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# The Enhancement of Cell Surface ICAM-I and HLA Cla I Antigens in Human Gastric Cancer Cell Lines by IFN-$\gamma$

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## Abstract

Cytotoxic lymphocytes, including natural killer cells, lymphokine-activated killer cells, and cytotoxic T lymphocytes, adhere to and lyse cancer cells by recognizing cell surface antigens. Among the cell surface antigens, intercellular adhesion molecule-1 (ICAM-1) and HLA class I antigen are important for the cytotoxic activity of lymphocytes. The ICAM-1 and HLA class I antigen were examined in gastric cancer cell lines MKN-28 and MKN-45 by flow cytometry to determine whether their expression on the cell surface is enhanced by interferon gamma (IFN-gamma). The cell expression rate [stained cells/10(4) cells x 100(%)] was only 10% in ICAM-1 and about 20% in HLA class I antigen without IFN-gamma, but reached 70% in ICAM-1 and up to 60% in HLA class I antigen after incubation with IFN-gamma for 24-96 h. This enhanced expression of cell surface ICAM-1 and HLA class I antigen by IFN-gamma might increase sensitivity for cytotoxic lymphocytes.

**KEYWORDS:** gastric cancer, ICAM-I, HLA class I IFN-?, biological response modifier

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# The Enhancement of Cell Surface ICAM-I and HLA Class I Antigens in Human Gastric Cancer Cell Lines by IFN-$\gamma$

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Cytotoxic lymphocytes, including natural killer cells, lymphokine-activated killer cells, and cytotoxic T lymphocytes, adhere to and lyse cancer cells by recognizing cell surface antigens. Among the cell surface antigens, intercellular adhesion molecule-1 (ICAM-1) and HLA class I antigen are important for the cytotoxic activity of lymphocytes. The ICAM-1 and HLA class I antigen were examined in gastric cancer cell lines MKN-28 and MKN-45 by flow cytometry to determine whether their expression on the cell surface is enhanced by interferon gamma (IFN-$\gamma$). The cell expression rate [stained cells/$10^4$ cells $\times$ 100(%)] was only 10% in ICAM-1 and about 20% in HLA class I antigen without IFN-$\gamma$, but reached 70% in ICAM-1 and up to 60% in HLA class I antigen after incubation with IFN-$\gamma$ for 24-96h. This enhanced expression of cell surface ICAM-1 and HLA class I antigen by IFN-$\gamma$ might increase sensitivity for cytotoxic lymphocytes.

**Key words:** gastric cancer, ICAM-I, HLA class I, IFN-$\gamma$, biological response modifier

The 5-year survival rate of patients with stage III gastric carcinoma is still only 30%, despite that 80% of them had undergone curative operation. Recurrences in these patients are initially found in the peritoneal cavity. Thus, an adjuvant immuno-chemotherapy should be considered in this stage (1).

Interferon gamma (IFN-$\gamma$) is well known for attacking cancer cells both indirectly by activating various immunocompetent cells as well as directly by its own cytotoxic activity (2). Although IFN-$\gamma$ appears to have little cytotoxic effect at low concentrations, it is considered to retain some influence on cancer cells such as the enhancement of various membranous surface antigens in melanoma cells (3). Moreover, a relationship between intercellular adhesion molecule-1 (ICAM-1) expression and autologous cytotoxic activity has been reported (4). The aim of this study was to clarify the relationship between the expression of ICAM-1 and HLA class I antigen, that are considered to be important for lymphocytes with killer activities to adhere and recognize to cancer cells, and the administration of IFN-$\gamma$ in gastric cancer cell lines using flow cytometry.

## Materials and Methods

***Cell preparations for flow cytometry.*** Two human gastric cancer cell lines, MKN-28 (well differentiated type) and MKN-45 (poorly differentiated type) ($3 \times 10^5$ cells), were inplanted in plastic plates and pre-incubated for 24h with RPMI 1640 medium (GIBCO, New York, USA) containing 10% fetal calf serum (FCS) (GIBCO), 25mM HEPES (Sigma, St. Louis, USA), streptomycin 0.1mg/ml, and penicillin G 100U/ml. The wells, except those for control, were then incubated with the recombinant IFN-$\gamma$ (Kyowa Hakko, Tokyo, Japan) at $10^2$, and $10^3$ U/ml concentrations for 24, 48, 72, and 96h. As a control, K562 (human chronic myelogenous leukemia cell line) ($3 \times 10^5$ cells) were also incubated with the IFN-$\gamma$ at the 0, $10^2$, and $10^3$ U/ml concentrations for 24 and 72h.

