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Cross-Linking of CD44 on Rheumatoid Synovial Cells Up-Regulates VCAM-1

Koichi Fujii,* Yoshiya Tanaka,2* Stefan Hubscher,† Kazuyoshi Saito,* Toshiyuki Ota,† and Sumiya Eto*†

CD44 is a ubiquitous molecule also known as hyaluronic acid or homing receptor. However, the cellular functions and its role in inflammation, for example, rheumatoid synovitis, are currently unknown. In this study, we propose a novel function for CD44. Using synovial cells from rheumatoid arthritis (RA) patients, we demonstrated that CD44 cross-linking and binding to hyaluronan augmented VCAM-1 expression and subsequently VCAM-1-mediated cell adhesion. Briefly, we found that 1) rheumatoid synovial cells highly expressed CD44; 2) cross-linking of CD44 markedly but transiently augmented VCAM-1 expression and its mRNA transcription much more than did IL-1β and TNF-α; 3) hyaluronan, especially when fragmented, also up-regulated VCAM-1; 4) CD44 activated the transcription factor AP-1; and 5) the integrin-dependent adhesive function of RA synovial cells to T cells was also amplified by CD44 cross-linking. These results indicate that the adhesion of RA synovial cells to matrices such as hyaluronan acid through CD44 could up-regulate VCAM-1 expression and VCAM-1-mediated adhesion to T cells, which might in turn cause activation of T cells and synovial cells in RA synovitis. We therefore propose that such cross-talking among distinct adhesion molecules may be involved in the pathogenesis of inflammation, including RA synovitis. The Journal of Immunology, 1999, 162: 2391–2398.

Inflammation is characterized by the accumulation of leukocytes and other mesenchymal cells in the extravascular space in response to multiple stimuli. Adhesion molecules play a fundamental role in inflammatory processes by mediating leukocyte-endothelial cell adhesion, leukocyte migration, and T cell/APC interactions. Ig superfamily members are involved in cell adhesion in many cell types, and on endothelial cells, several Ig family members, including ICAM-1, ICAM-2, ICAM-3, and VCAM-1, are recognized by leukocyte integrins such as LFA-1 and VLA-4 (1–3). VCAM-1 is highly inducible by several inflammatory cytokines on endothelial cells and restricted subsets of APCs, compared with a lack of such induction by resting endothelial cells and noninflammatory tissues. VCAM-1 thereby plays a pivotal role in lymphocyte migration/infiltration and lymphocyte activation by APC through its binding to the ligand VLA-4 and/or α6β1 in inflammatory processes (4, 5).

The expression of VCAM-1 is tightly regulated by locally produced inflammatory cytokines such as IL-1β, and TNF-α (4, 5). However, recent findings have indicated that certain adhesion molecules not only function as a glue, but also regulate several cellular functions by transducing signaling. The best-known molecules are LFA-1, VLA-4, CD2, CD28, and CD154, which induce costimulatory signals in T cell/APC binding via multiple cellular signaling molecules, including focal adhesion kinases, resulting in cell activation and cytokine production (6–8). We have reported previously that the engagement of CD31 on T cell subsets amplified integrin-dependent adhesion to endothelial ligands, and also that ICAM-1 cross-linking on rheumatoid synovial cells induced IL-1β transcription by activation of a nuclear factor, AP-1 (9, 10). Thus, in the new concept, adhesion molecules transduce certain activation stimuli independent of cytokine stimulation. This has prompted us to investigate whether stimulation of adhesion molecules also up-regulates other adhesion molecule expression, especially VCAM-1. From a survey of cross-linking of multiple adhesion molecules, including LFA-1, VLA-4, ICAM-1, and CD44, on rheumatoid synovial cells (a typical model of inflammation), we found that one such molecule, CD44, was unique in its remarkable up-regulation of VCAM-1 expression on synovial cells and also of their adhesion to T cells.

