Intercellular adhesion molecule 1 (ICAM-1) in the pathogenesis of mononuclear cell alveolitis in pulmonary sarcoidosis

Klaus Dalhoff, Sabine Bohnet, Jörg Braun, Burkhard Kreft, Karl J Wießmann

Abstract

Background—Alveolitis in pulmonary sarcoidosis is characterised by an accumulation of highly activated macrophages and CD4⁺ lymphocytes in the alveolar compartment. The role of intercellular adhesion molecule 1 (ICAM-1) expression on alveolar cells has been studied in this context.

Methods—Using a sandwich ELISA technique, ICAM-1 expression on alveolar macrophages from 17 consecutive untreated patients with pulmonary sarcoidosis and six healthy normal volunteers was quantified. In addition, parameters of macrophage activation (tumour necrosis factor α (TNF α) and superoxide anion release) were evaluated.

Results—Significantly elevated expression could be demonstrated on alveolar macrophages from patients with pulmonary sarcoidosis compared with healthy controls (mean (SD) 0.74 (0.24) ELISA units (EU) v 0.46 (0.12) EU). On subdividing the patients into those with active and those with inactive disease, only the former showed increased ICAM-1 levels on alveolar macrophages (0.82 (0.27) EU) compared with control alveolar macrophages. No differences were detected in serum levels of soluble ICAM-1 between patients and controls. ICAM-1 expression on alveolar macrophages from patients with sarcoidosis correlated with the spontaneous release of TNF α but not with the release of the superoxide anion by the activated macrophages. There was no correlation with the percentage of lymphocytes or the absolute number of CD4⁺ cells in bronchoalveolar lavage fluid.

Conclusions—Increased ICAM-1 surface expression on alveolar macrophages reflects disease activity in the pulmonary compartment. Considering the significance of adhesion molecules during antigen presentation and lymphocyte activation, ICAM-1 expression on alveolar macrophages may have an important role in the immune process of pulmonary sarcoidosis.

Leucocyte homing to sites of acute or chronic inflammation is a crucial step during the inflammatory response. Adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) play a major part in this process by mediating adherence of leucocytes to the endothelium and initiating the extravasation of those cells.¹ At the site of inflammation the interaction of ICAM-1 (CD54) with its ligands LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) provides an antigen independent mechanism of cell-cell activation in addition to the antigen dependent interaction mediated by molecules of the MHC class II complex and the T cell receptor.² Under normal conditions the β_2 integrins, which are restricted to leucocytes, exist in an inactive form. They are activated rapidly by a number of substances including lipopolysaccharide (LPS) and phorbol-12-myristate-13-acetate (PMA).³ In contrast, ICAM-1 is not normally present at the cell surface but can be induced within hours during inflammation.⁴⁻⁶ Signals which are transduced at least partially by the ICAM-1/ β_2 integrin pathway include lymphocyte proliferation, antigen processing, and phagocytosis.⁷

Pulmonary sarcoidosis represents an example of compartmentalised inflammation, with accumulation of activated macrophages and CD4⁺ lymphocytes in the lung.⁸⁻¹⁰ Central effector mechanisms of primed macrophages include the release of cytokines, reactive oxygen species, and proteases.¹¹⁻¹³ However, the mechanisms leading to local upregulation of the inflammation are not understood. Alveolar macrophages from patients with sarcoidosis have an increased ability to function as antigen presenting cells (APC) and to support antigen driven proliferation of autologous T lymphocytes, which is not due to an upregulation of HLA-DR molecules on the cell surface.¹⁴ Recently the expression of adhesion molecules on alveolar macrophages from patients with sarcoidosis has been reported.^{15,16} The present study was undertaken to elucidate the contribution of ICAM-1 to the inflammatory process in correlation with clinical and immunological parameters of disease activity in pulmonary sarcoidosis. Using a recently established ELISA method which allows semiquantitative analysis of ICAM-1 levels on alveolar macrophages as well as immunocytochemical

Department of
Internal Medicine,
Medical University of
Lübeck, Ratzeburger
Allee 160, D-23538
Lübeck, Germany
K Dalhoff
S Bohnet
J Braun
B Kreft
K J Wießmann

Reprint requests to:
Dr med K Dalhoff

Received 16 November 1992

Returned to authors

3 March 1993

Revised version received

13 April 1993

Accepted 11 August 1993

(Thorax 1993;48:1140-1144)

staining, we determined ICAM-1 expression in a group of untreated patients with pulmonary sarcoidosis and a group of healthy volunteers.

