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ICAM-1 (Intercellular Adhesion Molecule-1) Gene Transfection Inhibits Lymph Node Metastasis by Human Gastric Cancer Cells

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Lymph node metastasis is one of the prognostic factors in gastric cancer. We have previously reported that decreased intercellular adhesion molecule-1 (ICAM-1) expression on cancer cells is associated with lymph node metastasis using a gastric cancer cell. In this study, we transfected *ICAM-1* gene into a gastric cancer cell line, 2MLN, and analyzed the effect on lymph node metastasis *in vitro* and *in vivo*. A significantly greater amount of peripheral blood mononuclear cells (PBMC) adhered to ICAM-1 transfected 2MLN cells, 2MLN/ICAM cells, than to 2MLN/Vector cells. The lysis of 2MLN/ICAM cells by PBMC was significantly increased compared with that of 2MLN/Vector cells. The tumor growth rate of 2MLN/ICAM cells was significantly decreased *in vivo*. Lymph node metastases caused by 2MLN/ICAM cells were recognized as being fewer in number and smaller, while many lymph node metastases were caused by 2MLN cells. Histologic findings showed that leukocytes were heavily infiltrated in both the 2MLN/ICAM tumors and metastatic lesions, while only a few leukocytes were observed in the lesions associated with 2MLN cells. The above findings indicate that *ICAM-1* gene transduction could prove to be an effective gene therapy for lymph node metastasis of gastric cancer.

Key words: ICAM-1 — Lymph node metastasis — Gene therapy — Gastric cancer

In Japan, the survival rate after gastrectomy for gastric cancer has recently been steadily improving due to developments in diagnostic techniques and new operative procedures, including formal extended lymphadenectomy.[1, 2] However, gastric cancer is still the leading cause of death in Japan, and about 50 000 cancer-related deaths are reported annually. Metastases, such as lymph node metastasis, peritoneal metastasis and liver metastasis, are a common cause of death from gastric cancer. It has been reported that lymphatic involvement and lymph node metastasis are significantly related to the high rate of cancer death.[1, 3–5] Therefore, it is important to understand how to control lymph node metastasis in patients with gastric cancer by clarifying the mechanisms responsible for such metastasis.

Metastasis occurs subsequent to a sequential multistep process.[6] Previous studies of metastases have concentrated on invasion and adhesion ability of cancer cells.[7–11] Immune responses to cancer cells as well as the properties of cancer cells themselves might be responsible for metastases. However, few reports have discussed metastasis in terms of the immunological tolerance of cancer cells. Rejection of tumors is primarily mediated by cytotoxic lymphocytes (CTLs) and natural killer (NK) cells.[12, 13] These immune effector cells recognize cancer cells in two different ways, i.e., in a non-specific, non-major histocompatibility complex (MHC)-restricted manner and in an antigen-specific MHC-restricted manner. In non-MHC restricted recognition, intercellular adhesion molecule-1 (ICAM-1)/leukocyte function-associated antigen-1 (LFA-1) interaction plays an important role in adhesion to the target cells, which are lysed by NK cells and lymphocyte activated killer (LAK) cells.[14] In MHC-restricted recognition, not only the interaction of T-cell receptors with antigen/MHC molecule complex, but also additional second signal transmission through co-stimulatory molecules is required for optimal T-cell activation.[15–18] ICAM-1 is one of the co-stimulatory molecules; ICAM-1 thus plays an important role in both types of recognition. The decrease or loss of co-stimulatory molecules might help cancer cells to escape from the immunosurveillance system, thereby contributing to progression and metastasis.[19–21] We have immunohistologically investigated the correlation between the expression of ICAM-1 on cancer cells and clinicopathological factors of 274 patients with invasive breast cancer, and revealed that ICAM-1 expression was significantly decreased in the cases with lymph node metastasis.[22] We have also revealed that decreased ICAM-1 expression on cancer cells is associated with lymph node metastasis using a gastric cancer cell line in an experimental model with mice.[23] These findings suggest that ICAM-1 plays an important role in the process of lymph node metastasis as a signal transmitter of MHC and non-MHC-restricted immune response.

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In the last decade, there have been numerous studies of gene therapy for cancer; a variety of genes have been transduced. Most of these studies aimed at inhibiting tumor growth by transducing tumor suppressor genes such as *p53*. However, there have been few reports concerning gene therapy for cancer metastasis based on the metastatic mechanisms, especially for lymph node metastasis. In this study, we investigated the inhibitory effect on lymph node metastasis of the transduction of ICAM-1 into gastric cancer cells *in vitro* and *in vivo*.

MATERIALS AND METHODS

Mice Specific, pathogen-free, athymic BALB/c-*nu*/*nu* nude mice were purchased from Oriental Kobo (Tokyo). The mice were maintained under specific pathogen-free conditions and given sterile food and water *ad libitum*. Four- to six-week-old mice were used for the experiments.