***Cell preparation for immunohistochemical staining.*** The cell lines were digested with trypsin, suspended ($10^6$ cells/ml) and grown on glass slides placed in petri dishes. The cells were incubated in RPMI 1640 medium with 10% FCS for 24h and then in the medium containing IFN-$\gamma$ (102U/ml) for 48h. The slides were then immersed in fresh acetone (−20℃) for 5min and washed in phosphate-buffered saline (PBS) (pH7.4).

***Immunohistochemical staining.*** The slides were first incubated with a 1:100 diluted mouse anti-

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human ICAM-1 antibody (IgG1; Immunotech S.A., Marseille, France) for 1h at 37℃. Then, they were incubated with biotinated rabbit anti-mouse immunoglobulin for 20min and avidin labeled alkaline phosphatase (ALP) for 20min. ALP was visualized with fast red substrate, and the nuclei of cells were counter-stained by methyl green.

***Indirect immunofluorescence staining.*** All cells on the plates were removed by EDTA solution and an average of $10^6$ cells were incubated with ICAM-1 monoclonal antibody (IgG1, Immunotech S.A.) or mouse anti-human HLA-ABC monoclonal antibody (IgG2a, Chemicon, El Segundo, CA, USA) for 40min at 4℃, and washed twice by centrifugation (5min 1,500rpm) with RPMI 1640 medium. The cell suspensions were incubatd with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG1 (The Binding Site Ltd. Birmingham, England) or anti-mouse IgG2a (ICN ImmunoBiologicals, Lisle, IL, USA) antibody were added as second antibody and incubated for 40min at 4℃, and then washed twice in PBS. The cells were then re-suspended at concentration of $10^6$ cells/ml and analyzed by an EPICS flow cytometer (Coulter, Hialeah, FL, USA). In each group, $10^4$ cells were counted and the cell expression ratio (CER) was gained as the percentage of stained cells as calculated as follows.

$$\text{CER} = \frac{\text{No of flourescence-stained cells}}{\text{Total cells per group } (10^4)} \times 100$$

Dead or fragmented cells were excluded from analysis.

## Results

***Immunohistochemical staining of cell lines.*** MKN-28 and MKN-45 cells without incubation with IFN-$\gamma$ were poorly stained by anti-ICAM-1 antibody (Fig. 1a, c), but those incubated with IFN-$\gamma$ (Fig. 1b, d) showed a much greater staining intensity.

***Channel decision of fluorescence-stained cells.*** More than 99% of K562 cells were stained with ICAM-1 on their surface. Positive staining was determined above the channel 61 of intensity. More than 98% of K562 cells incubated without FITC had staining below 61 channels (Fig. 2).

***Cell surface ICAM-1 expression and its enhancement by IFN-$\gamma$.*** The CER of ICAM-1 was about 70% in untreated K562 cells and increased about 10% with $10^2$ and $10^3$ U/ml IFN-$\gamma$ for 48 and 72 h incubation. Although the number of positively stained K562 cells did not markedly increase, the fluorescence intensity of individual cells was enhanced (Fig. 3).
The CER of ICAM-1 was about 10% in MKN-28 and MKN-45 cells. The value increased to 66% and 74% respectively when treated with $10^2$ U/ml IFN-$\gamma$ for 24h. Enhancement of ICAM-1 expression peaked after 48h of treatment (85% in MKN-28 and 78% in MKN-45), and decreased thereafter. The CER of ICAM-1 increased about 5% at an IFN-$\gamma$ concentration of $10^3$ U/ml compared with $10^2$ U/ml (Fig. 4 and Table 1).

***Cell surface HLA class I expression and its enhancement by IFN-$\gamma$.*** The CER of HLA class

**Table 1** The cell surface expression rate of intercellular adhesion molecule-1 (ICAM-1) incubated with or without interferon-$\gamma$ (IFN-$\gamma$) in MKN-28, MKN-45, and K562 cell lines.

| Cell line | IFN-$\gamma$ concentration (U/ml) | Incubation time (h) | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 |
| MKN-28 | (−) | 8* | 15 | 11 | 12 |
| | $10^2$ | 66 | 84 | 68 | 74 |
| | $10^3$ | 72 | 87 | 72 | 54 |
| MKN-45 | (−) | 10 | 8 | 10 | 11 |
| | $10^2$ | 74 | 78 | 49 | 35 |
| | $10^3$ | 80 | 83 | 86 | 56 |
| K562 | (−) | 71 | — | 76 | — |
| | $10^2$ | 81 | — | 88 | — |
| | $10^3$ | 78 | — | 86 | — |

* The cell expression rate = (Fluorescence-staining cell/$10^4$ cells) × 100

**Table 2** The cell surface expression rate of HLA-ABC incubated with or without IFN-$\gamma$ in MKN-28 and MKN-45 cell lines

| Cell line | IFN-$\gamma$ concentration (U/ml) | Incubation time (h) | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 |
| MKN-28 | (−) | 21* | 21 | 16 | 21 |
| | $10^2$ | 49 | 59 | 70 | 66 |
| | $10^3$ | 42 | 71 | 73 | 69 |
| MKN-45 | (−) | 22 | 17 | 23 | 23 |
| | $10^2$ | 58 | 72 | 68 | 69 |
| | $10^3$ | 43 | 74 | 73 | 63 |

* The figures are values of the cell expression rate. IFN-$\gamma$: See Table 1.

