information to researchers and clinicians alike, the treatment of the disease still constitutes a major challenge. Most patients require a combination of different medications, including nonsteroidal anti-inflammatory drugs, early and aggressive application of disease modifying anti-rheumatics, and steroids. Using such regimens, slowing of disease progression can be observed in some patients, but frequently the treatment fails to achieve satisfactory results. Specifically, the progressive destruction of articular cartilage and bone is barely influenced at all by most of the approaches that we use routinely today. This is of particular concern because the joint destruction not only constitutes a most prominent feature of disease, but also determines its outcome in the majority of patients.

Therefore, the development of new 'biological' agents that interfere specifically with cytokine-mediated inflammatory pathways and activation of synovial cells has been followed as a novel therapeutic approach with great interest. Indeed, clinical studies that used monoclonal antibodies against tumour necrosis factor (TNF)-α, as well as different TNF receptor fusion proteins, have provided convincing results [1,2]. It was shown that the administration of such proteins effectively influences the clinical features of disease. Growing experience in using these agents has also unveiled some limitations, however. Not only have there been concerns about a potential immunogeneity and the development of autoantibodies, but also, and more importantly, the systemic inhibition of TNF-α over a long time may bear some as yet unknown risks.

In the search for alternatives, gene transfer has become an interesting novel approach for interfering with key processes in rheumatoid arthritis. Although gene transfer was developed originally to treat inherited diseases by correcting the underlying genetic abnormality, it has been understood that gene transfer also provides new opportunities for treating acquired disorders. In the field of rheumatoid arthritis, Evans has been the pioneer. Together with his colleagues, he was the first to demonstrate that gene transfer to synovial cells is a feasible tool for altering the course of disease in animal models of arthritis. His review article in this issue of *Arthritis Research* summarizes this experience [3]. Clearly, delivering the genes that encode therapeutic proteins rather than administering these proteins directly appears attractive for several reasons. In particular, gene transfer has been suggested to overcome major disadvantages of conventional pharmacology, and has been associated with a selective and highly specific targeting of disease mechanisms at the molecular level. Not surprisingly, expectations for gene therapy approaches have been high among both scientists and clinicians. Visible progress has been made mainly on the experimental, rather than clinical side so far. Although researchers have been using gene transfer widely to study the molecular mechanisms of disease and have made it a standard tool for investigating functional relationships in cells of the rheumatoid synovium, the therapeutic impact that can be expected from gene therapy is still a matter of debate. Specifically, the question of what molecules or pathways should be targeted to inhibit the joint destruction in rheumatoid arthritis has been controversial.

It is well established that inflammation constitutes a major feature of rheumatoid arthritis, and inflammatory

IL-1Ra = interleukin-1 receptor antagonist; MMP = matrix metalloproteinase; NF-κB = nuclear factor-κB; RA-SF = rheumatoid arthritis synovial fibroblast; SCID = severe combined immunodeficiency; TNF = tumour necrosis factor; VCAM = vascular cell adhesion molecule; VLA = very late antigen.