CD44 is a 90-kDa transmembrane glycoprotein widely distributed on T lymphocytes, granulocytes, monocytes, fibroblasts, keratinocytes, and epithelial cells (11). The principal known ligand of CD44 is hyaluronan (12). CD44 plays a major role in multiple physiologic functions, including cell-cell adhesion, cell-substrate interaction, lymphocyte recruitment to inflammatory sites, and tumor metastasis (13–16). Recently, the function of CD44 as a signaling molecule has also been demonstrated. Stimulation of CD44 with mAbs or hyaluronan transmits the signal into the cells, which leads to activation of T cells and cytokine or chemokine release from monocytes (17, 18). CD44 is overexpressed in inflammatory sites in proportion to the intensity of inflammation, implicating CD44 in the pathogenesis of inflammation (19).

This study demonstrates a role for CD44 and the potent ligand hyaluronan in VCAM-1 induction on rheumatoid synovial cells through activation of a nuclear transcription factor AP-1. We propose a model for the involvement of adhesion molecules per se (in...
**this study, CD44), in the induction or amplification of other adhesion molecules (in this study, VCAM-1) on the same cell and the subsequent mediation of cellular adhesion in inflammatory processes.**

### Materials and Methods

#### Synovial tissues and culture of synoviocytes

Synovial tissues were obtained from patients with active rheumatoid arthritis (RA) and osteoarthritis (OA), diagnosed according to the criteria of the American College of Rheumatology, (Atlanta, GA), who were treated by joint replacement surgery or synovectomy. The synovial membrane samples were snap frozen and later stained for immunohistochemical studies. Samples were also dissected under sterile conditions in PBS, and immediately prepared for culture of fibroblast-like synoviocytes. Briefly, the tissue sample was minced into small pieces and digested with collagenase (Sigma Aldrich Japan, Tokyo) in serum-free DMEM (Life Technologies, Grand Island, NY). After filtering through a nylon mesh, the cells were extensively washed and suspended in DMEM, supplemented with 10% FCS (Bio-Pro, Karlsruhe, Germany) and penicillin-streptomycin (10 U/ml; Sigma Aldrich). Finally, isolated cells were seeded in 25-cm² culture flasks (Falcon, Lincoln Park, NJ) and cultured in a humidified 5% carbon dioxide atmosphere. After overnight culture, nonadherent cells were removed, and further incubation of adherent cells was continued in fresh medium. At confluence, the cells were trypsinized, passaged at a 1:3 split ratio, and recultured. The medium was changed twice each week, and the cells were used after three to seven passages.

#### Reagents and mAbs

IL-1β, IL-6, and TNF-α (Cosmobio, Tokyo, Japan) were purchased. Fragmented and native hyaluronan were kindly donated by the Tokyo Research Institute of Seikagaku. The following mAbs were used as purified Igs: MHC class I (mAb W6/32), CD14 (mAb 63D3), anti-glycophorin (mAb 8G12), ICAM-1 (mAb LFA-1a), ICAM-2 (mAb TS1/22), ICAM-3 (mAb 3B1), MHC class II (mAb IVA12), CD16 (mAb 3G8), CD19 (mAb FMC63, from Dr. H. Zola, Bedford Park, Australia), CD106 (mAb 2392), CD11a (mAb 64D2), CD11b (mAb 34B12), CD44 (mAb 2D7, Dr. W. Newman, Rockville, MD), CD45 (mAb 304), ICAM-1 (mAb IV 35), VCAM-1 (mAb VA/L-14), and CD3 (mAb 176). Highly purified T cells were prepared from PBMC of healthy volunteers by exhaustive immunomagnetic negative selection, as previously described (9, 20, 21). We initially characterized cultured synovial cells derived from the synovial membrane of RA and OA patients. The cells were stimulated in 0.3 ml of buffer II with protease inhibitors (20 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 300 mM KCl, 0.1 mM EGTA, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT, and 0.5 mM PMSF) and gently rocked on a platform at 4°C for 30 min. After 30-min microcentrifugation at 4°C, supernatants were dialyzed against 50 vol of buffer III (20 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 300 mM KCl, 0.1 mM EGTA, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT, and 0.5 mM PMSF) and gently rocked on a platform at 4°C for 30 min. After 30-min microcentrifugation at 4°C, supernatants were dialyzed against 50 vol of buffer III (20 mM HEPES (pH 7.9), 100 mM KCl, 0.2 mM EDTA, 20% glycerol, 0.5 mM DTT, and 0.5 mM PMSF) for 24 h at 4°C. After microcentrifugation for 30 min at 4°C, protein concentrations in the supernatants were measured using the Bradford assay and stored at –80°C. EMSA was performed essentially as previously described. Briefly, 3 μg of nuclear extracts was preincubated for 20 min at room temperature in 15 μl of a buffer (10 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1 mM MgCl₂, 4% glycerol, and 40 mM NaCl) containing 0.5 μg of poly(dI–dC) and an oligonucleotide containing AP-1 or NF-κB binding sites (Promega, Madison, WI), which was labeled with T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Consensus oligonucleotides of AP-1 or NF-κB were used as cold competitors.

