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Effect of cytokine-induced soluble ICAM-1 from human synovial cells on synovial cell–lymphocyte adhesion

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SUMMARY

The present study was designed to establish (i) the effects of cytokines on soluble ICAM-1 (sICAM-1) production by human synovial cells (SC) and ICAM-1 expression on these cells, and (ii) the effects of sICAM-1 on lymphocyte–SC adhesion. sICAM-1 production was enhanced in parallel with ICAM-1 expression by IL-1β, TNF-α and IFN-γ. IL-4 showed no effects on ICAM-1 expression. In contrast with the transient elevation of cell-associated ICAM-1 by IL-1β, which peaked 36 h after stimulation and declined thereafter, sICAM-1 continued to accumulate in culture supernatants even after 48 h. Purified sICAM-1 was obtained from a 48 h culture synovial cell supernatant by affinity chromatography using ICAM-1 monoclonal antibody. The purified sICAM-1 significantly inhibited adhesion of lymphocytes and monocytes to cytokine-stimulated synovial cells. These results suggest that sICAM-1 may modulate chronic synovitis by inhibiting ICAM-1-mediated cell-to-cell adhesion.

Keywords synovial cells soluble ICAM-1 IL-1β interferon-gamma lymphocyte adhesion

INTRODUCTION

A primary event in the immune response to infectious agents is the recruitment of circulating inflammatory cells to inflammatory sites. Adhesion of these cells to the endothelium is a prerequisite step for margination and extravasation to the perivascular site. Cell surface adhesion molecules are thought to play an important role in establishing the intercellular contacts that are essential for various immunological reactions [1]. It has been shown that cytokines stimulate lymphocyte–endothelial cell (EC) adhesion, through the interaction of adhesion molecules [2,3]. One of these adhesion molecules expressed on EC is intercellular adhesion molecule-1 (ICAM-1) (CD54). ICAM-1 is a cell surface glycoprotein which has a molecular weight of 90 000 and which is composed of five Ig-like extracellular domains, a hydrophobic transmembrane domain, and a short cytoplasmic domain [4]. ICAM-1 is constitutively expressed or can be induced on a wide range of cells of haematopoietic and non-haematopoietic lineages such as fibroblasts, keratinocytes and melanoma cells by the pro-inflammatory cytokines, interferon-gamma (IFN-γ), IL-1β and tumour necrosis factor-α (TNF-α) [5,6]. ICAM-1 plays a role in lymphocyte migration to inflammatory sites through interaction with its ligand lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18) or Mac-1 (CD11b/CD18). Recently it has been shown that neutrophils may also interact with EC through the LFA-1/ICAM-1 adhesion [7].

T lymphocytes accumulate in the rheumatoid synovium as a prominent feature of the chronic inflammatory lesion which leads to the degradation of cartilage and bone [8,9]. The mechanisms by which T lymphocytes are recruited to the synovium involve the enhanced expression of adhesion molecules on leucocytes [10–12] and non-immune cells [13–15]. The involvement of adhesion molecules in the pathogenesis of RA, moreover, has received much attention as a crucial component in promoting and exacerbating immune-mediated chronic inflammation [16–20]. It has been demonstrated that human synovial cells stimulated with IL-1β, TNF-α, IFN-γ, or combinations of these cytokines, show increased expression of adhesion molecules such as ICAM-1 [21]. VCAM-1 is expressed on synovial cells [19] and its expression is enhanced by TNF-α [22]. The adhesion event in T lymphocyte–synovial cell interaction that is of particular interest is the ICAM-1 (synovial cells) and LFA-1 (T lymphocytes) interaction.

Recently, a soluble form of ICAM-1 (sICAM-1) has been demonstrated in considerable amounts in normal human serum, and sICAM-1 is increased in the serum of patients with leucocyte adhesion deficiency disease [23]. sICAM-1 has an apparent molecular weight by size exclusion chromatography of approximately 80 000 [23]. It not only binds LFA-1 but also blocks rhinovirus infection in which ICAM-1 acts as the cellular receptor for the virus [24]. Mononuclear cells and EC are thought to be sources of sICAM-1 [25,26]. Leeuwenberg *et al.*

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[27] have shown that sICAM-1 and soluble E-selectin (ELAM-1) are released from EC after activation by IL-1 or TNF-α, and that the amount of soluble adhesion molecules released was directly correlated with cell surface expression. Maximal release of E-selectin was observed 6–12 h after activation and decreased to non-detectable levels 24 h after activation, while release of ICAM-1 gradually increased with ICAM-1 cell surface expression, and reached a plateau after 24 h, which remained constant for 3 days [27]. Newman *et al.* [28], on the other hand, have shown that E-selectin was first detectable in supernatants of IL-1-stimulated EC at 24 h and increased slowly up until 72 h [28]. sICAM-1 is increased in concentration in the sera and synovial fluids of patients with RA [29,30]. sICAM-1 levels in RA sera correlate with the erythrocyte sedimentation rate (ESR), joint score [31] and the presence of vasculitis [29]. In another report correlation with ESR was not observed [30]. Comparison of sICAM-1 levels in serum and synovial fluid in RA patients has yielded variable results [30,31].

