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Role of ICAM-1 in the aggregation and adhesion of human alveolar macrophages in response to TNF-α and INF-γ

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Intracellular adhesion molecule-1 (ICAM-1)-mediated cell–cell adhesion is thought to play an important role at sites of inflammation. Recent evidence suggests that ICAM-1 surface expression on alveolar macrophages is increased in pulmonary sarcoidosis and that inflammatory granuloma formation is characterized by the aggregation of macrophages. The present study shows that ICAM-1 expression is significantly elevated on alveolar macrophages from patients with sarcoidosis in response to tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ) compared with healthy controls. Aggregation and adhesion were significantly increased in alveolar macrophages treated with TNF-α and INF-γ, and significantly inhibited in those pretreated with a monoclonal antibody to ICAM-1. Similarly, aggregation and adhesion were inhibited in macrophages treated with heparin, which then exhibited a wide range of biological activities relevant to inflammation. These results suggested that the surface expression of ICAM-1 on alveolar macrophages in response to TNF-α and INF-γ is important in mediating aggregation and adhesion. Additionally, heparin may be useful for developing novel therapeutic agents for fibrotic lung disease.

Key words: ICAM-1, Alveolar macrophage, TNF-α, INF-γ, Sarcoidosis

Introduction

Cell adhesion is an important event that plays a role in cell-to-cell communication mediated by inflammatory cytokines and adhesion molecules. Several chronic interstitial lung diseases, such as sarcoidosis and hypersensitivity pneumonitis, are characterized by interstitial cellular infiltration or alveolitis, granuloma and varying degrees of interstitial fibrosis.[1–3]

Granuloma is characterized as masses of macrophages, lymphocytes and epitheloid cells. At inflammatory sites, macrophages that can release many mediators, such as tumor necrosis factor-α (TNF-α), interleukin-1β, interferon-γ (IFN-γ), platelet-derived growth factor and others,[4–6] interact between various cell types. Both TNF-α and intracellular adhesion molecule-1 (ICAM-1) participate in lymphocyte activation and granuloma formation,[7,8] suggesting that one mechanism of TNF-α in granuloma develops through TNF-α-induced ICAM-1 expression in schistosome granulation.[9] Aggregation of rat alveolar macrophages is induced by TNF-α during the upregulation of ICAM-1 expression on alveolar macrophages.[5,8,10] Heparin, a major component of the mast cell granule matrix, is a polyanionic anticoagulant. Apart from this classical role, heparin and heparin-related complex have numerous other functions, among which are inhibition of fibrin deposition in tissues, proliferation of fibroblasts associated with proteases including tryptase and chymase, and regulation of T-cell or eosinophil infiltration-mediated allergic inflammation.[11–16] However, little evidence shows that heparin attenuates cell–cell interactions with adhesion molecules.

The present study investigates the roles of TNF-α and INF-γ on ICAM-1 expression of human alveolar macrophages, aggregation formation and the effect of heparin on aggregation of human alveolar macrophages.

Material and methods

Study population

Six patients with pulmonary sarcoidosis (four men and two women; mean age, 33 years) participated in the study. The diagnosis was based on consistent clinical features, along with biopsy evidence of non-caseating epithelioid cell granulation. Six control patients had no evidence of interstitial lung disease (five men and one woman). All were non-smokers undergoing routine bronchoscopy for suspected

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bronchial carcinoma. All X-ray findings appeared normal and bronchoalveolar lavage (BAL) cytology showed normal differential cell counts.

The study was approved by the local ethics committee, and all individuals gave informed consent.

