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Immunolocalization of Adhesion Molecules in Rheumatoid and Osteoarthritic Synovial Tissues

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To elucidate the potential role of adhesion molecules in the pathogenesis of rheumatoid arthritis (RA), we stained specimens of synovial tissue from patients with RA and osteoarthritis (OA) with monoclonal antibodies against adhesion molecules using an immunohistochemical method. Positive staining with anti-ICAM-1 monoclonal antibody was detected on the synovial lining cells, the sublining cells and the capillary endothelial cells in the synovium from patients with RA, and, to a lesser degree, in that from patients with OA. The capillary endothelial cells from patients with RA intensively expressed both ELAM-1 and VLA-5α molecules, in contrast to that from OA patients. The intensity of both ICAM-1 and ELAM-1 on the capillary endothelial cells in RA synovium was comparable to disease activity and to the degree of synovial proliferation. A high density of expression of LFA-1α , VLA-4α and VLA-5α was observed on the mononuclear cells that infiltrated the RA synovium, especially in the lesions with aggregated mononuclear cells. The findings clearly demonstrated an up-regulation of the expression of adhesion molecules on synovial cells, capillary endothelial cells and infiltrated mononuclear cells in the synovial tissues of patients with RA. This enhanced expression of adhesion molecules may play an important role in the migration of mononuclear cells into the synovial tissues and thus perpetuate the inflammatory response in these tissues.

Key Words: rheumatoid arthritis, osteoarthritis, adhesion molecule, synovium.

Introduction

Rheumatoid arthritis (RA) is associated with immune dysfunctions which contribute to its pathogenesis (1). The synovial membrane contains a dense infiltration of mononuclear cells, predominantly T cells (2, 3) that contribute to the intraarticular inflammation via the production of cytokine and the promotion of B cell hyperactivity. The result is an excess production of immunoglobulins and rheumatoid factors (4, 5). Under current investigation is the mechanism by which the T cells are continually recruited into the synovium to set up inflammatory lesions which eventually lead to the degradation of cartilage and bone.

Adhesion molecules play a fundamental role in diverse immunological functions, especially the presentation of antigen, the recognition of target cells by effector cells, and the migration and retention of mononuclear cells (6, 7). They may also mediate signal transduction, leading to the activation or proliferation of cells (8, 9). The molecules involved in such interactions consist of four superfamilies classified according to their molecular characteristics: the integrin family, the immunoglobulin (Ig) supergene family, the selectin family, and the H-CAM family (CD44). The adhesion-dependent interactions of lymphocytes in processes that are dependent on, or-independent of, antigen, are mediated via an interaction of the integrin lymphocyte function-associated antigen 1 (LFA-1) with the Ig supergene family intercellular adhesion molecule 1 (ICAM-1) (10, 11). Those include T-cell interactions with antigen-presenting cells, cytotoxity by T-lymphocytes, and binding of lymphocytes to the endothelium (12, 13). A second adhesion pathway via the binding of very late antigen 4 (VLA-4) to vascular cell adhesion molecule 1 (VCAM-1) and fibronectin (CS1) has been defined (14, 15). These molecules belong to the integrin, the Ig supergene family and the extracellular matrix, respectively, and mediate the adhesion of lymphocytes to the endothelium and α antigen-presenting cells (13, 14). A pathway via the binding of VLA-5 to fibronectin has also been described (12). A third pathway via sialyl Le^x and sialyl Le^a with endothelial-leukocyte adhesion molecule 1 (ELAM-1), which belong to the selectin family, can mediate the adhesion of neutrophils and monocytes, and of lymphocytes to the endothelium (16-20).

We investigated the expression of adhesion molecules on synovial tissues from patients with RA and the correlation of this expression with the presence of general or of local inflammatory disease. These findings were compared with those in specimens obtained from patients with OA.

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Materials and Methods

Patients.

Specimens of inflamed synovial membrane were obtained from patients who were undergoing total knee arthroplasty or synovectomy. We studied 10 patients (2 men and 8 women, mean age; 54.0 ± 11.6 (± SD), range; 36-72 years) who met the American College of Rheumatology criteria RA (21). Table 1 shows their clinical characteristics. At the time of study, all patients were taking non-steroidal anti-inflammatory drugs (NSAIDs). Four of them were also taking slow-acting anti-rheumatic drugs (SAARDs) including gold sodium thiomalate, methotrexate and D-penicillamine. In addition, 7 patients with RA were receiving prednisolone at a dose of less than 10mg daily. We also studied 4 patients with OA (1 man and 3 women, mean age; 58.4 ± 8.7 (± SD), range; 51-70 years). Specimens of normal synovial tissue were also obtained from 3 patients who were undergoing corrective surgery for joint trauma. Patients with RA were divided into 2 groups according to their inflammatory activity as follows (22): (a) duration of morning stiffness ≧ 45min; (b) erythrocyte sedimentation rate ≧ 28min/hour; and (c) the number of tender joints ≧ 9; (d) the number of swollen joints ≧ 6. Five patients fulfilled at least three of these four criteria and were classified as the active disease group, while 5 patients were classified as the inactive disease group. In addition, by arthroscopic examination, we graded the local inflammatory activity as follows: grade 1: mild synovial proliferation; grade 2: moderate synovial proliferation; and grade 3: severe synovial proliferation. The same rheumatologist evaluated the grades of the local inflammatory activity in all cases.