In the present study, we identified and quantified sICAM-1 in culture supernatants of synovial cells and investigated the mechanisms of sICAM-1 production by synovial cells by analysing the effects of various cytokines on this process. The effect of sICAM-1 purified by affinity chromatography from cell-free supernatants obtained from a 48 h culture of synovial cells in IL-1β on lymphocyte–synovial cell adhesion was also studied.

MATERIALS AND METHODS

Preparation of synovial cells

Synovial cells were cultured as previously described [32,33]. The synovial tissue obtained from RA patients at the time of knee replacement was minced and enzymatically dissociated using 0·2% collagenase (from *Clostridium histolyticum*, type 1; Sigma, St Louis, MO) and 0·2% trypsin (from porcine pancreas; Chiba, Ichikawa, Japan). The enzyme-dissociated cells suspended in RPMI 1640 supplemented with antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin, 0·25 ng/ml amphotericin B (Gibco, Grand Island, NY), 2 mM L-glutamine (Gibco) and 10% fetal bovine serum (FBS; Mitsubishi Oil Chemicals, Tokyo, Japan) were cultured in 10 cm Petri dishes (Falcon). The synovial cells proliferated rapidly and the dishes were stripped with trypsin for subculture. Cells taken at the second and third subcultures were used in this study.

Cytokines

Recombinant human IL-1β (2×10^7 U/mg, 1×10^6 U/ml) and recombinant human IFN-γ ($1{\cdot}25 \times 10^5$ U/ml) were kindly supplied by Ohtsuka Pharmaceutical Co. Ltd (Tokushima, Japan). Recombinant human IL-6 (5×10^6 U/mg) was a kind gift from Dr Toshio Hirano (Osaka University, Osaka, Japan) [34]. Recombinant human IL-4 (1×10^5 U/ml) and recombinant human TNF-α ($3{\cdot}2 \times 10^7$ U/mg) were purchased from Genzyme Corporation (Boston, MA). Recombinant human M-CSF (8×10^6 U/ml) was kindly supplied by Midorijyuji Co. Ltd. (Osaka, Japan). The biological activity of IL-1β was estimated by the mouse thymocyte ^{3}H-thymidine incorporation assay. The biological activity in half maximal units of IL-1 is defined by the reciprocal of IL-1 dilution supporting 50% of maximal ^{3}H-thymidine incorporation. The biological activity of IL-6 was determined by using IL-6-dependent SKW6-C14 cells. One unit per ml of IL-6 induces 50% of the maximum IgM production in 1×10^4 SKW6-C14 cells [34]. Bioactivity of IL-4 was determined using the IL-4-dependent proprietary cell line and one unit of bioactivity was the amount of IL-4 required to induce half-maximal ^{3}H-TdR incorporation by 4×10^3 cells. TNF-α activity was assayed on L-929 cells in the presence of actinomycin D. One unit of TNF-α equals the amount required to induce half maximal cytotoxic activity. Bioactivity of IFN-γ was determined by the ability to induce resistance of WISH cells to infections by vesicular stomatitis virus (VSV cells). One unit was defined as the amount of IFN-γ required to provide 50% inhibition of viral replication. Units are equivalent to those of NIH human IFN-γ reference standard. The colony-stimulating activity of M-CSF was assayed using C57Bl/6N mouse bone marrow cells. One unit was defined as the amount of M-CSF which stimulated the production of one colony per 10^5 plated cells. Endotoxin level, as determined by limulus lysate assay (Seikagaku Kogyo, Tokyo, Japan), was less than 100 pg/μg of protein in each cytokine solution.

Monoclonal antibody

Mouse anti-human ICAM-1 monoclonal antibody (MoAb) (100 μg/ml) was purchased from British Bio-technology Ltd (Abingdon, UK).