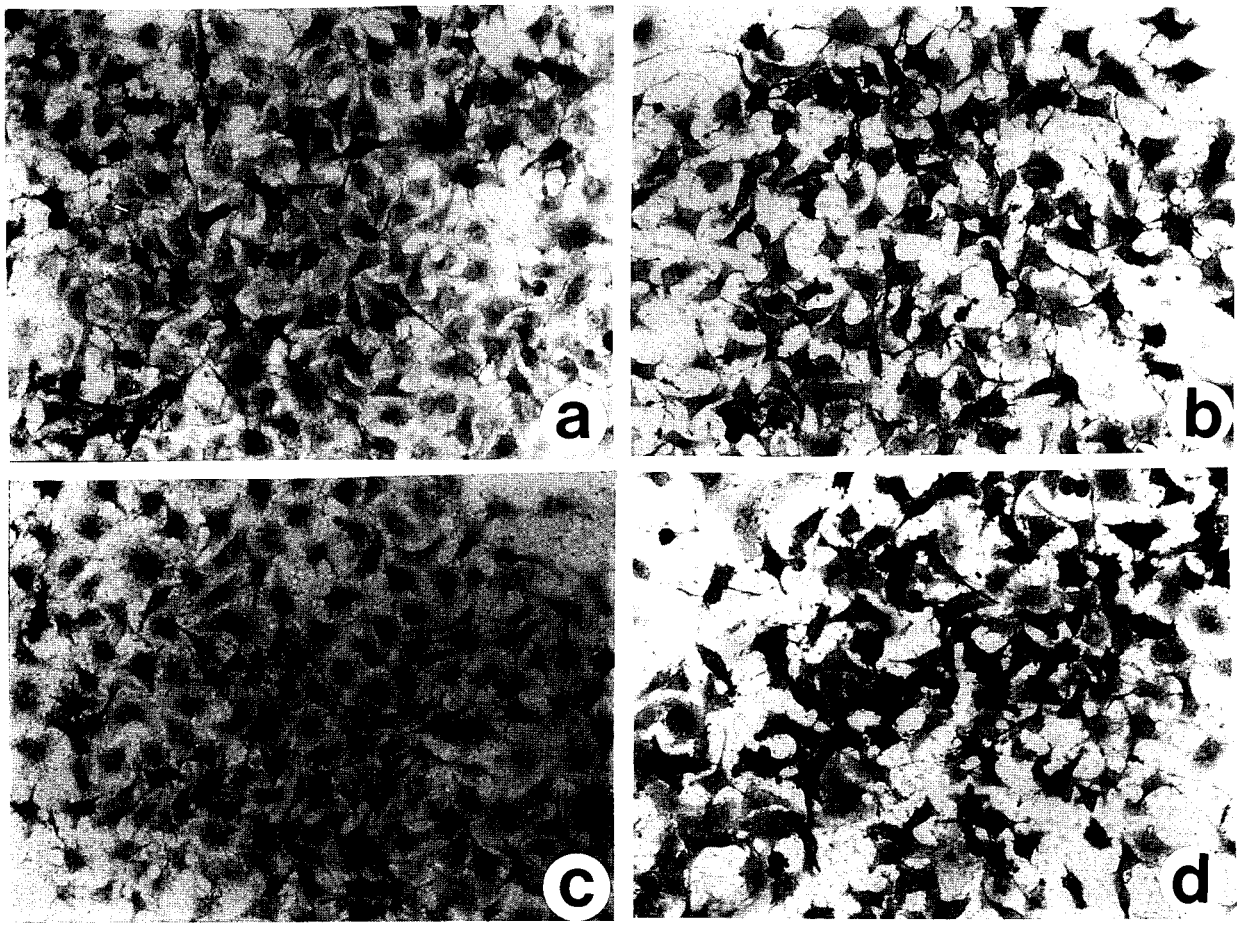


**Fig. 1** The immunohistochemical staining of cell lines with anti-intercellular adhesion molecule-1 (anti-ICAM-1) antibody. **a**) MKN-28 cell, **b**) MKN-28 cells incubated with IFN-$\gamma$ 100U/ml for 48h, **c**) MKN45 cells, **d**) MKN-45 cells incubated with interferon gamma (IFN-$\gamma$) 100U/ml for 48h. Both MKN-28 cells and MKN 45 cells were poorly stained in cytoplasm and surface with ICAM-1 antibody, but stained well by incubation with IFN-$\gamma$. ($\times$200)