Immunohistochemistry.

The synovia from 10 RA patients and 4 OA patients and 3 normal synovia were stained by an immunohistochemical technique described elsewhere (23). Fresh synovial sections were fixed in 4% paraformaldehyde solution (Wako Co., Tokyo, Japan) for 8 hours at 4℃ , then washed with phosphate-buffered saline solution (PBS) for 15 hours, or with 30% saccharose (Wako Co., Tokyo, Japan) in PBS for 30 minutes. The fixed tissues were embedded in O. C. T. compound and quickly frozen on glass slide coated with albumin. Sections at 6 μm thick were mounted on the coated slides and stained by the labeled-streptavidin biotin method (Histofine staining kit. Nichirei Co., Tokyo, Japan). Briefly, endogenous peroxidase was inactivated by emerging the section in a 3% H_2O_2 solution. Sections were next incubated with non-immune rabbit IgG, then with mouse monoclonal antibodies directed against adhesion molecules in a humidified chamber for 1 hour at room temperature. The monoclonal antibodies used in this experiment are characterized in Table 2. Sections were treated with biotinylated rabbit anti-mouse IgG for 30 miniutes. After being washed, they were incubated with peroxidase-conjugated streptavidin. Color was developed using 3.3'-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) and H_2O_2. Slides were counterstained with hematoxylin. Anti-factor VIII monoclonal antibody was used for staining as a marker of vascular endothelial cells. Control sections were treated with anti-insulin antibody instead of specific monoclonal antibody. The intensity of antigen expression was tentatively categorized as: (–): negative staining, (±): faint staining, (+): positive staining, and (++): very bright staining.

Table 1. Clinical characteristics of 10 patients with rheumatoid arthritis

Case	Age	Sex	Class	Stage	Morning stiffness (minutes)	Tender joints	Swollen joints	ESR (mm/h)	CRP (mg/dl)	Drug therapy	
										Current	Previous
1	65	F	3	3	120	9	1	40	2.1	PSL (2.5mg/d)	Gold. PSL
2	61	F	3	3	240	10	3	54	3.2	PSL (5.0mg/d)	PSL. MTX
3	36	M	2	2	0	2	0	36	2.0	PSL (10.0mg/d)	PSL
4	61	F	2	3	360	9	5	92	9.0	PSL (5.0mg/d)	Gold. D-P. CCA Bucil
5	46	F	2	4	0	1	1	11	0.0	Gold	
6	59	F	2	1	90	12	11	23	4.3	PSL (5.0mg/d)	PSL
7	59	F	3	4	5	12	10	26	3.8	PSL (2.5mg/d) MTX	PSL. MTX
8	72	F	3	2	240	14	5	107	10.5	PSL (2.5mg/d)	
9	40	M	2	3	0	3	3	58	3.1	MTX	Gold. D-P. Bucil. PSL
10	41	F	2	3	60	4	5	34	0.9	D-P	D-P.CCA. Bucil. PSL

ESR: erythrocyte sedimentation rate CRP: C-reactive protein PSL: prednisolone MTX: methotrexate
D-P: D-penicillamine CCA: lobenzarit disodium Bucil: bucillamine

Table 2. Murine monoclonal antibodies

Clone	Specificity	Dilution	Source
2002	Factor VIII	1:2	Nichirei Co., Tokyo Japan
84H10	ICAM-1	0.5 μg/ml	Immunotech, Marseiles, France
25, 3. 1	LFA-1 α	1 μg/ml	Immunotech
BBA-1	ELAM-1	5 μg/ml	British Biotechnology, Oxon, UK
1G11	VCAM-1	5 μg/ml	Immunotech
HP2.1	VLA-4 α	1 μg/ml	Immunotech
SAM1	VLA-5 α	1 μg/ml	Immunotech
2006	Insulin	1:2	Nichirei Co.,

ICAM-1 : intercellular adhesion molecule 1, LFA-1 α : lymphocyte function-associated antigen 1 α ,
ELAM-1 : endothelial-leukocyte adhesion molecule 1, VCAM-1 : vascular cell adhesion molecule 1,
VLA-4 α : very late antigen 4 α , VLA-5 α : very late antigen 5 α ,

Results

Expression of ICAM-1 and LFA-1 on synovial tissues from patients with RA and OA.