#### Immunohistochemistry of synovial tissue

Synovial tissue samples were obtained after total joint replacement from five patients diagnosed with active RA according to the criteria of the American College of Rheumatology, and from five patients with OA as controls. Tissues were snap frozen and later stained by a three-stage alkaline phosphatase/antialkaline phosphatase (APAAP) method with CD44 mAb and CD3 mAb.

#### Northern blot analysis

For Northern blot analysis, total cellular RNA was isolated from cultured RA synovial cells by a single-step isolation procedure. The RNA (10 μg) was electrophoresed through a 1% agarose gel and blotted onto nylon filters (Hybond N, Amersham, Arlington Heights, IL). VCAM-1 cDNA and β-actin cDNA (donated by Drs. A. Kitani and T. Matsuyama, Kagoshima, Japan) were labeled with [32P]dCTP (DuPont NEN, Boston, MA), and Northern blot analysis was subsequently performed.

#### Preparation of nuclear extracts and electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared by a previously reported method with some modifications. After harvest, 1 × 10⁶ cells were washed with cold PBS, and incubated on ice for 15 min in 5 ml of buffer I (10 mM HEPES (pH 7.9), 4°C, 1.5 mM MgCl₂, 10 mM KCl, 0.3 M sucrose, 0.1 mM EGTA, 0.5 mM DTT, and 0.5 mM PMSF containing 1 μg/ml of each of the protease inhibitors (antipain, aprotinin, chymostatin, leupeptin, and pepstatin A)). After centrifugation, the cells were resuspended in 1 ml of buffer I with protease inhibitors and then Dounce homogenized (20 strokes; Kontes, Vineland, NJ). The homogenate was microcentrifuged for 30 s, and nuclei were resuspended in 0.3 ml of buffer II with protease inhibitors (20 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 300 mM KCl, 0.1 mM EGTA, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT, and 0.5 mM PMSF) and gently rocked on a platform at 4°C for 30 min. After 30-min microcentrifugation at 4°C, supernatants were dialyzed against 50 vol of buffer III (20 mM HEPES (pH 7.9), 100 mM KCl, 0.2 mM EDTA, 20% glycerol, 0.5 mM DTT, and 0.5 mM PMSF) for 5 h at 4°C. After microcentrifugation for 30 min at 4°C, protein concentrations in the supernatants were measured using the Bradford assay and stored at –80°C. EMSA was performed essentially as previously described. Briefly, 3 μg of nuclear extracts was preincubated for 20 min at room temperature in 15 μl of a buffer (10 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1 mM MgCl₂, 4% glycerol, and 40 mM NaCl) containing 0.5 μg of poly(dI–dC) and an oligonucleotide containing AP-1 or NF-κB binding sites (Promega, Madison, WI), which was labeled with T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Consensus oligonucleotides of AP-1 or NF-κB were used as cold competitors.