Cells and culture conditions The human gastric cancer cell line, OCUM-2M LN (2MLN), was used in this study. 2MLN cells have high lymph node metastatic potential, as previously reported.[23] Cells were cultivated in medium (see below) in 100 mm culture dishes (Falcon, Lincoln Park, NJ), and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. The culture medium was composed of Dulbecco's modified Eagle's medium (DMEM; Bioproducts, Walkersville, MD) with 10% fetal bovine serum (FBS; GIBCO/BRL, Grand Island, NY), 100 IU/ml of penicillin (ICN Biomedicals, Costa Mesa, CA), 100 μg/ml of streptomycin penicillin (ICN Biomedicals), and 0.5 m*M* sodium pyruvate (Bioproducts).

ICAM-1 transfection and selection of clones Transfection was carried out by lipofection, according to the manufacturer's instructions. In brief, 1×10^5 2MLN cells were seeded in 2 ml of complete medium. After incubation and until the cells were 50% confluent, transfection was performed, under the conditions described above. One microgram of pICAM-1, a full-length ICAM-1 cDNA inserted in the CDM8 expression vector (generously provided by Dr. Timothy Springer, Center for Blood Research, Inc., Harvard Medical School, Boston, MA) and 0.1 μg of pCMV-Script mammalian expression vector, containing the neomycin- and kanamycin-resistance gene, (Stratagene) were mixed with 3 μg of 2 mg/ml LIPOFECTAMINE (GIBCO/BRL) in serum-free medium, and incubated at room temperature for 45 min to form DNA-liposome complexes. Fifty percent confluent cells on a 35 mm tissue culture plate were washed twice with serum-free medium and 1 ml of the DNA-liposome complex was added. The cells were incubated for 5 h at 37°C in a CO_2 incubator and then 1 ml of DMEM with 20% FBS was added. After incubation for 18 h at 37°C, the medium was replaced with 10 ml of fresh complete medium. To select the transfected cells, cells were passaged 1:10 at 72 h after transfection into the selection medium containing 500 μg/ml of "GENETICIN" (GIBCO/BRL), and expanded for 2 weeks. From these populations, many clones were obtained by the limiting dilution method. ICAM-1 expression was confirmed by FACScan Calibur (Becton Dickinson, San Jose, CA). A high-expressing clone was selected for this study, and was designated 2MLN/ICAM. 2MLN cells transduced with pCMV-expression vector only, designated as 2MLN/Vector, were used as the control cell line.

Flow cytometric analysis Individual cell lines, 2MLN/Vector and 2MLN/ICAM, were prepared as single cell suspensions by treatment with trypsin. The cells were washed twice with cold FACS buffer (phosphate-buffered saline, 0.01% sodium azide, 0.1% bovine serum albumin), and adjusted to 1×10^6 cells/test. Monoclonal antibodies (2 μg/200 μl) were added to each sample, and incubated for 30 min on ice. The cells were washed with cold FACS buffer, then incubated with 2 μg/200 μl of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Chemicon Int. Inc., Temecula, CA) for 30 min on ice. After additional washing, 1×10^4 cells were analyzed using FACScan. For the first antibody, the following monoclonal antibodies (mAbs) were used: monoclonal anti-ICAM-1 Ab (Immunotech, Marseille, France), monoclonal anti-LFA-3 Ab (Pharmingen, San Diego, CA), monoclonal anti-HLA class I Ab (DAKO, A/S, Copenhagen, Denmark), monoclonal anti-B7-1 Ab (Ancell, Bayport, MN), monoclonal anti-B7-2 Ab (Pharmingen). Monoclonal mouse IgG_1 (Serotec, Oxford, UK) was used as a control.

Isolation of the peripheral blood mononuclear cells (PBMC) Human PBMC were obtained from healthy volunteers using Mono-Poly Resolving Medium (Dainippon Pharmaceutical Co., Ltd., Tokyo) according to the manufacturer's instructions. Briefly, 7 ml of fresh anti-coagulated blood was laid gently onto 6 ml of the Resolving Medium in a 15 ml tube. The tube was centrifuged at 400*g* at room temperature for 40 min. After drawing off the plasma, the upper fraction containing mononuclear lymphocytes was transferred to a clean tube, and was washed with phosphate-buffered saline (PBS). The tube was centrifuged at 250*g* for 12 min, and the pellet was resuspended to a concentration of 5×10^6/ml in DMEM with 10% FBS.