Methods

STUDY POPULATION

The study population consisted of 17 consecutive untreated patients with biopsy proven pulmonary sarcoidosis (radiological type 0, n = 1; type I, n = 9; type II, n = 7). There were 10 patients with active and seven with inactive disease. The assessment of clinical disease activity was performed according to previously published criteria.⁸ Briefly, new or increasing respiratory or systemic symptoms, a reproducible decline in vital capacity and/or diffusing capacity of >10%, and new or progressing infiltrates in the chest radiograph were accepted as signs of active disease (table 1). Six healthy volunteers were studied as controls. There were no smokers in either group which is notable as smoking influences expression of adhesion molecules.¹⁷ Ethical committee approval and individual informed consent were obtained. Clinical data and bronchoalveolar lavage cell counts are summarised in table 2.

BRONCHOALVEOLAR LAVAGE AND CELL ISOLATION

Bronchoalveolar lavage was performed from the middle lobe using a flexible fiberoptic bronchoscope under standard conditions.¹⁸ The lavage volume was 200 ml with a mean recovery of 84%, which was similar in patients and controls. The first 20 ml aliquot of the lavage fluid which is known to be contaminated by bronchial secretions¹⁹ was discarded and aliquots were pooled. A total cell count was made by a haemocytometer

Table 1 Clinical characteristics of patients with active and inactive sarcoidosis

	Active	Inactive
Chest radiograph		
Type 0		
progressing	0	0
stable	1	0
Type I		
progressing	6	0
stable	1	2
Type II		
progressing	2	0
stable	0	5
Lung function*		
Vital capacity		
decreasing	5	0
stable	5	6
Diffusing capacity		
decreasing	7	0
stable	3	6
Respiratory symptoms	7	1
Systemic symptoms		
Fever	5	0
Erythema nodosum	4	0
Arthritis/arthralgia	8	0
Extrathoracic sarcoidosis		
Uveitis	1	0
Skin involvement	1	0

*Data available only for 16 patients.

Table 2 Age, sex, and cell counts in bronchoalveolar lavage (BAL) fluid of patients and healthy volunteers

	Patients (n = 17)	Controls (n = 6)
Mean (SD) age (years)	38.2(15)	27(4)
Sex (M:F)	6:11	6:0
BAL fluid		
Total cell count (× 10 ⁶)	23(21)	11(3)
Alveolar macrophages (%)	70(18)	90(7)
Granulocytes (%)	1(1)	1(1)
Eosinophils (%)	1(1)	
Lymphocytes (%)	28(19)	8(5)
CD4+ (%)	81(7)	
CD4+ /CD8+	8.8(4.6)	

(Neubauer), then lavage cell differentials were determined by Wright-Giemsa stains of cytocentrifuge preparations (Cytospin II, Shandon). Cell viability was assessed using trypan blue dye exclusion. For differentiation of lymphocyte subsets (CD3, CD4, CD8) fluorescein conjugated monoclonal antibodies (Ortho Diagnostics, Beerse, Belgium) were used. Lavage cells were washed twice with phosphate buffered saline and resuspended at a concentration of 1 × 10⁶ alveolar macrophages/ml in M199 supplemented with fetal calf serum (5%, Gibco), L-glutamine (2 mmol/l, Gibco), and penicillin/streptomycin (1 mg/ml, Gibco).