Table 3 summerizes the degrees of ICAM-1 and LFA-1 expression in the synovial tissues from patients with RA and OA that were stained with anti-ICAM-1 and anti-LFA-1, and concomitantly with anti-factor VIII monoclonal antibodies. No staining was observed when anti-insulin monoclonal antibody was used as control. As shown in Fig. 1A and 2A, positive staining for factor VIII was found on the capillary endothelial cells in the sublining layer of synovium from both the RA and OA patients. We detected the expression of ICAM-1 on the capillary endothelial cells in the sublining layer of RA synovium (Fig. 1B), and to a lesser extent, in the sublining layer of OA synovium (Fig. 2B). In patients with RA, the intensity of ICAM-1 expression on vascular endothelial cells were correlated with generalized disease activity and the grade of synovial proliferation. ICAM-1 positive staining were also observed on the infiltrated mononuclear cells in the sublining layer of RA patients (Fig. 1B) but not on that OA patients (Fig. 2B). RA and OA patients each showed an

Table 3. Expression of ICAM-1 and LFA-1 α on synovium from patients with rheumatoid arthritis and osteoarthritis, and healthy controls

Disease/Patients	Staining for ICAM-1*				Staining for LFA-1 α*				Arthroscopy findings**
	LC	SLC	EC	MNC	LC	SLC	EC	MNC	
Rheumatoid arthritis									
Case 1	++	+	++	+	–	–	–	+	+++
2	++	+	++	+	–	–	–	+	++
3	++	+	+	+	–	–	–	+	+
4	++	+	+	+–	–	–	–	+	+++
5	++	+	+	+–	–	–	–	+	+–
6	++	+	++	+	–	–	–	+	+++
7	++	+	+	+	–	–	–	+	+
8	++	+	++	+	–	–	–	+	++
9	++	+	++	+–	–	–	–	+	+
10	++	+	+	+–	–	–	–	+	+
Osteoarthritis									
Case 1	+	+	+	–	–	–	–	+	+–
2	++	+	++	–	–	–	–	+	+–
3	+	+	+	–	–	–	–	+	+–
4	+	+	+	–	–	–	–	+	+–
Healthy subjects									
Case 1	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–

LC : synovial lining cells, SLC : synovial sublining cells,
EC : capillary endothelial cells, MNC: mononuclear cells
*; (–) : negative staining, (+–): faint staining,(+): positive staining,
(++) : very bright staining
**; (–) : no synovial proliferation, (+–): faint synovial proliferation,
(+) : mild synovial proliferation, (++): moderate synovial proliferation,
(+++): severe synovial proliferation

enhanced expression of ICAM-1 on the synovial lining cells and synovial sublining cells The intensity of ICAM-1 expression on these synovial cells was greater in RA with a trend toward lesser amounts in OA (Table 3) An up-regulated expression of LFA-1 was indicated on the infiltrated mononuclear cells in synovial sections from patients with RA as well as from OA (Fig 1C and 2C, Table 3) LFA-1 positive mononuclear cells infiltrated the areas of aggregated mononuclear cells, especially around the ICAM-1-expressed vessels No staining with anti-ICAM-1 and anti-LFA-1 monoclonal antibodies was observed in the sections of normal synovium

Expression of ELAM-1, VLA-4 and VLA-5 on synovial tissue from RA and OA patients

Data on immunostaining of synovial tissues from patients with RA and OA are summarized in Table 4 Intense staining of ELAM-1 was detected on the capillary endothelial cells in sections of synovia from patients with RA (Fig 1D, Table 4) Expression of ELAM-1 on the capillary endothelial cells in the synovia from the patients with active RA was more extensive and intense than on those from the patients with inactive RA In addition, an up-regulated expression of ELAM-1 was indicated on the endothelial cells in the synovial sections obtained from RA patients with moderate or severe local inflammatory activity In contrast, the capilary endothelial cells of patinets with OA express no staining or stained only faintly for ELAM-1 expression (Fig 2D) Intense staining of VLA-4 was found on the infiltrated mononuclear cells in synovial sections from both patients with RA and OA (Fig 1E and 2E) VLA-4 positive mononuclear cells were especially evident in the areas of cell aggregation VLA-4 positive staining on the synovial sublining cells was also found in the RA synovium (Fig 1F), but not in the OA synovium (Table 4) In synovial sectios from patients with RA, staining with anti-VLA-5α monoclonal antibody was observed on the synovial lining cells, sublining cells, capillary endothelial cells and infiltrated mononuclear cells (Fig