#### Adhesion assay

The adhesion assay was performed as previously described (9, 20). Briefly, synovial cells were applied to 24-well culture plates (Nunc, Roskilde, Denmark) and cultured in DMEM (Life Technologies) with 10% heat-inactivated FCS. T cells were labeled with sodium ¹⁵¹Cr (DuPont NEN). A total of 1 × 10⁶ T cells in 1 mM PMA (Sigma Aldrich) in the presence or absence of relevant blocking mAb (10 μg/ml) was added, and the cells were incubated at 37°C for 30 min in PBS with 0.5% human serum albumin: nonadherent cells were washed off completely. Well contents were lysed with 1% Triton X-100 (Sigma Aldrich), and gamma emissions of adherent wells were determined.

#### Results

**RA synovial cells highly expressed CD44**

We initially characterized cultured synovial cells derived from the synovium of RA patients. The cells were spindle shaped and grew in a cobblestone pattern. Flow-cytometric analysis of these cells indicated that they lacked macrophage markers such as MHC class II Ags, CD14 and CD11b, but spontaneously expressed CD44 and perivascular areas in RA tissue (22). Immunohistochemical studies showed that CD44 was strongly expressed on synoviocytes

**Flow microfluorometry**

Staining and flow-cytometric analysis of synovial cells were conducted by standard procedures, as already described, using a FACScan (Becton Dickson, Mountain View, CA). Briefly, cells (2 × 10⁶) were incubated with specific mAbs and subsequent FITC-conjugated anti-goat IgG Ab or FITC-conjugated CD106 (VCAM-1) mAb at saturating concentrations in FACS medium consisting of HBSS (Nissui, Tokyo, Japan), 0.5% human serum albumin (Bio-Rad, Osaka, Japan), and 0.2% NaN₃ (Sigma Aldrich) for 30 min at 4°C. After three washes in FACS medium, the cells were analyzed with FACScan. Amplification of the mAb binding was provided by a three-decade logarithmic amplifier. Quantification of the cell surface Ags on one cell was performed using beads, QIFKIT (Dako Japan, Kyoto).
and weakly on the vessels of RA, whereas synoviocytes of OA only scarcely expressed CD44 (Fig. 2, A and B). Furthermore, a marked accumulation of mononuclear cells, most of which were CD3-positive T cells, was observed in RA synovium (Fig. 2C). These results suggest that the predominant difference in CD44 expression between active RA and OA is observed on synoviocytes, and that CD44-positive cells are seen in areas rich in T cells in RA synovium.

Cross-linking of CD44 on RA synovial cells up-regulated the expression of VCAM-1

To characterize the function of CD44, we assayed the cell surface molecule expression effects of CD44 cross-linking using a specific mAb and second cross-linker Ab. Flow cytometry showed that VCAM-1 was markedly induced or augmented by the CD44 cross-linking on synovial cells. As shown in Figs. 3 and 4, VCAM-1 was only slightly expressed on nonstimulated synovial cells. However, CD44 cross-linking significantly up-regulated VCAM-1 expression, whereas cross-linking of control anti-VLA-4 mAb had no effect. The results were consistent in five patients with RA. Time-course experiments showed that VCAM-1 expression on RA synovial cells reached maximum levels (approximately a 10-fold increase compared with nonstimulated synovial cells), within 6 h of CD44 cross-linking, but the expression returned to almost basal levels after 24 h of incubation (Fig. 5A). The results indicate that the CD44 cross-linking caused a marked but transient amplification of VCAM-1 expression on RA synovial cells.

Cross-linking of CD44 triggered VCAM-1 mRNA transcription

CD44 cross-linking also induced transcription of VCAM-1 mRNA in RA synovial cells. After CD44 cross-linking, RNA was extracted from RA synovial cells, and specific mRNA was detected by Northern blot analysis using primers specific for human VCAM-1. RA synovial cells slightly expressed VCAM-1 mRNA without stimulation. CD44 cross-linking markedly augmented VCAM-1 mRNA transcription, which was maximal within 2 h of stimulation, but subsequently gradually diminished (Fig. 5B).