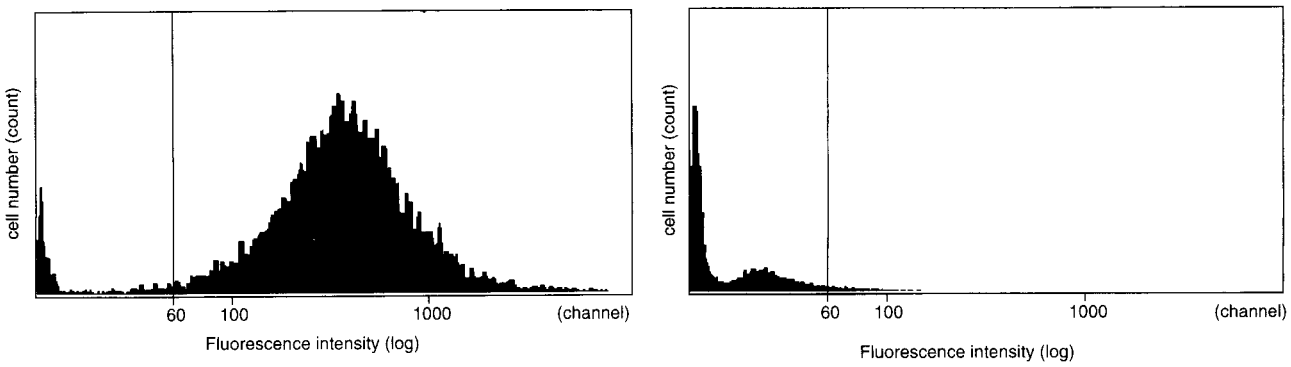


**Fig. 2** The fluorescence channels were determined with cells by fluorescein isothiocyanate (FITC) staining with ICAM-1 antibody in K562 (left column) and cells without staining by FITC in K562 (right column). Staining cells over 60 channels were evaluated to fluorescent cells in this study. ICAM-1: See Fig.1.

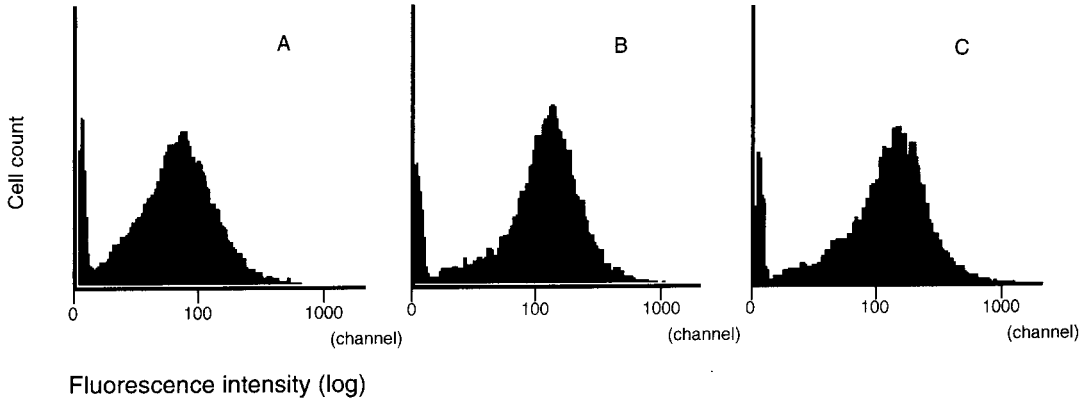


**Fig. 3** The fluorescence intensity of ICAM-1 in K562 cell surface without IFN-$\gamma$ incubation (**A**), and with IFN-$\gamma$ 100U/ml incubation (**B**) and 1000U/ml incubation (**C**) for 72h. The fluorescence intensity of each cell was more enhanced with IFN-$\gamma$ incubation than without IFN-$\gamma$, although the number of ICAM-1 expressed cells did not so increase. ICAM-1, IFN: See Fig. 1.