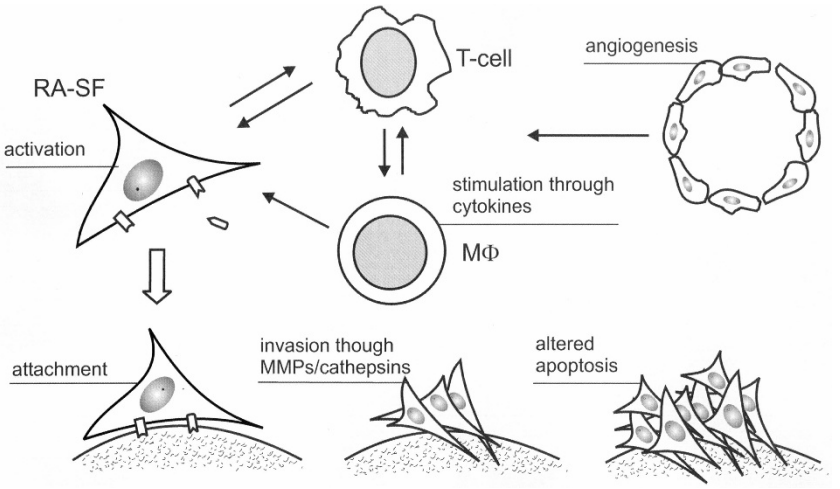
cytokines such as interleukin-1 and TNF-α are produced at high levels in the rheumatoid synovium. By activating receptor-induced intracellular signalling pathways, these cytokines trigger several disease-related pathways and can induce mediators of disease such as matrix metalloproteinases (MMPs) [4]. This understanding, together with the promising data from novel anticytokine therapies, have made proinflammatory cytokines a major target also for gene therapy [1,2]. As outlined by Evans *et al* in this issue of *Arthritis Research* [3], there has been considerable progress in delivering antagonists of proinflammatory cytokines, as well as of anti-inflammatory mediators, to the synovium of different animals, and it has been shown that targeting inflammatory pathways substantially reduces the severity of antigen-induced arthritis. In addition, it was demonstrated in the severe combined immunodeficiency (SCID) mouse model of rheumatoid arthritis that overexpression of the interleukin-1 receptor antagonist (IL-1Ra) in human rheumatoid arthritis synovial fibroblasts (RA-SFs) has chondroprotective effects [5]. In that study, RA-SFs were transduced with the gene for IL-1Ra or a control (*LacZ*) gene and coimplanted with normal human cartilage under the renal capsule of SCID mice. Because SCID mice lack a functional immune system, they do not reject the implants and permit study of the invasive behaviour of genetically modified and control RA-SFs. Interestingly, overexpression of the IL-1Ra in RA-SFs, did not reduce significantly the invasiveness of these cells into the cartilage. Instead, the chondroprotective effect was due to an inhibition of the perichondrocytic matrix degradation. Conversely, gene transfer of the interleukin-10 gene significantly reduced the invasion of RA-SFs into the coimplanted cartilage, but had little effect on the perichondrocytic degradation [6]. It has to be noted, however, that in spite of their indisputable value for research, animal models do not reflect the complete reality of human rheumatoid arthritis. This is demonstrated by recent findings indicating that the combination treatment with anti-TNF-α antibodies and methotrexate clearly retard the progression of radiological changes in rheumatoid arthritis (Maini RN, personal communication).

Inhibition of a single proinflammatory cytokine may not be sufficient to inhibit inflammatory pathways in rheumatoid arthritis in the long run. Rather, it appears necessary that the genes for two or even more antagonists of proinflammatory cytokines need to be delivered at the same time using appropriate vectors and expression cassettes. Notably, the simultaneous inhibition of several proinflammatory cytokines may constitute an advantage of gene transfer as compared with the delivery of the individual recombinant proteins. Based on this understanding, Evans *et al* [7] initiated the first clinical trial of gene transfer for human rheumatoid arthritis. In that study, *ex vivo* gene transfer with a retroviral vector carrying the IL-1Ra gene was used to transduce synovial fibroblasts from nine patients before a scheduled joint replacement. It needs to be stressed that this trial was not designed to assess the clinical efficacy of gene transfer with the IL-1Ra gene, but to demonstrate the feasibility of this approach.