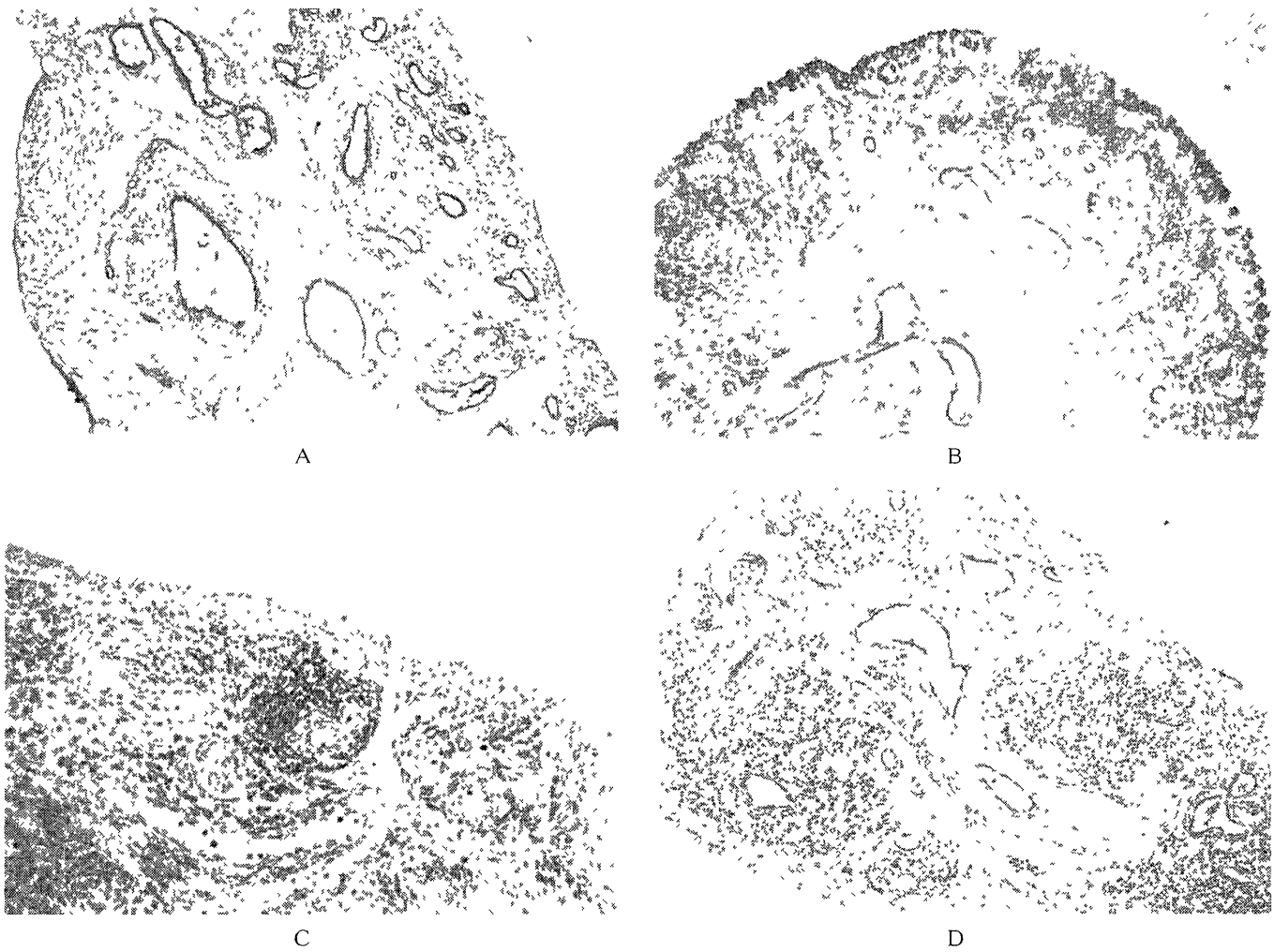

Fig. 1. (to be continued)