Adhesion assay The *in vitro* adhesiveness of PBMC to cancer cells was assessed as previously described, with some modification.[24, 25] Briefly, cancer cells were plated onto 0.01% Poly Lysine (Sigma Chemical Co., St. Louis, MO)-precoated 96-well plates (Costar Co., Cambridge, MA) and cultured until confluent. The 96-well plates were placed on ice before addition of the PBMC. The PBMC (2×10^5) were added to each well in a final volume of 200 μl. The plates were placed on ice for 30 min, which

allowed the PBMC to sink to the bottom. Then the plates were rapidly warmed to 37°C and incubated for 30 min or for 60 min. Non-adherent cells were washed with PBS. Cellular adhesion was quantified by 3-(4,5-dimethyl-2-thiazol)-2H tetrazolium bromide (MTT; Wako Pure Chemical Industries, Ltd., Tokyo) colorimetric assay, which is designed to measure the formazan product of MTT, using an MTP-120 microplate reader (Corona Electric Co., Ibaraki) to measure absorbance at 550 nm. The percentage of adhering PBMC with respect to all PBMC (% adhesion) was calculated as follows: % adhesion=$(A-B)/T\times100$, where A=absorbance of experimental wells and B=absorbance of cancer cell wells, and T=absorbance of total PBMC added to each well.

***In vitro* cytotoxicity assay** We investigated the non-MHC restricted cytotoxicity by PBMC. Target cancer cells and PBMC were placed into a 96-well plate at an effector to target (E/T) ratio of 5, 10 and 20. Cells were incubated for 12 h at 37°C. For analysis of cytotoxicity, the Cyto Tox 96 assay (Promega, Madison, WI) was used, which quantitatively measures lactate dehydrogenase released upon cell lysis. Absorbance data at 490 nm were obtained using the MTP-120 microplate reader. The percentage of cancer cell lysis was calculated as follows: % cytotoxicity=$(A-B-C)/(D-E)\times100$, where A=experimental lysis, B=effector spontaneous lysis, C=target spontaneous lysis, D=target maximum lysis, and E=target spontaneous lysis.

Tumor growth of xenograft 2MLN/Vector cells or 2MLN/ICAM cells (5×10^6) in 200 μl of DMEM were inoculated subcutaneously into each nude mouse's lateral abdominal wall. Tumor areas were measured with a caliper every 2 days.

Tumor growth following treatment with anti-asialo G_{M1} antibody Nude mice were treated by intraperitoneal injection of 40 μl/mouse of anti-asialo G_{M1} Ab on days −2, 0, 2, 4, 7, 10, and then every 3 days during observation. 2MLN/ICAM cells were inoculated into the subcutis of nude mice on day 0. This protocol is effective in depleting NK cells in mice.[26)]

Lymph node metastatic ability following orthotopic implantation of gastric cancer cells *in vivo* Each mouse was anesthetized with diethyl ether (Wako Pure Chemical Industries, Ltd.) and placed in the supine position. An upper-midline incision was made and the stomach was exposed. Using a 30-gauge needle, tumor cell suspensions (1×10^7 cells in 20 μl of DMEM) were inoculated into the middle of the greater curve of the glandular stomach. The stomach was then returned into the peritoneal cavity, and the abdominal wall was closed. The mice were killed 4 weeks after the implantation, and metastatic lymph nodes were excised and weighed. Primary tumors and metastatic lymph nodes were fixed in 10% formalin neutral buffer solution (Wako Pure Chemical Industries, Ltd.) and were stained with hematoxylin and eosin (H & E).

Statistical analysis Differences between the control and experimental groups were analyzed using Student's t test. A P-value of less than 0.05 was considered statistically significant.

RESULTS

***ICAM-1* transfectants exhibit an increase of cell surface ICAM-1 expression** In order to verify cell surface ICAM-1 expression, transfected cells were analyzed using FACS. The mean fluorescence was measured by FACS analysis. Mean values reflect the amount of fluorescent activity of each cell line, which represents the relative degree of binding of the monoclonal antibodies to ICAM-1. The results are shown in Fig. 1. While the mean fluorescence of the 2MLN/Vector cells was 45.1 and the per-

