RAPID COMMUNICATION

ICAM-1 and integrin expression on isolated human   alveolar type II pneumocytes

J. Guzman*, T. Izumi**, S. Nagai**, U. Costabel†

ICAM-1 and integrin expression on isolated human alveolar type II pneumocytes. J. Guzman, T. Izumi, S. Nagai, U. Costabel. ©ERS Journals Ltd.
ABSTRACT: Adhesion molecules are involved in the recruitment of leucocytes to sites of inflammation. In this study, we determined the expression of several adhesion molecules on isolated human alveolar type II pneumocytes.

Type II pneumocytes were isolated from 10 normal lung specimens, by enzymatic digestion with dispase, followed by metrizamide gradient centrifugation and panning on immunoglobulin G (IgG)-coated plastic dishes. With the freshly isolated type II cells, immunostaining was performed using a sensitive immunoperoxidase slide technique.

In all cases, 60–90% of type II cells were positive for intercellular adhesion molecule-1 (ICAM-1) (CD54). A minor portion of type II cells expressed the alpha 4 (CD49d) subunit of the β_1-integrins, and the alpha-v (CD51) subunit of the vitronectin receptor. CD11a, CD11b, CD11c, CD18, CD49b, CD49e and CD49f failed to demonstrate any immunostaining with type II cells.

In conclusion, the observation of the expression of ICAM-1 and, to a lesser degree, of some integrin subunits, may indicate that alveolar type II cells participate in local immune and inflammatory responses.
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* Allg., insb. Experimentelle Pathologie, Ruhr-Universität Bochum, Germany. ** Chest Disease Research Institute, Kyoto University, Japan. †Abt. Pneumologie und Allergologie, Ruhrlandklinik, Essen, Germany.

Correspondence: J. Guzman y Rotaeche, Allgemeine, insbesondere Experimentelle Pathologie, Gebäude MA 01-596, Ruhr-Universität Bochum, Universitätstrasse 150 D-44780 Bochum, Germany.

Intercellular adhesion molecule-1 (ICAM-1) is an adhesion molecule of the immunoglobulin superfamily. It serves as a ligand for the leucocyte function associated antigen-1 (LFA-1) (CD11a/CD18) and Mac-1 (CD11b/CD18) integrin on human T-cells, but is also involved in adhesion of other cells. ICAM-1 can be expressed on many cells [1].

The integrins are members of a supergene family of heterodimeric receptors present on the surface of various cells [2]. They apparently function to link the extracellular environment to cytoskeletal structures. Cell-to-cell interactions and adhesion of cells to the extracellular matrix are of fundamental importance for many immunological processes in the lung. Increased adhesiveness of activated leucocytes may contribute to damage of the lung parenchyma in several pathological processes.

Recent evidence suggests that alveolar type II pneumocytes play an indirect role in immunological functions. Their major products, surfactant phospholipids and proteins, have been shown to modulate the activity of immune cells. Surfactant protein A may enhance certain macrophage activities, including phagocytosis and intracellular killing of bacteria, migration, and cytotoxicity against tumour cells. Surfactant phospholipids, on the other hand, are capable of suppressing several lymphocyte functions, including the proliferative response to mitogens, immunoglobulin production, and natural killer

cell activity [3, 4]. Whether alveolar type II cells are also directly involved in immunological processes is, at present, unknown.

In the context of the new knowledge concerning the role of adhesion molecules in cell-to-cell and cell-to-extracellular matrix interactions, the purpose of the present study was to investigate the expression of adhesion molecules on human alveolar type II cells isolated from normal lung tissue. We chose to use isolated type II cells for this purpose, since a recent study indicated that precise identification of the individual cell type within the alveolar septae is not possible by light microscopy with the relatively thick sections used for immunohistochemistry of frozen tissue [5].

Material and methods

Isolation of alveolar type II cells

Normal lung specimens (30g) were obtained from 10 patients (6 smokers and 4 nonsmokers) undergoing lobectomy or pneumonectomy for primary lung cancer. Preoperative lung function of these subjects was normal. The area from which the lung parenchyma for cell isolation was taken was macroscopically and histologically

free of emphysema or other pathological changes. The cell isolation was performed immediately after surgical resection. Human type II pneumocytes were isolated as described previously [6], using a combination of the procedures published by Dobbs *et al.* [7], Edelson *et al.* [8], and Papadopoulos *et al.* [9]. The isolation procedure included enzymatic digestion with dispase, and enrichment of type II cells by metrizamide gradient centrifugation, followed by panning on immunoglobulin G (IgG)-coated plastic dishes. Immunocytochemistry was performed with the freshly isolated type II cells.

