Gastric Cancer (1999) 2: 221–225



*Original article*

# Decrease in ICAM-1 expression on gastric cancer cells is correlated with lymph node metastasis

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**Abstract:**
***Background.*** **Lymph node metastasis is a frequent type of metastasis in patients with gastric cancer. The mechanisms responsible for this type of metastasis, however, are not clearly understood. We hypothesize that the immunosurveillance system between cancer cells and lymphocytes may be associated with the lymph node metastatic process. In this study, we examined the correlation between lymph node metastasis and intercellular adhesion molecule-1 (ICAM-1), which mediates the immunosurveillance system between tumor cells and cytotoxic lymphocytes, in gastric cancer.**
***Methods.*** **One hundred and forty-three specimens resected from patients with gastric cancer were investigated by staining with a monoclonal antibody against ICAM-1. We studied the correlation between the expression of ICAM-1 and various clinicopathologic factors, as well as infiltration of tumor-infiltrating lymphocytes (TILs).**
***Results.*** **ICAM-1 expression on gastric cancer cells was significantly decreased in patients with lymph node metastasis. The infiltration of TILs was associated with ICAM-1 expression level. The prognosis of patients with ICAM-1-negative tumors was poorer than the prognosis of those with ICAM-1-positive tumors.**
***Conclusions.*** **These findings suggest that ICAM-1 expression on cancer cells is closely associated with lymph node metastasis in gastric cancer, under the influence of the host immunosurveillance system.**

**Key words:** ICAM-1, gastric cancer, lymph node metastasis, immunosurveillance system, TIL

## Introduction

Lymph node metastasis is a frequent type of metastasis in patients with gastric cancer, and is one of the reasons for their poor prognosis. However, the mechanisms responsible for lymph node metastasis are not clearly understood. Although lymph nodes have the potential for immune responses to tumor cells, gastric cancer cells frequently metastasize to lymph nodes. We hypothesized that the lymph node metastatic process may be associated with the impairment of immunosurveillance systems between cancer cells and lymphocytes.

Cellular adhesion and recognition mechanisms are considered to be basic requirements for immunosurveillance systems [1,2]. Antigen-dependent and/or-independent interactions between target cells and lymphocytes are an initial step in immune responses to tumor cells. Cytotoxic lymphocytes, including natural killer (NK) cells, lymphokine-activated killer cells, and cytotoxic T-lymphocytes (CTLs), adhere to cancer cells and recognize the various molecules expressed on the tumor surface. It has been reported that both the first signal by the histocompatibility locus antigen and the co-stimulatory signal are important for the process of tumor rejection. Intercellular adhesion molecule-1 (ICAM-1) has been shown to play an important role as a co-stimulatory signal in mediating the cytotoxic effects of lymphocytes [3–5]. However, the function of ICAM-1 during the progression of gastric cancer is not clearly understood [6–10]. In this study, we investigated the correlation between ICAM-1 expression on tumor cells and clinicopathologic factors in gastric cancer.

## Materials and methods

### *Clinical material*

Resected specimens from 143 patients with gastric carcinoma who underwent gastrectomy at our institution were studied. The patients ranged in age from 26 to 83 years (mean age, 61.5 years); 94 were men and 49 were women (Table 1). No patient had received chemotherapy or radiation therapy before surgery. The *gen-*

*Offprint requests to:* M. Yashiro
Received on Aug. 20, 1999; accepted on Jan. 5, 2000

**Table 1.** Characteristics of 143 patients studied

| | |
|---|---|
| Age in years (mean) | 26–83 (61.5) |
| Sex (number of patients) | |
| Male | 94 |
| Female | 49 |
| Stage (number of patients) | |
| I | 58 |
| II | 16 |
| III | 49 |
| IV | 20 |
| Operation (number of patients) | |
| Curative | 114 |
| Non-curative | 29 |

*eral rules for gastric cancer study* of the Japanese Research Society for Gastric Cancer [11] were used for the pathologic diagnosis and classification of variables. Tumors were divided into two histologic subgroups: differentiated type (which consisted of papillary and tubular adenocarcinomas) and undifferentiated type (which included poorly differentiated adenocarcinomas, signet ring cell carcinomas, and mucinous adenocarcinomas). All patients were observed for at least 5 years after surgery and routinely studied by diagnostic imaging (computed tomography, ultrasonography, or magnetic resonance imaging) once or twice a year. Specimens were fixed in 10% formaldehyde solution and embedded in paraffin. Four-μm-thick sections were cut and mounted on glass slides.