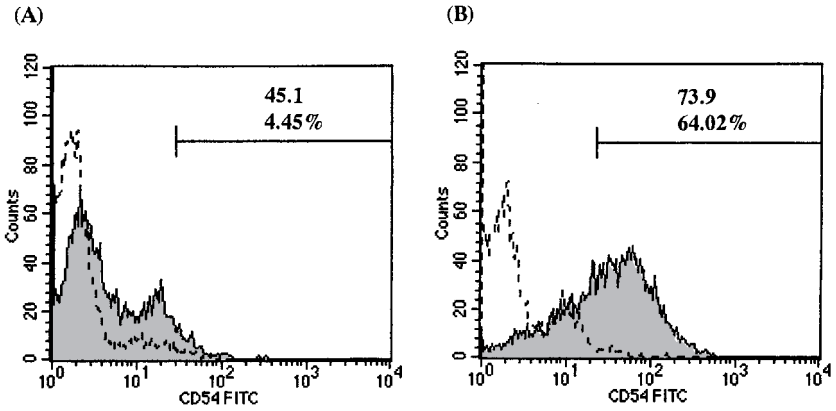

Fig. 1. ICAM-1 expression on 2MLN/Vector cells (A) and 2MLN/ICAM cells (B) by FACScan analysis. Cells were stained as described in "Materials and Methods." Shaded histograms indicate stained cells, and empty histograms show the negative control. FACS analysis revealed that ICAM-1 expression was increased in 2MLN/ICAM cells. Values are mean fluorescence and percentages of positive cells.

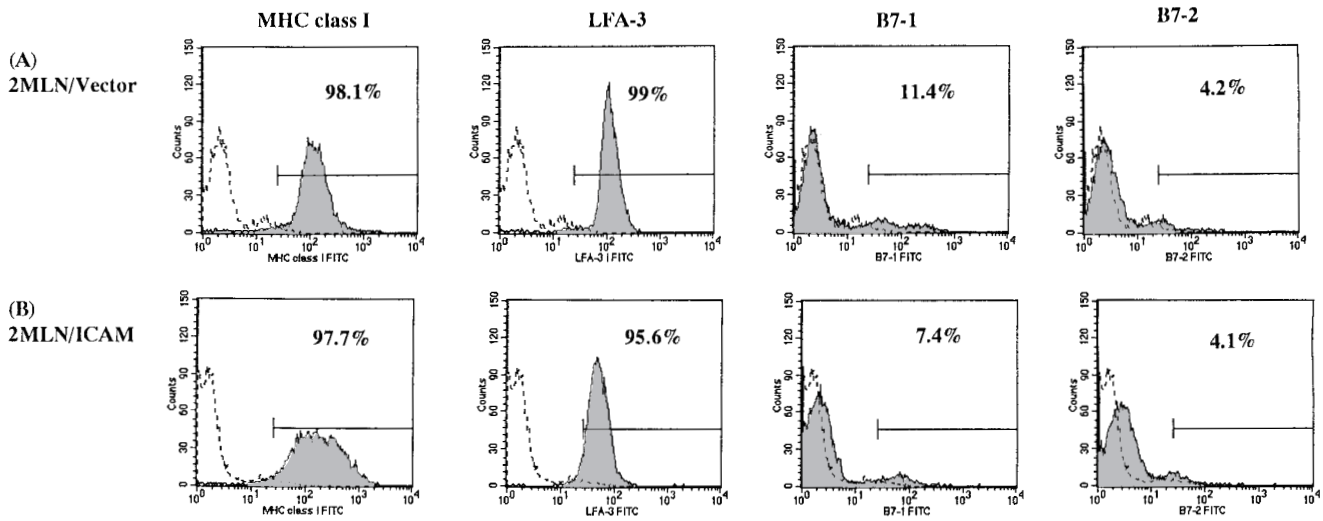

Fig. 2. Expression of MHC class I, LFA-3, B7-1, and B7-2 molecules on 2MLN/Vector cells (A) and 2MLN/ICAM cells (B). FACS analysis revealed that there was no difference in the expression of each molecule between the two cell lines.

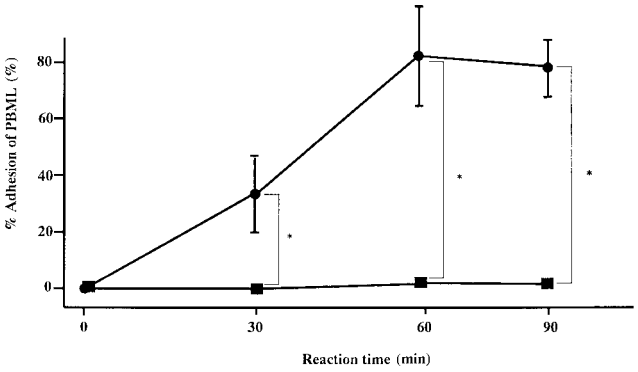

Fig. 3. Effect of ICAM-1 expression on adhesion ability between PBMC and cancer cells. The adhesion ratio was calculated at 30, 60 and 90 min after allowing PBMC to adhere to cancer cells. A significantly greater number of PBMC adhered to 2MLN/ICAM cells, as compared with adherence to 2MLN/Vector cells at each time point. 2MLN/Vector cells (■), 2MLN/ICAM cells (●). The results are presented as the mean of four samples and the bars indicate the SD. * $P<0.01$.