ELISA methods for sICAM-1 and ICAM-1 expression

Synovial cells at confluence were removed from culture flasks with 0·125% trypsin and 1 mM EDTA, centrifuged and resuspended in RPMI 1640 containing 10% heat-inactivated FBS supplemented with antibiotics at a density of 5×10^4/ml. Aliquots of 0·1 ml were seeded into 96-well flat-bottomed microtitre plates (Falcon, CA) previously coated with 1% gelatin and allowed to reach confluence. The medium was replaced with 0·1 ml of fresh medium. Then 0·02 ml of each cytokine or medium were added to each appropriate well to yield a final volume of 0·12 ml per well. Unless otherwise specified, IL-1β, IL-4, IL-6, TNF-α and IFN-γ were used at final concentrations of 1, 10 100 U/ml. TNF-α was also used at 1000 U/ml. M-CSF was used at concentrations of 10, 100 and 1000 U/ml. The cultures were incubated for 16 h at 37°C in 5% CO_2 in air, and the supernatants obtained by centrifugation were kept at −80°C until the sICAM-1 assay. sICAM-1 was measured by ELISA as previously described [23,25]. The cells in each well were washed and fixed with 1% glutaraldehyde for 10 min at room temperature. In a separate experiment, the cells in 96-well plates in the presence or absence of 10 U/ml IL-1β were cultured for various time intervals, and fixed at each time. After washing the fixed plates three times with RPMI, 0·1 ml of RPMI 1640 containing 2% bovine serum albumin (BSA) was added to each well to block unbound sites and incubated at 37°C for 1 h. After the blocking solution was removed, 0·05 ml of mouse anti-ICAM-1 MoAb (4 μg/ml) (0·2 μg/well) was added to each well and the plates were incubated at 37°C for 1 h. The culture wells were washed three times with RPMI 1640, and then 0·1 ml of a 1:10 000 dilution of the horseradish peroxidase-conjugated anti-mouse IgG (Cappel) in 1% BSA-RPMI 1640 was added to each well. The plates were then incubated for 1 h at 37°C, the enzyme conjugate removed and the culture plates washed four times with RPMI 1640. Then 0·1 ml of 1 : 1 mixed 3, 3′, 5, 5′-tetramethylbenzidine substrate solution (TMB, from Kirkegaard & Perry Labs, Gaithersburg,

MD) and 0·02% hydrogen peroxide in a citric acid buffer (0·1 M, pH 5·0, Kirkegaard & Perry Labs) were added to each well and the plate was incubated at 37°C. Appropriate controls were included in each assay. These included omitting the first antibody and/or the second antibody. The wells without synovial cells were also used as controls. The absorbance at 450 nm was measured using an ELISA reader (Wako, Japan) at 10-min intervals from 20 to 180 min or until maximum absorbance was obtained [5,26].

Preparation of neutrophils, monocytes and lymphocytes

Ten ml of heparinized venous blood were collected from healthy volunteers. Neutrophils were obtained as previously described [35]. Mononuclear cells obtained by density gradient centrifugation were resuspended in RPMI 1640 containing 10% FBS and antibiotics and incubated in Petri dishes (Falcon, 3002) which had been coated with FBS for 1 h at 37°C in a CO_2 incubator. Adherent cells obtained by gentle agitation and non-adherent cells gently poured from the dishes were washed with medium, resuspended in fresh medium at 1×10^6/ml, and used as monocytes and lymphocytes, respectively [36]. The percentages of monocytes and lymphocytes as judged by Giemsa and esterase staining averaged 95% and 93%, respectively.

Lymphocyte adhesion assay

For adhesion assays, synovial cells removed from the culture flask with trypsin and EDTA were resuspended in medium. Synovial cell suspension, 0·4 ml, was plated into 24-well plates and cultured until confluence was reached. Synovial cells in each well were cultured with 100 U/ml IL-1β for 16 h. At the end of incubation, cell layers were washed with complete medium before the addition of 0·5 ml of lymphocyte or monocyte suspension (1×10^6/ml), which had been pretreated with purified sICAM-1 (2 ng/ml), synovial cell conditioned medium (concentration of sICAM-1, 5·8 ng/ml) or medium alone for 45 min and washed with medium. After a co-culture period of 90 min at 37°C, non-adherent lymphocytes or monocytes were removed by gentle washing of the monolayers with medium. Quantification of adherent cells was obtained by microscopic examination of random high power fields in each well using phase contrast microscopy. Adhesion was expressed as the number of adherent cells per high power field by counting four high power fields and presented as mean ± s.d. of four separate experiments. Adhesion of neutrophils was also measured similarly.

RESULTS

Effects of cytokines on ICAM-1 expression and sICAM-1 production in synovial cells

IL-1β, 10 and 100 U/ml, induced significant expression of surface ICAM-1 and release of sICAM-1 into the cell supernatant. IL-4, 100 U/ml, significantly enhanced sICAM-1 production, but IL-4 at any concentration showed no effects on ICAM-1 expression. On the contrary, 10 and 100 U/ml IL-6 significantly enhanced ICAM-1 expression, while IL-6 at any concentration showed no effects on sICAM-1 production (Fig. 1). TNF-α (1–1000 U/ml) and 1–100 U/ml IFN-γ significantly enhanced ICAM-1 expression and sICAM-1 production. A concentration of 1000 U/ml M-CSF significantly enhanced ICAM-1 expression (Fig. 2).