### *Immunohistochemical techniques*

Immunohistochemical studies were performed using commercially available antibodies and the catalyzed signal amplification (CSA) System (Dako, Carpinteria, CA, USA) in routinely formaldehyde-fixed, paraffin-embedded tissue sections. Sections were dewaxed and washed in phosphate-buffered saline (PBS). As a primary antibody against ICAM-1 CD54 (IgG1κ; Genzyme, Cambridge, MA, USA) was used. Specimens were incubated with a 1:400 dilution of the antibody for 15 min at room temperature. The sections were then processed according to the manufacturer's instructions in the CSA kit. Finally, slides were counterstained with hematoxylin, and mounted. Normal mouse immunoglobulin-G was substituted for primary antibody as the negative control. For positive controls of ICAM-1, staining patterns of endothelial cells or monocytes were checked. Slides were interpreted for antigen expression by two investigators without knowledge of the corresponding clinicopathologic data.

### *Immunohistochemical determination of ICAM-1*

The degree of monoclonal antibody reactivity with individual tissue sections was considered positive if unequivocal staining of the membrane or the cytoplasm was seen in more than 50% of tumor cells, with the staining as strong as that of the germinal center of lymphatic follicle and vessel endothelium.

### *Evaluation of the infiltration of tumor-infiltrating lymphocytes (TILs)*

The methods for evaluating the infiltration of TILs were described previously [12]. The stained sections were screened at ×5 magnification to identify the areas of the highest infiltration of TILs within the tumor. These highly infiltrated areas could occur anywhere within the tumor, but were most frequent at the margins of the carcinoma. The TILs were counted in the three areas of highest infiltration at ×400 magnification (×40 objective and ×10 ocular). The TIL count was expressed as the mean number of TILs in these areas. Infiltration of TILs was considered positive if the mean number of TILs was more than 200.

### *Statistical methods*

The relationship between ICAM-1 expression and various clinicopathologic factors was examined by $\chi^2$ (Fisher; Nakayama Shyoten, Tokyo, Japan) or logistic regression analysis [13]. The relationship between ICAM-1 expression and survival was examined by constructing Kaplan-Meier survival curves and analyzing differences by the log-rank test (Stat View; Abacus Concepts, Berkeley, CA, USA). Two-tailed $P$ values less than 0.05 were considered significant.

## Results

### *ICAM-1 expression in gastric cancer*

Normal gastric mucosa was not immunoreactive with the anti-ICAM-1 antibody. However, the germinal center of lymphatic follicles and vessel endothelium in normal gastric tissue was strongly immunoreactive with the anti-ICAM-1 antibody. As demonstrated in Fig. 1, ICAM-1 was mainly localized in the cytoplasma or the membrane of the carcinoma cells.

### *ICAM-1 expression and clinicopathologic factors*

Table 2 shows the correlation between ICAM-1 expression and the various clinicopathologic factors studied. There were significant differences between the expression of ICAM-1 according to clinicopathologic factors, including histological stage and lymph node metastasis. ICAM-1 had a negative relationship to the histological

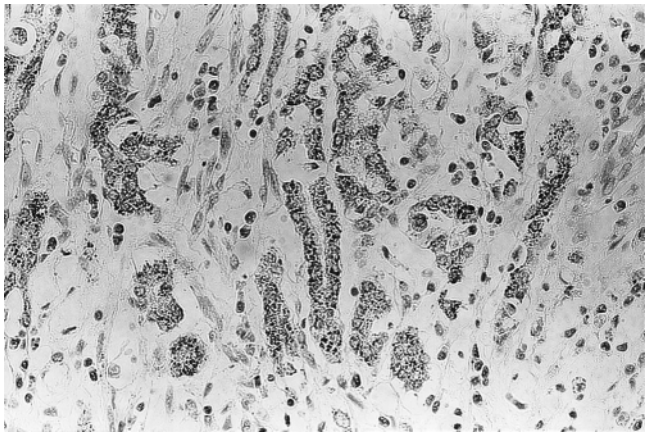

**Fig. 1.** Intercellular adhesion molecule 1 (ICAM-1) immunostaining of gastric carcinoma. ×400

stage ($P < 0.01$). The number of ICAM-1-positive tumors had a negative relationship to lymph node metastasis ($P < 0.01$). Also, differentiated type cancer cells had a higher rate of ICAM-1 expression than undifferentiated type cancer cells ($P < 0.01$). There was a significant correlation between the expression of ICAM-1 and lymph node metastasis in patients with the same histologic type of gastric cancer ($P < 0.05$). On the other hand, no relationship was found between ICAM-1 expression and in depth of wall invasion, or pressnce of lymphatic invasion and venous invasion (Table 2).

