Interleukin 4 increases human synovial cell expression of VCAM-1 and T cell binding

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Abstract

Objective—The effects were studied of interleukin 4 (IL-4) on T cell-synovial cell adhesion and on the expression of adhesion molecules on the surface of synovial fibroblast-like cells.

Methods—The adhesion of T cells toward the synovial cells were measured by ⁵¹chromium-labelled adhesion assay. The expression of adhesion molecules on synovial cells were analysed by flowcytometry.

Results—Stimulation of synovial cells with IL-4 increased T cell-synovial cells adhesion in a time- and dose-dependent manner. IL-4 considerably enhanced the expression of VCAM-1 on the surface of synovial cells, but not the expression of ICAM-1 and ELAM-1. The combination of IL-1 β and IL-4 had no effect on the expression of ICAM-1 or VCAM-1 on the surface of synovial cells. The increased adhesion of T cells to IL-4 stimulated synovial cells was inhibited significantly by adding anti-VCAM-1 or anti-CD29 monoclonal antibody. Furthermore, anti-VLA-4 α or the combination of anti-VLA-4 α and anti-VCAM-1 antibodies blocked completely T-cell binding to IL-4 stimulated synovial cells.

Conclusions—These results suggest that the increased adhesion of T cells to IL-4-stimulated synovial cells is mediated by VLA-4/VCAM-1 pathway.

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The inflammatory cells in the synovium and synovial fluid in rheumatoid arthritis (RA) are thought to pass through the vasculature from the peripheral blood. The mononuclear cells that are recruited continually to the synovium may engage in cellular interactions that lead to the proliferation of T cells, production of autoantibodies and secretion of proinflammatory mediators such as cytokines.¹ The persistence of the synovitis leads to destruction of cartilage and bone.^{2,3} The cellular interactions among the infiltrating lymphoid cells, cells of the monocyte/macrophage series, synovial cells, vascular endothelial cells, and extracellular matrix may play an important role in the initiating and perpetuating autoimmune processes in the synovium of patients with RA.⁴

Adhesion molecules play a fundamental role in many diverse immunological functions,

especially antigen presentation, recognition of target cells by effector cells, and migration and retention of mononuclear cells. Many interactions that are mediated by adhesion molecules and their ligands may also mediate signal transduction leading to cellular activation or proliferation.⁵

Recent interest in the synovium from RA patients has focused on the possible role of adhesion molecules.^{6–9} Vascular cell adhesion molecule 1 (VCAM-1) was originally identified on cytokine-treated endothelial cells.¹⁰ The ligand for VCAM-1 is the integrin very late antigen 4 (VLA-4),¹¹ a heterodimer comprised of α 4- and β 1-chains, that is expressed on T cells and monocytes but not on neutrophils.¹² VCAM-1 is now known to be present on dendritic cells.¹³ VCAM-1 is also implicated in CD3 dependent T cell proliferation, in conjunction with VLA-4,¹⁴ and in B cell adherence to dendritic cells.¹⁵ Increased expression of VCAM-1 has been demonstrated in synovial tissue from RA patients.^{6–9,15} Recently, bright VCAM-1 staining has been detected on synovial intimal fibroblast-like cells, vascular wall cells outside the endothelial layer, scattered stromal cells with cytoplasmic processes, and cells resembling follicular dendritic reticulum cells in lymphoid aggregates with germinal centres. Some macrophages of the synovial intima show weak VCAM-1 staining. Endothelial cell staining was seen but it was consistently weaker than the staining of vascular wall cells.^{7,8}

T cell interactions with fibroblast-like cells in RA synovium have been observed by electron microscopy. Mitogen-activated peripheral blood T cells, but not fresh peripheral blood T cells, are reported to bind to synovial fibroblasts in vitro.¹⁷ We demonstrated previously that stimulation of synovial fibroblasts with interferon γ (IFN- γ) or interleukin 1 β (IL-1 β) considerably increases the binding of peripheral T cells to synovial fibroblasts.¹⁸

Interleukin 4 (IL-4), a T cell-derived lymphokine, was initially described as B cell stimulating factor 1 (BSF-1¹⁹), but has been found to have a variety of effects on other haematopoietic and nonhaematopoietic cells.^{20–22} It has been reported that IL-4 induces the VCAM-1 expression on human umbilical endothelium cells (HUVEC).²³

The present study was performed to further characterise the effect of IL-4 on synovial fibroblast-like cell activation by studying its action on T-cell adhesion and on the expression of adhesion molecules.

