



Interaction of VLA-5 Molecule With Rheumatoid Articular Cartilage Surface : An Electron Microscopic Evidence of Expression of VLA-5 on Pannus Invading Cells

Ishikawa, Hitoshi
Hirata, Souichirou
Saura, Ryyuichi
Andoh, Yoshihiro
Mizuno, Kosaku

(Citation)

Bulletin of health sciences Kobe, 14:25-33

(Issue Date)

1998-12-26

(Resource Type)

departmental bulletin paper

(Version)

Version of Record

(JaLCD0I)

<https://doi.org/10.24546/00181746>

(URL)

<https://hdl.handle.net/20.500.14094/00181746>



Interaction of VLA-5 Molecule With Rheumatoid Articular Cartilage Surface: An Electron Microscopic Evidence of Expression of VLA-5 on Pannus Invading Cells

Hitoshi Ishikawa¹, Souichirou Hirata¹, Ryuuichi Saura¹,
Yoshihiro Andoh², and Kosaku Mizuno³

Pannus is made up mainly of fibroblasts, macrophages and lymphocytes. VLA-5 positive cells are present in the pannus in large numbers. It is likely that the tissue distribution of infiltrated cells derived from post-capillary venules is influenced by the ECM of the pannus and the ability of these cells to interact with the ECM through surface receptor expression. VLA-5 molecules are the predominant $\beta 1$ integrins expressed by synovial pannus. Since the VLA integrins function as fibronectin receptors, it is possible that the fibronectin-rich environment of the rheumatoid cartilage surface effectively traps pannus cells expressing high levels of these molecules. Furthermore, an increase in $\beta 1$ integrins may be necessary for the growth of the pannus and also for the upregulation of the VLA molecules, leading secondarily to increased attachment.

Key Words

pannus formation,
synovial cell attachment,
adhesion molecules,
VLA-5 integrin,
cell-ECM interaction.

Introduction

Rheumatoid arthritis (RA) is characterized by two distinct types of pathological responses in the joint: an exudative phase involving the synovial lining layer and the synovial effusion, which are associated with an immune complex induced reaction, and by a cellular immune

response in the sublining tissue¹⁻⁴⁾.

As a part of the synovial tissue reaction, proliferating synovial cells penetrate the cartilage in the form of a pannus, and cartilage destruction takes place in the zone between the cells and cartilage⁵⁻¹³⁾. The mechanisms responsible for pannus formation are not fully understood, but there is general agreement as to the role of pannus, growing over the cartilage surface and invading the cartilage matrix⁴⁻¹³⁾, in the production of cartilage injury. Little is known about when pannus first appears and its initial localization, but in established disease, the pannus is commonly regarded as an extension of synoviocytes from the joint lining layer expanding under the influence of an immunologically mediated process¹²⁾.

The cellular origin of rheumatoid pannus has been debated by many investigators¹⁴⁻¹⁷⁾, based upon their histologic

Faculty of Health Sciences Kobe University School of Medicine¹, Ishikawa Hospital², Himeji, and Department of Orthopaedic Surgery, Kobe University School of Medicine³, Kobe, Japan.

analysis of pannus specimens from patients with RA (Figure 1). It is accepted that fibroblast proliferation, endothelial cell proliferation and monocyte chemotaxis are probably involved. Recently, it has been noted that synovio-
cytes may be stimulated by cytokines and growth factors released by the activated macrophages and synovio-
cytes of the chronically infiltrated synovial tissue¹⁸⁻²⁴.