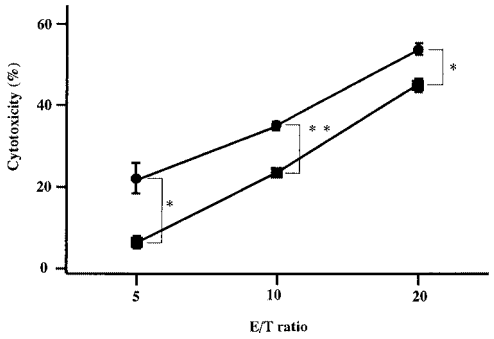

Fig. 4. Effect of ICAM-1 expression on cytotoxicity of PBMC to cancer cells. 2MLN/ICAM cells were significantly more susceptible to cell death by PBMC than were 2MLN/Vector cells at each time point. 2MLN/Vector cells (■), 2MLN/ICAM cells (●). Data points show the mean % cytotoxicity from four experiments. The bars indicate the SD. * $P<0.01$, ** $P<0.05$.

centage of positive cells was 4.45%, the 2MLN/ICAM cells showed much higher values, at 73.9 and 64.02%, respectively. No difference was found in the expression of HLA-class I, LFA-3, B7-1, or B7-2 between 2MLN/Vector cells and 2MLN/ICAM cells (Fig. 2).

Enhanced ICAM-1 expression on 2MLN/ICAM cells increases the adhesiveness of PBMC to 2MLN/ICAM cells We investigated the effect of enhanced ICAM-1 expression on adhesiveness of PBMC to cancer cells. Fig. 3 shows the results of the adhesion assay. PBMC hardly adhered to 2MLN/Vector cells at any time point, whereas significantly larger numbers of PBMC adhered to 2MLN/ICAM cells at 30, 60 and 90 min later, at 31%, 82% and 78%, respectively.

PBMC show significantly increased lysis of 2MLN/ICAM cells Next, we investigated the effect on PBMC cytotoxicity. The results are shown in Fig. 4. The data reveal that 2MLN/ICAM cells were lysed to a significantly greater degree than 2MLN cells at each E/T ratio of 5–20.

Enhanced expression of ICAM-1 on 2MLN/ICAM cells reduces tumor growth rate The doubling times of 2MLN/Vector cells and 2MLN/ICAM cells were 11.2 and 11.9 h, respectively. Therefore, the transduction procedure did not alter the *in vitro* growth rate of 2MLN/ICAM cells. To investigate the effect of enhanced ICAM-1 expression on the tumor growth rate, 2MLN/Vector cells and 2MLN/ICAM cells were injected subcutaneously into athymic BALB/c nude mice. The results are presented in Fig. 5. 2MLN/Vector tumors began to grow at day 9 after inoculation, and continued to grow throughout the observation period. On the other hand, 2MLN/ICAM tumors began to grow at day 9, but growth reached a plateau at day 15. At any time, the tumor sizes following the inoculation of 2MLN/ICAM cells were significantly smaller than following that of 2MLN/Vector cells. We then investigated whether NK cells contributed to the significant effect on tumor growth in nude mice. Nude mice were treated with anti-asialo G_{M1} antibody, which eliminates NK activity, then 2MLN/ICAM cells and 2MLN/Vector cells were injected subcutaneously into athymic BALB/c nude mice. The results are shown in Fig. 5. This treatment significantly enhanced the tumor growth rate of 2MLN/ICAM cells.

Enhanced ICAM-1 expression of transfected cells inhibited lymph node metastasis in an orthotopic implantation model The primary purpose of this study was to clarify whether or not the decreased ICAM-1 expression on cancer cells is responsible for the escape of such cells from the immunosurveillance system; cancer cells would then be free to metastasize to the lymph nodes. In order to perform this investigation, we used a spontaneous lymph node metastatic model with orthotopic implantation. The results are shown in Table I. Tumorigenesis in the stomach was recognized in all inoculated mice. Lymph node metastasis by 2MLN cells and 2MLN/Vector cells was recognized in all inoculated mice, whereas 2MLN/ICAM cells induced lymph node metastasis in 6 of 7 inoculated mice. As shown in the photograph in Fig. 6A, lymph node metastases caused by 2MLN cells were recognized at the greater omentum as a large, protuberant and irregularly shaped nodule. In addition, several metastatic nodules were recognized at the hylus of the spleen, the lesser omentum, or the mesenterium. The primary lesion was concealed by these nodules. Metastases caused by 2MLN/Vector cells as well as 2MLN cells were found at distant lymph nodes. In contrast, lymph node metastases caused by 2MLN/ICAM cells were recognized as being fewer in number and smaller; metastasis was confined to the lesser omentum (Fig. 6B). The primary lesions of the 2MLN/ICAM cells were also smaller than those of the 2MLN cells. The average weight of the metastatic lymph nodes was 29, 670 and 745 mg/mouse in the case of the 2MLN/ICAM cells, 2MLN cells and 2MLN/Vector cells, respectively. The lymph nodes were significantly lighter in the group associated with 2MLN/ICAM cells, compared with 2MLN cells or 2MLN/Vector cells (Table I).

