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Interferon-alpha and dexamethasone inhibit adhesion of T cells to endothelial cells and synovial cells

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SUMMARY

We investigated whether interferon-gamma (IFN-γ), interferon-alpha (IFN-α) and glucocorticoids affected the adhesion of T cells to human umbilical endothelial cells or human synovial cells. About 30% of peripheral blood T cells could bind to unstimulated endothelial cells, but only a few T cells could bind to unstimulated synovial cells. When both endothelial cells and synovial cells were cultured with recombinant IFN-γ (rIFN-γ), the percentage of T cell binding to both types of cells increased in a dose-dependent manner. rIFN-α and dexamethasone blocked the T cell binding to unstimulated endothelial cells. Furthermore, rIFN-α and dexamethasone suppressed T cell binding to both endothelial cells and synovial cells stimulated by IFN-γ, and also inhibited intercellular adhesion molecule-1 (ICAM-1) expression on both endothelial cells and synovial cells stimulated by IFN-γ. These results suggest that IFN-α and glucocorticoids may inhibit T cell binding to endothelial cells or synovial cells by modulating adhesion molecule expression on these cells.

Keywords endothelial cells synovial cells adhesion molecules interferon-alpha dexamethasone

INTRODUCTION

Synovial tissue from patients with rheumatoid arthritis (RA) is characterized by pronounced hyperplasia of the synovium, generation of new blood vessels and infiltration of mononuclear cells. Cellular interactions among infiltrating mononuclear cells, vascular endothelial cells and synovial cells have demonstrated importance in the triggering and perpetuation of autoimmune processes in synovial tissues from patients with RA. Recently, the cell adhesion molecules that mediate interactions between lymphocytes and various accessory or target cells have been described [1,2]. Many of these interactions mediated by adhesion molecules and their ligands may have functional consequences for T cells as well as for the cells to which they bind. Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3) are expressed on the surface of macrophage-like type A synovial cells and synovial fibroblasts, as well as on tissue macrophages and endothelial cells [3]. The adhesion of leucocytes to vascular endothelium is the first step in their migration from the blood into inflammatory tissue. *In vitro* studies with cultured human vascular endothelial cells have shown that the adhesiveness of lymphocytes to cultured endothelial cells can be increased by IL-1 [4,5], tumour necrosis factor (TNF) [6], lipopolysaccharide (LPS) [5], interferon-gamma (IFN-γ) [7,8] and IL-4 [9]. Moreover, IFN-γ and IL-4 selectively enhance endothelial cell adhesiveness for T cells, but not for polymorphonuclear cells [9]. These data suggest that the binding of lymphocytes to endothelial cells and synovial cells may play an important role in the inflammatory processes in synovial tissue from patients with RA. If lymphocyte binding to these cells can be inhibited by cytokines and glucocorticoids, it is possible that the inflammatory response can be dampened. The present study was undertaken, therefore, to determine whether interferon-alpha (IFN-α) and dexamethasone inhibit T cell binding to either endothelial cells or synovial cells.

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MATERIALS AND METHODS

Preparation of human umbilical vein endothelial cells

Endothelial cells were obtained from human umbilical veins as described elsewhere [10]. Briefly, the umbilical vein was perfused with PBS to wash out residual blood. Both ends of the cord were clamped and then infused with 100 μg/ml collagenase (Sigma Chemical Co., St Louis, MO) in HBSS. After a 10-min

incubation at room temperature, the solution containing the detached endothelial cells was flushed out with PBS, after which it was centrifuged and the cells were suspended in RPMI 1640 supplemented with 20% heat-inactivated fetal bovine serum (FBS; GIBCO, Grand Island, NY), 25 μg/ml endothelial cell growth supplement (ECGS, Sigma Chemical Co.), 5 U/ml heparin and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin), and incubated in culture dishes (Falcon 3003; Becton Dickinson, Oxnard, CA) precoated with 15 μg/ml fibronectin (Sigma Chemical Co.). The endothelial cells were serially passed by brief exposure to trypsin–EDTA–HBSS and were used at the third passage for the experiments described hereafter. Endothelial cells were identified by their characteristic morphology (a cobblestone appearance under phase contrast microscopy) and by an enzyme-labelled antibody technique that used mouse monoclonal anti-human von Willebrand factor (Immunoteck, Marseille, France). The endothelial cell preparation was 99% reactive with the antibody as determined by an immunohistological method (avidin–biotin immunoperoxidase technique).