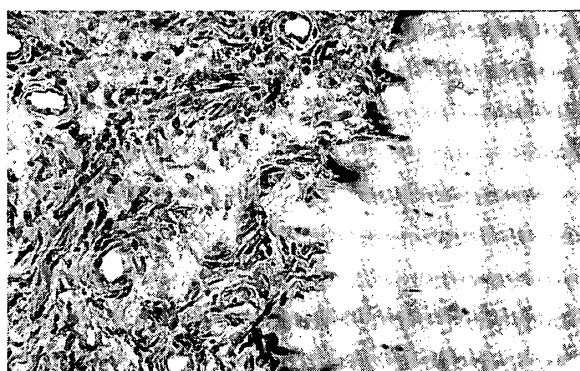


Figure 1. Pannus-cartilage junction stained with anti-VLA-5 antibody, showing almost all cells of the invading into cartilage showed strong VLA-5 staining.

Zvaifler and Firestein identified cells which are unlike typical fibroblast-like synovio-
cytes, and designated these as pannocytes. These cells have attributes in common with the primitive mesenchymal cells responsible for the initiation of cartilage damage in RA¹².

We have previously shown that when rheumatoid articular cartilage samples are incubated with normal polymorphonuclear leukocytes (PMN) in vitro, large numbers of PMN are seen to be attached to the articular surface²⁵. As observed by electron microscopy, significant numbers of these cells invaded the cartilage tissue and phagocytosed amorphous material which presumably contained immune complexes. These results demonstrated

that immune complexes trapped in the superficial region of rheumatoid articular cartilage^{26, 27} may play an important role in the destruction of cartilage by the release of lysosomal enzymes from the PMN.

Although Shiozawa et al. observed that the pannus-free surface of cartilage contained deposits of immunoglobulin, areas covered by pannus showed none²⁸. These authors concluded that rheumatoid pannus invasion of the free cartilage surface containing trapped immune complexes results in the disappearance of such complexes.

In a previous study²⁹, we have shown when rheumatoid articular cartilage samples are incubated with normal peripheral blood monocytes and cultured synovial cells in the presence of recombinant human IL-1 in vitro, large numbers of monocytes and cultured synovial cells are seen to be attached to the articular surface. There was a significantly greater attachment of monocytes to rheumatoid than to normal cartilage. These results indicated that increased levels of IL-1 in the rheumatoid joint may play an important role in joint destruction by stimulation of pannus formation through induction of synovial cell attachment to the articular surface. Synovial cell attachment to cartilage may be the initial step in pannus formation. Our data suggested that the elevated levels of collagenase and IL-1 present in synovial fluid may contribute to pannus formation in the rheumatoid joint by stimulation of the attachment of monocytes and synovial cells to cartilage matrix proteins.

Recent studies^{30, 31} have demonstrated interactions between adhesion molecules of the β 1 group of the integrin superfamily, specifically VLA-4 and VLA-5, as well as other adhesion receptors pre-

sent on nucleated hematopoietic cells, with collagen, fibronectin and laminin ligands of the connective tissue matrix. It is likely that binding of synovial cells to cartilage in the presence of IL-1, as observed in our experiments described above²⁹, results from stimulation of interactions between VLA receptors on the synovial cell and these matrix protein ligands. Furthermore, we have recently shown that the increased expression of VLA-5 and the intercellular adhesion molecule-1 (ICAM-1) at the cartilage-pannus junction may result from the interaction of synovial mononuclear cells with matrix proteins³²⁻³⁹. These findings have supported the role of adhesion molecules in pannus formation and its attachment to cartilage.

In the present study, an immunoperoxidase staining method has been used to determine whether adhesion molecules and ligand expressed on pannus and cartilage respectively play a role in pannus formation.

Materials and Methods

Twenty-eight samples of rheumatoid articular cartilage covered with pannus from 28 patients were obtained during synovectomy or joint replacement surgery. All patients were rheumatoid factor seropositive and fulfilled the ACR 1987 diagnostic criteria for rheumatoid arthritis. Several samples of pannus-cartilage junction from the same patients were selected to contain active pannus on the basis of naked eye examination, and this was confirmed by light microscopy using haematoxylin and eosin staining. Fibrous pannus was discarded on the basis of lack of cellularity. Each specimen was treated immediately after collection.