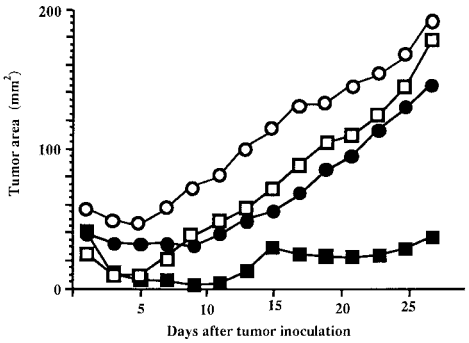

Fig. 5. Effect of ICAM-1 expression and anti-asialo G_{M1} treatment on xenografted tumor growth. Cancer cells (5×10^6) were inoculated subcutaneously into nude mice (5 mice/group). The growth rate of 2MLN/ICAM cells was significantly reduced. Treatment with anti-asialo G_{M1} caused the reduced growth of 2MLN/ICAM tumor to recover significantly. 2MLN/Vector tumor (■), 2MLN/ICAM tumor (●) without anti-asialo G_{M1} treatment. 2MLN/Vector tumor (□), 2MLN/ICAM tumor (○) without anti-asialo G_{M1} treatment. Data points show the mean tumor size in squared millimeters.

Table I. Growth and Metastasis of 2MLN Cells in an Orthotopic Implantation Model

Cell line[a)]	Primary tumors	Lymph node metastasis		
	Incidence	Incidence	Weight (mg)	
2MLN	7/7[b)]	7/7[c)]	670±340[d)]	
2MLN/Vector	4/4	4/4	745±756.6	$P<0.05$
2MLN/ICAM	7/7	6/7	28.6±12	

The incidences of primary tumor and lymph node metastasis and the weight of metastatic lymph nodes were evaluated after 4 weeks.
a) 2MLN cells, 2MLN/Vector cells and 2MLN/ICAM cells (1×10^7 cells/20 μl of DMEM) were implanted into the stomach of nude mice.
b) Data are shown as the number of mice bearing primary tumor/total number of mice.
c) Data are shown as the number of mice bearing lymph node metastasis/total number of mice.
d) Figures represent means±SD.

Histological sections of primary tumors and metastatic lymph nodes are shown in Fig. 7. The orthotopically implanted tumors in the stomach showed diffuse infiltration of cancer cells with abundant fibrous stroma. Only a few leukocytes were present in the 2MLN tumors with necrotic lesions (Fig. 7A), whereas heavy infiltration of

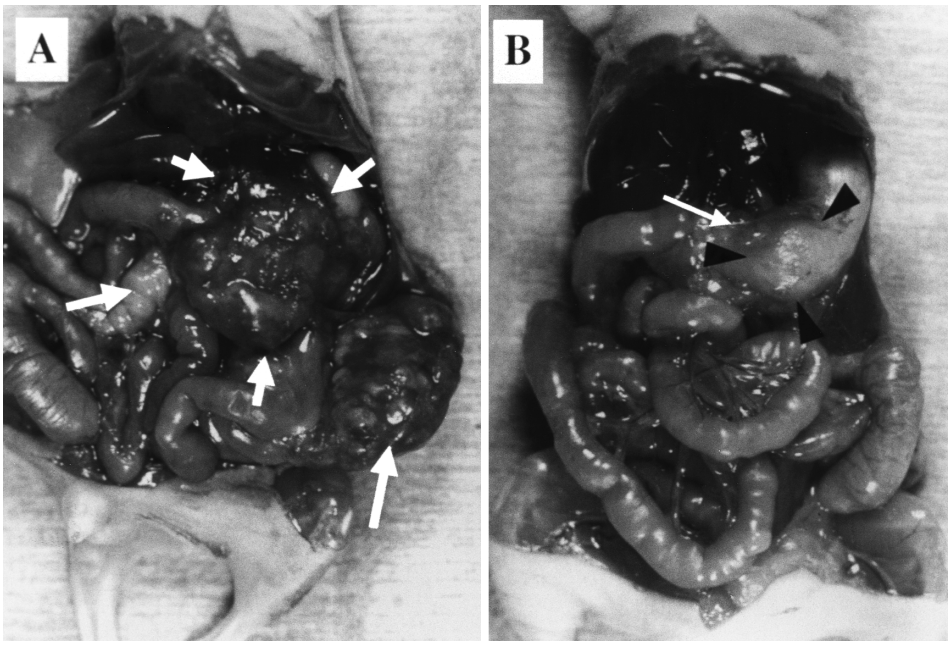

Fig. 6. Effect of ICAM-1 transduction on lymph node metastasis in an orthotopic implantation model. Lymph node metastases associated with 2MLN cells (A) appeared as large masses or small nodules in several areas, whereas lymph node metastasis associated with 2MLN/ICAM cells (B) is seen as a small nodule with a small area. Arrowheads indicate the primary tumor and arrows indicate the metastatic lymph nodes. The photographs were taken 4 weeks after orthotopic implantation.