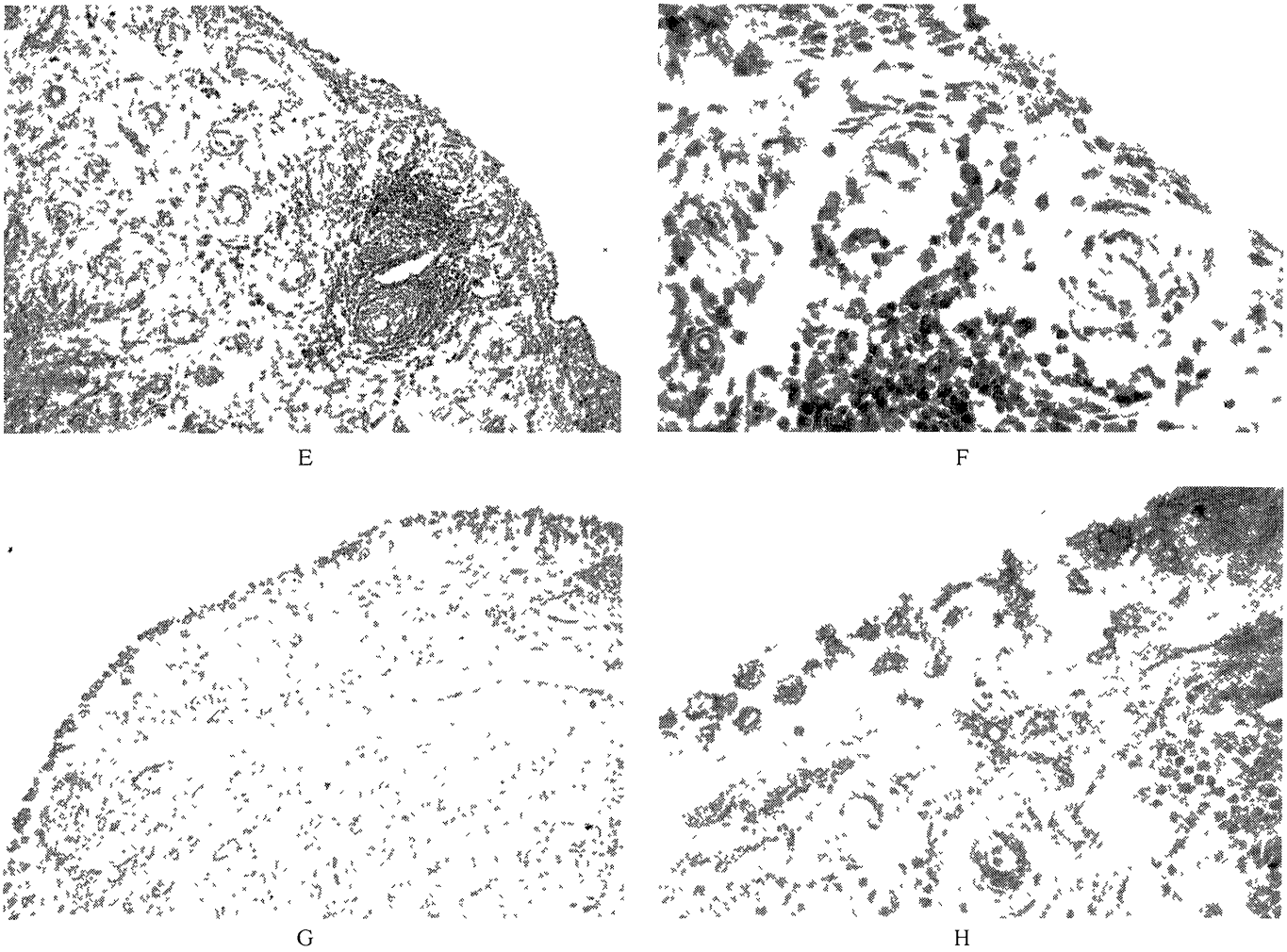

Fig. 1. Expression of factor VIII, ICAM 1, LFA 1, ELAM-1, VLA-4 and VLA 5 on synovial tissues from patients with RA Synovial specimens were stained with the following monoclonal antibodies by an immunoperoxidase technique
(A) anti factor VIII monoclonal antibody (X100) (B) anti-ICAM 1 monoclonal antibody (X100)
(C) anti LFA 1α monoclonal antibody (X100) (D) anti-ELAM-1 monoclonal antibody (X100)
(E) anti VLA 4α monoclonal antibody (X100) (F) anti VLA 4α monoclonal antibody (X400)
(G) anti VLA-5α monoclonal antibody (X100) (H) anti VLA 5α monoclonal antibody (X100)

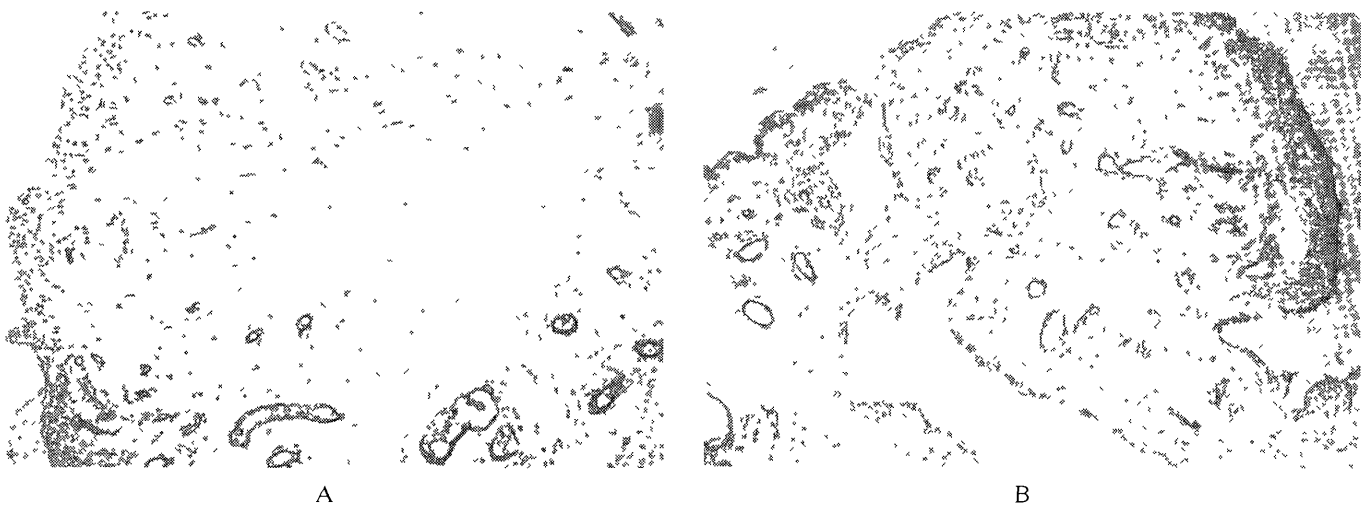

Fig. 2. (to be continued)