ICAM-1 EXPRESSION ON ALVEOLAR MACROPHAGES AND LYMPHOCYTES

ICAM-1 expression on alveolar macrophages was determined by a sandwich ELISA technique. Mononuclear lavage cells containing viable alveolar macrophages at a density of 1 × 10⁶/ml were seeded into 96-well flat bottom microtitre plates (Greiner, Nürtingen, Germany) for three hours (37°C, 5% carbon dioxide), allowing the macrophages to adhere. Non-adherent cells were removed by gentle washing and serum-supplemented M199 was added. Incubation was continued for 16 hours at 37°C in a total humidified atmosphere containing 5% carbon dioxide. Cells were then fixed with 1% paraformaldehyde (one hour, 21°C) and free binding sites were blocked by adding a 2% solution of bovine serum albumin (BSA) diluted in phosphate buffered saline (PBS, 0.1 mol/l, pH 7.2) for one hour (37°C). The monoclonal mouse anti-ICAM-1 antibody (clone 84H10, Dianova, Hamburg, Germany, 1:1000 in 0.1% BSA/PBS) was added for two hours at 37°C. Peroxidase conjugated goat antimouse antibody (Sigma, Deisenhofen, Germany, 1:1000 in 0.1% BSA/PBS) was used as developing antibody. Finally, ABTS (Sigma, 1 mg/ml) and hydrogen peroxide (2.8 µl, 30% H₂O₂/10 ml) in 0.1 mol/l sodium acetate (pH 4.2) was added. The colour was allowed to develop for 120 minutes and plates were read with a microplate reader (Behring EL 311) 405 nm.

Results were expressed as ELISA units (EU) from quadruplicate wells after subtraction of non-specific background absorbance which was determined in the absence of the

primary antibody. The number of adherent cells at the time of the ELISA was assessed in parallel wells by crystal violet staining. Using this method we found no differences between the three groups of our study (data not shown).

In addition, ICAM-1 expression on bronchoalveolar cells was determined by immunocytochemical staining on cytocentrifuge preparations using the alkaline phosphatase monoclonal mouse antialkaline phosphatase (APAAP) complex as described.²⁰

SOLUBLE ICAM-1 (sICAM-1) SERUM LEVELS

Serum was collected at the time of bronchoalveolar lavage and frozen at -20°C . sICAM-1 levels were determined with a commercially available kit (Serva, Heidelberg, Germany).

TNF α BIOASSAY

Tumour necrosis factor α (TNF α) activity was measured with an L929 fibroblast lytic assay as previously described.²¹ Briefly, L929 cells (6×10^4 well) were cultured in 96-well flat bottom microtitre plates (Nunc) containing serial dilutions of conditioned supernatant of sarcoid alveolar macrophages in the presence of actinomycin D. After 20 hours of incubation the remaining cells were stained with crystal violet. The TNF α concentration

of the samples was quantified by comparing the results with the linear portion of a standard curve obtained with human recombinant TNF α (Serva, Heidelberg, Germany, specific activity 6.6×10^6 units/mg). The specificity of this bioassay was tested by neutralising peak samples with a goat anti-human TNF α antibody (obtained through H Biermann, Bad Nauheim, Germany from British Biotechnology).

SUPEROXIDE ANION

The production of superoxide anion was determined by a ferricytochrome c microassay.²² Briefly, 10^5 alveolar macrophages were incubated in a 96-well microtitre plate with $100 \mu\text{mol}$ ferricytochrome c $\pm 0.2 \text{ mg/ml}$ superoxide dismutase, with or without 10 mmol sodium fluoride or 100 nmol PMA. All experiments were done in triplicate. Optical density was determined at 550 nm over a period of two hours at 30 minute intervals on a microplate reader.

STATISTICS

Non-parametric statistics were used throughout the study. The Mann-Witney U test was used for independent samples. Correlations were made with the Spearman rank correlation, using the median as a marker of central tendency. Differences were considered significant at $p < 0.05$.²³

Results

TOTAL AND DIFFERENTIAL CELL COUNT

Table 2 shows the cell counts in bronchoalveolar lavage fluid. The total number of cells recovered from the bronchoalveolar lavage fluid was increased in patients with pulmonary sarcoidosis. Furthermore, the percentage of lymphocytes was significantly increased compared with the healthy controls ($p < 0.005$).