CD44 was the most potent stimulator of VCAM-1 expression among surface molecules and cytokines

In the next series of experiments, we compared the magnitude of CD44-induced VCAM-1 expression with that induced by other stimuli, including several cytokines and cell surface molecules expressed on RA synovial cells. When ICAM-1, LFA-1, or VLA-4 Ag was cross-linked by their specific mAb and second cross-linker Ab, the expression of VCAM-1 on RA synovial cells was unchanged or slightly increased, whereas CD44 cross-linking markedly augmented VCAM-1 expression (Fig. 6A). It is noteworthy that multiple inflammatory cytokines such as IL-1β and TNF-α, which are well known to exist in high concentrations in RA synovium and to be involved in the pathogenesis of RA synovitis, had much less effect on VCAM-1 expression than CD44 cross-linking at 6 h of stimulation of RA synovial cells (Fig. 6B). Up-regulation of VCAM-1 induced by these cytokines took 24–48 h and remained still slight level at 6-h stimulation by cytokines (data not shown). All of the kinetic studies were reproducible among three different RA patients. Thus, CD44 stimulation and cytokine stimulation require a clearly different kinetics, namely CD44 functions much faster than cytokines, when they induce VCAM-1 expression. These results suggest that CD44 appears to play a pivotal role in the induction of VCAM-1 on the cell surface.
VCAM-1 expression was stimulated by fragmented hyaluronan

Hyaluronic acids are major ligands for cell surface CD44. We assessed the biological activity of hyaluronan on VCAM-1 expression on RA synovial cells. As shown in Fig. 7, soluble full-length hyaluronan markedly augmented VCAM-1 expression. Fragmented hyaluronan is thought to be more relevant to inflammation including RA synovitis. Of several hyaluronan fragments, the 6.9-kDa fragment had the most marked effect on VCAM-1 expression, approximately twice the effect of native hyaluronan. VCAM-1 up-regulation by hyaluronan also reached maximum level within 6 h. This suggests that hyaluronan, especially when fragmented, is a possible ligand involved in CD44-mediated VCAM-1 expression on RA synovial cells.

AP-1 activation triggered by cross-linking of CD44

We also examined the nuclear transcription factors involved in CD44-mediated signaling by EMSA using a probe containing a palindromic AP-1 and NF-κB binding element. Nuclear extracts were prepared from RA synovial cells 60 min after CD44 cross-linking. As shown in Fig. 8, RA synovial cells exhibited low basal binding to AP-1 and NF-κB oligonucleotides. However, after CD44 cross-linking, the intensity of the complex of nuclear extracts with AP-1 oligonucleotides, but not with NF-κB, was markedly enhanced, which was completely competed away by excess amounts of unlabeled AP-1 oligonucleotide. Furthermore, mAb specific for c-jun produced clearly defined supershift complex in AP-1/DNA complex induced by CD44 stimulation (Fig. 8). These results were reproducible in three separate experiments. This result suggests that CD44 cross-linking results in a specific activation of the transcription factor AP-1.
Finally, we investigated whether CD44-induced VCAM-1 up-regulation on RA synovial cells was involved in their adhesion to T cells using three different RA patients. RA synovial cells adhered to highly purified peripheral PMA-stimulated T cells without any stimuli. However, stimulation of synovial cells by CD44 cross-linking for 12 h resulted in doubling the adhesion rate relative to control (Fig. 9). Adhesion of both nonstimulated and CD44-cross-linked RA synovial cells to PMA-activated T cells was significantly inhibited by either anti-VLA-4 or anti-LFA-1 mAbs alone or in combination. However, there was an interesting and consistent tendency for the anti-LFA-1 mAb to inhibit adhesion of nonstimulated synovial cells better than the anti-VLA-4 mAb, whereas the anti-VLA-4 mAb more effectively reduced the adhesion of CD44-stimulated synovial cells. Thus, the adhesion of synovial cells to T cells is mediated by integrins LFA-1 and VLA-4, but the VLA-4/VCAM-1 pathway appeared to be centrally involved in CD44-stimulated RA synovial cells.