When addressing rheumatoid joint destruction, however, we have to remember that inflammation reflects only one aspect of the pathogenesis of rheumatoid arthritis. Not only have clinical data provided evidence that the pathological mechanisms of inflammation and articular damage may differ [8], but experiments in the SCID mouse model of rheumatoid arthritis, as well as *in vitro* data, have demonstrated that the aggressive potential of RA-SFs is maintained in the absence of inflammatory cells [9]. It has been established by numerous studies that the activation of synovial fibroblasts in the lining layer of the rheumatoid synovium plays a pivotal role in the progressive degradation of cartilage. When compared with normal synovial fibroblasts, these activated RA-SFs exhibit a different morphology and significant alterations in their behaviour. Molecular changes in RA-SFs comprise the upregulation of several proto-oncogenes as well as the downregulation and functional inactivation of potentially protective (tumour suppressor) genes. Firestein *et al* [10] have demonstrated convincingly that somatic mutations in the p53 tumour suppressor occur in a number of patients with rheumatoid arthritis, with the predicted amino acid substitutions being similar to those observed in tumors. Although such mutations appear not to be present in some other populations of rheumatoid arthritis patients [11], it has been established that nonfunctional p53 may account for altered signalling in RA-SFs. As reported in the next issue of *Arthritis Research* [12], aggressive RA-SFs also lack the expression of the tumour suppressor *PTEN*, which exhibits tyrosine phosphatase activity as well as homology to the cytoskeletal proteins tensin and auxilin [13]. Interestingly, mutations in *PTEN* resulting in a nonfunctional molecule have also been described in several human cancers and are associated with the invasive and metastatic properties of malignancies [14].

It is now established that the activation of synovial fibroblasts in the rheumatoid arthritis synovium alters the regulation of various genes and ultimately results in the progressive destruction of cartilage in rheumatoid arthritis. In this context, three distinct processes have been shown to contribute to the aggressive behaviour of RA-SF: upregulation of adhesion molecules that mediate the attachment of RA-SFs to cartilage; expression of matrix degrading enzymes such as MMPs and cathepsins; and prolonged survival of RA-SF because of inhibited apoptotic pathways. These changes are highly complex, and one has to be aware that our understanding of signalling pathways in RA-SFs, and particularly the role of individual molecules, is still rather fragmented. Despite major advances in our understanding of specific pathways, it has

Figure 1



Targeting joint destruction in rheumatoid arthritis requires the inhibition of several pathways. Apart from interrupting the effect of macrophage-derived proinflammatory cytokines that stimulate the rheumatoid arthritis synovial fibroblasts (RA-SFs), the specific properties of these cells need to be targeted. This comprises the inhibition of cellular activation of RA-SFs, their attachment to the cartilage, the invasion into the cartilage through the release of matrix-degrading enzymes, altered apoptosis and angiogenesis.

become evident that activation of synovial cells in rheumatoid arthritis involves the whole complexity of cellular signalling, and not just two or three paths. Therefore, targeting rheumatoid joint destruction requires several distinct approaches to be followed (Fig. 1). Apart from interrupting the effect of macrophage-derived proinflammatory cytokines that stimulate the RA-SF, the specific properties of these cells need to be targeted.

Among the different molecules that have been implicated in the activation of RA-SF, upregulation of the transcription factors AP-1 and NF-κB have become of particular interest. The AP-1 transcription factor consists of the c-Fos and c-Jun elements, and appears to constitute a key element in the signalling pathways that ultimately lead to the degradation of cartilage and bone in rheumatoid arthritis. Not only are c-Fos and c-Jun overexpressed in the rheumatoid arthritis synovium [15–17], but the promoters of several MMP genes contain consensus binding sites for the transcription factor AP-1. In addition, AP-1 sites have been shown to be involved in tissue-specific expression of MMPs [18]. Therefore, interfering with pathways that upregulate the AP-1 complex may inhibit the rheumatoid joint destruction [19]. In this context, the delivery and overexpression of genes that encode nonfunctional signalling molecules (dominant-negative mutants) not only permits investigation of the specific role of such molecules, but also permits assessment of the therapeutic potential of their inhibition.

Using this technique, we recently investigated the effect of retroviral gene transfer with dominant negative mutants of Raf-1 on the invasiveness of RA-SFs, as well as on the expression of AP-1-related proteins and MMPs [20]. It was demonstrated, in RA-SFs, that Raf-1 is involved in signalling cascades that upregulate disease-relevant MMPs though the activation of c-Jun. Despite an effect on the expression of c-Jun, as well as on the expression of MMP-1 and MMP-13, however, invasiveness of RA-SFs was not inhibited completely in the SCID mouse model. Also, no complete inhibition of the MMP production was observed. These data demonstrate the complexity of signalling pathways in RA-SFs and suggest that Raf-independent pathways are involved critically in the activation of these cells. Moreover, these data confirm other observations suggesting that AP-1 sites do not regulate the transcription of MMPs alone. Rather, there are essential interactions with other *cis*-acting sequences in the promoters and with transcription factors that bind to these sequences [18].

