Circulating Intercellular Adhesion Molecule-1 in Patients with Lung Cancer

Osamu Taguchi, Esteban Cesar Gabazza, Tetsu Kobayashi, Masamichi Yoshida, Hiroki Yasui and Hiroyasu Kobayashi

Recently, abnormal expression of a great variety of adhesion molecules has been reported in malignancy. Of these adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) has been suggested to play an important role in the process of tumor invasion and distant metastasis. The purpose of this investigation was to assess the peripheral blood levels of soluble ICAM-1 and the effect of cytotoxic therapy upon these circulating molecules in a cohort of patients with lung cancer. This study comprised 19 lung cancer patients hospitalized in our institution (males 16 and females 3, mean age 60 years old). Serum concentration of soluble ICAM-1 was measured using a commercially available enzyme immunoassay test kit. These measurements were done before the initiation of any therapy and on day 5 of chemotherapy. Samples taken from healthy volunteers were available for comparison. Soluble ICAM-1 serum concentration was significantly higher (p<0.0001) in the cancer patients as compared to that of the control group. Serum levels of ICAM-1 were more significantly (p<0.02) elevated in patients with advanced stages of disease. This study suggests the presence of an increased expression of circulating adhesion molecules in lung cancer. The concentration of this adhesion molecule was correlated with the clinical stage of the malignant disease, but did not change significantly after multidrug cytotoxic therapy.

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Introduction

Intercellular adhesion molecule-1 (ICAM-1) is a 74–114 KDa transmembrane glycoprotein that belongs to the immunoglobulin supergene family (1). ICAM-1 together with its integrin ligands the leukocyte function-related antigen-1 (LFA-1, CD1la/CD18) and the macrophage antigen Mac-1 (CD11b/CD18), plays important role during the immune response in inflammatory and neoplastic processes (2, 3). ICAM-1 mediates the adherence of leukocytes to vascular endothelial cells and their subsequent emigration into the sites of inflammation (4). The molecular interaction between ICAM-1 and LFA-1 is a crucial step for triggering the signals leading to cytotoxic and helper lymphocyte functions (5, 6). ICAM-1 has been also found to play significant roles in tumorigenesis and metastatic spread of malignant tumors (7, 8).

ICAM-1 has been isolated from most tissues and cells. It is expressed on the surface of normal hematopoietic and non-hematopoietic cells, vascular endothelium and in a great number of tumors (9–14). This expression of ICAM-1s can be up-regulated by inflammatory cytokines such as gamma-interferon, tumor necrosis factor alpha, and by lipopolysaccharides (15, 16). The significance of the in situ expression of ICAM-1 in malignant tumors remains controversial. While local induction of ICAM-1 is related to most susceptibility of tumor cells to lysis by the host cellular immune system, increased histological expression of this adhesion molecule is correlated with disease progression and poor prognosis in some patients, particularly in patients with malignant melanoma (17, 18).

Recently, the existence of a soluble form of ICAM-1 has been described in human serum (19). It was also found to be elevated in a variety of benign and malignant disorders (20). The biological and clinical significance of this circulating ICAM-1 has not yet been elucidated. Preliminary studies demonstrated a positive correlation of the serum ICAM-1 levels with the stage of disease and survival time of patients with melanoma, hepatocellular carcinoma, Hodgkin’s disease, Ewing’s sarcoma and Wilms’ tumor (21, 22).
The objective of present study was to investigate the serum concentration of ICAM-1 before and after systemic administration of cisplatin-based anticancer therapy and its relation with the clinical stage in patients with lung cancer. To the best of our knowledge, this is the first study to report the levels of circulating ICAM-1 in lung cancer patients.

Materials and Methods

This study comprised 19 consecutive patients with lung cancer (male 16, female 3, mean age 60±10 y-o). Most of the patients (n=16) were smokers. There were 16 patients with non-small cell lung cancer (adenocarcinoma, 10; squamous cell carcinoma, 6 cases) and 3 with small cell lung carcinoma (SCLC). The distribution of disease stage was as follows: stage I (4 patients), stage II (3 patients), stage IIIa (4 patients), stage IIIb (5 patients), stage IV (3 patients). Staging was based on the new international staging system (23). The staging procedure included a clinical examination, standard chest radiography, computed tomography scan of the chest, upper abdomen and brain, fiber optic bronchoscopy, liver ultrasonography, and bone scintigraphy. The diagnosis of lung cancer was confirmed by the examination of biopsy and cytologic specimens. Serum ICAM-1 was measured by an enzyme immunoassay using a commercially available ICAM-1 test kit (T Cell Diagnostics, Inc., Cambridge MA). Briefly, serum samples were added to microplate wells coated with murine monoclonal antibody to human ICAM-1. Then, a horseradish peroxidase-conjugated murine monoclonal antibody to human ICAM-1 was added and incubated at room temperature for 2 hours. After extensive washing, chromogenic solution containing o-phenylenediamine was pipetted into each microplate well and absorbance was read at 490 nm in a microplate reader after stopping the reaction with sulfuric acid. The detection limit of the assay was 0.3 ng/ml. The intra-assay and the inter-assay coefficient of variations were 2 and 3%, respectively. Values of ICAM-1 in 31 healthy volunteers were available for comparison.