ICAM-1 ON ALVEOLAR CELLS AND SERUM LEVELS OF sICAM-1

ICAM-1 expression could be detected on alveolar macrophages in all cases (fig 1). Significantly elevated expression was seen on alveolar macrophages from patients with sarcoidosis compared with controls (mean (SD) $0.74 (0.24) \text{ EU}$ v $0.46 (0.12) \text{ EU}$, $p < 0.05$). Only patients with apparently active disease showed significantly increased ICAM-1 levels on alveolar macrophages ($0.82 (0.27) \text{ EU}$) compared with alveolar macrophages from the controls.

Immunocytochemical staining confirmed the results of the ELISA for the macrophage population in the bronchoalveolar lavage fluid. Using the APAAP complex, ICAM-1 expression could also be demonstrated on alveolar lymphocytes from patients with sarcoidosis (fig 2).

Serum levels of sICAM-1 ranged from 200 to 700 ng/ml and were similar in patients and controls ($490 (130) \text{ ng/ml}$ v $320 (90) \text{ ng/ml}$).

Figure 1 ICAM-1 expression of alveolar macrophages from patients ($n = 17$) with active ($n = 10$) or inactive ($n = 7$) disease and healthy volunteers ($n = 6$) as determined by ELISA. $**p < 0.05$ v control.

Figure 2 Immunoalkaline phosphatase (APAAP) staining on cytopspin preparations of alveolar cells from patient with sarcoidosis. Original magnification $\times 630$, reduced to 82% during origination.

Table 3 Mean (range) data of macrophage activation in bronchoalveolar lavage fluid of patients with active and inactive sarcoidosis and healthy controls

	Control	Sarcoidosis		
		Total	Active	Inactive
ICAM-1 (EU/10 ⁵ AM)	0.46 (0.29–0.57)	0.78 (0.42–1.17)	0.82 (0.44–1.17)	0.64 (0.42–0.84)
TNF α (units/10 ⁶ AM)	3 (0–10)	239 (5–1345)	319 (6–1345)	59 (5–141)
O ₂ ⁻ (nmol/10 ⁵ AM)	0.76 (0–1.40)	2.59 (0.37–4.79)	2.52 (1.0–4.79)	2.75 (0.37–3.97)

ICAM-1—intercellular adhesion molecule 1; EU—ELISA unit; TNF α —tumour necrosis factor α ; O₂⁻—superoxide anion; AM—alveolar macrophage.

No correlation between sICAM-1 levels and ICAM-1 surface expression on alveolar macrophages was demonstrated.

RELEASE OF TNF α AND OXIDANTS BY ALVEOLAR MACROPHAGES

All alveolar macrophages from patients with sarcoidosis spontaneously released TNF α and free oxygen radicals whereas only a small amount of activity was detected in the supernatants of controls (table 3). TNF α release of alveolar macrophages correlated strongly with the ICAM-1 surface expression of those cells ($r = 0.69$, $p < 0.05$; fig 3). The spontaneous release of free oxygen radicals was significantly elevated in patients and did not differ between subjects with active and inactive disease (table 3). No correlation with ICAM-1 expression could be shown.

The intensity of the lymphocytic alveolitis as assessed by absolute numbers and percentage of CD4+ lymphocytes in bronchoalveolar lavage fluid was not related to the enhanced expression of ICAM-1 on alveolar macrophages.

Discussion

Pulmonary sarcoidosis is an inflammatory disorder of unknown aetiology, characterised by a mononuclear cell alveolitis and non-caseating granulomas, leading in some cases to interstitial fibrosis.^{24–26} To elucidate the possible role of adhesion molecules in this disease, we investigated the contribution of ICAM-1 to the process of recruitment and activation of inflammatory cells in the lung.

ICAM-1 levels on alveolar macrophages were elevated in patients with pulmonary sarcoidosis. This increase was statistically significant only in patients with active disease.

No other statistical difference between the two groups of patients could be established, which is probably due to the limited number of subjects in our study.

There are several possible mechanisms leading to the increased ICAM-1 levels we measured. One could be enhanced recruitment of blood monocytes into the pulmonary compartment. In a recent study by Melis *et al*, however, no relation between ICAM-1 expression and different blood monocyte associated markers on alveolar macrophages was found.¹⁵ Furthermore, they found no difference between ICAM-1 expression on blood monocytes from patients with sarcoidosis and from healthy volunteers. This is in line with our findings that serum levels of sICAM-1 were similar in our patients and controls.