Kinetics of ICAM-1 expression and sICAM-1 production in synovial cells

To investigate the kinetics of ICAM-1 expression and sICAM-1 production in synovial cells, ICAM-1 expression and

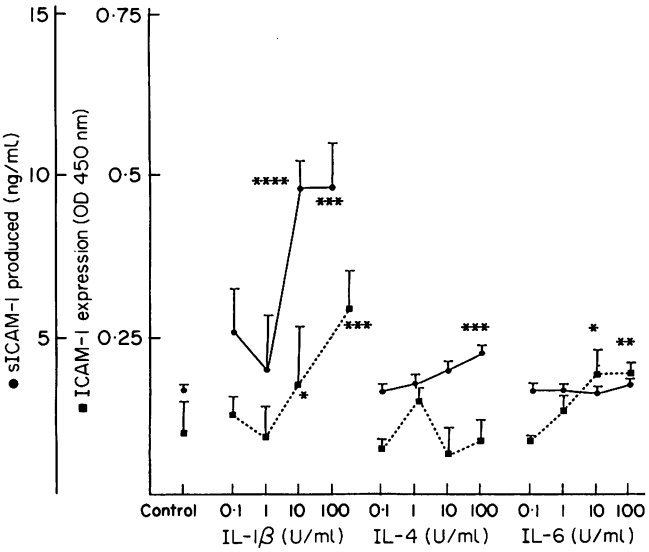

Fig. 1. Effects of IL-1β, IL-4 and IL-6 on ICAM-1 expression and sICAM-1 production by human synovial cells. Synovial cells, 0·1 ml, were plated into gelatin-coated 96-well plates and precultured. Cytokines were added to each well at the concentrations indicated and the plates were subsequently cultured for 16 h. The supernatants obtained were measured for sICAM-1 by ELISA (●). The plates were fixed with 1% glutaraldehyde, and then assayed for ICAM-1 expression (■) as in Materials and Methods. Each point is the mean and s.d. of four separate experiments. $*P < 0·05$; $**P < 0·02$; $***P < 0·01$; $****P < 0·001$ *versus* control (Student's *t*-test).

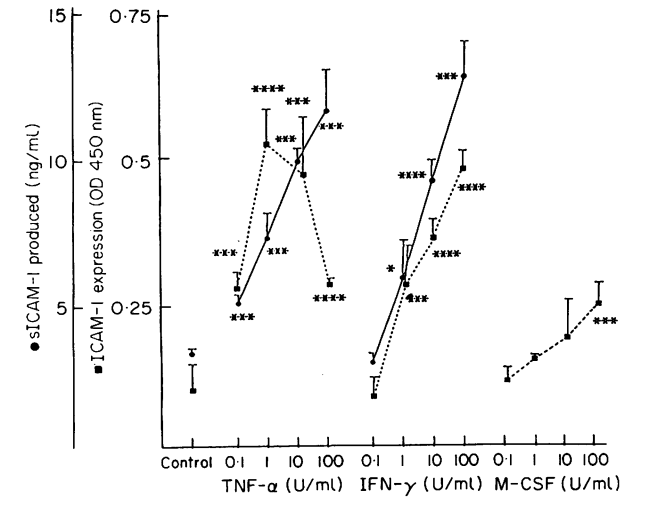

Fig. 2. Effects of TNF-α, IFN-γ and M-CSF on ICAM-1 expression and sICAM-1 production in synovial cells. Effects of TNF-α, IFN-γ, and M-CSF on ICAM-1 expression and sICAM-1 production in synovial cells were tested as in Fig. 1.

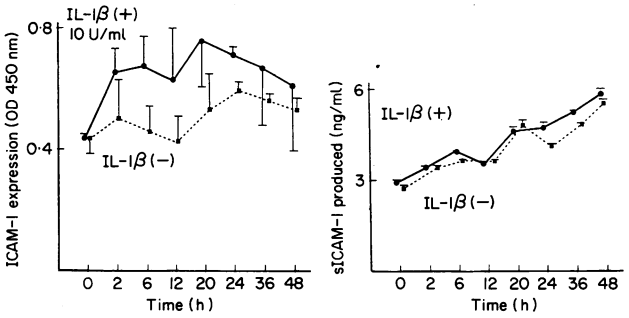

Fig. 3. Kinetics of ICAM-1 expression and sICAM-1 production by synovial cells cultured with or without IL-1β. Synovial cells were cultured in 96-well plates with or without 10 U/ml IL-1β for varying time intervals indicated. At each time period, the supernatants were obtained and the corresponding wells fixed with 1% glutaraldehyde. The supernatants were measured for sICAM-1 and the cells were quantified for ICAM-1 expression as in Fig. 1 after all wells had been fixed.