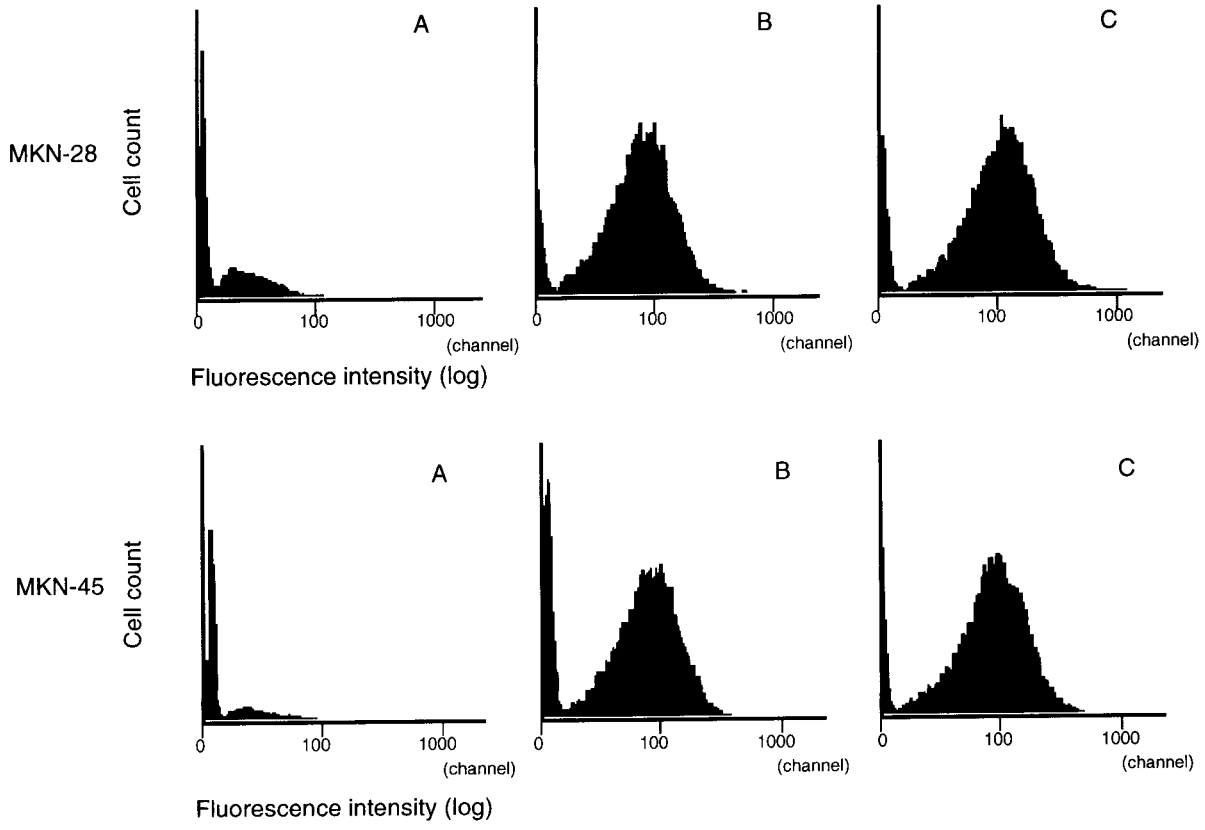


**Fig. 4** The fluorescence intensity of ICAM-1 in MKN-45 cell surface without IFN-$\gamma$ incubation (**A**), incubated with IFN-$\gamma$ 100U/ml for 48h (**B**), and incubated with IFN-$\gamma$ 1000U/ml for 48h (**C**). According to IFN-$\gamma$ concentration, the number of ICAM-1 expressed cell increased and fluorescence intensity of each cells was enhanced. ICAM-1, IFN: See Fig. 1.