Preparation of synovial cells

Synovial tissues were obtained from patients with RA, osteoarthritis (OA) and from healthy subjects who were undergoing corrective surgery. The methods used for synovial cell preparation were as described previously in detail [11]. The synovial membranes were minced aseptically, then dissociated enzymatically with 100 μg/ml collagenase (Sigma Chemical Co.) and 3·33 mg/ml dispase (Godo Shusei Co., Tokyo, Japan) in HBSS for 15 min at 37°C. The dispersed cells were plated in culture dishes with RPMI 1640 supplemented with 10% heat-inactivated FBS and antibiotics, and were allowed to adhere. To eliminate non-adherent cells from the synovial cell preparations, the plated cells were cultured for 18 h, then washed extensively with HBSS. The synovial cells obtained were used at the fourth or fifth passage for the experiments. The synovial cell preparations were less than 1% reactive with the MoAbs CD3 (Coulter Immunology, Hialeah, FL), Leu M3 (Becton Dickinson), CD20 (Coulter Immunology) and anti-human von Willebrand factor, which, respectively, define an antigen on all mature T cells, on monocyte/macrophages, on *pan*-B cells and on vascular endothelial cells.

Separation of mononuclear cells

Heparinized peripheral blood was obtained from normal subjects. Mononuclear cells were isolated from the peripheral blood by Ficoll–Conray gradient centrifugation (Daiichi Pharmaceutical Co., Tokyo, Japan). The mononuclear cells were depleted of adherent cells by incubating the cell suspensions on culture dishes for 2 h at 37°C. T cell-enriched populations were then prepared from the non-adherent cells by rosetting with 5% sheep erythrocytes (Nippon Biotest Laboratory, Tokyo, Japan). In each experiment, the purity of the T cell population was determined using anti-CD2, anti-CD20 and anti-MY4 MoAbs (Coulter Immunology). T cell-rich populations contained more than 95% T cells, less than 1% B cells and less than 1% monocytes, and were defined as T cells.

Endothelial cell or synovial cell monolayer adhesion assay

The adhesion of T cells to human endothelial cell monolayer was examined. The methods used for the adhesion assay have been described elsewhere [10]. Briefly, endothelial cells (2×10^4/well) were cultured for 24 h in triplicate in fibronectin-coated 96-well flat-bottomed microtitre plates (Costar, Cambridge, MA) in 200 μl of culture medium in the presence of varying concentrations of reagents. The reagents used in this study were as follows: rIFN-γ (Shionogi Co., Osaka, Japan), rIFN-α (Japan Roche Co., Tokyo, Japan) and dexamethasone (Banyu Pharmaceutical Co., Tokyo, Japan). After incubation, each endothelial cell monolayer was washed thoroughly with RPMI 1640 containing 2% FBS. T cells were radiolabelled with $Na_2{}^{51}CrO_4$ (Amersham International, Amersham, UK) at 37°C for 2 h with occasional shaking. They were then washed three times with RPMI 1640 containing 2% FBS and were finally resuspended at 1×10^6/ml in RPMI 1640 supplemented with 20% FBS. Chromium-labelled T cells (2×10^5/0·2 ml) were dispensed on the endothelial cell monolayer, and the mixture was incubated for 2 h at 37°C. To remove the non-attached T cells, microtitre plates were washed five times. The adherent T cells were lysed by adding of 200 μl of a 1% solution of Triton-X. The data were expressed as the percentage of T cells bound, calculated according to the following formula:

$$\% \text{ adhesion} = \frac{\text{ct/min in lysate}}{\text{ct/min in original T cell suspension}} \times 100\%$$

The adhesion of T cells to synovial cells was also investigated using a similar method used for T cell adhesion to endothelial cells. In some experiments, the adhesion assay was performed in the presence of anti-HLA-ABC (Dakopatts, Glostrup, Denmark) and anti-HLA-DR (Coulter Immunology) MoAbs.