Bronchoalveolar lavage cells

BAL cells were obtained as described[17], with minor modification. Two 50-ml aliquots of saline were injected into the right middle lobe with immediate aspiration with a plastic sterile syringe. After filtration through a double layer of surgical gauze, total cells were counted in a small aliquot using a hemocytometer. We count differential cells by identifying at least 200 cells on Diff-Quick stained cytocentrifuge preparations (Cytospine II; Shando Instruments, Runcorn, UK). Alveolar macrophages (AMs) obtained via BAL were resuspended in RPMI 1640 medium and allowed to adhere to plastic Petri dishes (Nunc, Roskilde, Denmark) at 37°C, in a 5% CO_2 atmosphere for 1 h. Thereafter, non-adherent cells were removed by gently flooding dishes with phosphate-buffered saline, while adherent cells were detached by a 5 min exposure to 0.02% ethylenediamine tetraacetic acid. Enriched preparations contained > 95% AMs. Cell viability was > 98%, assessed using Tripan blue exclusion. Cytocentrifuge preparations of 0.1 ml were placed on slides and air-dried at room temperature overnight. The slides were fixed in cold acetone for 5 min at 4°C, wrapped in plastic film and stored at −20°C.

Immunocytochemical staining

To detect ICAM-1, CD54 monoclonal antibodies were used in conjunction with the alkaline phosphatase/anti-alkaline phosphatase (APAAP) procedure. To evaluate the reaction, the slides were viewed under light microscopy and 200 cells were counted in each area.

In vitro aggregation of AMs by cytokines

Human lung fibroblasts (CCL-153) obtained from the American Type Culture Collection were cultured in modified Ham's F12 (Sigma, USA) supplemented with 10% fetal calf serum at 37°C and 5% CO_2, then seeded at a density of 1.0×10^5 cells in Tissue-Tek slides. AMs were suspended in chamber slides at a density of 1×10^3/well. Cells were cultured with medium alone, TNF-α or INF-γ, and then incubated at 37°C for 24 h in Tissue-Tek slides coated with CCL-153 cells. Thereafter, either anti-ICAM-1, anti-leukocyte function associated antigen-1 (anti-LFA-1), anti-Mac-1 or isotype control antibody was added. After 24 h of culture, cells were fixed and stained. Aggregation was measured by counting the number of aggregates of > 5 cells per 200 × field (agg/hpf).

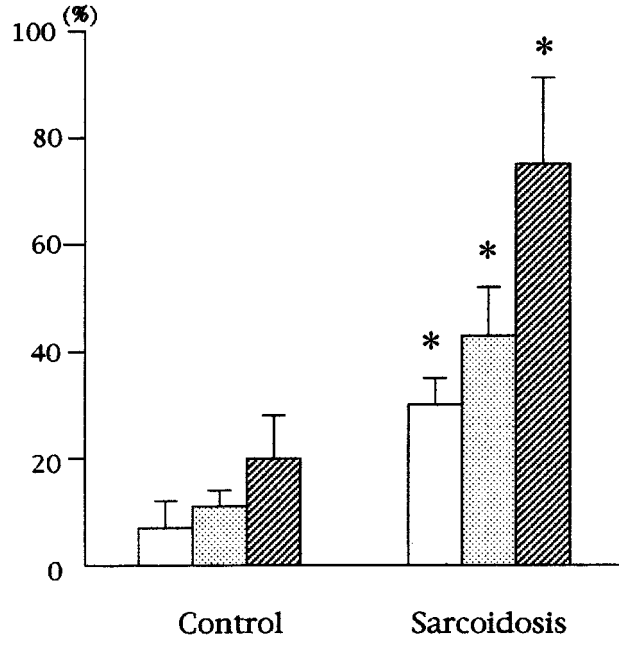

FIG. 1. Regulation of ICAM-1 expression on AM by TNF-α. AM cultured with non-treated (□), medium alone (▩) or TNF-α (▨). $P < 0.05$, compared with control.

Statistics

Statistical significance was determined using Student's *t*-test and significance was determined at $p < 0.05$.

Results

Total and differential cell counts

The total number of cells recovered from the patient with pulmonary sarcoidosis ($1.42 \pm 0.31 \times 10^5$/ml) was increased compared with healthy controls ($0.99 \pm 0.15 \times 10^5$/ml). Furthermore, the percentage of lymphocytes was significantly increased in pulmonary sarcoidosis compared with controls. The CD4/CD8 ratio in BALF from patients with pulmonary sarcoidosis was 2.9 ± 0.5.