Purified anti-human monoclonal anti-

body denoted CDw 49 e ($\alpha 5 \beta 1$, VLA-5) was obtained from Immunotech (Marseille, Cedex, France). Purified mouse IgG was obtained from Cappel Laboratories (Cochranville, Pa.). Avidin biotinylated peroxidase (ABC-kit) was obtained from Vector Laboratories (Burlingame, Calif.), and 3-3'-diaminobenzidine was purchased from Sigma Chemical Co. (St. Louis, Mo.). Frozen sections, about 4-6-mm thick, were cut on a cryostat (Bright, Huntington, England) at -20°C , and mounted on gelatin and egg albumin coated slides. After drying at room temperature, the sections were washed with phosphate-buffered saline (PBS). Normal goat serum, diluted 1:200, was applied to the sections for 20 min. After washing, they were incubated with 100-200 μl of diluted monoclonal antibody for 60 min. After washing with PBS, biotinylated peroxidase conjugated goat anti-mouse IgG antibody was added, and this was followed by an avidine peroxidase complex. The tissue was then incubated with 3 mg of 3-3'-diaminobenzidine in 10 ml of Tris HCl buffer, pH 7.5, for 10 min. The specimens were then washed in PBS and dried at room temperature. For electron microscopic examination, the sections were fixed with 1% osmium tetroxide (OsO_4) for 1 h and washed in PBS and that was dehydrated in graded alcohol to 100%. While the sections were still wet, plastic capsules filled with Epon 812 were inverted over the sections. After polymerization of the Epon 812, the slides were heated on a hot plate and the sections were removed from the slides.

Fields were chosen for electron microscopic study by examining the light microscopic pictures on a dissecting microscope. Sections were cut on an LKB

microtome and examined in a Hitachi H-300 electron microscope.

Results

When the synovial pannus cartilage tissue samples were stained with antibody to VLA-5, almost all cells, including the synovial lining cells, mononuclear cells and endothelial cells of the post-capillary venules showed strong VLA-5 staining (Figure 1). Some degree of hyperplasia of the synovial lining cells was observed. In agreement with previous report, the cellular components of the rheumatoid pannus varied in their numbers^{5, 14)}. VLA-5-positive cells were observed in a linear distribution along the border between the synovium and cartilage (Figure 2 and 3). In the electron microscopic examination, the electron-dense materials were

observed in a patchy distribution on the cell membrane, and these materials were observed to be in contact with cartilage matrix (Figure 4). The cell membrane of the chondrocytes located at or close to the pannus-cartilage junction also showed strong anti-VLA staining when examined under the electron microscope (Figure 5).

Discussion

The integrins are a family of ubiquitous cell-surface receptors that bind to extracellular matrix (ECM) protein. Recent studies have demonstrated an interaction between adhesion molecules of $\beta 1$ group of the integrin supergene family and fibronectin, collagen and laminin of the ECM^{30, 35)}, and stimulation of synovio-cyte proliferation by a reaction between