In all patients, the adhesion molecule was determined before the initiation of any therapy. In 11 patients treated with cisplatin-based combination chemotherapy, ICAM-1 was measured before starting and after completing the administration of the full dose of cytotoxic drugs. Chemotherapeutic agents combination and schedule were as follows: (a) for non-SCLC: cisplatin 80 mg/m² day 1, mitomycin 8 mg/m² day 1, vindesine 3 mg/m² days 1, 8, and 15; (b) for SCLC: cisplatin 80 mg/m² day 1, etoposide 100 mg/m² day 1, 3 and 5. Patients were assigned to two groups according to the response to anticancer therapy. Responders showed a 50% or more decrease in the product of the longest perpendicular diameters of measurable tumor lesions lasting at least 4 weeks after chemotherapy. Patients with increased tumor size or with diminution of tumor volume less than 50% were classified as non-responders. Blood samples were drawn after patients rested in supine position for 30-min. After centrifuging the blood samples at 3,000 rpm for 20-min, the serum fractions were separated in small aliquots and stored at −20°C until use. All blood samples were taken after obtaining written and formal consent from the patients. The investigation was approved by our Institution Ethic Committee and it was carried out in accordance with the stipulations of the Helsinki Declaration.

Statistical analysis

Data are expressed as means±SD, unless otherwise specified. The difference between the means of variables was analyzed by the Mann-Whitney U test. The strength of correlation was calculated by the Spearman coefficient of correlation (24).

Results

There was no significant difference in age and sex between lung cancer patients and healthy controls. Although there were more smokers among patients with lung cancer than in control subjects, there was no significant difference in the serum concentration of ICAM-1 between smokers and nonsmokers (data not shown).

The serum concentration of ICAM-1 was significantly higher (p<0.0001) in patients with lung cancer (494.2±33.3 ng/ml vs. 280.2±21.3 ng/ml) than in the healthy control group (Fig. 1). For evaluating the influence of clinical progression on the serum expression of ICAM-1, the patients were assigned to two groups: a group with malignant lesions restricted to one hemithorax (stages I, II, IIIa) and another with contralateral or distant metastasis to other organs (stage IIIb, IV). Patients in stages IIIb, IV (554.9±44.1 ng/ml vs 410.8±34.7 ng/ml) showed a significantly (p<0.03) increased serum concentration of ICAM-1 compared to those in stages I, II, IIIa (Fig. 2). The serum levels of the adhesion molecule in patients of each clinical stage, stage I (414.5±50.5 ng/ml, p=0.03), stage III (525.7±42.3 ng/ml, p<0.0001) and stage IV (474.3±110.2, p=0.01) were significantly higher than in the healthy control group.

Analysis of the serum levels of ICAM-1 in each histological type, adenocarcinoma (514.6±62.3 ng/ml), squamous cell carcinoma (586.5±100.1 ng/ml) and small cell carcinoma (592.5±27.5 ng/ml) did not reveal any statistically significant difference among the three histological groups (p=0.3). The administration of combination chemotherapy in 11 patients did not significantly change (p=0.6) the serum levels of ICAM-1 (510.8±46.3 ng/ml vs 535.5±52.9 ng/ml). Also there was no significant difference in the serum ICAM-1 levels between non-responder and responder groups (500.3±71.8 ng/ml vs 516.6±60.3 ng/ml, p=0.5).

Discussion

The current work showed for the first time the presence of elevated levels of circulating ICAM-1 and their correlation with the clinical stage of disease in patients with lung cancer. The cellular source of ICAM-1 in lung cancer is not clear. It may result from shedding by normal host cells or by tumor cells. ICAM-1 is secreted by fibroblasts, inflammatory and endothelial cells as well as by various lung tumor cells of different histological types, including adenocarcinoma, large and squa-
The serum concentration of ICAM-1 was significantly higher in patients with lung cancer than in the healthy group. Bars indicate the mean values.