sICAM-1 production were assayed at varying time intervals. In the absence of IL-1β, ICAM-1 expression increased gradually with time, and sICAM-1 production paralleled ICAM-1 expression. On the other hand, ICAM-1 expression showed an accelerated increase, attaining a maximum at 20 h post-stimulation and declining thereafter, in the presence of IL-β. sICAM-1 production by IL-1β-stimulated synovial cells increased cumulatively in the supernatants even after 48 h post-stimulation, in contrast to a decline of ICAM-1 expression (Fig. 3).

Lymphocyte adhesion to IL-1β-stimulated synovial cells and the effect of sICAM-1 or synovial-cell-conditioned medium

Synovial cells were stimulated with 100 U/ml IL-1β for 16 h and then tested for lymphocyte adhesion after the medium was replaced with fresh medium. To confirm a possible inhibitory effect of sICAM-1 on adhesion of inflammatory cells to IL-1β-stimulated synovial cells, lymphocytes were tested for the ability to adhere to cytokine-stimulated synovial cells after the lymphocytes had been pretreated with sICAM-1 purified on an ICAM-1-MoAb-coupled Sepharose 4B column. Synovial cell conditioned medium containing sICAM-1 was also tested for its inhibitory effect on lymphocyte–synovial cell adhesion. In the presence of 2 ng/ml purified sICAM-1 or synovial cell conditioned medium (concentration of sICAM-1, 5·8 ng/ml), lymphocyte adhesion was significantly decreased compared with that in the presence of fresh medium alone ($P < 0{\cdot}01$) (Fig. 4). Mean percentage inhibition of lymphocyte adhesion was 35·6% for purified sICAM-1 and 33·8% for synovial cell conditioned medium. Similarly, the effects of purified sICAM-1 or synovial cell conditioned medium on neutrophil adhesion to cytokine-stimulated synovial cells were tested. Neutrophil adhesion to IL-1β-stimulated synovial cells was significantly suppressed in the presence of synovial cell conditioned medium but not in the presence of 2 ng/ml purified sICAM-1 (Fig. 4). Pretreatment of monocytes with purified sICAM-1 (2 ng/ml) significantly inhibited monocyte adhesion to IL-1β-stimulated synovial cells ($P < 0{\cdot}001$) (Fig. 4).

DISCUSSION

The effects of cytokines on ICAM-1 expression in synovial cells were investigated. It has already been shown that dose and time-dependent increases in class I and class II MHC antigens and ICAM-1 expression by human synovial cells occur in response to a variety of cytokines such as IL-1β, TNF-α and IFN-γ [21]. Our data are in accord with this report. In the present study, the effects of M-CSF on ICAM-1 expression in synovial cells were also studied. M-CSF showed an enhancing effect on ICAM-1 expression in synovial cells, suggesting that M-CSF which is derived from proliferated synovial microvasculature may augment synovial inflammation.

sICAM-1 was detected in supernatants of synovial cells cultured without cytokine. IL-1β, TNF-α and IFN-γ significantly enhanced sICAM-1 production but IL-6 did not show a significant effect. Interestingly, the effects of IL-1β, TNF-α and IFN-γ on ICAM-1 expression paralleled the sICAM-1 production induced by the corresponding cytokines. We have shown that ICAM-1 expression in EC was significantly enhanced by IFN-γ, whereas sICAM-1 production was not influenced by IFN-γ at all concentrations, suggesting that response of sICAM-1 production to IFN-γ is different in EC and synovial cells [26]. There were discrepancies between ICAM-1 expression and sICAM-1 production in synovial cells cultured with IL-4 or IL-6, sICAM-1 production increasing with increasing IL-4, and ICAM-1 expression increasing with increasing IL-6 concentration. The molecular mechanisms of these variable changes require clarification. In synovial cells, as previously observed with CE [26], ICAM-1 expression showed accelerated increase, attaining a maximum at 20 h after stimulation and