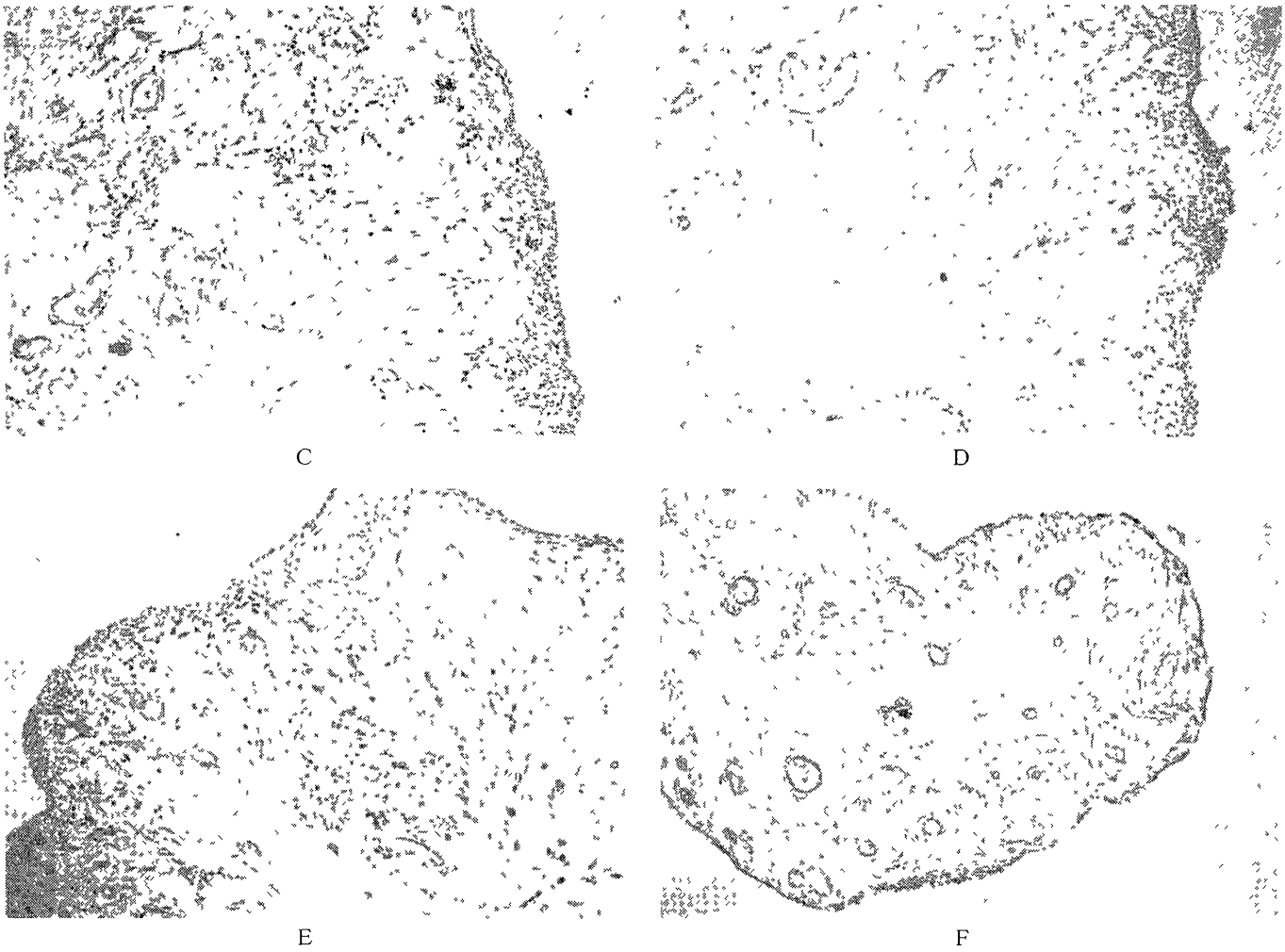

Fig. 2. Expression of adhesion molecules on synovial tissues from patients with OA Synovial sections were stained with the following monoclonal antibodies as used Fig 1

(A) anti factor VIII monoclonal antibody (X100)
(B) anti-ICAM 1 monoclonal antibody (X100)
(C) anti LFA-1α monoclonal antibody (X100)
(D) anti-ELAM-1 monoclonal antibody (X100)
(E) anti-VLA-4α monoclonal antibody (X100)
(F) anti-VLA 5α monoclonal antibody (X400)