Immunocytological staining of AMs and human lung fibroblasts

AMs were identified according to morphologic criteria, and red staining of the cell membrane recognized positive cells. Data are expressed as positivity (%) of total AMs. The percentages of AMs expressing ICAM-1 in control and patients with pulmonary sarcoidosis are shown in Fig. 1. The ratio of ICAM-1-positive AMs was significantly higher in patients with pulmonary sarcoidosis than in controls. In both groups, the percentage of ICAM-1-positive AMs treated with TNF-α was significantly higher than those treated with medium alone. INF-γ also stimulated AM ICAM-1 expression in both groups. Figure 2 shows

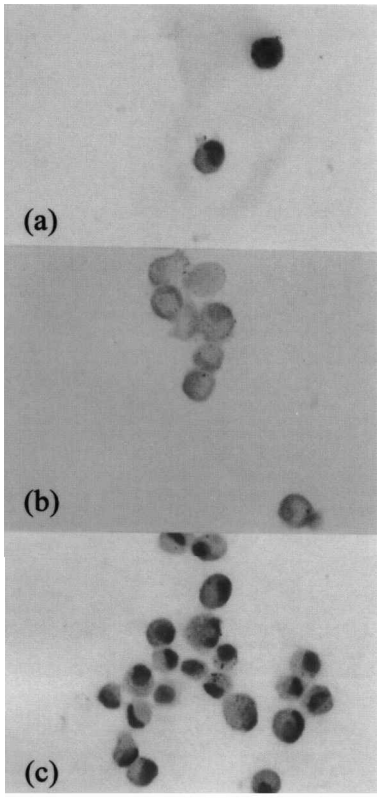

FIG. 2. Immunocytochemical staining of ICAM-1 on alveolar macrophages (AM) (A), and after treatment with TNF-α (B) from a patient with sarcoidosis. Staining for IgG_1, as a negative control (C), on AM treated with TNF-α (× 100). Using APAAP complex, ICAM-1 expression on AM from a patient with sarcoidosis was demonstrated. Staining of AM from a patient treated with TNF-α resulted in more intense coloring of the cell surface than staining of control AM.

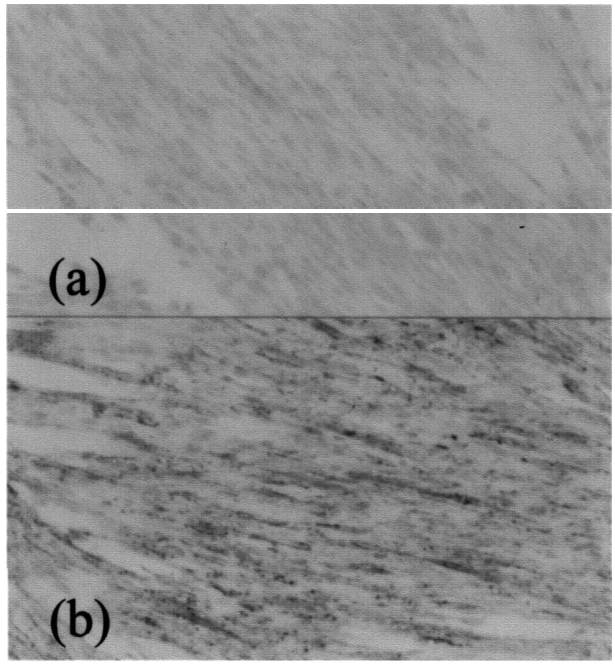

FIG. 3. APAAP stains for ICAM-1 on human fibroblasts treated with medium alone (A) or TNF-α (B) (× 100). Human fibroblasts expressed ICAM-1, showing a weak diffuse positivity on their surface. ICAM-1 expression on the surface of human fibroblasts increased after stimulation with TNF-α.

sarcoid AMs stained with anti-ICAM-1 monoclonal antibody (mAb). Human lung fibroblasts cultured with medium alone weakly expressed ICAM-1. TNF-α stimulated ICAM-1 expression on human lung fibroblasts. Figure 3 shows human lung fibroblasts stained with anti-ICAM-1 mAb.