The transcription factor nuclear factor-κB (NF-κB) is also highly activated in the synovial membrane of rheumatoid arthritis patients, particularly in RA-SFs [21]. NF-κB can be induced through proinflammatory cytokines, but, conversely, NF-κB activation induces the transcription of such cytokines, as well as of adhesion molecules. Therefore, the activation of NF-κB in synovial cells appears to be an important factor in the perpetuation of rheumatoid arthritis [22] and to play a major role in synovial inflammation. A study by Makarov *et al* [23] most recently demonstrated that inhibition of NF-κB through gene transfer with IκB may constitute a feasible tool for inhibiting the inflammatory response.

Interfering with the attachment of RA-SFs to the cartilage constitutes another major target for gene transfer approaches to alter the specific pathology of RA-SFs. Integrins have been implicated most frequently in the attachment of RA-SFs to cartilage, and several studies have demonstrated the expression of β_1-integrins such as very late antigen (VLA)-3, VLA-4 and VLA-5 on synovial

fibroblasts [24]. In addition, anti-β_1 integrin antibodies inhibit, at least in part, the binding of synovial fibroblasts to extracellular matrix [25]. However, integrins have become of interest for rheumatoid arthritis not only because of their function as receptor molecules, but also because of their interaction with several signalling pathways and cellular proto-oncogenes [26]. Specifically, the expression of early cell cycle genes such as c-*fos* and c-*myc* is also stimulated by integrin-mediated cell adhesion, and gene expression driven by the *fos* promoter shows strong synergistic activation by integrin-mediated adhesion [27,28]. Therefore, inhibiting the expression of integrins directly in RA-SFs through gene transfer appears much more promising than the delivery of antibodies to block integrins on the cell surface.

Apart from the integrins, vascular cell adhesion molecule (VCAM)-1 has been characterized as an important adhesion molecule associated with the activated phenotype of RA-SF. Several studies [29–31] have revealed high expression of VCAM-1 in RA-SFs, particularly in the lining layer. Although the specific role of VCAM-1 in the attachment of these cells to the cartilage needs to be established, a recent study [32] demonstrated that soluble VCAM-1 exhibits chemotactic activity to T cells that express high affinity VLA-4. Interestingly, the chemotactic activity of soluble VCAM-1 is inhibited in the presence of anti-VCAM-1 and anti-VLA-4 [32], suggesting that VCAM-1 produced by RA-SFs contributes to the development of the synovial inflammation by attracting T cells to the joint. As demonstrated by other studies VCAM-1 may also contribute to T-cell anergy [33] and the induction of angiogenesis [34]. From these studies it can be anticipated that inhibiting the expression of VCAM-1 in RA-SFs may lead to several beneficial effects in rheumatoid arthritis. Angiogenesis, a very early feature of rheumatoid arthritis [35], appears to be a target for gene transfer by itself, however. By inhibiting angiogenesis, the influx of inflammatory cells to the joint might be reduced significantly [34].

Another promising approach that is aimed at modulating the terminal phase of MMP and cathepsin upregulation by cleaving the messenger RNA for these enzymes. This can be achieved by the delivery and expression of ribozymes to RA-SFs. Ribozymes are RNA molecules that are able to cleave RNA in a site-specific manner. Such ribozymes can be used to destroy messages inside cells. RA-SFs in which ribozymes capable of cleaving collagenase are expressed will therefore produce no or only limited amounts of this enzyme. The usefulness of this mode to reduce the invasiveness of RA-SFs is currently being evaluated [36].