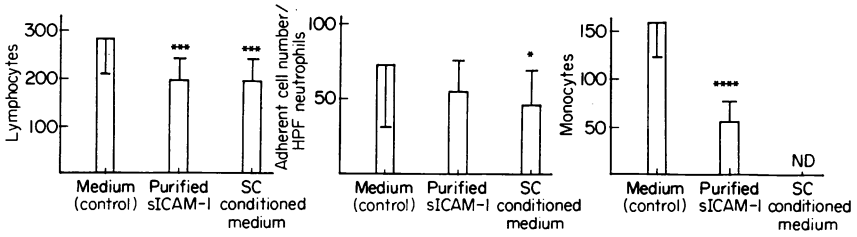

Fig. 4. Effects of purified sICAM-1 or synovial cell conditioned medium on inflammatory cell adhesion to IL-1β stimulated synovial cells. Lymphocytes, monocytes or neutrophils, which had been pretreated with purified sICAM-1, synovial cell conditioned medium or medium alone for 45 min, were added to each well or synovial cell cultures and incubated for 90 min (lymphocytes and monocytes) or 30 min (neutrophils). After the wells were gently washed three times with medium, the wells were fixed with 1% glutaraldehyde. Each point is mean and s.d. of the numbers of lymphocytes adherent to the synovial cells in each random high power field (HPF). SC, Synovial cells; ND, not done; $*P < 0{\cdot}05$; $***P < 0{\cdot}01$; $****P < 0{\cdot}001$ *versus* medium alone.

declining thereafter, and ICAM-1 production by IL-1β-stimulated synovial cells increased cumulatively in the supernatants even after 48 h after stimulation, in contrast to decline of ICAM-1 expression. These data imply that the ICAM-1 molecule is shed from the cell surface after its expression reaches a maximum level. The effects of TNF-α on ICAM-1 expression are stimulatory at lower concentration, but decrease at high concentration. At 1000 U/ml, TNF-α did not show any effect. It is possible that at such high concentration, TNF-α exerts a toxic effect on synovial cells, although no evidence of cytotoxicity was observed by phase contrast microscopy at the end of culture.

To further establish the biological function of sICAM-1, this purified sICAM-1 was tested for its ability to influence leucocyte adhesion to the IL-1β-stimulated synovial cells. Purified sICAM-1 at a final concentration of 2 ng/ml significantly inhibited adhesion of lymphocytes and monocytes. The conditioned medium of synovial cells, which contains considerable amounts of sICAM-1, significantly inhibited the adhesion of lymphocytes and neutrophils to IL-1β-stimulated synovial cells. These data strongly suggest that sICAM-1 blocks ICAM-1 ligands expressed on lymphocytes and thus inhibits lymphocyte–synovial cell adhesion through the ICAM-1/LFA-1 adhesion system. On the other hand, neutrophil–synovial cell adhesion was significantly inhibited by synovial cell conditioned medium but not by purified sICAM-1.

It is possible that the blocking effect of sICAM-1 was neutralized by ELAM-1, expressed by the stimulated synovial cells [19], interacting with its ligand sialyl Lewis X (SLX) on neutrophils [37].

Shedding of ICAM-1 from the cell surface of synovial cells may be suspected from the kinetics of the expression and production of this molecule after IL-1β treatment. It has been suggested that ICAM-1 is shed from melanoma cell lines after IFN-γ and TNF-α treatment [38]. Moreover, it has been shown that sICAM-1 produced by melanoma cells retains all, or most, of the structural features necessary for binding to LFA-1, and is potentially active, as an LFA-1 ligand [23]. Thus sICAM-1 appear to retain most of the structure and function of the extracellular portion of cell-bound ICAM-1. It seems likely that sICAM-1 is released from the cell membrane via a proteolytic cleavage of cell-bound ICAM-1. sICAM-1 release could also be the result of an alternatively spliced messenger RNA which effectively deletes the transmembrane and cytoplasmic domains. It has been shown that the soluble form of L-selectin (LECAM-1), believed to be shed from the neutrophil membrane as a result of cleavage at the cell membrane, is present in neutrophil culture supernatants [39], and that soluble E-selectin is found in supernatants of IL-1-activated EC [28], suggesting that proteolytic cleavage of the membrane form of ICAM-1 appears more likely.

In rheumatoid synovitis, enhanced expression of ICAM-1 in synovial cells and EC induced by IL-1 [21,40] and increased expression of LFA-1 on synovial tissue T lymphocytes [18,41] may play an important role in the development and exacerbation of chronic synovitis by recruitment and retention of T lymphocytes. Enhanced production of sICAM-1 may exert a modulating anti-inflammatory effect by blocking T lymphocyte–synovial cell interaction mediated by the LFA-1/ICAM-1 pathway. sICAM-1 could compete with membrane ICAM-1 on synovial cells for one or more members of the CD18 family of lymphocyte adhesion molecules, thus preventing attachment or promoting detachment, and permitting these cells to enter the recirculating pool [23]. It has been suggested also that sICAM-1 abrogates the non-MHC-restricted cytotoxicity mediated by NK and lymphokine-activated killer cells [38]. In addition, sICAM-1 inhibits rhinovirus infection [24]. Finally, it has also been suggested that sICAM-1 inhibits not only lymphocyte–EC adhesion but also T cell receptor mediated antigen recognition and co-stimulatory signalling of T cell activation [42]. Thus, sICAM-1 produced by synovial cells may play an important role in the modulation of the functions of haematopoietic cells in the inflammatory response.