Figure 1. Serum concentration of ICAM-1 in patients with lung cancer and in control. The levels of ICAM-1 were significantly higher in patients with lung cancer than in the healthy group. Bars indicate the mean values.

Figure 2. ICAM-1 serum level by disease clinical stage. Patients in stage IIIb-IV showed a significantly increased concentration of ICAM-1 as compared to those in stage I–IIIa.

Lung tumors have been shown to produce a number of cytokines. Thus, cytokine-induced expression and secretion of ICAM-1 by tumor or normal cells may also explain the markedly increased levels of ICAM-1 in lung cancer patients. Direct evidence of tumor cell secretion of ICAM-1 has been reported in some types of malignancy such as Hodgkin’s disease and ovarian carcinoma.

Our current preliminary study showed that, the serum concentration of ICAM-1 is elevated even at stage I in lung cancer patients, indicating that stimulating activities for ICAM-1 secretion start in the early clinical stages of disease. Further, increased circulating ICAM-1 was related with the progression of the malignant disease. This increase in the peripheral expression of ICAM-1 with the advance of malignancy may represent either a major tumor burden or an increased inflammatory immune response of the host to malignant cells. In this connection, enhancement of soluble ICAM-1 from human tumors has been observed to occur in association with the grade of tumor growth and with the presence of inflammatory processes.

Confirmation of this serum ICAM-1 and clinical stage correlation by additional investigations in a larger population of patients might allow the potential application of this in vitro test for the follow-up of disease progression in patients with lung cancer.

The clinical and biological significance of the shedding of ICAM-1 into the systematic circulation in malignancy is still a matter of controversy. Some evidence has shown that ICAM-1 might have profound implications in tumor progression and in the development of metastatic foci. Augmented shedding of soluble ICAM-1 into the systemic circulation might bind and saturate the LFA-1 and thus block the adhesion between leukocyte LFA-1 and membrane-bound ICAM-1 on tumor cells. In this way, ICAM-1 may promote metastasis by allowing circulating neoplastic cells to escape from the immune surveillance mediated by cytotoxic T cells and natural killer and lymphokine-activated killer cells. It has also been hypothesized that the in situ overexpression of ICAM-1 may increase the binding of lymphocytes to locally growing tumor cells and may consequently interfere with the homotypic aggregation of malignant cells. This effect would facilitate the detachment of cancer cells from the primary tumor leading to an increased metastatic spread.
metastatic activity. These assumptions are supported by a recent in vitro finding that ICAM-1 shed from melanoma cells lines or purified from the serum of patients with melanoma inhibits the binding of effector cells to malignant cells and by the observation that ICAM-1 serum levels are correlated with the state of clinical progression and prognosis of patients with certain types of tumors such as melanoma and Hodgkin’s lymphoma (32–34).

The regulatory effect of various cytokines including interferon-gamma, interleukin-1, interleukin-7 and tumor necrosis factor alpha and beta on the expression of ICAM-1, suggests the possibility of designing anticancer therapy based on the use of cytokines alone or in combination with other cytotoxic agents (35). However, conflicting results have been reported so far in the literature. Some studies have shown that the induction of de novo ICAM-1 expression by pretreating cancer cell lines with cytokines renders the cells more susceptible to the cytolytic activity mediated by tumor associated lymphocytes and monocytes (36, 37). By contrast, other investigations presented data showing that gamma-interferon and interleukin-1 promote invasiveness and metastasis of tumor cells (38). Furthermore, sensitivity of tumor cells to anticancer cytotoxic drugs has been shown to depend on the level of ICAM-1 expression; the multidrug-resistant cells have a more significantly increased level of adhesion molecule expression as compared to sensitive cells (39, 40). If this were the case, cancer patients that respond to anticancer treatment might theoretically have a lower concentration of soluble ICAM-1 in their serum than those non-responders to chemotherapy. This potential usefulness of circulating ICAM-1 as a marker of response to chemotherapy in patients with malignancy was assessed in the current study. ICAM-1 serum levels were not significantly different between responder and non-responder patients. However, a prospective study with a larger number of patients is required to clarify this issue.

In brief, the results of this investigation suggest the existence of an increased expression and shedding of ICAM-1 in lung cancer and that the serum concentration of ICAM-1 is positively correlated with the clinical stage of disease. Serum levels of ICAM-1 might not be good indicators of the chemotherapy response in these patients.

References