Enzymatic digestion. The lung was cut into small pieces (less than 0.5 cm³), which were washed thoroughly in solution I (7.95 g NaCl + 0.4 g KCl + 1.11 g D (+) glucose·1H$_2$O + 0.46 g Na$_2$HPO$_4$·2H$_2$O + 2.38 g hydroxyethylpiperazine ethane sulphate acid (HEPES) and 1,000 ml aqua bidest) to remove excess blood; followed by incubation with 80 ml of dispase solution (0.2 g dispase in 80 ml solution II: solution I + 0.28 g CaCl$_2$·2H$_2$O$_2$ + 0.32 g MgSO$_4$·7H$_2$O$_2$, pH 7.4) for 45 min at 37°C with continuous stirring. After further mincing of the digested tissue, the suspension was filtered through a series of 100, 50 and 20 µm nylon meshes, centrifuged at 160×g for 10 min at 4°C, and resuspended in solution II.

Metrizamide gradient centrifugation. The cells were layered on a discontinuous metrizamide gradient with the upper and lower solutions having densities of 1.040 and 1.090, respectively. After centrifugation at 160×g for 20 min, the cells that accumulated at the interphase were removed and washed in 50 ml solution II containing desoxyribonuclease (1 mg·ml^{-1}) to prevent cell clumping.

Panning on IgG-coated dishes. Macrophages and blood leucocytes were removed from the cell suspension by differential adherence to 100 mm plastic Petri dishes coated with human IgG (500 µg·ml^{-1}) during incubation for 60 min at 37°C (7, 8). The nonadherent cells, mainly type II cells, were pooled and centrifuged at 160×g for 5 min at 4°C. Total cell counts were performed using a Neubauer chamber. Identification and purity of type II cells were assessed on cytocentrifuge preparations using the modified Papanicolaou stain [10].

Immunocytochemistry

The expression of ICAM-1 (ligand for β$_2$-integrins), β$_1$-integrins (extracellular matrix receptors), β$_2$-integrins (leucocyte cell adhesion molecules) and β$_3$-integrins (cytoadhesins) on type II cells was analysed using the immunoperoxidase adhesive slide assay, as described previously [11]. Briefly, 10 µl of cell suspension, at a concentration of 2×10^6 cells·ml^{-1} was added to each of the reaction areas of adhesive slides (Bio-Rad, Germany). After 10 min, the cells were settled and firmly attached to the glass surface. The cells were then fixed with 0.05% glutaraldehyde solution for 5 min. The surface marker staining was performed using a sensitive four layer peroxidase anti-peroxidase technique, followed by postfixation with OsO$_4$. The monoclonal antibodies

Table 1. – Monoclonal antibodies used in this study

Monoclonal antibody	Source	Antigen
IOL 54	Dianova	CD54 (ICAM-1)
β1-integrins		
IOP 49b	Dianova	CD49b (alpha-2), collagen receptor
IOP 49d	Dianova	CD49d (alpha-4), ligand for VCAM-1
IOP 49e	Dianova	CD49e (alpha-5), fibronectin receptor
IOP 49f	Dianova	CD49f (alpha-6), laminin receptor
β2-integrins		
IOT 16	Dianova	CD11a (LFA-1)
OKM 1	Ortho	CD11b (Mac 1)
Ki-M1	Behring	CD11c (CR 4)
IOT 18	Dianova	CD18 (β-chain)
β3-integrin		
IOP 51	Dianova	CD51 (alpha-v), vitronectin receptor

ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule; LFA: leucocyte function associated antigen.

(MoAbs) used in this study are listed in table 1. The percentage of positively reacting type II cells was determined for each antigen.

Results

The percentage of isolated type II cells ranged between 80–95% of total cells isolated (fig. 1). The cell viability was 95%. The total number of isolated type II cells ranged between 1.5-3.0x10^7 cells per 30 g lung tissue.