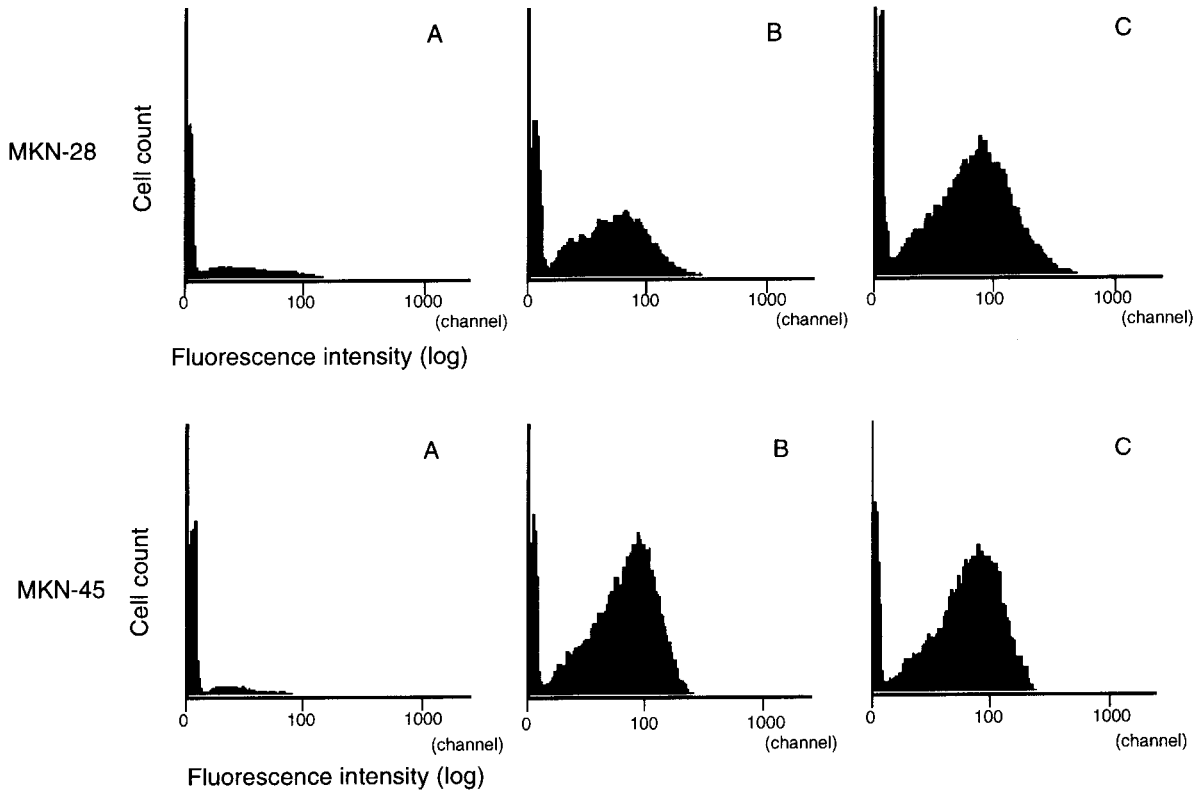


**Fig. 5** The fluorescence intensity of HLA class I in MKN-28 and MKN-45 cell surface. (**A**) without IFN-$\gamma$ incubation, (**B**) incubated with IFN-$\gamma$ 100U/ml for 48h, and (**C**) incubated with IFN-$\gamma$ 1000U/ml for 48h. According to IFN-$\gamma$ concentration, the number of HLA class I positive cells increased and fluorescence intensity of each cell was enhanced like as ICAM-1 expression with IFN-$\gamma$.

I was about 20 % in MKN-28 and MKN-45 cells without IFN-$\gamma$ treatment, but increased to 49 % in MKN-28 and 58 % in MKN-45 when incubated with $10^2$ U/ml IFN-$\gamma$ for 24 h. The peak of enhancement was 70 % in MKN-28 after 72 h and 73 % in MKN-45 after 48 h. The CER of HLA class I at a concentration of $10^3$ U/ml IFN-$\gamma$ was lower than that at a concentration of $10^2$ U/ml after incubation for 24 h, but increased by about 3–12 % after incubation for 48, 72, and 96 h. For HLA class I antigen, decrease in the CER values by the time was unremarkable (Fig. 5 and Table 2).

## Discussion

Because it takes a long time to develop a tumor mass, the host should have enough time to mount immunocompetent cells and attack an antigenic tumor. Low antigenic tumors, however, remain unaffected keeping their growth rates (5). Reduced expression of HLA class I, II antigens have been reported in many tumors (6, 7). A therapeutic strategy for treating such low antigenic tumors is to increase their antigenecity of tumor by anti-cancer-agents. It was reported that HLA class I and II, ICAM-1, NCAM, LFA-3, glycoprotein, glycolipid, and carcinoembryonic antigen (8–13) are expressed on the surface of cancer cells. They were considered to be potential targets for immunocompetent cells, including lymphocytes (14, 15). Particularly, it is known that non-specific cell-cell adhesion for example by binding of LFA-1 on lymphocyte to ICAM-1 on target cells, are required for effective recognition by T-cells (16). Thus, the LFA-1/ICAM-1 interaction stimulates the T-cell mediated activation (17, 18). The relationship between cell surface ICAM-1 expression and susceptibility of cell-

mediated lysis has already been established (9). Its expression as well as expression of MHC class I product, on human tumor cells is necessary for activation of autologous lymphocytes in the mixed lymphocyte-tumor cell culture (MLTC). Also, anti-ICAM-1 antibody has been shown to inhibit the lysis of neoplastic cells (19). In contrast, the expression of HLA class I antigen, especially the HLA-A allele (20), is an important factor for adherence of cancer cells to $CD8^+$ cytotoxic lymphocytes. Indeed, tumors with low expression of HLA class I can escape from $CD8^+$ T cell-dependent cytolysis *in vivo* (21). Cerosaletti *et al.* reported that increased class I antigen expression in mice treated with dimethyl sulfoxide (DMSO) and class I gene transfection decreases the metastatic potential of lung carcinoma cells (22).