Aggregation of AMs *in vitro*

Incubation of AMs from the patients with pulmonary sarcoidosis for 24 h resulted in the spontaneous formation of large cellular aggregates (Fig. 4A). In contrast, AMs obtained from control individuals formed small clumps (Fig. 4B). *In vitro* exposure, TNF-α or INF-γ increased the numbers of aggregates in AMs from the control (Figs. 5 and 6). The effects of TNF-α and INF-γ did not differ over a period of 24 h. However, aggregation significantly increased in AMs treated with TNF-α compared for 48 h with INF-γ. Both processes were significantly inhibited in AMs pretreated with anti-ICAM-1 mAb or anti-LFA-1 mAb (Figs. 5 and 6). Antibody type (anti-ICAM-1 mAb versus anti-LFA-1 mAb) had no significantly different effects. Aggregation was not inhibited when AMs were pretreated with anti-MAC-1 mAb or anti-IgG_1 mAb. Heparin, which may act as an anti-inflammatory, also inhibited the effects of TNF-α-induced or INF-γ-induced aggregation (Figs. 5 and 6). The inhibitory effect of heparin was weaker than that of both anti-ICAM-1 mAb and anti-LFA-1 mAb.

Discussion

Cell adhesion is an important event that plays a role in cell-to-cell communication mediated by inflammatory cytokines and adhesion molecules. Several chronic interstitial lung diseases, such as pulmonary sarcoidosis and interstitial pneumonia, are characterized by interstitial cellular infiltration or alveolitis, granuloma and varying degrees of interstitial fibrosis.[1-3,18] Persistent upregulation of adhesion molecules and cytokines could lead to chronic cell activation, inflammation, and pathology. Members of the integrin family, such as ICAM-1 and LFA-1, seem to be critical to the cell-to-cell interaction of immune cells, including AMs.[19,20] These glycoproteins play roles not only in cellular adhesion, but probably also in signal transduction.[21] The results of the present study indicate that several early events initiate and regulate *in vitro* aggregation formation. High levels of ICAM-1 were expressed on AMs from the patient with pulmonary sarcoidosis. Alveolar macrophages cultured with TNF-α and INF-γ expressed significantly more ICAM-1 than those cultured with medium alone. These findings

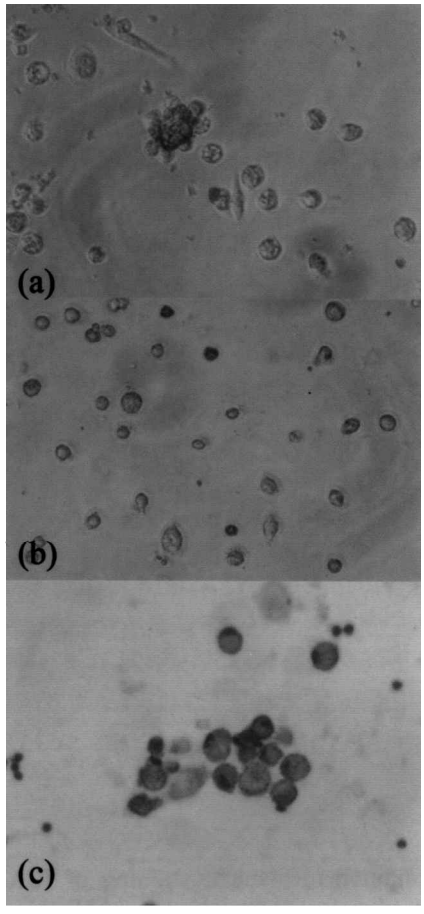

FIG. 4. Aggregation of AM from a patient with pulmonary sarcoidosis, treated with TNF-α (A) or TNF-α anti-ICAM-1 (B) (both × 50). Aggregation of AM was abolished by treatment with antibody to ICAM-1. APAAP stains for ICAM-1 on AM treated with TNF-α (C) (× 100). ICAM-1 expression was indicated by strong positivity in the cytoplasm and on the membrane of AM.