In all cases, immunostaining of type II cells for ICAM-1 was observed (fig. 2). Sixty to ninety percent of type II cells per case were positive. In many instances, the positive reaction for ICAM-1 was located at one side of the cell surface (fig. 3); mostly corresponding to the area closer to the nucleus. This staining pattern was in contrast to the positive reaction with an epithelial marker, HEA 125 (Progen), in which the reaction was homogeneously distributed on the cell surface.

A negative reaction of type II cells was found for CD11a, CD11b, CD11c, CD18, CD49b, CD49e, CD49f.
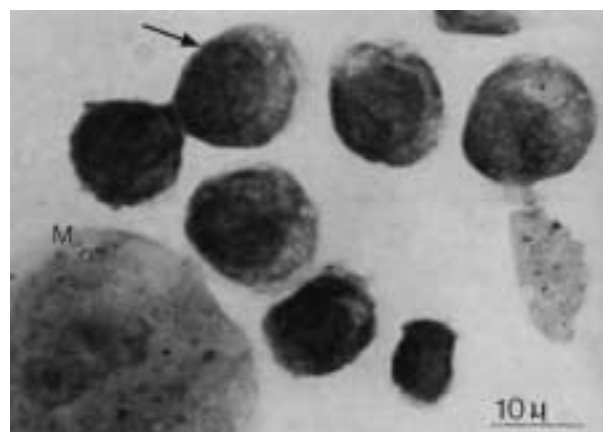

Fig. 1 – Freshly isolated human type II cells (arrow). Macrophage (M). May-Grünwald Giensa stain; original magnification ×100.
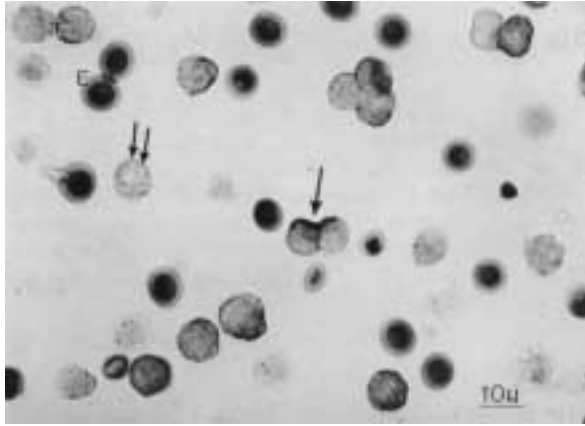
Fig. 2 – Cell surface immunostaining of isolated human type II cells for intercellular adhesion molecule-1 (ICAM-1) (CD54). Positive reacting type II cells (one arrow), negative type II cell (two arrows), erythrocytes with pseudoendogenous peroxidase reaction (E). In many instances the positive reaction of type II cells for ICAM-1 is located at one side of the cell surface. Peroxidase-antiperoxidase adhesive slide assay for the detection of cell surface antigens. (No nuclear counterstain; Original magnification ×400).
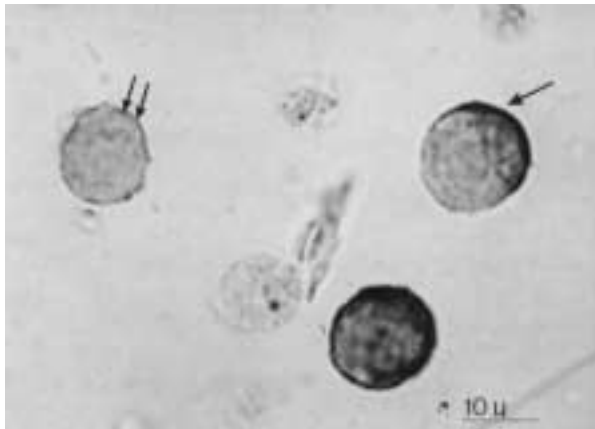

Fig. 3 – Cell surface immunostaining of isolated human type II cells for intercellular adhesion molecule-1 (ICAM-1) (CD54). In many instances the positive reaction is located at one side of the cell surface (arrow). Negative type II cell (two arrows). Peroxidase-antiperoxidase adhesive slide assay for the detection of cell surface antigens. (No nuclear counterstain. Original magnification ×1,000).

A weak reaction of only few type II cells (5–10%) was found for CD49d in 6 out of 10 cases, and for CD51 in 6 out of 10 cases. No significant differences were observed in the reaction pattern of type II cells between nonsmokers and smokers.