Flow cytometric analysis

Human umbilical cord vein endothelial cells and synovial cells were cultured with cytokines and/or dexamethasone. After incubation, the cells were washed once with PBS without calcium or magnesium, and were detached from the plates with 0·265 mM EDTA. The detached cells were immediately placed in PBS containing 20% FBS and were washed. The cells were then incubated with anti-ICAM-1 MoAb (CD54; Cosmo Bio Co., Tokyo, Japan). The cells were washed intensively and incubated with FITC-conjugated goat anti-mouse IgG (Tago, Burl-

Table 1. Time course of T cell adhesion to synovial cells stimulated with rIFN-γ

Incubation time (h)	T cell adhesion to IFN-γ-stimulated synovial cells (%)
0	5·5 ± 1·0
24	19·2 ± 1·2*
48	20·2 ± 2·0*
72	20·0 ± 1·8*
96	18·5 ± 2·0*

Synovial cells were cultured with 100 U/ml IFN-γ for varying periods. After incubation, the percentage of T cell adhesion to synovial cells was determined. The results from three separate experiments are expressed as the mean ± s.d.

* $P < 0.01$ *versus* T cell adhesion to synovial cells with incubation time 0 h.

Table 2. Effects of IFN-γ on T cell adhesion to synovial cells

rIFN-γ concentrations (U/ml)	T cell adhesion to synovial cells (%)
0	6·8±0·5
50	11·2±1·0*
100	19·0±2·0*
500	20·2±2·2*
1000	21·0±2·5*

Synovial cells were cultured for 24 h with varying concentrations of rIFN-γ. After incubation, the percentage of T cell adhesion to synovial cells was determined. The results from three separate experiments are expressed as the mean ±s.d.

* $P<0·01$ *versus* T cell binding to synovial cells cultured in the absence of rIFN-γ.

ingame, CA) for 45 min on ice. Samples were analysed on a flow cytometer (FACScan; Becton Dickinson).

Statistical analysis

The data were analysed using Student's *t*-test. $P<0·05$ was chosen as the level of significance.

RESULTS

Effects of rIFN-γ on T cell binding to endothelial cells or synovial cells

Previously we have demonstrated that treatment of endothelial cells with 50 U/ml rIFN-γ enhanced T cell binding to endothelial cells and reached a maximum after 24 h of culture [10]. We therefore investigated whether rIFN-γ increased T cell binding to synovial cells. When synovial cells obtained from patients with RA were incubated with 100 U/ml of rIFN-γ, the percentage of T cell binding to synovial cells also increased significantly and reached a plateau at 24 h of culture (Table 1). Next, synovial cells were cultured with varying concentrations of rIFN-γ, and T cell binding to synovial cells was detected on day 1 of culture. Treatment of synovial cells with rIFN-γ increased T cell binding to synovial cells dose dependently and reached a maximum at the concentration of 100 U/ml rIFN-γ (Table 2). When synovial cells were cultured in the absence of rIFN-γ for 24 h, only a few T cells adhered to the untreated synovial cells. Furthermore, T cell binding to rIFN-γ-stimulated synovial cells from patients with OA or healthy normal subjects was similar to that of patients with RA (data not shown). We previously demonstrated that the T cell binding to endothelial cells stimulated with either rIFN-γ or rIL-1 was independent of cell surface expression of HLA-DR antigen and HLA-ABC antigen [10]. We therefore investigated whether HLA-DR and HLA-ABC antigen expression on synovial cells was involved in the adhesion of T cells to synovial cells. Treatment of synovial cells with anti-HLA-DR or anti-HLA-ABC antigen MoAb could not block T cell binding to rIFN-γ-stimulated synovial cells (data not shown).