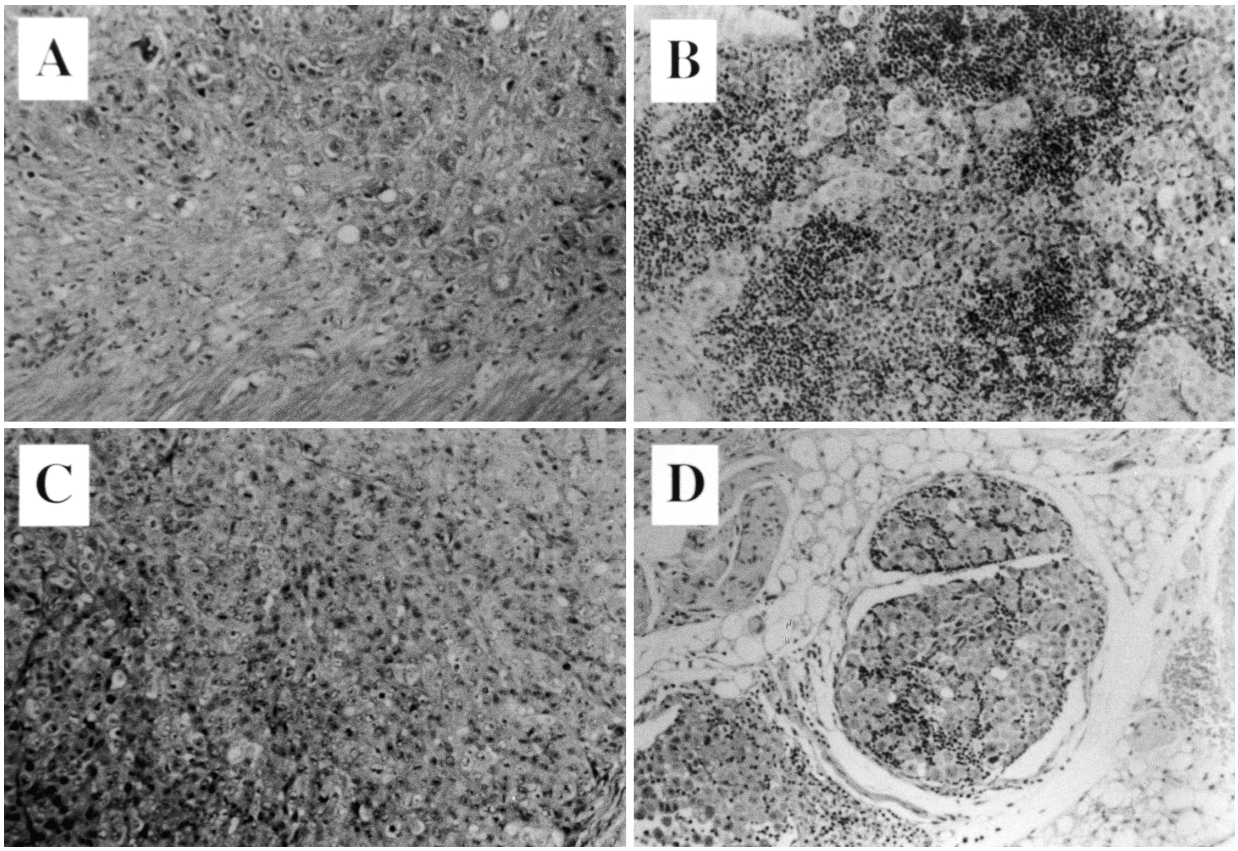

Fig. 7. Histological findings of an orthotopically implanted tumor and metastatic lymph node (H & E, ×200). Few leukocytes were observed around the 2MLN tumors (A), whereas the 2MLN/ICAM tumors (B) were heavily infiltrated with leukocytes. Among metastatic lymph nodes, lesions associated with 2MLN cells (C) were surrounded with few leukocytes, and lesions associated with 2MLN/ICAM cells (D) were infiltrated with many leukocytes.

leukocytes was observed surrounding cancer cells in the 2MLN/ICAM tumors (Fig. 7B). The infiltrating leukocytes were mononuclear. Metastatic lymph nodes associated with 2MLN cells were occupied with cancer cells, and few leukocytes were observed (Fig. 7C). In the case of 2MLN/ICAM cells, many leukocytes were recognized, gathering around cancer cells (Fig. 7D).