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REFERENCES

1 Larson RS, Springer TA. Structure and function of leukocyte integrins. Immunol Rev 1990; **114**:181–217.
2 Cavender D, Haskard D, Foster N, Ziff M. Superinduction of T lymphocyte–endothelial cell (EC) binding by treatment of EC with interleukin 1 and protein synthesis inhibitors. J Immunol 1987; **138**:2149–54.
3 Yu CL, Haskard DO, Cavender AR, Johnson AR, Ziff M. Human gamma interferon increases the binding of T lymphocytes to endothelial cells. Clin Exp Immunol 1985; **62**:554–60.
4 Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen (LFA-1). Cell 1987; **51**:813–19.
5 Rothlein R, Czajkowski M. O'Neili MM, Marlin SD, Mainolfi E, Merluzzi VJ. Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. J Immunol 1988; **141**:1665–9.
6 Dustin ML, Singer KH, Tuck DT, Springer TA. Adhesion of T lymphocytes to epidermal keratinocytes is regulated by interferon gamma and is mediated by intercellular adhesion molecule-1 (ICAM-1). J Exp Med 1988; **167**:1323–40.
7 Smith CW, Marlin SD, Rothlein R, Toman C. Anderson DC. Cooperative interaction of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils *in vitro*. J Clin Invest 1989; **83**:2008–17.
8 Van Boxel JA, Paget SA. Predominantly T cell infiltration in rheumatoid synovial membranes. N Engl J Med 1975; **293**:517–20.
9 Morimoto C, Romain PL, Fox DA, Anderson P, Dimaggio M, Levine H, Schlossmann SF. Abnormalities of $CD4^+$ T lymphocyte subsets in inflammatory rheumatic diseases. Am J Med 1988; **84**:817–25.
10 Haskard D, Cavender D, Beatty P, Springer T, Ziff M. T lymphocyte adhesion to endothelial cells: mechanisms demonstrated by anti-LFA-1 monoconal antibodies. J Immunol 1986; **137**:2901–6.
11 Springer TA, Dustin ML, Kishimoto TK, Marlin SD. The lymphocyte function-associated LFA-1, CD-2 and LFA-3 molecules: cell adhesion receptors of the immune system. Ann Rev Immunol 1987; **5**:223–252.
12 Springer TA. Adhesion receptors of the immune system. Nature 1990 **346**:425–34.
13 Cavender D, Haskard DO, Joseph B, Ziff M. Interleukin-1 increases the binding of human T and B lymphocytes to endothelial cell monolayers. J Immunol 1986; **136**:203–7.
14 Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA.

Induction by IL-1 and interferon-gamma: tissue distribution, biochemistry, and function of natural adherence molecules (ICAM-1). J Immunol 1986; **137**:245–54.

15 Rothlein R, Czajkoski M, O'Neill MM, Marlin SD, Mainolfi E, Merluzzi VJ. Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines: regulation by pharmacologic agents and neutralizing antibodies. J Immunol 1988; **141**:1665–9.

16 Haynes BF, Grover BJ, Whichard LP, Hale LP, Nunley JA, McCollum DE, Singer KH. Synovial microenvironment–T cell interactions: human T cells bind to fibroblast like synovial cells *in vitro*. Arthritis Rheum 1988; **31**:947–55.

17 Hale LP, Martin ME, McCollum DE, Nunley JA, Springer TA. Singer KH, Haynes BF. Immunohistologic analysis of the distribution of cell adhesion molecules within the inflammatory synovial microenvironment. Arthritis Rheum 1989; **32**:22-30.

18 Haynes BF, Hale LP, Denning SM, Le PT, Singer KH. The role of leukocyte adhesion molecules in cellular interactions: implications for the pathogenesis of inflammatory synovitis. Springer Semin Immunopathol 1989; **11**:163–85.

19 Koch AE, Burrows JC, Haines GK, Carlos TM, Harlan JM, Leibovich SJ. Immunolocalization of endothelial and leukocyte adhesion molecules in human rheumatoid and osteoarthritic synovial tissues. Lab Invest 1991; **64**:313–20.

20 Oppenheimer-Marks N, Ziff M. Binding of normal human mononuclear cells to bind blood vessels in rheumatoid arthritis synovial membrane. Arthritis Rheum 1986; **29**:789–92.

21 Chin JE, Winterrowd GE, Krzesicki RF, Sanders ME. Role of cytokines in inflammatory synovitis: the coordinate regulation of intercellular adhesion molecule 1 and HLA class 1 and class II antigens in rheumatoid synovial fibroblasts. Arthritis Rheum 1990; **33**:1776–86.