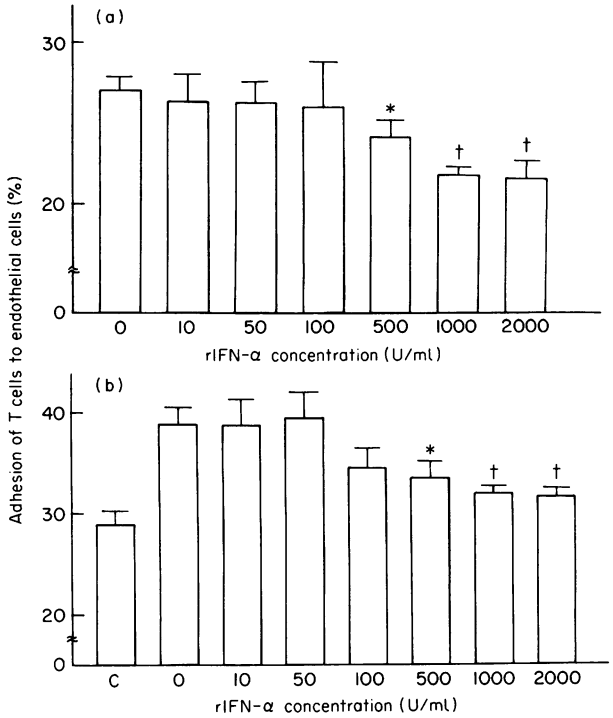

Fig. 1. Effect of rIFN-α on T cell adhesion to endothelial cells. Endothelial cells were cultured with varying concentrations of rIFN-α in the absence or presence of 50 U/ml rIFN-γ for 24 h. After incubation, the percentage of T cell binding to endothelial cell monolayer was determined. The results from four separate experiments are expressed as the mean ±s.d. (a) T cell binding to endothelial cell monolayer in the absence of rIFN-γ. * $P<0·05$ *versus* the percentage of T cell binding to endothelial cells in the absence of rIFN-α. † $P<0·01$ *versus* the percentage of T cell binding to endothelial cells in the absence of rIFN-α; (b) T cell binding to endothelial cells stimulated with rIFN-γ. C, control, cultured in the absence of rIFN-γ and rIFN-α. * $P<0·05$ *versus* the percentage of T cell binding to endothelial cells in the absence of rIFN-α. † $P<0·01$ *versus* the percentage of T cell binding to endothelial cells in the absence of rIFN-α.

Effects of rIFN-α on T cell binding to endothelial cells or synovial cells

Previously it has been reported that IFN-α has an antagonistic effect on Ia antigen expression of macrophages stimulated by IFN-γ [12,13]. We therefore investigated whether IFN-α inhibited T cell binding to either endothelial cells or synovial cells. Initially, we examined the effects of IFN-α on T cell binding to untreated endothelial cells. When endothelial cells were cultured with various amounts of rIFN-α for 24 h, rIFN-α inhibited the percentage of T cell binding to untreated endothelial cells dose-dependently (Fig. 1a). Next, endothelial cells were cultured with 50 U/ml rIFN-γ in the presence of varying concentrations of rIFN-α for 24 h. As shown in Fig. 1b, the percentage of T cell binding to rIFN-γ-stimulated endothelial cells was significantly blocked by the addition of 500 U/ml rIFN-α. In addition to endothelial cells, we also studied the effects of rIFN-α on T cell binding to synovial cells stimulated with rIFN-γ. Synovial cells were cultured with 100 U/ml rIFN-γ in the presence of varying concentrations of rIFN-α for 24 h. As shown in Fig. 2, rIFN-α was able to inhibit T cell binding to synovial cells stimulated with rIFN-γ in a dose-dependent fashion. The viability of endothelial cells and synovial cells was measured by a trypan