In this study, we have examined the expression of ICAM-1 and HLA class I antigen in gastric cancer cell lines on their cell surface that appears to be importance for the cytotoxic activity of lymphocyte. Many agents such as interleukin-1 (IL-1) (23), tumor necrosis factor-$\alpha$(TNF-$\alpha$) (8), IFN-$\beta$, $\gamma$ (24, 25), DMSO and transfected gene (22) are reported to enhance the expression of membranous surface antigens (5). We used IFN-$\gamma$ produced by monocytes stimulated with biological response modifiers (*i.e.*, OK-432, PSK). IFN-$\gamma$ is produced at 30-1,500 U/ml by addition of OK-432 to monocytic cells in peripheral blood and 30-100 U/ml by PSK stimulation (26). At these concentrations, IFN-$\gamma$ increased the expression of HLA class I antigen by 30-40 %, and the expression of ICAM-1 by 50-70 % in this study. Furthermore, increased mRNA and protein within the cell and aggregation of intercellular microtubules around membranes are considered to be involved in the enhancement of surface antigens by the addition of IFN-$\gamma$ (27, 28).

It has been reported that the expression of ICAM-1 antigen increases according to the tumor growth, and that cases with distant metastasis have also shown increased levels of ICAM-1 expression (29). This may be resulted from up-regulation of the cell surface ICAM-1 expression by cytokines and immunocompetent cells stimulated by tumor cell antigens. In fact, lymphocytes with killer activity can be identified around tumor cells, and they seem to increase the expression of ICAM-1.

It has been investigated whether tumors with high expression of ICAM-1 have good prognosis. Not only ICAM-1 but also HLA class I is up-regulated by IFN-$\gamma$, which stimulates tumor-specific cytotoxic lymphocytes that are necessary for the killing of cancer cells. Agents that induce production of a variety of cytokines (biological response modifiers) may be effective in the up-regulation of both the host defense mechanism and the expression of tumor antigens.

Our results demonstrated that the expression of cell surface ICAM-1 and HLA class I antigens was enhanced up to 70 % and 60 %, respectively. This enhancement might result in increased sensitivity for cytotoxic lymphocytes and could be the basis for the effect of biological response modifier on cancer cells. It may be part of the host mechanism that decreases the incidence, the occurrence of micro-metastases to regional lymph nodes, and eventually improves prognosis in patients with gastric cancer as previously reported (30, 31).

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## References

1. Miwa K: Cancer of the stomach in Japan. Gann Monogr Cancer Res (1979) **22**, 61-759.
2. Clemens MJ and McNurlan MA: Regulation of cell proliferation and differentiation by interferons. Biochem J (1985) **226**, 345-360.
3. Mortani R, Belli F, Parmiani G and Anichini A: Cytokine-mediated modulation of HLA-Class 2, ICAM-1, LFA-3, and tumor associated antigen profile of melanoma cells: Comparison with anti-proliferative activity by rIL1-b, rTNF-a, rIFN-$\gamma$, rIL4 and their combinations. Int J Cancer (1990) **45**, 334-341.
4. Marley GM, Doyle LA, Ordonez JV, Sisk A, Hussain A and Yen RWC: Potentiation of interferon induction of class I major histocompatibility complex antigen expression by human tumor necrosis factor in small cell lung cancer cell lines. Cancer Res (1989) **49**, 6232-6236.
5. Fidler IJ and Kripke ML: Tumor cell antigenicity, host immunity, and cancer metastasis. Cancer Immunol Immunother (1980) **7**, 201-205.
6. Momburg F, Degener T, Bacchus E, Moldenhauer G, Hammerling G, and Moller P: Loss of HLA-ABC and de novo expression of HLA-D in colorectal carcinoma. Int J Cancer (1986) **37**, 179 184.
7. McDougall CJ, Ngoi SS, Goldman IS, Godwin T, Felix J, DeCosse JJ and Rigas B: Reduced expression of HLA class I and class II antigens in colon cancer. Cancer Res (1990) **50**, 8023-8027.
8. Pfizenmaier K, Scheurich P, Schluter C and Kronke M: Tumor necrosis factor enhances HLA-A, B, C and HLA-DR gene expression in human tumor cells. J Immunol (1987) **138**, 975-980.
9. Anichini A, Mortarini R, Supino R and Parmiani G: Human melanoma cells with high susceptability to cell-mediated lysis can be identified on the basis of ICAM-1 phenotype, VLA profile and invasive ability. Int J Cancer (1990) **46**, 508-515.
10. Mechtersheimer G, Staudter M and Moller P: Expression of the Natural Killer cell-associated antigens CD56 and CD57 in human neural and striated muscle cells and in their tumors. Cancer Res (1991) **51**, 1300-1307.
11. Roder JC, Ahrlund-Richter L and Jondal M: Target-effector interaction in the human and murine natural killer system. J Exp Med (1979) **150**, 471-481.