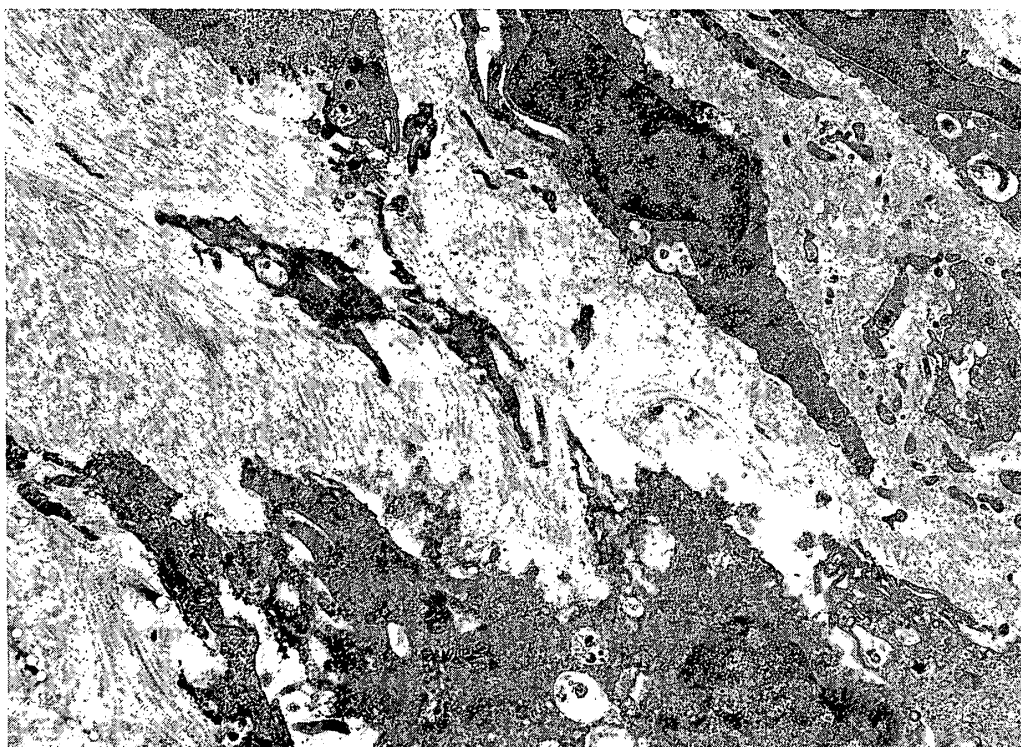


Figure 2. Immuno-peroxidase staining of VLA-5 at rheumatoid pannus-cartilage junction. Most of the cells located at the cartilage border show strong staining with anti VLA-5 antibody.

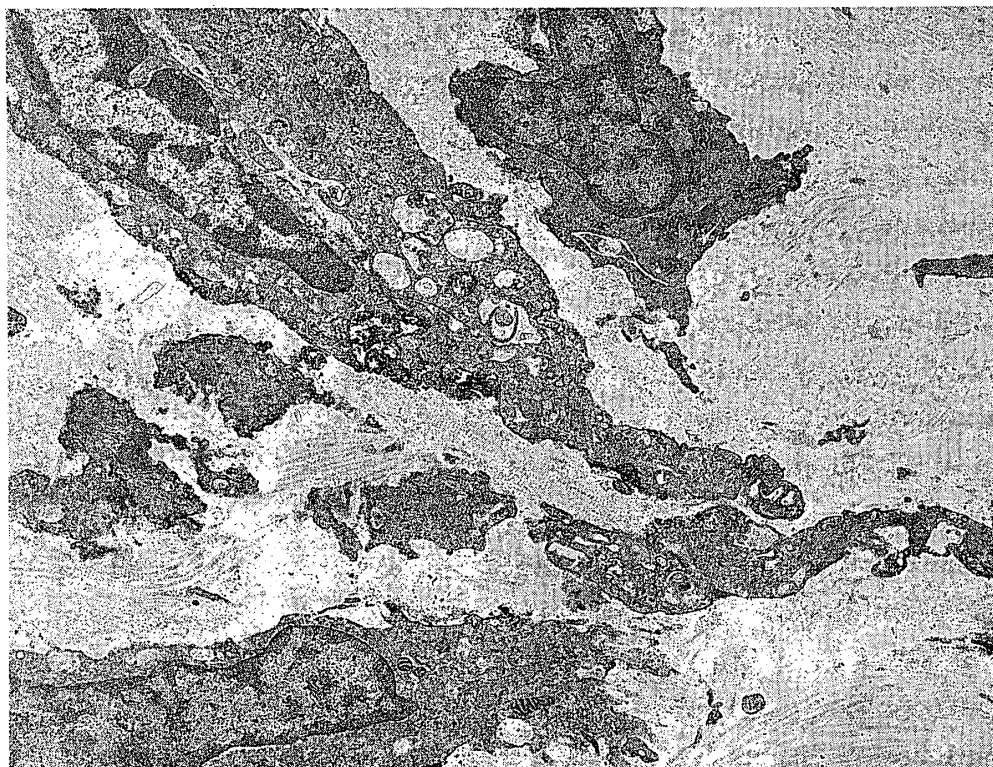


Figure 3. VLA-5 positive cells were observed in a linear distribution along the border between the synovium and cartilage.