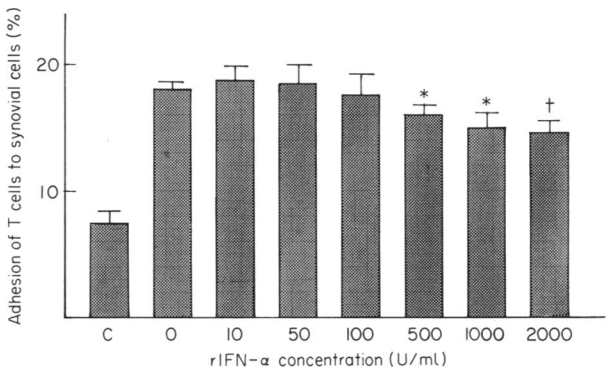

Fig. 2. Effect of rIFN-α on T cell adhesion to synovial cells. Synovial cells were cultured with 100 U/ml rIFN-γ in the presence of varying concentrations of rIFN-α for 24 h; the percentage of T cell adhesion to synovial cells was then calculated. Results from three separate experiments are expressed as the mean ±s.d. C, control, cultured in the absence of rIFN-γ and rIFN-α. * $P < 0{\cdot}05$ *versus* the percentage of T cell binding to rIFN-γ-stimulated synovial cells in the absence of rIFN-α. † $P < 0{\cdot}01$ *versus* the percentage of T cell binding to rIFN-γ-stimulated synovial cells in the absence of rIFN-α.

blue dye exclusion test. Neither rIFN-α alone nor combinations of rIFN-α and rIFN-γ were cytotoxic to endothelial cells or synovial cells in these experiments (data not shown).

Effects of dexamethasone on T cell binding to endothelial cells or synovial cells

Glucocorticoids have also been shown to inhibit Ia antigen expression on the surface of IFN-γ-induced macrophages [14,15]. We attempted to determine whether dexamethasone decreased T cell binding to endothelial cells and synovial cells. Initially, the percentage of T cell binding to endothelial cells was determined after endothelial cells were cultured with varying amounts of dexamethasone for 24 h. As shown in Fig. 3a, the suppressive effect of dexamethasone was detected at a concentration of 10^{-8} M dexamethasone, and dose-dependent inhibition was seen. Next, when endothelial cells were cultured with 50 U/ml rIFN-γ in the presence of varying concentrations of dexamethasone, the percentage of T cell binding to IFN-γ-stimulated endothelial cells was significantly inhibited by addition of 10^{-8} M dexamethasone. Dexamethasone also suppressed the T cell binding to endothelial cells dose dependently (Fig. 3b). We investigated the effects of dexamethasone on T cell binding to synovial cells in the same manner. Synovial cells were cultured with 100 U/ml rIFN-γ in the presence of varying amounts of dexamethasone for 24 h. After incubation, the percentage of T cell binding to synovial cells was assayed. As shown in Fig. 4, the percentage of T cell binding to synovial cells was markedly suppressed by the addition of 10^{-8} M dexamethasone. Dexamethasone also inhibited the T cell binding to rIFN-γ-stimulated synovial cells in a dose-dependent manner. The viability of endothelial cells and synovial cells was measured by trypan blue dye exclusion. Neither dexamethasone alone nor combinations of dexamethasone and rIFN-γ was cytotoxic for endothelial cells or synovial cells in these experiments (data not shown).