TNF α activity in the supernatant of alveolar macrophages correlated strongly with ICAM-1 surface expression of those cells. It has been shown previously that TNF α and other cytokines induce ICAM-1 on endothelial cells, whereas airway epithelial cells are predominantly responsive to τ -interferon.²⁷ We recently showed that TNF α induces ICAM-1 on control alveolar macrophages *in vitro* (unpublished observation). Thus, it seems likely that in sarcoidosis ICAM-1 expression on alveolar macrophages is regulated in an autocrine manner, supporting the concept that alveolar macrophages are pivotal cells in the pathogenesis and maintenance of the inflammatory process in the pulmonary compartment. Further support for a local upregulation of ICAM-1 was that serum levels of sICAM-1 were not related to the activation demonstrated on alveolar macrophages.

Few studies exist which describe elevated ICAM-1 levels on alveolar macrophages in patients with sarcoidosis.^{15 16} However, to our knowledge this is the first description of increased ICAM-1 expression in correlation with other parameters of immunological activity. We found no correlation between upregulated ICAM-1 expression of alveolar macrophages to the number of CD4+ lymphocytes in bronchoalveolar lavage fluid, which is in agreement with previous work.¹⁵ Elevated ICAM-1 surface expression was not restricted to alveolar macrophages in patients with sarcoidosis, however, but could also be demonstrated on alveolar lymphocytes.²⁸ This suggests that the interaction between ICAM-1 and its ligands on macrophages and CD4+ lymphocytes functions reciprocally.

Figure 3 Correlation between the spontaneous release of TNF α and ICAM-1 surface expression of alveolar macrophages in sarcoidosis.

Considering the strong correlation we found between ICAM-1 expression and TNF α secretion of the activated macrophages, we conclude that upregulated ICAM-1 levels reflect disease activity in the pulmonary compartment. The fact that we could not show a correlation with the number of CD4+ lymphocytes agrees with other studies, suggesting different mechanisms of activation for the lymphocytic and macrophage component of the mononuclear cell alveolitis in pulmonary sarcoidosis.^{15,24}

It is not clear whether upregulated ICAM-1 expression on alveolar macrophages is an early event during the development of the mononuclear cell alveolitis or a result of the inappropriate cytokine secretion of alveolar macrophages and lymphocytes in the pulmonary compartment. Further research is needed to characterise the exact position of ICAM-1 in the inflammatory cascade in sarcoidosis.

The authors acknowledge the expert technical assistance of Birgitta Karlberg and Monika Losch.