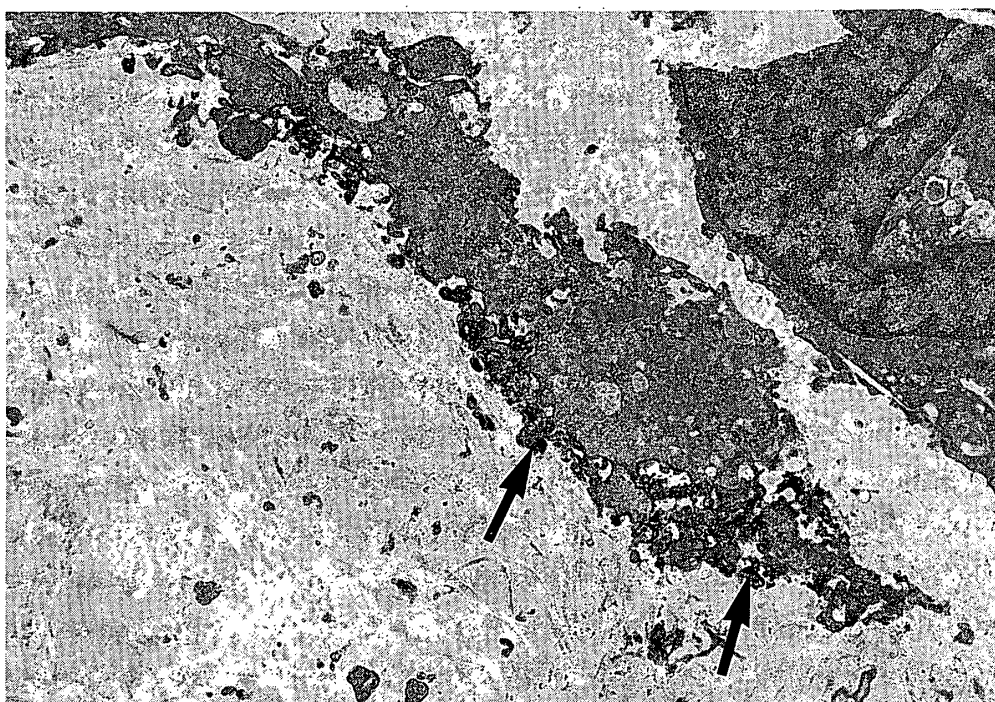


Figure 4. The electron dense materials were observed in a patchy distribution (arrows) on the cell membrane.