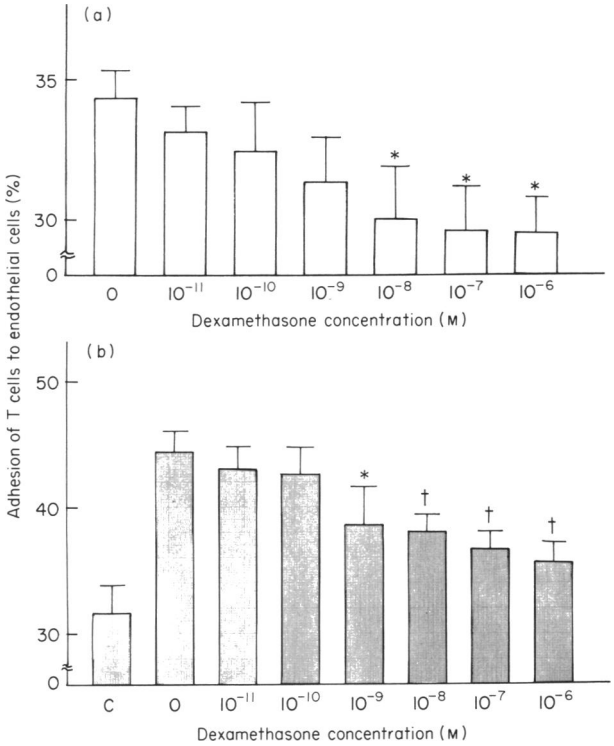

Fig. 3. Effect of dexamethasone on T cell binding to endothelial cells. Endothelial cells were cultured with varying concentrations of dexamethasone in the presence or absence of 50 U/ml rIFN-γ. After incubation, the percentage of T cell adhesion to endothelial cells was determined. The results from three separate experiments are expressed as the mean ±s.d. (a) T cell adhesion to endothelial cells in the absence of rIFN-γ. * $P < 0{\cdot}05$ *versus* percentage of T cell binding to endothelial cells in the absence of dexamethasone; (b) T cell adhesion to endothelial cells stimulated with rIFN-γ. C, control, cultured in the absence of rIFN-γ and dexamethasone. * $P < 0{\cdot}05$ *versus* the percentage of T cell binding to rIFN-γ-stimulated endothelial cells in the absence of dexamethasone. † $P < 0{\cdot}01$ *versus* the percentage of T cell binding to rIFN-γ-stimulated endothelial cells in the absence of dexamethasone.

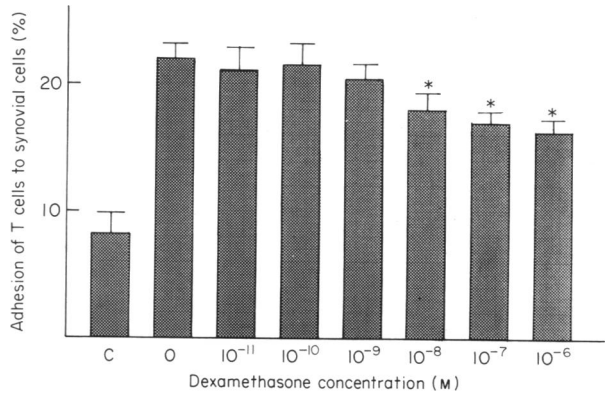

Fig. 4. Effect of dexamethasone on T cell adhesion to synovial cells. Synovial cells were cultured with varying concentrations of dexamethasone in the presence of 100 U/ml rIFN-γ for 24 h. After incubation, the percentage of T cell binding to synovial cells was determined. The results from three separate experiments are expressed as the mean ±s.d. C, control, cultured in the absence of IFN-γ and dexamethasone. * $P < 0{\cdot}05$ *versus* the percentage of T cell binding to IFN-γ-stimulated synovial cells in the absence of dexamethasone.