### *ICAM-1 expression and infiltration of TILs*

Table 3 shows the correlation between ICAM-1 expression and infiltration of TILs. Specimens positive for

**Table 2.** Correlation between expression of ICAM-1 and clinicopathologic factors

| | ICAM-1 expression | | |
|---|---|---|---|
| Variable | Negative | Positive | *P* value |
| Lymph node metastasis | | | |
| Negative ($n = 58$) | 27 (46.6%) | 31 (53.4%) | |
| Positive ($n = 85$) | 61 (71.8%) | 24 (28.2%) | <0.01 |
| Histologic type | | | |
| Differentiated ($n = 69$) | 33 (47.8%) | 36 (52.1%) | |
| n-Negative | 10 (33.3%) | 20 (66.7%) | |
| n-Positive | 23 (59%) | 16 (41%) | <0.05 |
| Undifferentiated ($n = 74$) | 55 (74.3%) | 19 (25.7%) | <0.01[a] |
| n-Negative | 17 (60.7%) | 11 (39.3%) | |
| n-Positive | 38 (82.6%) | 8 (17.4%) | <0.05 |
| Lymphatic invasion | | | |
| Negative ($n = 80$) | 52 (65%) | 28 (35%) | |
| Positive ($n = 63$) | 36 (57.1%) | 27 (42.9%) | NS |
| Venous invasion | | | |
| Negative ($n = 82$) | 51 (62.2%) | 31 (37.8%) | |
| Positive ($n = 61$) | 37 (60.7%) | 24 (39.3%) | NS |
| Depth of invasion | | | |
| m ($n = 1$) | 1 (100%) | 0 (0%) | |
| sm ($n = 55$) | 27 (49.1%) | 28 (50.9%) | |
| mp ($n = 20$) | 14 (70%) | 6 (30%) | |
| ss ($n = 6$) | 4 (66.7%) | 2 (33.3%) | |
| se ($n = 52$) | 35 (67.3%) | 17 (32.7%) | |
| sei ($n = 9$) | 7 (77.8%) | 2 (22.2%) | NS |
| Liver metastasis | | | |
| Negative ($n = 139$) | 87 (62.6%) | 52 (37.4%) | |
| Positive ($n = 4$) | 1 (25%) | 3 (75%) | NS |
| Stage | | | |
| I ($n = 58$) | 27 (46.6%) | 31 (53.4%) | |
| II ($n = 16$) | 11 (68.7%) | 5 (31.3%) | |
| III ($n = 49$) | 35 (71.4%) | 14 (28.6%) | |
| IV ($n = 20$) | 15 (75%) | 5 (25%) | <0.05 |

[a] Statistical analysis was performed between differentiated type and undifferentiated type ICAM-1, Intercellular adhesion molecule-1; n-negative; lymph node metastasis-negative; n-positive; lymph node-positive; m, mucosal neoplastic involvement; sm, submucosal neoplastic involvement; mp, muscle layer neoplastic involvement; se, serosal neoplastic involvement; sei, serosal involvement with direct infiltration of other organs beyond serosa; NS, not significant

ICAM-1 expression showed significantly higher infiltration of TILs than specimens negative for ICAM-1.

*ICAM-1 expression and survival*

Figure 2 shows the survival rates of ICAM-1-positive and -negative groups. The prognosis of patients with ICAM-1 negative tumors was significantly poorer compared with prognosis in those with ICAM-1-positive tumors ($P < 0.05$).