DISCUSSION

It has been reported that decreased ICAM-1 expression on cancer cells is closely associated with increased lymph node metastasis.[19–21] In this study, we transfected *ICAM-1* genes into 2MLN cells to analyze the usefulness of gene therapy for lymph node metastasis of gastric cancer. A FACS analysis revealed that the transfected 2MLN/ICAM cells had expressed a greater amount of ICAM-1 molecules; the positive ratio of ICAM-1-expressing cells was increased. Using an ICAM-1 transfected cell line, 2MLN/ICAM, we investigated the effect on immunogenicity and metastatic potential of ICAM-1 transduction.

An adhesion assay showed that a significantly greater amount of PBMC adhered to 2MLN/ICAM cells than to 2MLN/Vector cells. These findings indicate that ICAM-1 plays an important role in adhesion between cancer cells and PBMC. The lysis of 2MLN/ICAM cells by PBMC was significantly increased compared with that of 2MLN cells. Increased adhesion might allow PBMC more opportunity to lyse target cancer cells. These findings indicate that the transduction of *ICAM-1* into cancer cells could be effective for inducing an enhanced immune response. The ICAM-1/LFA-1 interaction has been reported to participate in non-MHC restricted cytotoxicity mediated by NK cells[27, 28] and monocytes.[29] This interaction has also been reported to serve as a co-stimulus in the activation of CTLs by adhesion and also by signal transduction.[30] The effect of increased ICAM-1 expression could be amplified by not only MHC-restricted immune cells, but also by non-MHC-restricted immune cells.

The tumor growth of xenografted 2MLN/ICAM cells *in vivo* was significantly reduced compared with that of 2MLN/Vector cells. No difference in the *in vitro* growth rate was found between 2MLN/Vector cells and 2MLN/ICAM cells, which indicates that the reduction of the tumor growth rate is not a direct result of the transfection process. The lower growth of 2MLN/ICAM cells *in vivo* was diminished by pretreatment of mice with anti-asialo G_{M1} Ab, which eliminates NK activity. These findings indicate that the regression of tumors is partly mediated by NK cells, and that ICAM-1 plays an important role in cytolysis by NK cells. It has been reported that human ICAM-1 has a 53% structural homology with murine ICAM-1.[31] There has been a report on the therapeutic efficacy of increasing ICAM-1 expression on sarcoma cells by transfecting human *ICAM-1* gene using a murine model.[32] This paper and our result may suggest that human ICAM-1 can bind to murine LFA-1.

Lymph node metastatic ability of 2MLN/ICAM cells was significantly decreased compared with that of 2MLN cells and 2MLN/Vector cells in an orthotopic implantation model. Our model exhibited spontaneous lymph node metastasis and should thus be a useful model for analyzing the efficacy of various therapies for lymph node metastasis. The number of metastatic lymph nodes associated with 2MLN/ICAM cells was low or zero, and these metastases were only recognized in nearby regional lymph nodes, whereas 2MLN/Vector cells metastasized to several distant lymph nodes, like 2MLN cells. The total weight of metastatic nodes associated with 2MLN/ICAM cells was significantly less than that of metastatic nodes associated with 2MLN/Vector cells. Histological findings showed many infiltrating leukocytes in both 2MLN/ICAM tumors and metastatic lesions, whereas only a few leukocytes in the lesions associated with 2MLN cells. The above findings indicate that the loss or decrease of ICAM-1 expression on cancer cells is responsible for cancer cells' escaping from immunosurveillance. These findings might explain the mechanism of the relationship between decreased ICAM-1 expression on cancer cells and increased lymph node metastasis. The growth of xenografted tumor of 2MLN/ICAM was significantly reduced, which might be one of the reasons for the reduction of lymph node metastasis. However, histologic findings show that many more leukocytes were infiltrating metastatic lesions of 2MLN/ICAM cells than those of 2MLN/Vector cells, as well as primary lesions, which could also be one of the reasons for the reduction of lymph node metastasis, and suggests that enhancing ICAM-1 expression inhibits the process of lymph node metastasis. In view of the fact that lymph node metastasis could be inhibited by enhancing ICAM-1 expression on cancer cells, *ICAM-1* gene transduction could prove to be an effective gene therapy for metastasis of gastric cancer. Moreover, this would also be the case for other types of cancer which have frequent lymph node metastasis, such as breast or colon cancer, and have the same relationship between ICAM-1 expression on cancer cells and lymph node metastasis.[21, 22] Therefore, *ICAM-1* gene transduction could also be a useful gene therapy in cases of lymph node metastasis associated with those types of cancers.

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