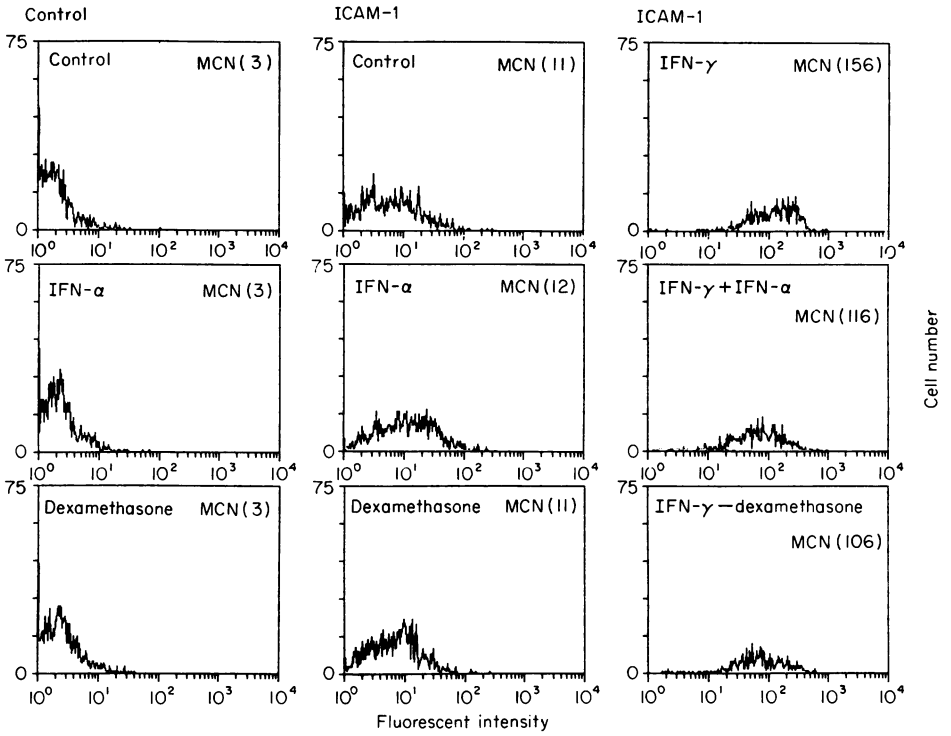

Fig. 5. Flow cytometric analysis of ICAM-1 expression in synovial cells cultured with cytokines and dexamethasone. The synovial cells were incubated with 1000 U/ml rIFN-α or 10^{-7} M dexamethasone in the presence or absence of 100 U/ml rIFN-γ. Histograms of flow cytometric analysis of ICAM-1 expression in synovial cells show a representative result of four experiments. Background staining was determined using isotype-matched irrelevant antibodies (left lane); middle lane, ICAM-1 expression on unstimulated synovial cells; right lane, ICAM-1 expression on rIFN-γ-stimulated synovial cells. MCN, mean channel number.

Effects of rIFN-α and dexamethasone on ICAM-1 expression of synovial cells and endothelial cells stimulated by rIFN-γ

We sought to determine whether rIFN-α and dexamethasone inhibited the surface expression of ICAM-1 on rIFN-γ-stimulated synovial cells and endothelial cells. Initially, synovial cells were cultured with 1000 U/ml rIFN-α or 10^{-7} M dexamethasone in the presence or absence of 100 U/ml rIFN-γ. Neither rIFN-α nor dexamethasone had any effect on the ICAM-1 expression of unstimulated synovial cells (Fig. 5, middle). The synovial cell ICAM-1 expression was markedly increased by rIFN-γ. Both IFN-α and dexamethasone inhibited ICAM-1 expression of synovial cells stimulated by rIFN-γ (Fig. 5, right). Furthermore, the ICAM-1 expression of endothelial cells stimulted by rIFN-γ was also suppressed by rIFN-α and dexamethasone (data not shown).