## Discussion

In this study, ICAM-1 expression was found to be significantly associated with lymph node metastasis. It has been reported that the histological growth pattern of carcinomas is associated with lymph node metastasis [14]; however, no relationship was found between lymph node metastasis and the histologic type on gastric cancer in this study (data not shown). In contrast, a significant correlation ($P < 0.05$) between the expression of ICAM-1 and lymph node metastasis was found in the patients with the same histologic type on gastric cancer. These findings suggest that ICAM-1 expression was associated with lymph node metastasis independent of the histologic type of gastric cancer. When tumor cells without ICAM-1 expression metastasize to lymph nodes, these cells can grow without recognition by hostile cytotoxic lymphocytes. We have previously reported that decreased ICAM-1 expression on gastric cancer cells decreased the immune responses mediated through leukocyte function-associated antigen 1 (LFA-1)-dependent effector cell adhesion, and that this escape may be associated with lymph node metastasis [15]. In this study, ICAM-1 expression on tumor cells was significantly lower in patients with lymph node metastasis, and infiltration of TILs was associated with ICAM-1 expression. It has also been reported that TILs infiltrating to tumors mainly consist of $CD4^+$ or $CD8^+$ T cells, but not NK cells, which induce non-adoptive anti-tumor immunoresponses [16]. The LFA-1/ICAM-1 system produces co-stimulatory signals and plays a key role in CTL and NK cell responses [17–19]. These findings suggested that ICAM-1 expressed on cancer cells may act as a suppressor of lymph node metastasis under the influence of the host immune surveillance system.

In contrast, ICAM-1 expression was not associated with lymphatic vessel infiltration. In the process of lymphogenous metastasis, cancer cells initially infiltrate into lymph vessels and then metastasize to lymph nodes. Patients with lymphatic infiltration by cancer cells do not always develop lymph node metastasis. Consequently, there may be different mechanisms for lymphatic infiltration and lymph node metastasis. Infiltration into lymphatic vessels from primary tumors may be associated with matrix metalloproteinase or adhesion molecules on the cancer cell surface, as previously reported [20]. After infiltrating into lymphatic vessels, cancer cells without ICAM-1 expression may have the capacity to metastasize to lymph nodes.

ICAM-1 was found to have a negative relationship to the histological stage. Of the relationship between ICAM-1 expression and the prognosis of patients, it has been reported that increased ICAM-1 expression in malignant tumors is associated with both poorer prog-

**Table 3.** Correlation between the expression of ICAM-1 and infiltration of tumor infiltrating lymphocytes (TIL)

| | ICAM-1 expression | | |
|---|---|---|---|
| TIL infiltration | Negative ($n = 88$) | Positive ($n = 55$) | $P$ value |
| High | 7 | 37 | |
| Low | 81 | 18 | $P < 0.001$ |

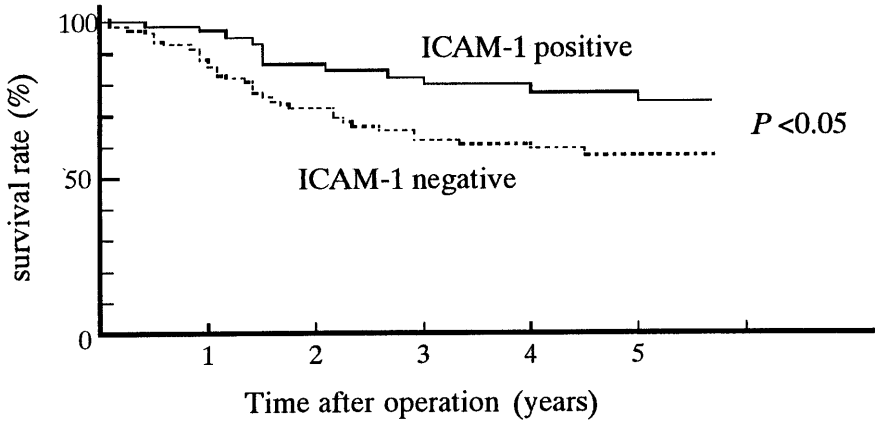


**Fig. 2.** Postoperative survival curves for 112 patients who underwent curative resection for gastric cancer. Patients with positive ICAM-1 expression in the tumor (*solid line*; $n = 46$), and patients with negative ICAM-1 expression in the tumor (*broken line*; $n = 68$). There was a significant difference between the two groups ($P < 0.05$, by Cox-Mantel test)

nosis [21] and better prognosis [22]. In this study, patients with gastric cancer with ICAM-1-positive tumors had a better prognosis than those with ICAM-1-negative tumors, while no significant relation was found between ICAM-1 expression and prognosis according to stage (data not shown). These findings suggest that ICAM-1 expression is not a prognostic indicator, but rather, that ICAM-1 expression in gastric cancer plays an important role in the mechanism of lymph node metastasis under the influence of the host immunosurveillance system.

**Acknowledgments** This study was supported in part by a Grant-in Aid for Osaka City University Medical Research Foundation, by a Grant-in Aid for Scientific Research (B) from the Ministry of Education, Science, Sports and Culture of Japan, and by a Grant in aid for Cancer Research from the Ministry of Health and Welfare of Japan.

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