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Materials and methods

PATIENTS AND HEALTHY SUBJECTS

Synovium was provided by the Department of Orthopaedics, National Ureshino Hospital. It was obtained from six patients with RA [one man and five women, mean (SD) age 55 (12) years] at the time of surgery for total knee replacement. All of the patients fulfilled the American College of Rheumatology criteria for RA.²⁴ In some of the experiments, synovial cells from patients with osteoarthritis (OA) were used. Peripheral blood samples were obtained from 12 healthy subjects [six men and six women, mean (SD) age 30 (5) years].

SEPARATION OF T CELLS FROM PERIPHERAL BLOOD

Mononuclear cells were isolated from heparinized blood of healthy donors by Ficoll-Conray gradient centrifugation (Daiichi Pharmaceutical Co, Tokyo, Japan). These cells were depleted of adherent cells by incubating the cell suspensions in Petri dishes (Falcon 3003, Becton Dickinson, Oxnard, CA) for two hours at 37°C. A T-cell-enriched population was prepared from the non-adherent cells by rosetting with 5% sheep red blood cells (SRBC, Nippon Biotest Laboratory, Tokyo, Japan). In each experiment, T cell purity was determined with anti-CD2, anti-CD20 and anti-MY4 monoclonal antibodies (Coulter Immunology, Hialeah, FL), which react, respectively, with mature T cells, pan B cells and monocytes. T-cell rich populations were comprised of more than 95% T cells, less than 1% B cells and less than 1% monocytes.

PREPARATION OF SYNOVIAL CELLS

The method used to prepare synovial fibroblasts has been described elsewhere.²⁵ In brief, the synovial tissue was trimmed of fat, minced with scissors, and then added to a mixture of collagenase (Sigma Chemical Co, St Louis, MO) and dispase (Godo Shusei Co, Tokyo, Japan). The tissue mixture was digested for 15 minutes during gentle stirring at 37°C. The suspension was then passed through a nylon mesh and the resulting cells were allowed to adhere to Petri dishes (Falcon 3003) by culturing for 18 hours at 37°C in RPMI 1640 supplemented with 10% fetal bovine serum (FBS, GIBCO, Gland Island, NY). To eliminate nonadherent cells, the plated cells were washed extensively with phosphate-buffered saline solution (PBS). The synovial cells were then cultured with RPMI 1640 supplemented with 10% FBS at 37°C in a humidified atmosphere at 5% CO₂ in air. The resulting synovial cells were used after their third or fourth passage for the following experiments.

SYNOVIAL CELL MONOLAYER ADHESION ASSAY

The method used to study adhesion of T cells to synovial cells has been described elsewhere.²⁶ In brief, synovial cells (2×10^4 /well) were cultured in quadruplicate in 96-well flat-

bottomed microtitre plates (Costar, Cambridge, MA) for 24 hours with various concentrations or recombinant interleukin 4 (rIL-4, Ono Pharmaceutical Co, Osaka, Japan) or recombinant interleukin 1 β (rIL-1 β , Otsuka Pharmaceutical Co, Tokushima, Japan). After incubation, each synovial cell monolayer was washed thoroughly with RPMI 1640 containing 5% FBS. T cells were radiolabelled with Na₂ ⁵¹Cr O₄ (Amersham International, Amersham, UK) at 37°C for two hours with occasional shaking, then were washed three times with RPMI 1640 containing 5% FBS. The washed cells were resuspended at 1×10^6 /ml in RPMI 1640 supplemented with 10% FBS. Chromium-labelled T cells (2×10^5 /200 μ l) were added to the synovial cell monolayer; this mixture was incubated for two hours at 37°C in a humidified atmosphere at 5% CO₂ in air. The microtitre plates were then washed five times to remove nonadherent T cells. The adherent T cells were lysed by adding 200 μ l of a 1% solution of polyoxyethylene octylphenyl ether. The data were expressed as percentage bound T cells, determined according to the following formula:

$$\text{Percent bound T cells} = \frac{\text{cpm in lysate}}{\text{cpm in original T cell suspension}} \times 100$$

In some experiments, monoclonal antibodies to adhesion molecules were added to the synovial cell monolayer adhesion assay culture and incubated for two hours at 37°C in a humidified atmosphere at 5% CO₂ in air.