22 Marlor CW, Webb DL, Bobvara MP, Greve JM, Blue ML. Expression of vascular cell adhesion molecule-1 in fibroblast like synoviocytes after stimulation with tumor necrosis factor. Am J Pathol 1992; **140**:1055–60.

23 Rothlein R, Mainolfi EA, Czajkowski M. Marlin SD. A form of circulating ICAM-1 in human serum. J Immunol 1991; **147**:3788–93.

24 Marlin SD, Staunton DE, Springer TA, Stratowa C, Sommergruber W, Merluzzi VJ. A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. Nature 1990; **344**:70–2.

25 Shingu M, Hashimoto M, Nobunaga M, Isayama T, Yasutake C, Naono T. Production of soluble ICAM-1 by mononuclear cells from patients with rheumatoid arthritis patients. Inflammation 1994; **18**:23–34.

26 Hashimoto M, Shingu M, Ezaki I, Nobunaga M, Minamihara M, Kato K, Sumioki H. Production of soluble ICAM-1 from human endothelial cells induced by IL-1β and TNF-α. Inflammation 1994; **18**:163–73.

27 Leeuwenberg JF, Smeets EF, Neefjes JJ, Schcaffer MA, Clinek T, Jeunhomme TM, Adhern TJ, Buurman WA. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. Immunology 1992; **77**:543–9.

28 Newman W, Beall LD, Carson CW, Hunder GG, Graben N, Randhawa ZI, Gopal TV, Wiener-Kronish J, Matthay MA. Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock. J Immunol 1993; **150**:644–54.

29 Hashimoto M, Shingu M, Wada T, Nobunaga M. Elevated soluble intercellular adhesion molecule-1 (sICAM-1) in sera and synovial fluids (SF) of patients with rheumatoid arthritis (RA). Arthritis Rheum 1992; **35**(Suppl):s230.

30 Mason JC, Kapahi P, Haskard DO. Detection of increases levels of circulating intercellular adhesion molecule a in some patients with rheumatoid arthritis but not in patients with systemic lupus erythematosus. Arthritis Rheum 1993; **36**:519–27.

31 Cush JJ, Rothlein R, Lindsley HB, Mainolfi EA, Lipsky PE. Increased levels of circulating intercellular adhesion molecule 1 in the sera of patients with rheumatoid arthritis. Arthritis Rheum 1993; **36**:1098–1102.

32 Dayer J-M, DeRochemoneix B, Burrus B, Demczuk S, Dinarello CA. Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by human synovial cells. J Clin Invest 1986; **77**:645–8.

33 Shingu M, Nagai Y, Isayama T, Naono T, Nobunaga M, Nagai Y. The effects of cytokines on metalloproteinase inhibitors (TIMP) and collagenase production by human chondrocytes and TIMP production by synovial cells and endothelial cells. Clin Exp Immunol 1993; **94**:145–9.

34 Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S, Nakajima K, Koyama K, Iwamatsu A, Tsunasawa S, Sakiyama F, Matsui H, Takahara Y, Taniguchi T, Kishimoto T. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature 1986; **324**:73–6.

35 Shingu M, Todoroki T, Sakai K, Nobunaga M. Suppression of Fc receptor and C4/C3 receptors of the granulocytes from patients with systemic lupus erythematosus. J Rheumatol 1981; **8**:910-16.

36 Fujii I, Shingu M, Nobunaga M. Monocyte activation in early onset rheumatoid arthritis. Ann Rheum Dis 1990; **49**:497–503.

37 Luscinskas FW, Cybulsky MI, Kiely J-M, Peckins CS, Davis VM, Gimbrone MA. Jr. Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. J Immunol 1991; **146**:1617–25.

38 Becker JC, Dummer R, Hartmann AA, Burg G, Schmidt RE. Shedding of ICAM-1 from human melanoma cell lines induced by IFN-γ and tumor necrosis factor-α. Functional consequences on cell-mediated cytotoxicity. J Immunol 1991; **147**:4398–401.

39 Kishimoto TK, Jutila MA, Berg EL, Butcher EC. Neutrophils Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. Science 1989; **245**:1238–40.

40 Abbot SE, Kaul A, Stevens CR, Blake DR. Isolation and culture of synovial microvascular endothelial cells: characterization and assessment of adhesion molecule expression. Arthritis Rheum 1992; **35**:401–6.

41 Cush JJ, Lipsky PE. Phenotypic analysis of synovial tissue and peripheral blood lymphocytes isolated from patients with rheumatoid arthritis. Arthritis Rheum 1988; **31**:1230–8.

42 Van Seventer GA, Shimizu Y, Horgan KJ, Shaw S. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. J Immunol 1990; **144**:4579–86.