- 1 Wawryk SO, Novotny JR, Wicks IP, Wilkinson D, Maher D, Salvaris E, et al. The role of the LFA-1/ICAM-1 interaction in human leukocyte homing and adhesion. *Immunol Rev* 1989;108:135-61.
- 2 Makgoba MW, Bernard A, Sanders ME. Cell adhesion/signalling: biology and clinical applications. *Eur J Clin Invest* 1992;22:443-53.
- 3 Springer TA. Adhesion receptors of the immune system. *Nature* 1990;346:425-34.
- 4 Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990;247:456-9.
- 5 Malizia G, Calabrese A, Cottone M, Raimondo M, Treidosiewicz LK, Smart CJ, et al. Expression of leukocyte adhesion molecules by mucosal mononuclear phagocytes in inflammatory bowel disease: immunohistological evidence for enhanced antigen presenting capacity. *Gastroenterology* 1991;100:150-9.
- 6 Müller GA, Markovic-Lipovski J, Müller CA. Intercellular adhesion molecule-1 expression in human kidneys with glomerulonephritis. *Clin Nephrol* 1991;36:203-8.
- 7 Arnaout MA. Structure and function of the leukocyte adhesion molecules CD11/CD18. *Blood* 1990;75:1037-50.
- 8 Müller-Quernheim J, Pfeifer S, Männel D, Strausz J, Ferlinz R. Lung-restricted activation of the alveolar macrophage/monocyte system in pulmonary sarcoidosis. *Am Rev Respir Dis* 1992;145:187-92.
- 9 Crystal RG, Bitterman PB, Rennard SI, Hance AJ, Keogh BA. Interstitial lung disease of unknown cause: disorders characterized by chronic inflammation of the lower respiratory tract. *N Engl J Med* 1984;310:154-66, 235-44.
- 10 Semenzato G. The immunology of sarcoidosis. *Semin Respir Med* 1986;8:17-29.
- 11 Bachwich PR, Lynch JP III, Larrick JW, Spengler M, Kunkel SL. Tumor necrosis factor production by human sarcoid alveolar macrophages. *Am J Pathol* 1986;125:421-5.
- 12 Aerts C, Wallaert B, Grobois JM, Voisin C. Release of superoxide anion by alveolar macrophages in pulmonary sarcoidosis. *Ann NY Acad Sci* 1986;465:192-200.
- 13 Dalhoff K, Braun J, Lipp R, Schnabel A, Wießmann KJ. Sauerstoffradikalbildung bei pulmonaler Sarkoidose. *Dtsch Med Wschr* 1992;117:887-92.
- 14 Ina Y, Takada K, Yamamoto M, Morishita M, Miyachi A. Antigen-presenting capacity in patients with sarcoidosis. *Chest* 1990;98:911-6.
- 15 Melis M, Gjomarkaj M, Pace E, Malizia G, Spatafora M. Increased expression of leukocyte function associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) by alveolar macrophages of patients with pulmonary sarcoidosis. *Chest* 1991;100:910-6.
- 16 Stritz I, Wang YM, Kalaycioglu O, Costabel U. Expression of alveolar macrophage adhesion molecules in pulmonary sarcoidosis. *Chest* 1992;102:882-6.
- 17 Hoogsteden HC, van Hal PTW, Wijkhuijs JM, Hop W, Verkaik APK, Hivering C. Expression of the CD11/CD18 cell surface adhesion glycoprotein family on alveolar macrophages in smokers and nonsmokers. *Chest* 1991;100:1567-71.
- 18 Crystal RG, Reynolds HY, Kalica AR. Bronchoalveolar lavage. *Chest* 1996;90:120-33.
- 19 Kelly CA, Ward C, Stenton SC, Hendrick DJ, Walters EH. Assessment of pulmonary macrophage and neutrophil function in sequential bronchoalveolar lavage aspirates in sarcoidosis. *Thorax* 1988;43:787-91.
- 20 Erber WN, Pinching AJ, Mason DY. Immunocytochemical detection of T and B cell populations in routine blood smears. *Lancet* 1984;i:1042-6.
- 21 Feist W, Ulmer AJ, Musehold J, Brade H, Kusumoto S, Flad H-D. Induction of tumor necrosis factor-alpha release by lipopolysaccharide and defined lipopolysaccharide partial structures. *Immunobiology* 1989;179:293-307.
- 22 Pick E. Microassay for superoxide anion and hydrogen peroxide production and nitroblue tetrazolium reduction using an enzyme immunoassay microplate reader. *Method Enzymol* 1986;132:407-21.
- 23 Sachs L. *Angewandte Statistik*. Berlin: Springer Verlag, 1984:230-8.
- 24 Keogh A, Hunninghake GW, Line BR, Crystal RG. The alveolitis of pulmonary sarcoidosis. *Am Rev Respir Dis* 1983;128:256-65.
- 25 Thomas PD, Hunninghake GW. Current concepts in the pathogenesis of sarcoidosis. *Am Rev Respir Dis* 1987;135:747-60.
- 26 Saboor SA, McI Johnson N, McFadden J. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. *Lancet* 1992;339:1012-5.
- 27 Look DC, Rapp SR, Keller BT, Holtzmann MJ. Selective induction of intercellular adhesion molecule-1 by interferon-U in human airway epithelial cells. *Am J Physiol* 1992;263:L79-L87.
- 28 Nagai S, Satake N, Kaneshima H, Kawatani A, Takeuchi M, Izumi T. Expression of adhesion molecules on lung T cells and macrophages in patients with pulmonary sarcoidosis. *Eur Respir J* 1992;5(Suppl):501s.