MONOCLONAL ANTIBODIES

The murine monoclonal antibody 25.3.1, a mouse IgG1 directed against the α -chain (CD11a) of LFA-1, was purchased from Immunotech SA (Marseille, France). Anti-CD29 (4B4), a mouse IgG1 directed against the β -chain of the integrin VLA, was a gift from Dr Chikao Morimoto, Division of Tumor Immunology, Dana-Faber Cancer Institute, Boston, MA. The monoclonal antibody HP 2/1, a mouse IgG1 directed against the $\alpha 4$ subunit (CD49d) of VLA-4, was purchased from Immunotech SA. The monoclonal antibody 84H10, a mouse IgG1 directed against ICAM-1 (CD54), was also purchased from Immunotech SA. The monoclonal antibody BBA-1 is a mouse IgG specific for ELAM-1, and both BBA-5 and BBA-6 (mouse IgG1) bind VCAM-1. These monoclonal antibodies were purchased from Biotec G, Abingdon, Oxon, UK.

INDIRECT IMMUNOFLUORESCENCE METHOD

The expression of adhesion molecules by synovial cells was examined by an indirect immunofluorescence technique. Synovial cells (3×10^5 /2 ml) were cultured in 6-well plates (Costar) with various amounts of rIL-4 at 37°C in humidified 5% CO₂ in air. After incubation, the cells were washed twice with PBS without calcium or magnesium and then incubated with 0.02% EDTA solution for six minutes at 4°C. The synovial cells were detached from the plates using a rubber policeman and an

autopipetter. The resulting cells were washed with ice-cold PBS containing 1% FBS and 0.1% NaN₃, and resuspended to 1×10^6 /ml. To prevent nonspecific binding of Fc fragments to the Fc receptor on synovial cells, the cells were incubated with human gamma-globulin for 30 minutes at 4°C. The cells were washed twice and then allowed to react with monoclonal antibodies for 30 minutes at 4°C. After washing, FITC-conjugated affinity-purified goat IgG F(ab')₂ anti-mouse Ig (Zymed, San Francisco, CA) was added to each sample. Cell number and mean fluorescence intensity were measured with a flow cytometer (FACScan, Becton Dickinson, Mountain View, CA). Mouse IgG (Tago Inc, Burlingame, CA) was used as a control. Forward and right angle scatter gates were set for synovial cells, and fluorescence signals were acquired using a log scale.

STATISTICS

Statistical analysis was performed using Student's *t* test or the paired *t* test, and any $p < 0.05$ was considered significant.

Results

EFFECTS OF IL-4 ON T-CELL BINDING TO HUMAN SYNOVIAL CELLS

At first we investigated whether rIL-4 increases T-cell binding to synovial fibroblast-like cells. The kinetics of rIL-4-induced T-cell-synovial cell adhesion are shown in fig 1A. When synovial cells from patients with RA were cultured with 250 U/ml of rIL-4 for various time periods up to 96 hours, the percentage of T cells that adhered to the synovial cells increased significantly within three hours of culture. This percentage reached a maximum at 24 hours of culture and was maintained through 96 hours of culture. As a result of this finding, T-cell binding to synovial cells was determined in subsequent experiments at 24 hours of culture. The percentage of peripheral blood T-cell binding to untreated synovial cells was $7 \pm 1\%$.