Recent data have also provided evidence for changes of apoptotic pathways in the rheumatoid arthritis synovium. Specifically, it has been demonstrated that apoptosis is a rare event among RA-SFs. This applies predominantly to the lining layer, and the changes appear closely linked to the activated phenotype of RA-SF. As for possible mechanisms that may extend the life-span of lining layer RA-SFs, we have demonstrated most recently [37] that the novel antiapoptotic molecule sentrin is strongly expressed in rheumatoid arthritis synovium. Sentrin has been shown to interact with the signal competent forms of Fas/Apo-1 and TNF receptor-1, and thereby to protect cells against anti-Fas and TNF-induced cell death. Interestingly, sentrin is found mainly in rheumatoid arthritis synovial lining cells, whereas normal synovium cells express only very little sentrin messenger RNA [37]. Therefore, it is suggested that inhibiting the expression of sentrin in RA-SFs may reduce their invasiveness through interfering with altered apoptosis and normalizing the life-span of these cell. Because somatic mutations in p53 tumour suppressor gene in RA-SF and NF-κB-mediated pathways also contribute to the reduced apoptosis of RA-SFs, gene transfer approaches targeting these molecules may also affect the life-span of RA-SFs beneficially.

The clinical application of these approaches faces some major problems, however. As emphasized in a recent article by Jorgensen and Gay [36], premature application of gene therapy without appropriate delivery techniques and protocols may do more harm than good. Clearly, clinical trials that are aimed at demonstrating the efficacy of gene therapy approaches in rheumatoid arthritis have to use its fundamental advantages rather than just providing another vehicle. In this context, the design and utilization of vectors that ensure safe and effective delivery of potential transgenes, as well as their long-lasting expression, needs further research. Evans *et al* have addressed this problem extensively in this issue of *Arthritis Research* [3], and it has been suggested that the most appropriate strategy is the direct intra-articular injection of appropriate vectors. Indeed, the development of vectors for *in vivo* gene transfer has been favoured, not only because of their ease of use, but also and more importantly because of cost effectiveness. Today, most approaches focus on the use of vectors that at least bear the potential for *in vivo* use, and several studies have demonstrated that synovial cells can be transduced effectively by the intra-articular injection of different (viral) vectors. As for the aforementioned targets, there is yet another argument for the *in vivo* approach. Transducing a limited number of isolated cells *ex vivo* and reinjecting them into the joint may be sufficient to ensure the continuous local production of secreted proteins; however, altering intracellular signalling pathways or gene expressions will not be effective unless the majority of RA-SFs is targeted. On the other hand, one has to bear in mind that genetic modifications to signalling cascades may result in severe side effects when cells other than the pathological ones are affected. Therefore, it is anticipated that *in vivo* gene transfer for rheumatoid arthritis that is

aimed at modifying the intrinsic properties and the invasiveness of RA-SFs will not be successful without cell-specific delivery of the transgene.

In conclusion, there is a great potential for gene therapy approaches in rheumatoid arthritis, not only for the site specific and long-lasting delivery of therapeutic proteins, but also for the specific targeting of disease processes that are characteristic and unique for rheumatoid arthritis.