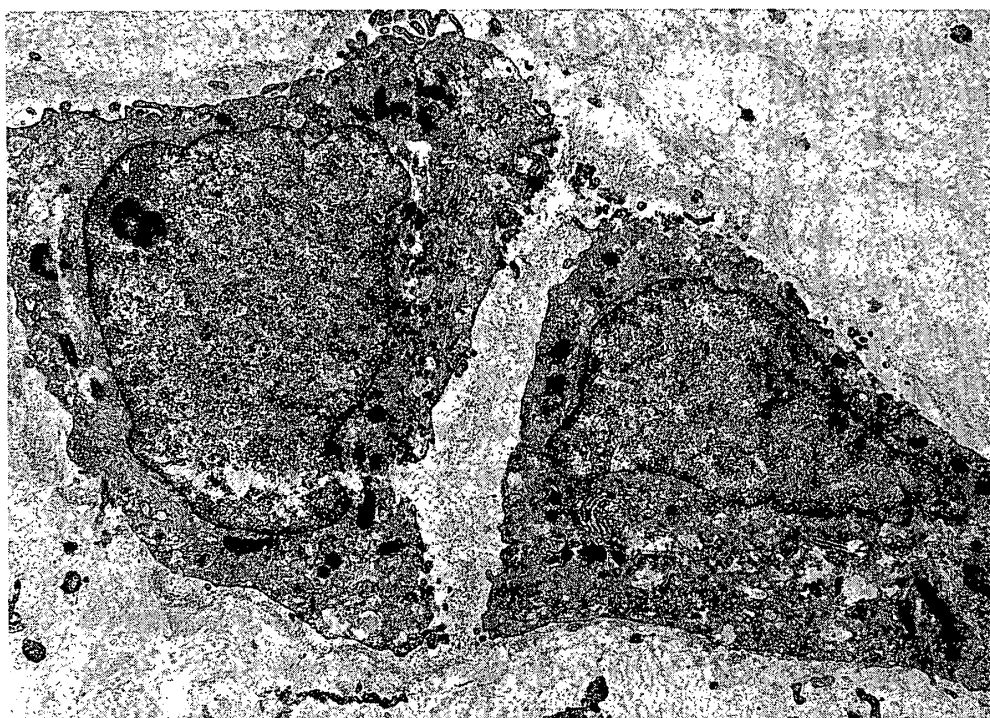


Figure 5. The cell membrane of the chondrocytes located at the pannus junction also showed anti-VLA-5 staining.

membrane-bound VLA-5 and fibronectin^{30, 39)}. Rheumatoid synovial tissue, moreover, is rich in lymphocytes expressing high levels of integrins³⁶⁻³⁸⁾. We have raised the possibility that VLA-5 may facilitate the growth of pannus by virtue of its ability to react with fibronectin with resulting proliferation of the synoviocytes of the pannus³²⁾.

Adhesion molecules are critically important for the binding of inflammatory cells to ECM proteins, such as fibronectin and collagen, via the $\beta 1$ integrins^{39, 40)}. Thus it is likely that the tissue distribution patterns of infiltrated cells derived from post-capillary venules are influenced by the ECM of the pannus and the ability of these cells to interact with ECM through cell surface receptor expression.

The strong expression of VLA-5 on chondrocytes³⁹⁾ would suggest that interactions between chondrocytes and fi-

bronectin may occur in the course of pannus formation through the activation of chondrocytes by various cytokines^{29, 41)}. Many of the known ligands for integrins, including collagen, fibronectin, laminin and thrombospondin, are present at the pannus-cartilage junction in RA. Their presence may be related to increased de novo synthesis by chondrocytes⁴²⁾. Furthermore, type II collagen binding proteins including anchorin, have been identified on chondrocytes. However, the role of integrins in collagen-chondrocyte interaction is, as yet, not clarified⁴³⁾. The finding of VLA-5 molecules in a pericellular as well as an interterritorial matrix distribution in our study³⁹⁾ strongly suggests that a receptor-ligand interaction between VLA-5 and cartilage matrix may occur at the early stage of pannus formation.

Acknowledgement

The authors wish to thank Dr. Morris Ziff, PhD., M.D. Ashbel Smith Profes-

sor Emeritus of Internal Medicine, the University of Texas, Southwestern Medical Center at Dallas, for help with this manuscript.