Next, synovial cells were cultured with various amounts of rIL-4, and T-cell binding to synovial cells was detected at 24 hours of culture. Ten U/ml of rIL-4 enhanced significantly T-cell binding to synovial cells (fig 1B). Maximum binding occurred with 50 U/ml of rIL-4 and was maintained up to 1000 U/ml of rIL-4. In addition, T-cell binding to synovial cells from two patients with OA was similar to that of patients with RA. To determine whether autologous T cells adhere to cells like allogeneic T cells, T cells were isolated from RA patients and normal individuals. There was no significant difference between autologous and allogeneic T-cell binding to rIL-4 treated synovial cells (data not shown). We therefore used allogeneic T cells in the following experiments.

We then investigated whether IL-4-stimulated T cells bind more strongly to synovial cells pretreated with rIL-4. T cells isolated from peripheral blood were cultured

Figure 1 Effects of rIL-4 on T-cell adhesion to synovial fibroblast-like cells. (A) the kinetics of T-cell adhesion to rIL-4 treated synovial cells. Synovial cells were pretreated with 250 U/ml of rIL-4 for 0 to 96 hours. After incubation, the synovial cells were washed and incubated with ⁵¹Cr-labelled T-cells for 2 hours. Percentage T-cell binding to synovial cells was determined as described in Materials and methods. Each point represents the mean of three individual experiments. Bars depict mean (SD).

* $p < 0.01$ v percentage T-cell binding to unstimulated synovial cells.

** $p < 0.001$ v percentage T-cell binding to unstimulated synovial cells; (B) T-cell adhesion to synovial cells pretreated with various concentrations of rIL-4. Synovial cells were cultured for 24 hours with 0 to 1000 U/ml of rIL-4. The data are the mean (SD) percentage of three individual experiments. Bars depict mean (SD).

* $p < 0.01$ v percent T-cell binding to unstimulated synovial cells.

** $p < 0.001$ v percent T-cell binding to unstimulated synovial cells.

with or without rIL-4 for five hours. After incubation, the T cells were added to synovial cell monolayers pretreated or not with rIL-4. As shown in fig 2, treatment of T cells with rIL-4 did not increase T cell adhesion to unstimulated synovial cells, and did not increase T-cell adhesion to IL-4-stimulated synovial cells. This finding demonstrated clearly that T-cell binding to synovial cells is increased by pretreatment of synovial cells, but not T-cells, with IL-4.

EXPRESSION OF ADHESION MOLECULES ON SYNOVIAL CELLS PRETREATED WITH IL-4

The treatment of synovial cells with IL-4 increased the percentage of T-cell binding to synovial cells. We tried to discover therefore which adhesion molecules on synovial cells mediate the increased T-cell binding to IL-4-stimulated synovial cells. Synovial fibroblast-like cells were stimulated with 200 U/ml of rIL-4 for various time periods. The surface expression of three adhesion molecules, ICAM-

Figure 2 Binding of T cells pretreated with rIL-4 to synovial cells treated with rIL-4. The synovial cells were preincubated for 24 hours with or without 200 U/ml of rIL-4. T cells isolated from peripheral blood were also preincubated for five hours in the presence or absence of 200 U/ml rIL-4. After incubation, T cells pretreated with or without rIL-4 were added to synovial fibroblast-like cells and the mixture was incubated for two hours. Percentage T-cell binding to synovial cells was determined using the ^{51}Cr -labelled adhesion assay. The data represent the mean (SD) of three experiments.

* $p < 0.01$ v percentage T-cell binding to untreated synovial cells.

** $p < 0.01$ v percentage rIL-4 pretreated T cell binding to unstimulated synovial cells.

1, VCAM-1 and ELAM-1 was measured by flow cytometry. Unstimulated synovial fibroblast-like cells expressed constitutively the ICAM-1 molecule, but not ELAM-1 molecule. A small percentage of synovial fibroblast like cells usually expressed VCAM-1 on their surface. IL-4 enhanced significantly VCAM-1 expression by synovial cells at three hours of culture. Figure 3 shows a representative histogram of VCAM-1 expression on IL-4-stimulated synovial cells. The intensity of VCAM-1 expression on synovial cells stimulated by rIL-4 increased in a time-dependent manner. Maximum expression was attained after eight hours of synovial cell stimulation with rIL-4 and was maintained for at least 36 hours. In contrast to VCAM-1, rIL-4 had no effect on the expression of either ICAM-1 or ELAM-1 molecules (fig 4).