Discussion

In this study, we determined the expression of several adhesion molecules on isolated human type II cells obtained from normal lung tissue of smokers and non-smokers. A previous immunohistochemical study of the distribution of various integrins in normal human lung tissue was not able to differentiate the various cellular components within the alveolar septae at the light microscopic level using frozen tissue sections [5]. Our

approach, using isolated alveolar type II cells, avoided this problem.

Our results show that the majority of human alveolar type II cells express ICAM-1, a minor portion also the alpha-4 (CD49d) subunit of the β1-integrins and the alpha-v (CD51) subunit of the vitronectin receptor. This may indicate that type II cells play a direct role in immune and inflammatory responses of the lung, because it is known that ICAM-1 is actively involved in the recruitment of lymphocytes, neutrophils and eosinophils to sites of inflammation [1].

The expression of ICAM-1 may enable type II cells to bind to leucocytes through LFA-1, which is one of the ligands for ICAM-1. This may be an important mechanism in the regulation of the access of leucocytes to the lung parenchyma during pulmonary immune and inflammatory reactions, including idiopathic pulmonary fibrosis, sarcoidosis, and other interstitial lung diseases. ICAM-1 is also constitutively expressed in human bronchial epithelium [12], and its expression increases after allergen challenge in human asthma, in parallel with an increase in neutrophils, eosinophils, T-cells, and mast cells [13]. In addition, ICAM-1 is known to be the major surface receptor for rhinoviruses, which may produce lung diseases [14].

Several cytokines, including interferon-γ (IFN-γ), interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α), are able to upregulate the expression of ICAM-1 on leucocytes, endothelial and epithelial cells [1]. Whether this is also valid for human type II cells is, at present, unknown. A recent study showed that, in mice, ICAM-1 expression on alveolar type II cells can be markedly induced by hyperoxic exposure [15]. ICAM-1 is involved not only in adhesive properties of activated cells, but also represents an important co-stimulatory signal to T-cell proliferation [16]. In this regard, we recently reported that human leucocyte antigen-DR (HLA-DR) antigens, which are other major molecules supporting T-cell proliferation, are also constitutively expressed on human type II cells [6]. However, the functional activity of alveolar type II cells in relation to antigen T-cell activation is still unknown [17].

Whereas, our study clearly demonstrated ICAM-1 expression on freshly isolated alveolar type II cells obtained from normal human lung parenchyma, CHRISTENSEN et al. [18] reported that in the rat ICAM-1 was not detected on type II cells immediately after isolation, but appeared over 48 h, and was maintained during 7 days in culture; suggesting that ICAM-1 expression might be a differentiation-related characteristic associated with the type I cell phenotype. By using electronmicroscopic immunocytochemistry, KANG et al. [15] showed, in normal mice, that type II cells had considerably lower expression of ICAM-1 than type I cells. Apparently, these divergencies may be explained by species specific differences, with detectable ICAM-1 expression on human alveolar type II cells, as seen in our study, and little or no detectable expression in the mouse [15] and the rat [18]. Since CHRISTENSEN et al. [18] and our group used similar isolation techniques, including enzymatic digestion and panning, it is unlikely that in vitro activation

of type II cells during the isolation procedure may have influenced our results.

Obviously, some differences are apparent in the expression of integrins between bronchial epithelial cells and alveolar type II cells. While, previous studies have shown that bronchial epithelial cells express CD49b, the alpha-2 chain of the collagen receptor, and CD49f, the alpha-6 chain of the laminin receptor [5, 19], these two β_1-integrin subunits were not expressed by alveolar type II cells in our study. On the other hand, CD49d, the alpha-4 subunit, which can serve both as a fibronectin receptor and as ligand of vascular cell adhesion molecule (VCAM-1), was not expressed by the bronchial epithelium [5] but by a few type II cells in our study. The CD51 (alpha-v) subunit of the vitronectin receptor, however, stained positive with the bronchial epithelium in the previous study [5], and some alveolar type II cells in our study. The biological basis for these differences is uncertain.

In conclusion, this report provides new insights into the phenotype of alveolar type II cells. The observation of the expression of ICAM-1, and also, but to a lesser degree, of some integrin subunits may indicate that these cells participate in local immune and inflammatory responses. The functional correlations and the regulatory mechanisms involved in integrin expression on alveolar type II cells remain areas for further research.

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