Discussion

Adhesion molecules are fundamental to mechanisms of normal immunity and also contribute to the pathogenesis of autoimmune diseases. Definition of the molecular basis of cellular adhesion and its importance in cell-cell and cell-matrix interactions has progressed during the last decade. The expression and function of

FIGURE 6. VCAM-1 up-regulation by cross-linking of adhesion molecules and cytokine stimulation. RA synovial cells were cross-linked with 10 μg/ml of the indicated mAb (A) or stimulated with 20 ng/ml of the indicated cytokine (B) for 6 h. VCAM-1 expression was analyzed by FACScan. Data are expressed from a representative experiment of three RA patients.

FIGURE 7. VCAM-1 up-regulation by fragmented hyaluronan stimulation. RA synovial cells were incubated with 100 ng/ml of fragmented hyaluronan for 6 h. The molecular mass of hyaluronan fragments were 1.7 kDa, 6.9 kDa, 40 kDa, and native. VCAM-1 expression was determined by FACScan. Data are expressed from a representative experiment of three RA patients.

FIGURE 8. EMSA for determination of transcription factors activated by cross-linking of CD44. RA synovial cells were incubated with control medium (lanes A and F), or cross-linked with anti-CD44 mAb (lanes B–E and G–J) for 1 h, and nuclear extracts were collected. Nuclear extracts were incubated with labeled AP-1 (lanes A–E) or labeled NF-κB (lanes F–J) oligonucleotide in the presence of unlabeled AP-1 (lane C) or NF-κB (lane H) oligonucleotide for cold competition and mAbs specific for c-fos (lane D), c-jun (lane E), p50 (lane I), and p65 (lane J) for supershift analysis.

FIGURE 9. Adhesion assay of CD44-cross-linked synovial cells and PMA-activated T cells. RA synovial cells were incubated with control medium (open bars), or cross-linked with CD44 mAb (solid bars) or MHC class I mAb (hatched bars) for 12 h, and sodium 51Cr-labeled T cells were added in the presence or absence of the indicated blocking mAb. Data are representative of four different experiments.
adhesion molecules are tightly regulated by several cellular stimuli. Cytokines are potent inducers of adhesion molecule expression. Among them, IL-1β or TNF-α, which are abundantly produced at inflammatory sites, contributes to the up-regulation of Ig-superfamily adhesion molecules, such as ICAM-1 and VCAM-1 (23). However, on the basis of the results presented in the current study, we propose a new concept, that stimulation of the adhesion molecule CD44 per se plays a pivotal role in the regulation of VCAM-1 expression. We deduce this from the following novel findings: 1) CD44 cross-linking on RA synovial fibroblast-like cells up-regulated VCAM-1 expression, more and faster than did stimulation with inflammatory cytokines including IL-1β and TNF-α; 2) fragmented hyaluronan effectively increased VCAM-1 expression compared with native hyaluronan; 3) integrin-dependent adhesion of RA synovial cells to PMA-activated T cells was markedly increased by the CD44 cross-linking on synovial cells; and 4) CD44 stimulation activated the nuclear transcription factor AP-1.