We then studied the dose-dependency of IL-4 on the expression of adhesion molecules. Synovial cells were stimulated for 18 hours with various concentrations up to 1000 U/ml of rIL-4. The increased VCAM-1 expression was observed at the concentration of 1 U/ml rIL-4. Peak expression was attained at the concentration of 10 U/ml and was maintained at the concentration of 1000 U/ml rIL-4 (fig 5).

COMBINED IL-1 β AND IL-4 INDUCTION OF ICAM AND VCAM EXPRESSION ON SYNOVIAL CELLS
We reported previously that stimulation of synovial cells with IL-1 β increases ICAM-1 expression and T cell synovial cell adhesion.¹⁸

Figure 3 IL-4 treatment of fibroblast-like cell increases VCAM-1 expression. Fibroblast-like cells were cultured for 24 hours with control medium alone or control medium containing IL-4. Cell number and fluorescence intensity were measured with FACScan. Mouse IgG control (A). VCAM-1 expression on fibroblast-like cells cultured with control medium alone (B) or control medium containing IL-4 (C).

The present study was therefore performed to investigate synergy between cytokines in the induction of adhesion molecule expression by human synovial cells. The synovial cells were stimulated with 10 U/ml of rIL-1 β in the presence or absence of rIL-4. As shown in table, rIL-1 β increased the intensity of ICAM-1, but not VCAM-1 expression on synovial cells. Stimulation of synovial cells with rIL-1 did not enhance ICAM-1 expression by IL-1 β -stimulated synovial cells. Furthermore, there was neither a synergistic nor additive effect on VCAM-1 expression by synovial cells when IL-1 β and IL-4 were used in combination.

EFFECTS OF MONOCLONAL ANTIBODIES SPECIFIC FOR ADHESION MOLECULES ON T CELL-SYNOVIAL CELL ADHESION
Stimulation of human synovial fibroblast-like cells with IL-4 enhanced their expression of

Figure 4 Expression of adhesion molecules on synovial cells stimulated with rIL-4. The synovial fibroblast-like cells were stimulated with 250 U/ml of rIL-4 for various time periods. After incubation, the cells were collected using an autopipetter, and then treated with monoclonal antibodies to ICAM-1, VCAM-1 and ELAM-1. The mean fluorescence intensity was determined using a flow cytometer. Background staining was determined using an isotype-matched irrelevant monoclonal antibody. Mean fluorescence intensity is shown on the ordinate, and incubation time is shown on the abscissa. The data are the mean of three individual experiments. ■—■ expression of ICAM-1; ●—● expression of VCAM-1; ○—○ expression of ELAM-1; □ control.

VCAM-1 and increased their adherence to T-cells. We therefore tested the ability of antibodies against ICAM-1 (84H10), VCAM-1 (BBA6), VLA-β (CD29) or VLA-4α (HP 2/1) to inhibit T cell-synovial cell adhesion. As shown in fig 6, stimulation of synovial cells with rIL-4 considerably increased the binding of T cells to the synovial cells. Anti-CD26 (4EL) and anti-LFA-1α monoclonal antibodies did not inhibit T cell-synovial cell adhesion. Anti-ICAM-1 (84H10) monoclonal antibody reduced slightly T-cell binding to synovial cells, but this effect was not significant. T cell-synovial cell adhesion was considerably blocked by anti-VCAM-1 and anti-CD29 monoclonal antibodies. The blocking of T cell-synovial cell adhesion was shown to be about 75%. Furthermore, either anti-VLA-4α or combination of anti-VLA-4α and anti-VLA-β

Figure 5 Expression of adhesion molecules on synovial cells treated with IL-4. Synovial fibroblast-like cells were incubated for 18 hours with various amounts of rIL-4. The expression of adhesion molecules on the synovial cells was analysed with a flow cytometer. Bars represent the mean (SD) of three individual experiments. Mean fluorescence intensity is shown on the ordinate, and the concentration of rIL-4 is shown on the abscissa. ■—■ expression of ICAM-1; ●—● expression of VCAM-1; ○—○ expression of ELAM-1; □ control.