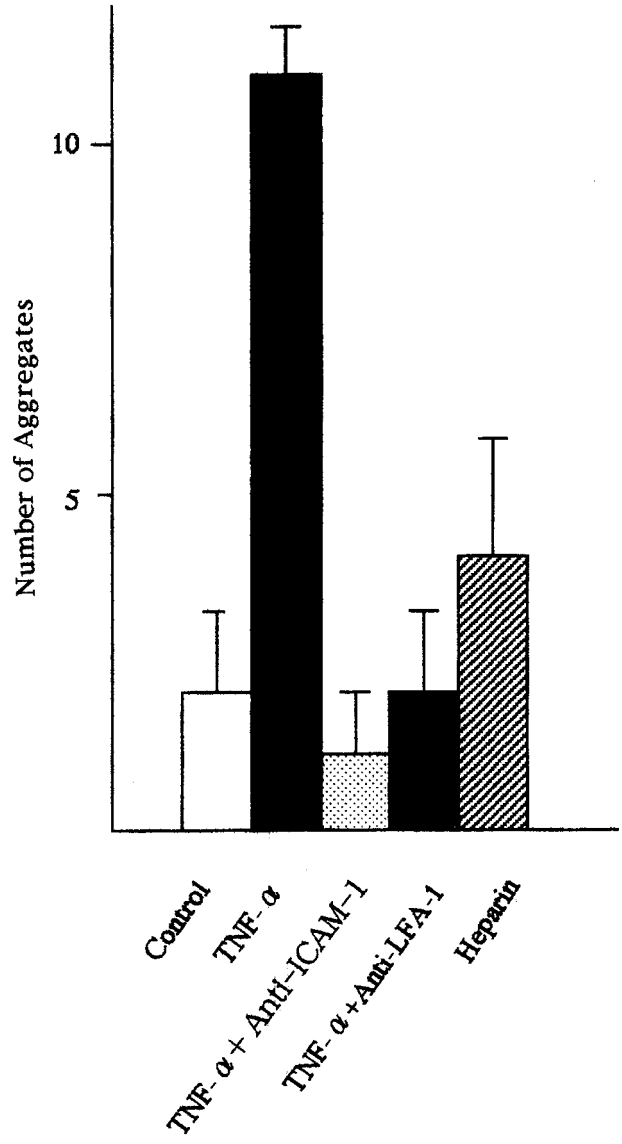

FIG. 5. Effect of TNF-α, TNF-α + Anti-ICAM-1 Ab, TNF-α + Anti-LFA-1 Ab and heparin on AM aggregation *in vitro*. Data are presented as the mean ±SEM of the number of cells comprising each aggregate.