The fact that stimulation of one adhesion molecule up-regulated another prompted us to investigate how this phenomenon might contribute to the inflammatory process. We used RA synovial fibroblast-like cells, because RA synovium is one of the most typical models of inflammation, and because CD44 is predominantly expressed on RA synovial cells and plays a major role in the pathogenesis of rheumatoid synovitis (19, 24). CD44 is found on the surface of most leukocytes, fibroblasts, keratinocytes, and epithelial cells, and is implicated in multiple physiologic cellular functions, including cell-cell and cell-matrix adhesion, lymphocyte homing, and tumor metastasis, by binding to its ligand (16, 24–27). However, the function of CD44 and relevance to the pathogenesis of inflammation are unknown at present. Recent reports suggest that CD44 functions not just as an adhesion molecule, but also as a signaling molecule. For instance, T cell activation and cytokine release from monocytes are induced by the regulation of VCAM-1 compared with native hyaluronan. These results demonstrate that hyaluronan is far from an inert space filler, but has an important role in biological activities, such as regulation of adhesion molecules, and that degraded products of the extracellular matrix at inflammatory sites are as important as cytokines in sustaining the inflammatory process. Our results also demonstrated that CD44 cross-linking by anti-CD44 mAb was more effective in VCAM-1 up-regulation than hyaluronan stimulation. Recent reports suggest that epitope 1 of CD44 (NIH 44-1 mAb binding site) is more effectively involved in CD44-induced cell-cell adhesion than the epitope 2 (hyaluronan binding site) (42, 43). The different ability in VCAM-1 up-regulation between CD44 mAb and hyaluronan might result from the difference of stimulated epitope.

Adhesion molecules play a fundamental role in many diverse immunologic functions, especially Ag presentation, recognition of target cells by effector cells, migration and retention of mononuclear cells, and signal transduction leading to cellular activation or proliferation (17, 19, 25, 44–46). VCAM-1 was originally reported to be expressed on endothelium, but we and others have shown that VCAM-1 is also expressed on dendritic cells and activated macrophages, and that cellular adhesion mediated by VCAM-1 and ICAM-1 is involved in signal transduction in the immune system (47, 48). Increased expression of VCAM-1 has been demonstrated in synovial tissue from RA patients: 1) VCAM-1 is highly 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 centers; 2) there is a marked accumulation of T cells in the perivascular area around VCAM-1-positive synoviocytes, including both fibroblast-like and dendritic-like cells; and 3) the prominent distinctive feature of the cells identified from a localized region of pannus in and around large cartilage erosions from RA patients is their abundant surface display of VCAM-1 (49). These findings indicate that the adhesion of T cells to synoviocytes or endothelium through VCAM-1 and its ligand VLA-4, which is a major T cell integrin, could contribute to the activation of interacting cells (both T cells and synoviocytes) and further release of cytokines and degradative enzymes from them. Furthermore, CD44 is characteristically expressed on fibroblast-like and dendritic-like cells as well as endothelium in rheumatoid synovium (19).

The concomitant expression of CD44 and VCAM-1 in the inflammatory pannus in RA and our current report that the stimulation of CD44 by cross-linking and ligation by fragmented hyaluronan up-regulated VCAM-1 expression on rheumatoid fibroblast-like cells and increased cell adhesion, implicate CD44 in an important role in synovial cells. Taken together, these results implicate CD44 in the pathogenesis of rheumatoid synovial inflammation and articular bone and cartilage destruction, which are characterized by the accumulation and proliferation of T cells and synovial cells and the abundant production of cytokines and degradative enzymes. The VCAM-1 gene promoter possesses binding sites for two major nuclear transcription factors, NF-κB and AP-1 (50). Initiation of VCAM-1 mRNA transcription induced by IL-1β and TNF-α has been reported to result from the activation of NF-κB (51–53). However, we observed that CD44 stimulation activated AP-1, but not NF-κB, in nuclear extracts of RA synovial cells, and confirmed this by EMSA. Thus, CD44 and these cytokines appear to differentially activate distinctive transcription factors leading to transcription of VCAM-1 mRNA. Furthermore, a clear difference of kinetics of VCAM-1 up-regulation was observed between CD44 stimulation and cytokine stimulation, namely its expression reached maximum level within 6 h by CD44 stimulation, whereas its up-regulation by cytokines took at least 24 h, indicating that CD44 may play a pivotal role in VCAM-1 expression on synoviocytes.
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