Combined effect of IL-1β and IL-4 on ICAM-1 and VCAM-1 expression by synovial fibroblast-like cells

Cytokine	rIL-4	Mean fluorescence intensity ICAM-1	Mean fluorescence intensity VCAM-1
—	—	13.5	5.2
10 U/ml	—	39.2	5.4
—	10 U/ml	14.3	12.0
10	10	37.5	10.4
10	50	38.5	10.1
10	100	42.2	10.1
10	250	38.8	10.5

(CD29) could block completely the binding of T cells to synovial cells.

Discussion

IL-4 is a T-cell-derived lymphokine with a variety of biological activities.²⁰⁻²² This cytokine, originally described as a B cell stimulating factor 1 (BSF-1), can cause IgM-stimulated B cells to progress from the G1 phase of the cell cycle to the S phase.¹⁹ IL-4 has subsequently been shown to stimulate B cell proliferation,¹⁹ IgG₁ and IgE production, and class II MHC antigen expression on B cells.^{27 28} IL-4 effects on T cells include enhanced proliferation and increased survival. Receptors for IL-4 have been detected on a variety of haematopoietic cells, fibroblasts and epithelial cells.²⁰⁻²² Although IL-4 may enhance lymphocyte function, this cytokine has complex and conflicting effects on several monocyte/macrophage functions. IL-4 increases T-cell adhesion to human umbilical vein endothelial cells (HUVEC) and induces VCAM-1 expression by HUVEC.^{23 29} In our experiments, IL-4 increased rapidly T-cell adhesion to the synovial fibroblasts as it increased VCAM-1 expression on the surface of synovial fibroblasts.

Adhesion molecules are not only involved extensively in cell adhesion, but are also

Figure 6 Inhibition of T-cell binding to IL-4-stimulated synovial cells by monoclonal antibodies to adhesion molecules. Synovial fibroblast-like cells were treated with or without rIL-4 (250 IU/ml) for 18 hours. The synovial cell monolayer adhesion assay was performed in the presence of anti-LFA-1α (CD11a, 25.3.1), anti-ICAM-1 (84H10), anti-VCAM-1 (BBA6), anti-CD29 (4B4), anti-VLA-4α (CD49d, HP 2/1) or anti-CD26 (4EL) as the control antibody. Chromium labelled T cells were preincubated with monoclonal antibodies for 30 minutes at room temperature and added directly to the previously washed synovial cell monolayers which were preincubated with or without monoclonal antibodies. Experiments were performed in triplicate, and the results expressed as the mean (SD) of T-cell binding to human synovial cells. *p < 0.001 v percentage T-cell binding to untreated synovial cells.

important in transduction of signals which activate cells or cause them to proliferate.^{30, 31} The molecules involved in these interactions are divided into four major superfamilies based on their molecular characteristics: the integrin family, the immunoglobulin (Ig) supergene family, the selectin family and the H-CAM family (CD44).³² A wide variety of adhesion-dependent interactions of lymphocytes, both in antigen-dependent and -independent processes, are mediated through interactions of LFA-1 (CD11a/CD18) with ICAM-1, and LFA-2 (CD2) with LFA-3.³³ Those interactions include T-cell interactions with antigen-presenting cells, cytotoxic T cell-mediated killing, and lymphocyte binding to the endothelium. A second adhesion pathway involving VLA-4 binding to VCAM-1 and the CS-1 peptide from alternatively spliced fibronectin has been described.^{10, 11, 34} VLA-4, which is comprised of the $\alpha 4$ (VLA-4 α) and β_1 (CD29) subunits of the integrin family, is expressed on a variety of cell types, including lymphocytes, monocytes and eosinophils, but not neutrophils.¹² These molecules may mediate lymphocyte adhesion to endothelial and antigen-presenting cells.³⁵⁻³⁷ A third pathway through sialyl Le^x and sialyl Le^a with ELAM-1 can mediate adhesion of neutrophils, monocytes, and lymphocytes to the endothelial cells.^{38, 39}