showed that TNF-α and INF-γ can induce ICAM-1 expression on AMs and may be correlated with ICAM-1 expression on AMs from the patients with pulmonary sarcoidosis. Levels of TNF-α in the BAL fluid were measured using an enzyme-linked immunosorbent assay. The concentration of TNF-α was not high enough to activate AMs. In patients with pulmonary sarcoidosis, AMs are primed to become more responsive than usual. TNF-α might account for peripheral blood monocyte recruitment to the alveolar spaces of patients with pulmonary sarcoidosis. ICAM-1 is expressed only at low levels in resting cells, but it is strongly upregulated on activation by cytokines. TNF-α may be one mediator of the regulation of ICAM-1 expression on AMs in pulmonary sarcoidosis. In chronic interstitial lung disease, mast cells infiltrate the parenchyma. The role of mast cells is not well understood in lung fibrosis, but mast cell tryptase stimulates the synthesis of type I collagen and the proliferation of lung fibroblasts.[22] Heparin, a major component of the mast cell granule matrix, is a polyanionic anticoagulant. However, both heparin and heparin-related complex have several other functions, among which are inhibition of fibrin deposition in the tissues, proliferation of fibroblasts associated with proteases including tryptase and chymase, and the regulation of T-cell or eosinophil infiltration-mediated allergic inflammation.[12–15] More recent studies have demonstrated that heparin can modulate the TNF-α-induced inflammatory response through a CD11b-dependent mechanism and interaction with matrix metalloproteinase 2.[23–25] Our previous study demonstrated that matrix metalloproteinase (MMP)-1 and MMP-9 were strongly positive in human lung fibroblasts and retained MMP-1 and MMP-2/MMP-9 activity.[26] We showed that heparin inhibited aggregation formation, but to a lesser extent than anti-ICAM-1 mAb or anti-LFA-1 mAb.

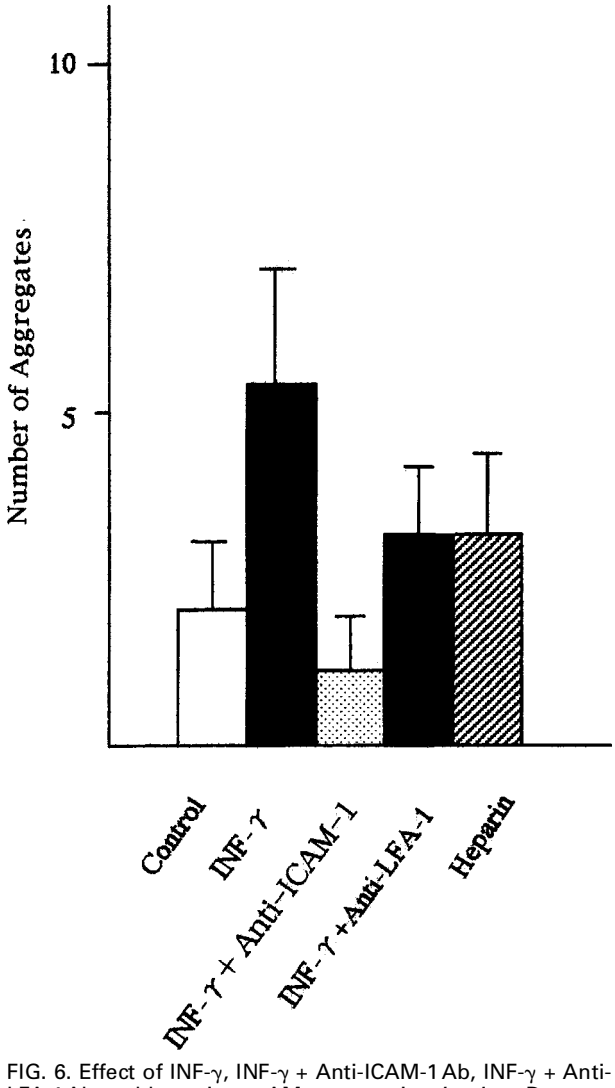

FIG. 6. Effect of INF-γ, INF-γ + Anti-ICAM-1 Ab, INF-γ + Anti-LFA-1 Ab and heparin on AM aggregation *in vitro*. Data are presented as the mean ±SEM of the number of cells comprising each aggregate.

We found no evidence supporting the notion that heparin attenuates the role of cell adhesion molecules. Although recent findings suggest that heparin disrupts cell–collagen attachment, our results do not show direct interaction of AMs but rather interaction between AMs and fibroblasts. They also show that heparin and MMPs act together to induce conformational change of the affinity sites of adhesion molecules.

More studies are required to define the mechanism of inhibitory effects of ICAM-1 and heparin, as well as to determine their suitability as therapeutic agents with which to treat pulmonary fibrosis.

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