1G and 1H) The intensity of VLA-5α expression on capillary endothelial cells in RA synovium paralled the severity of local inflammatory activity (Table 4) The lining cells in the OA synovium reacted with anti-VLA-5α monoclonal antibody (Fig 2F) There was no increased expression of VLA-5 on synovial sublining cells, capillary endothelial cells or infiltrated mononuclear cells of synovium from patients with OA (Fig 2F) No staining with anti-ELAM-1, anti-VLA-4α anti-VLA-5α monoclonal antibodies was observed in the normal synovium

Table 4. Expression of ELAM-1, VLA-4α and VLA-5α on synovium from patients with rheumatoid arthritis and osteoarthritis, and healthy controls

Disease/Patients	Staining for ELAM-1*				Staining for VLA-4α*				Staining for VLA-5α*				Arthroscopy findings**
	LC	SLC	EC	MNC	LC	SLC	EC	MNC	LC	SLC	EC	MNC	
Rheumatoid arthritis													
Case 1	–	–	+	–	–	+	–	++	++	++	++	+	+++
2	–	–	+	–	–	+	–	++	++	+	++	+	++
3	–	–	+	–	–	+	–	++	++	+	+	+	+
4	–	–	+	–	–	+	–	++	++	+	++	+	+++
5	–	–	+–	–	–	+	–	++	++	+	++	+	+–
6	–	–	+	–	–	+	–	++	++	+	++	+–	+++
7	–	–	+	–	–	+	–	+–	++	+	+–	+	+
8	–	–	+	–	–	+	–	++	+	+	++	+–	++
9	–	–	+	–	–	+	–	+	++	+–	+–	+–	+
10	–	–	+–	–	–	+	–	+	+	–	+–	–	+
Osteoarthritis													
Case 1	–	–	+–	–	–	+	–	+	++	+–	–	+–	+–
2	–	–	+–	–	–	–	–	+–	+	–	–	–	+–
3	–	–	–	–	–	–	–	+	+	–	–	–	+–
4	–	–	+–	–	–	–	–	+	++	+–	+–	–	+–
Healthy subjects													
Case 1	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–	–	–	–

LC: synovial lining cells, SLC: synovial sublining cells, EC: capillary enbothelial cells, MNC: mononuclear cells
*; (–) : negative staining, (+–): faint staining, (+): positive staining, (++): very bright staining
**; (–) : no synovial proliferation, (+–): faint synovial proliferation, (+): mild synovial proliferation
(++): moderate synovial proliferation, (+++): severe synovial proliferation

Discussion

In the synovial micro-environment, multiple cell-cell and cell-extracellular matrix interactions are influenced by the local release of soluble mediators. The attachment of T cells to endothelial cells leads to their subsequent migration between the endothelial cells into the surrounding tissue, and is critically important. Studies suggest that at least three molecular pathways of adhesion mediate the binding of T-cells to human umbilical vein endothelial cells in culture (HUVEC), LFA-1 integrin mediates the adhesion of lymphocytes to HUVEC by binding its ligand, ICAM-1 (10, 24, 25). Various studies suggest that the adhesion of T-cells to HUVEC is also mediated by an LFA-1-independent mechanism, as has been confirmed by the identification of two LFA-1-independent pathways: one pathway is mediated by the inducible vascular cell adhesion molecule 1 (VCAM-1); second is the lymphocyte molecule that binds VCAM-1 has been identified as of VLA-4 integrin, which also mediates adhesion to the connecting segment 1 (CS-1) of fibronectin (26, 27); third is the inducible endothelial cell surface protein ELAM-1, which also mediates the adheion of granulocytes to activated HUVEC (16-19). The ELAM-1 pathway is now thought to be important in the initial attachment of memory T cells to the inflamed endothelium and in the preferential migration of memory T cells into tissues and inflammatory sites (14, 20).

We have shown that synovial tissues from patients with RA demonstrate the intensive expression of ICAM-1 and ELAM-1 on vascular endothelial cells in the synovial sublining layer. This finding agrees with a previous report showing that ELAM-1 has endothelial reactivity, being present mainly on the venules and capillaries, and staining more blood vessels in RA than in OA (28). VCAM-1 is present on the synovial tissue endothelial cells of venules, capillaries, and arterioles in both RA and OA (28, 29). Like ELAM-1, VCAM-1 was present more often on the vascular endothelial cells in RA than OA tissues (28, 29).

There is increasing evidence that the adhesion of T-cell to endothelial cells can be regulated in several ways. First, the activation of endothelial cells can be regulated by inflammatory cytokines such as IL-1β and TNF-α , resulting in a rapid increase in the expression of ICAM-1 and the induction of the expression of both VCAM-1 and ELAM-1 on the surface of endothelial cells (30, 31). Because IL-1 and TNF-α are found in both the synovial fluid and tissues of patients with RA and, to a lesser extent, in those with OA (32, 33). They may induce the expression of adhesion molecules on vascular endothelial cells. Thus, adhesion molecules are expressed mainly on endothelial cells in the synovial tissues of patients with RA and, to a lesser extent, of those with OA.