References

1. Maini RN, Elliott M, Brennan FM, Williams RO, Feldmann M: **TNF blockade in rheumatoid arthritis: implications for therapy and pathogenesis.** *APMIS* 1997, **105**:257–263.
2. Moreland LW, Baumgartner SW, Schiff MH, *et al*: **Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein.** *N Engl J Med* 1997, **337**: 141–147.
3. Evans CH, Ghivizzani SC, Lechman ER, *et al*: **Lessons learned from gene transfer approaches [review].** http://arthritis-research.com/14oct99/ar0101r01
4. Arend WP, Dayer JM: **Inhibition of the production and effects of interleukin-1 and tumor necrosis factor alpha in rheumatoid arthritis.** *Arthritis Rheum* 1995, **38**:151–160.
5. Müller-Ladner U, Roberts CR, Franklin BN, *et al*: **Human IL-1Ra gene transfer into human synovial fibroblasts is chondroprotective.** *J Immunol* 1997, **158**:3492–3498.
6. Müller-Ladner U, Evans CH, Franklin BN, *et al*: **Gene transfer of cytokine inhibitors into human synovial fibroblasts in the SCID mouse model.** *Arthritis Rheum* 1999, **42**:490–497.
7. Evans CH, Robbins PD, Ghivizzani SC, *et al*: **Clinical trial to assess the safety, feasibility, and efficacy of transferring a potentially anti-arthritic cytokine gene to human joints with rheumatoid arthritis.** *Hum Gene Ther* 1996, **7**:1261–1280.
8. Mulherin D, Fitzgerald O, Bresnihan B: **Clinical improvement and radiological deterioration in rheumatoid arthritis: evidence that the pathogenesis of synovial inflammation and articular erosion may differ.** *Br J Rheumatol* 1996, **35**:1263–1268.
9. Müller-Ladner U, Kriegsmann J, Franklin BN, *et al*: **Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human cartilage when engrafted into SCID mice.** *Am J Pathol* 1996, **149**:1607–1615.
10. Firestein GS, Echeverri F, Yeo M, Zvaifler NJ, Green DR: **Somatic mutations in the p53 tumor suppressor gene in rheumatoid arthritis synovium.** *Proc Natl Acad Sci USA* 1997, **94**:10895–10900.
11. Kullmann F, Judex M, Neudecker I, *et al*: **Analysis of the p53 tumor suppressor gene in rheumatoid arthritis synovial fibroblasts.** *Arthritis Rheum* 1999, **42**:1594–1600.
12. Pap T, Franz JK, Hummel KM, Jeisy E, Gay R, Gay S: **Activation of synovial fibroblasts in RA: lacking expression of the tumor suppressor PTEN at sites of invasive growth and destruction.** *Arthritis Research* in press.
13. Pap T, Hummel KM, Franz JK, *et al*: **Downregulation but no mutation of novel tumor suppressor PTEN in aggressively invading rheumatoid arthritis fibroblasts (RA-SF).** *Arthritis Rheum* 1998, **41 (suppl 9)**:S239–S239.
14. Li J, Yen C, Liaw D, *et al*: **PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer.** *Science* 1997, **275**:1943–1947.
15. Müller-Ladner U, Kriegsmann J, Gay RE, Gay S: **Oncogenes in rheumatoid synovium.** *Rheum Dis Clin North Am* 1995, **21**:675–690.
16. Dooley S, Herlitzka I, Hanselmann R, *et al*: **Constitutive expression of c-fos and c-jun, overexpression of ets-2, and reduced expression of metastasis suppressor gene nm23- H1 in rheumatoid arthritis.** *Ann Rheum Dis* 1996, **55**:298–304.
17. Trabandt A, Aicher WK, Gay RE, *et al*: **Spontaneous expression of immediately-early response genes c-fos and egr-1 in collagenase-producing rheumatoid synovial fibroblasts.** *Rheumatol Int* 1992, **12**:53–59.
18. Benbow U, Brinckerhoff CE: **The AP-1 site and MMP gene regulation: what is all the fuss about?** *Matrix Biol* 1997, **15**:519–526.
19. Asahara H, Fujisawa K, Kobata T, *et al*: **Direct evidence of high DNA binding activity of transcription factor AP-1 in rheumatoid arthritis synovium.** *Arthritis Rheum* 1997, **40**:912–918.
20. Nawrath M, Hummel KM, Pap T, *et al*: **Effect of dominant negative mutants of raf-1 and c-myc on rheumatoid arthritis synovial fibroblasts in the SCID mouse model.** *Arthritis Rheum* 1998, **41 (suppl 9)**:S95–S95.