12. Young WW Jr, Durdik JM, Urdal D, Hakomori S and Henny CS: Glycolipid expression in lymphoma cell variants: Chemical quantity, immunologic reactivity, and correlations with susceptibility to natural-killer cells. J Immunol (1981) **126**, 1-6.
13. Harris JF, Chin J, Jewett MAS, Kennedy M and Gorczynsky RM: Monoclonal antibodies against SSEA-I antigen: Binding properties and inhibition of human natural killer cell activity against target cells bearing SSEA-I antigen. J Immunol (1984) **132**, 2502-2509.
14. Tanaka K, Yoshida T, Bierberich C and Jay G: Role of the major histocompatibility complex class I antigens in tumor growth and metastasis. Ann Rev Immunol (1988) **6**, 359-380.
15. Hammerling GJ, Klar D, Pulm W, Momburg F and Moldenhauer G: The influence of major histocompatibility complex class I antigens on tumor growth and metastasis. Biochem Biophys Acta (1987) **907**, 245-259.
16. Dustin ML and Springer TA: T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. Nature (1989) **341**, 619-624.
17. van Kooyk Y, van de Wiel-van Kemenade P, Weder P, Kuijpers TW and Figdor CG: Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. Nature (1989) **342**, 811-813.
18. van Seventer GA, Shimizu Y, Horgan KJ and Shaw S: The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T-cell receptor-mediated activation of resting T cells. J Immunol (1990) **144**, 4579-4586.
19. Vanky F, Wang P, Patarroyo M and Klein E: Expression of the adhesion molecule ICAM-1 and major histocompatibility complex class I antigens on human tumor cells is required for their interaction with autologous lymphcytes *in vitro*. Cancer Immunol Immunother (1990) **31**, 19-27.
20. Crowley NJ, Darrow TL, Quinn-Allen MA and Seigler HF: MHC-restricted recognition of autologous melanoma by tumor specific cytotoxic T cells. J Immunol (1991) **146**, 1692-1699.
21. Ward PL, Koeppen HK, Hurtean T, Rowley DA and Screiber H: Major histocompatibility complex class I and unique antigen expression by murine tumors that escaped from CD8 T-cell-dependent surveillance. Cancer Res (1990) **50**, 3851-3858.
22. Cerosaletti KM, Blieden TM, Harwell LW, Welsh KM, Frelinger JG and Lord EM: Alteration of the metastatic potential of line I lung carcinoma cells: Opposite effects of class I antigen induction by interferons versus DMSO and gene transfection. Cell Immunol (1990) **127**, 299 310.
23. Dustin ML, Rothlein R, Bahn AK, Dinavello CA and Springeer TA: Induction by IL-1 and interferon-g: Tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J Immunol (1986) **137**, 245-254.
24. Carrel S, Scmidt-Kessen A and Giuffre L: Recombinant interferon-g can induce the expression of HLA-DR and -DC on DR-negative melanoma cells and enhance the expression of HLA-ABC and tumor associated antigens. Eur J Immunol (1985) **15**, 118-123.
25. Weber JS and Rosenberg SA: Modulation of murine tumor major histocompatibility antigens by cytokines *in vivo* and *in vitro*. Cancer Res (1988) **48**, 5818-5824.
26. Nakayama F, Gouchi A, Ishii H, Aoyama M, Fuchimoto S and Orita K: Sensitivity of PMBC from gastric cancer patients when co-culture PMBC with various BRM (Abst. in English). Biotherapy (1991) **5**, 595-599.
27. Kuppner MC, van Meir E, Hamou MF and De Tribolet N: Cytokine regulation of intercellular adhesion molecule-I (ICAM-I) expresstion on human glioblastoma cells. Clin Exp Immunol (1990) **81**, 142-148.
28. Rosa MF and Fellous M: Regulation of HLA-DR gene by IFN-$\gamma$. Transcriptional and post-transcriptional control. J Immunol (1988) **140**, 1660-1664.
29. Natali P, Nicotra MR, Cavaliere R, Bigotti A, Romano G, Temponi M and Ferrone S: Differential expression of intercellular adhesion molecule I in primary and metastatic melanoma lesions. Cancer Res (1990) **50**, 1271-1278.
30. Gochi A, Orita K, Fuchimoto S, Konaga E, Okamura S, Sato K, Mannami T, Ohara T and Ogawa N: Reduction of lymphnode metastasis and improvement of survival by preoperative endoscopic intratumoral injection of OK-432, streptococcal preparation, in gastric cancer patients; in Frontiers of Mucosal Immunology, M. Tsuchiya, Nagura, Hibi, and Moro eds, Excerpta Medica, Amsterdam-New York-Oxford. (1991) pp453-456.
31. Tsujitani S, Okamura T, Baba H, Korenaga D, Haraguchi M and Sugimachi K: Endoscopic intratumoral injection of OK-432 and Langerhans' cells in patients with gastric carcinoma. Cancer (1988) **61**, 1749-1753.

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