Our study showed that infiltrated mononuclear cells had an enhanced expression of LFA-1α , VLA-4α and VLA-5α molecules. Synovium from the OA patients contained a lower expression of VLA-4α and VLA-5α molecules

than did the RA synovium. The infiltrated mononuclear cells that intensively expressed these adhesion molecules were present around the vascular endothelial cells that expressed the ICAM-1 and ELAM-1. We previously demonstrated that the T cells that infiltrate the synovium of RA patients are dominantly memory T cells which bear the markers of CD45RO and CD29 (34). Circulating memory T cells reportedly have an increased expression of adhesion molecules, and have greater ability to adhere to HUVEC, than the naive cells that bear CD45RA molecules (35, 36). Furthermore, mononuclear cells from the RA synovium show activated markers such as HLA-DR and CD26, and an enhanced expression of adhesion molecules including LFA-1α, VLA-4α and VLA-5α as compared with the circulating mononuclear cells (37). These observations indicate that the circulating activated T cells, especially the memory T cells had an increased expression of adhesion molecules, resulting in the migration of T-cells through the endothelium into the site of joint inflammation.

We found that the intensity of the expression of adhesion molecule was paralled generalized disease activity and the localized proliferation of synovial cells. Walters et al (38) and Rooney et al (39) described a decrease in the number of lymphocyte in the synovial membrane associated with an improvement in the variables that reflected disease activity during treatment with slow-acting anti-rheumatic drugs (SAARD). The improvement in RA during gold treatment is accompanied by a reduction in the numder of synovial blood vessels that express ELAM-1 (40). We previously demonstrated that lobenzarit disodium and bucillamine inhibit the adhesion of T cell to HUVEC in vitro (41, 42). The change in expression of adhesion molecules may be secondary to a decreased production of such inflammatory cytokines as IL-1β and TNF-α, or may instead be a direct effect on the vascular endothelial cells in the inflamed joint.

Positive staining with-anti-ICAM-1 monoclonal antibody was detected on the lining cells and the sublining cells in the synovium from RA patients and, to a lesser extent, in the synovium from OA patients. Synovial cells from these patients intensely express VCAM-1 as well as ICAM-1 molecules (28, 29). The expression of both molecules on the fibroblast like synovial cells is induced by the exposure to proinflammatory cytokines including IL-1β, TNF-α and IFN-γ (29). The cytokines are synergistic in this respect. We (43) and other investigators (44) demonstrated previously that the stimulation of synovial cells with cytokine also increases the binding of circulating T cells. Furthermore, the memory T cells bearing CD45RO and CD29 are more adhesive to the IL-1β-stimulated synovial cells than the naive T cells bearing CD45RA. The mean fluorescent intensities of LFA-1 and CD2 molecules on adherent T cell population are significantly exceeded those on nonadherent T-cell population. These findings agree with those showing that the memory T-cell population have an enhanced expression of such adhesion molecules as LFA-1, CD2, VLA-4 and VLA-5 as compared with naive T-cell population (12). The adhesion of T cells to cytokine-activated synovial cells is partially inhibited by anti-LFA-1, anti-ICAM-1, anti-VCAM-1, anti-VLA-4 and CS-1 peptide from alternatively spliced fibronectin, another VLA-4 ligand (29, 43, 44). These results suggest that the binding of T cells to cytokine-activated synovial cells is mediated via several pathways such as ICAM-1/LFA-1, VCAM-1/VLA-4, and fibronectin/VLA-4.

We also demonstrated that VLA-4α was expressed on the majority of infiltrated mononuclear cells in RA synovium, and to a lesser degree, on the synovial sublining cells. In contrast to VLA-4α, the VLA-5 positive staining was present diffusely on the synovial lining cells, sublining cells, vascular endothelial cells and infiltrated mononuclear cells. It has been reported that VLA-5α is expressed on the synovial cells, vascular endothelial cells and infiltrated mononuclear cells (45), which suggests that the VLA expressed on these cells may interact with the extracellular matrix fibronectin. However, the precise role of VLA-5 expression on synovial cells nad endothelial cells remains to be clarified.

In summary, vascular endothelial cells in the sublining layer from RA synovium expressed ICAM-1 and ELAM-1. These expression of these adhesion molecules appeared to be correlated with disease activity and synovial proliferation. The infiltrated mononuclear cells had an increased expression of LFA-1, VLA-4, VLA-5 and ICAM-1. Synovial lining cells and sublining cells were stained by anti-ICAM-1 and anti-VLA-5 monoclonal antibodies. Adhesion molecules thus appear to play an important role in the migration of lymphocytes into synovial tissues, and in the interaction between lymphocytes and synovial cells. This may result in the activations and stimulation of inflammatory response in the RA synovium.

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