Using a range of double-labelling techniques, bright VCAM-1 staining has been detected on vascular wall cells outside on the endothelial layer. These cells may belong to a variety of nonendothelial resident populations such as smooth muscle cells and pericytes, and play an important role in the behaviour of lymphocytes subsequent to the initial phase of trans-endothelial migration.^{7, 8} The bright VCAM-1 staining is shown on synovial intimal fibroblast-like cells, scattered stromal cells with cytoplasmic processes and cells resembling follicular dendritic reticulum cells in lymphoid aggregates with germinal centres.^{7, 8, 14}

Large lymphocyte aggregates developing in rheumatoid synovial tissues mimic the development of lymphoid follicles in terms of the central accumulation of B cells and the presence of VCAM-1 -positive cells.⁸ The lymphoid follicles provide a specialised micro environment for the induction of antibody production, affinity maturation and memory B cell formation.⁴⁰ These adhesion molecules probably play a critical role in cellular interactions among B cells, T cells and VCAM-1-positive cells in the lymphoid follicles.

We studied adhesion molecule expression on cultured synovial cells, which are derived presumably from fibroblast-like cells. ICAM-1 was constitutively expressed by synovial cells but ELAM-1 was not expressed on resting synovial cells. The expression of ICAM-1 and ELAM-1 on the cultured synovial cells did not enhance by addition of IL-4. It was reported that a small percentage of both RA- and OA-derived synovial fibroblasts (3rd to 9th passage) expresses VCAM-1 on the surface.⁶ In our experiment, synovial cells from RA and OA synovium expressed constitutively VCAM-1 on their surface, but the staining is weak.

We demonstrated that stimulation of synovial fibroblast with IL-4 increased T cell-synovial cell adhesion as it induced VCAM-1 expression by synovial fibroblast. The kinetics of increasing T-cell adhesiveness and induction of VCAM-1 on IL-4-stimulated synovial cells were similar to those reported for IL-4-stimulated HUVEC. However, IL-4 is shown to regulate the expression of adhesion molecules on HUVEC already activated by IL-1, TNF, or IFN- γ .²⁹ In our experiments, the combination of IL-4 and IL-1 β did not affect the expression of ICAM-1 and VCAM-1 on the surface of synovial fibroblast.

The present study demonstrated that IL-4 enhanced T-cell binding to synovial fibroblast. Blocking studies demonstrated clearly that addition of the anti-VCAM-1 monoclonal antibody decreased weakly the adhesion of T cells to IL-4-stimulated synovial cells. To our surprise, anti-VLA-4 α monoclonal antibody blocked completely T cell binding to IL-4-stimulated synovial cells. The CS-1 peptide in an alternatively spliced form of fibronectin, like VCAM-1, is a ligand for VLA.³⁴ This molecule is expressed in synovial lining cells and on blood vessels in RA synovium.¹¹ These results suggest that T-cell adhesion to IL-4-stimulated synovial cells is mediated through the VLA-4/fibronectin pathway and the VLA-4/VCAM-1 pathway.

While much has been written regarding the role of the VLA-4/VCAM-1 pathway in recruiting lymphocytes into the synovium,³⁶ less is known about its potential role in retaining cells and in the interactions between lymphocytes and synovial cells. We demonstrated previously that memory cells bearing CD45RO and CD29 antigens are more adherent to IL-1 β -stimulated synovial cells than are naive T cells bearing the CD45RA antigen.¹⁸ The intensity of adhesion molecule expression on adherent T cells is significantly higher than on nonadherent T-cells. These results are in accordance with findings that the memory T-cells have enhanced expression of adhesion molecules including LFA-1, CD2, VLA-4 and VLA-5 when compared with naive T-cells.³⁷ The infiltrated T cells, which are predominantly memory T cells, express an abundance of these adhesion molecules compared with peripheral blood T cells.⁴¹

Taken together, the present data suggest that T-cell adhesion to synovial cells is mediated through the LFA-1/ICAM-1, VLA-4/VCAM-1, and VLA-4/fibronectin pathways. These interactions between lymphocytes and synovial cells may play an important role in activating and perpetuating immune responses or in retaining inflammatory cells in the RA synovium.

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