21. Miagkov AV, Kovalenko DV, Brown CE, *et al*: **NF-kappaB activation provides the potential link between inflammation and hyperplasia in the arthritic joint.** *Proc Natl Acad Sci USA* 1998, **95**:13859–13864.
22. Han Z, Boyle DL, Manning AM, Firestein GS: **AP-1 and NF-kappaB regulation in rheumatoid arthritis and murine collagen-induced arthritis.** *Autoimmunity* 1998, **28**:197–208.
23. Makarov SS, Johnston WN, Olsen JC, *et al*: **NF-kappa B as a target for anti-inflammatory gene therapy: suppression of inflammatory responses in monocytic and stromal cells by stable gene transfer of I kappa B alpha cDNA.** *Gene Ther* 1997, **4**:846–852.
24. Ishikawa H, Hirata S, Andoh Y, *et al*: **An immunohistochemical and immunoelectron microscopic study of adhesion molecules in synovial pannus formation in rheumatoid arthritis.** *Rheumatol Int* 1996, **16**:53–60.
25. Rinaldi N, Schwarz EM, Weis D, *et al*: **Increased expression of integrins on fibroblast-like synoviocytes from rheumatoid arthritis *in vitro* correlates with enhanced binding to extracellular matrix proteins.** *Ann Rheum Dis* 1997, **56**:45–51.
26. Schwartz MA: **Integrins, oncogenes, and anchorage independence.** *J Cell Biol* 1997, **139**:575–578.
27. Dike LE, Farmer SR: **Cell adhesion induces expression of growth-associated genes in suspension-arrested fibroblasts.** *Proc Natl Acad Sci USA* 1988, **85**:6792–6796.
28. Dike LE, Ingber DE: **Integrin-dependent induction of early growth response genes in capillary endothelial cells.** *J Cell Sci* 1996, **109**:2855–2863.
29. Morales DJ, Wayner E, Elices MJ, *et al*: **Alpha 4/beta 1 integrin (VLA-4) ligands in arthritis. Vascular cell adhesion molecule-1 expression in synovium and on fibroblast-like synoviocytes.** *J Immunol* 1992, **149**:1424–1431.
30. Kriegsmann J, Keyszer GM, Geiler T, *et al*: **Expression of vascular cell adhesion molecule-1 mRNA and protein in rheumatoid synovium demonstrated by in situ hybridization and immunohistochemistry.** *Lab Invest* 1995, **72**:209–214.
31. Matsuyama T, Kitani A: **The role of VCAM-1 molecule in the pathogenesis of rheumatoid synovitis.** *Hum Cell* 1996, **9**:187–192.
32. Kitani A, Nakashima N, Izumihara T, *et al*: **Soluble VCAM-1 induces chemotaxis of Jurkat and synovial fluid T cells bearing high affinity very late antigen-4.** *J Immunol* 1998, **161**:4931–4938.
33. Kitani A, Nakashima N, Matsuda T, *et al*: **T cells bound by vascular cell adhesion molecule-1/CD106 in synovial fluid in rheumatoid arthritis: inhibitory role of soluble vascular cell adhesion molecule-1 in T cell activation.** *J Immunol* 1996, **156**:2300–2308.
34. Koch AE: **Review: angiogenesis: implications for rheumatoid arthritis.** *Arthritis Rheum* 1998, **41**:951–962.
35. Neidhart M, Wehrli R, Brühlmann P, *et al*: **Synovial fluid CD146 (MUC18), a marker for synovial membrane angiogenesis in rheumatoid arthritis.** *Arthritis Rheum* 1999, **42**:622–630.
36. Jorgensen C, Gay S: **Gene therapy in osteoarticular diseases: where are we?** *Immunol Today* 1998, **19**:387–391.
37. Franz JK, Pap T, Hummel KM, *et al*: **Expression of sentrin, a novel anti-apoptotic molecule at sites of synovial invasion in rheumatoid arthritis.** *Arthritis Rheum* 1999, in press.

Author address: WHO Collaborating Center for Molecular Biology and Novel Therapeutic Strategies for Rheumatic Diseases, Department of Rheumatology, University Hospital, Zurich, Switzerland

Correspondence: Thomas Pap, MD, WHO Collaborating Center for Molecular Biology and Novel Therapeutic Strategies for Rheumatic Diseases, Department of Rheumatology, University Hospital, Gloriastrasse 25, CH-8091 Zurich, Switzerland. Tel: +41 1 255 2962; fax: +41 1 255 4170; e-mail: exprheum@ruz.unizh.ch