DISCUSSION

We have demonstrated that freshly isolated peripheral blood T cells bound significantly to IFN-γ-stimulated synovial cells, but only a few bound to unstimulated synovial cells. Treatment of synovial cells with IFN-γ increased T cell binding in a dose dependent manner. In contrast to synovial cells, peripheral blood T cells were able to adhere to unstimulated endothelial cells. Human thymocytes and concanavalin-A-activated T cells have been shown to bind to unstimulated synovial fibroblast-like cells *in vitro*, whereas fresh peripheral blood T cells do not [16]. Neither anti-HLA-ABC nor anti-HLA-DR MoAb blocked the binding of peripheral blood T cells to IFN-γ-treated synovial cells. These results are in accordance with previous data showing that neither anti-MHC class I nor anti-MHC class II antibody inhibits the binding of thymocyte–synovial cells and activated T cell–synovial cells [16]. These results suggest that the HLA class I and HLA class II antigens on the surface of IFN-γ-stimulated synovial cells are not involved in the binding of untreated T cells to these cells.

Adhesion molecules are thought to be involved in necessary cellular interactions for the development of effective immune responses [17–19]. The adhesion of T cells to endothelial cells has been proved to be enhanced by pre-incubation of endothelial cells with IL-1 [4,5], TNF [5,6], lipopolysaccharide (LPS) [5], IFN-γ [7,8] and IL-4 [9]. The adhesion molecules ICAM-1 and LFA-3 are present on macrophage-like synovial cells, synovial fibroblasts, macrophages and endothelial cells in the inflammatory synovial micro-environment [3]. The expression of ICAM-1 on human synovial fibroblasts, adenocarcinoma cell line and endothelial cells is induced by IFN-γ, IL-1 and TNF-α [20–22]. LFA-1 molecules have been shown to be ligands for ICAM-1 and ICAM-2 [23], and LFA-3 is an endogenous ligand for CD2 molecules [24,25]. Furthermore, synovial tissues from patients with RA have increased percentages of T cells which express high densities of LFA-1 and very late antigen-1 (VLA-1) [26]. Adhesion of T cells toward synovial fibroblasts is shown to be only partially inhibited by anti-ICAM-1, anti-LFA-1 or anti-CD18 [22]. These findings suggest the existence of ICAM-1-independent and CD11/CD18-independent adhesion mechanism. Recently it became apparent that the VLA-4/VCAM-1 receptor–ligand pair represents a major lymphocyte/endothelial

adhesion pathway, independent of the LFA-1 pathway [27]. From the above findings, it is suggested that interactions between adhesion molecules on infiltrating lymphocytes and these ligands on endothelial cells or synovial cells may play an important role in the perpetuation of RA synovitis.

We have demonstrated that IFN-α and dexamethasone inhibited T cell binding to unstimulated endothelial cells. Dexamethasone and IFN-α were also able to suppress T cell binding to IFN-γ-stimulated endothelial cells or synovial cells. Furthermore, we showed that dexamethasone and IFN-α inhibited ICAM-1 expression on the surface of synovial cells stimulated by IFN-γ. Dexamethasone has been shown to inhibit ICAM-1 expression at doses as low as 10^{-8} M in human skin fibroblast and some tumour cell lines stimulated by IFN-γ, TNF-α and IL-1 [20]. The doses of dexamethasone that inhibited T cell binding to endothelial cells were equivalent to those that suppressed the expression of ICAM-1 [20]. It seems likely that dexamethasone and IFN-α may inhibit T cell binding to IFN-γ-stimulated endothelial cells or synovial cells by suppression of ICAM-1 expression on these cells.

RA synovitis is characterized by monononuclear cell infiltration of synovial tissue. The infiltrating mononuclear cells emigrate from blood through endothelial cells of high endothelial venules in the sublining layer [2,3,28,29]. The adhesion of lymphocytes to endothelial cells is essential for the emigration of T cells through endothelial cells. Furthermore, it is now apparent that the cellular interaction between infiltrating lymphocytes and synovial cells may occur and participate in the maintenance of chronic synovial inflammation [3,16]. It is suggested that steroids and IFN-α may down-regulate T cell binding to endothelial cells and synovial cells by modulation of adhesion molecule expression, resulting in inhibition of an already protracted inflammatory response in the RA synovium.

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