References

1. Ishikawa H, Ziff M: Electron microscopic observations of immunoreactive cells in the rheumatoid synovial membrane. *arthritis Rheum* 19: 1-14, 1975
2. Ishikawa H: Relationship between HLA-DR positive cells and lymphocytes of different subsets in rheumatoid synovial membrane: an immunohistochemical and immunoelectron microscopic study. *J orthop Rheum* 1: 146-158, 1988
3. Ziff M: Immunological aspect of rheumatoid synovitis-25 years. *Arthritis Rheum* 20: S 31-33, 1977
4. Zvaifler NJ: The immunopathology of joint inflammation in rheumatoid arthritis. *Adv Immunol* 16: 265-336, 1977
5. Kobayashi I, Ziff M: Electron microscopic studies of the cartilage-pannus junction in rheumatoid arthritis. *Arthritis Rheum* 475-483, 1975
6. Shiozawa S, Shiozawa K, Fujita T: Morphologic observations in the early phase of the cartilage-pannus junction. *Arthritis Rheum* 472-478, 1983
7. Ciobanu A, Ciobanu IR, Halalau F et al: Histological and histoenzymological investigations of the rheumatoid articular cartilage. *Rom J Morphol Embryol* 40: 23-27, 1994
8. Xue C, Takahashi M, Hasunuma T et al: Characterization of fibroblast-like cells in pannus lesions of patients with rheumatoid arthritis sharing properties of fibroblasts and chondrocytes. *Ann Rheum Dis* 56: 262-267, 1997
9. Hauselmann HJ: Mechanism of cartilage destruction and nonsurgical therapeutic strategies to retard cartilage injury in rheumatoid arthritis. *Curr Opin Rheumatol* 9: 241-250, 1997
10. Kunkel SL, Lukacs N, Kasama T et al: The role of chemokines in inflammatory disease. *J Leukoc Biol* 59: 6-12, 1996
11. Mohr W: The pathogenesis of joint destruction in chronic polyarthritis. *Radiologe* 36: 593-599, 1996
12. Zvaifler NJ, Firestein GS: Pannus and pannocytes; Alternative models of joint destruction in rheumatoid arthritis. *Arthritis Rheum* 37: 783-789, 1994
13. Hino K, Shiozawa S, Kuroki Y et al: EDA-containing fibronectin is synthesized from rheumatoid synovial fibroblast-like cells. *Arthritis Rheum* 38: 678-683, 1995
14. Fassbender HG: Is pannus a residue of inflammation? *Arthritis Rheum* 27: 956-957 (letter), 1984
15. Cooke TDV: Rheumatoid arthritis pannus; true or false? *Arthritis Rheum* 28: 1195-1197 (letter), 1985
16. Mitrovic D: The mechanism of cartilage destruction in rheumatoid arthritis. *Arthritis Rheum* 28: 1192-1193 (letter), 1985
17. Lavietes BB, Diamond HS, Carsons SE et al: Possible contribution of cartilage to fibrous pannus. *Arthritis Rheum* 30: 119-120, 1987
18. Glant T, Hadas E, Nagy M: Cell-mediated and humoral responses to cartilage antigenic components. *Scand J Immunol* 9: 39-44, 1979
19. Leizer T, Clarris BJ, Ash PE et al: Interleukin-1 and interleukin-1 stimulate the plasminogen activator activity and prostaglandin E₂ levels of human synovial cells. *Arthritis Rheum* 30: 562-566, 1987
20. Dayer J-M, Stephenson ML, Schmidt E et al: Purification of a factor from human blood monocyte-macrophages which stimulates the production of collagenase and prostaglandin E₂ by cells cultured from rheumatoid synovial tissues. *FEBS* 124:

- 253-256 (letter), 1981
21. Hamilton JA, Slywka J: Stimulation of human synovial fibroblast plasminogen activator production by mononuclear cell supernatants. *J Immunol* 126: 851-855, 1981
22. Dayer J-M, Demczuk S: Cytokines and other mediators in rheumatoid arthritis. *Springer Semin Immunopathol* 7: 387-413, 1984
23. Dayer J-M, Beutler B, Cerami A: Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E₂ production by human synovial cells and dermal fibroblasts. *J Exp Med* 162: 2163-2168, 1985
24. Chin JA, Lin Y: Effects of recombinant human interleukin-1 on rabbit articular chondrocytes. Stimulation of prostanoid release and inhibition of cell growth. *Arthritis Rheum* 31: 1290-1296, 1988
25. Ugai K, Ishikawa H, Hirohata K et al: Interaction of polymorphonuclear leukocytes with immune complexes trapped in rheumatoid articular cartilage. *Arthritis Rheum* 26: 1434-1441, 1983
26. Cooke TD, Hurd ER, Jasin H et al: Identification of immunoglobulins and complement in rheumatoid articular collagenous tissue. *Arthritis Rheum* 18: 541-551, 1975
27. Ishikawa H, Smiley JD, Ziff M: Electron microscopic demonstration of immunoglobulin deposition in rheumatoid cartilage. *Arthritis Rheum* 18: 565-576, 1975
28. Shiozawa S, Jasin HE, Ziff M: Absence of immunoglobulins in rheumatoid cartilage-pannus junctions. *Arthritis Rheum* 23: 816-821, 1980
29. Ishikawa H, Ohno O, Saura R et al: Cytokine enhancement of monocyte/synovial cell attachment to the surface of cartilage: a possible trigger of pannus formation in rheumatoid arthritis. *Rheumatol Int* 11: 31-36, 1991
30. Elices MJ, Hemler ME: The human integrin VLA-2 is a collagen receptor on some cells and a collagen/laminin receptor on others. *Proc Natl Acad Sci USA* 86: 9906-9910, 1989
31. Hemler ME, Takada Y, Elices M et al: Structure and functions for the adhesion receptor VLA-2 and VLA-4; comparisons to other members of the integrin superfamily. In: Springer TA, Anderson DC, Rosenthal AS, Rothlein R (eds) *Leukocyte adhesion molecules; structure, function and regulation*. Springer, Berlin Heidelberg New York, pp 44-57, 1989
32. Ishikawa H, Hirata S, Nishibayashi Y et al: The role of adhesion molecules in synovial-pannus formation in rheumatoid arthritis. *Clin Orthop Rel Res* 300: 297-303, 1994
33. Ishikawa H, Hirata S, Andoh Y et al: The role of adhesion molecules in synovial pannus formation in rheumatoid arthritis: an immunohistochemical and immunoelectron microscopic study. *Arthritis Rheum* 37: 9(S), 311, 1994
34. 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* 16: 53-60, 1996
35. Elices MJ, Osborn L, Takada Y et al: VCAM-1 on activated endothelium interacts with the leukocytes integrin VLA-4 at a site distinct from VLA-4/fibronectin binding site. *Cell* 60: 577-584, 1990
36. Ishikawa H, Hirata S, Nishibayashi Y et al: Role of adhesion molecules in the lymphoid cell distribution in rheumatoid synovial membrane. *Rheumatol Int* 13: 229-236, 1994
37. Hanley J, Pledger D, Parkhill W et al: Phenotypic characterization of dissociated mononuclear cells from rheumatoid synovial membrane. *Rheumatol Int* 17: 1274-1279, 1990
38. Laffon A, Garcia-Vincuna R, Humbria A et al: Upregulated expression and function of VLA-4 fibronectin receptors on human activated T cells in rheumatoid arthritis. *J Clin Invest* 88: 546-552, 1991
39. Pitzalis C, Kingsley G, Panayi G: Adhesion molecules in rheumatoid arthritis: role in the pathogenesis and prospects for therapy. *Ann Rheum Dis* 53: 287-288, 1994

40. Rodriquets RM, Pitzalis C, Kingsley GH et al: T-lymphocyte adhesion to fibronectin: a possible mechanism for T cell accumulation in the rheumatoid joint. *Clin Exp Immunol* 89: 439-445, 1992
41. Salter DM, Hughes DE, Simpson R et al: Integrin expression by human articular chondrocytes. *Br J Rheumatol* 31: 231-234, 1992
42. Brown RA, Fones KC: The synthesis and accumulation of fibronectin by human articular cartilage. *J Rheumatol* 17: 65-72, 1990
43. Mollenhauer J, Bee JA, Lixarbe MA et al: Role of anchorin C II, a 31,000-mol-wt membrane protein, in the interaction of chondrocytes with type II collagen. *J Cell Biol* 98: 1572-1578, 1984