RAPID COMMUNICATION Jpn. J. Cancer Res. 85, 131–134, February 1994

Immunosuppressive Effect of Shedding Intercellular Adhesion Molecule 1 Antigen on Cell-mediated Cytotoxicity against Tumor Cells

Shohei Koyama

Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, Ten-nodai 1-1-1, Tsukuba, Ibaraki 305

We have examined whether shedding intercellular adhesion molecule-1 (ICAM-1) antigen from cultured tumors is able to inhibit the leukocyte function-associated antigen-1 (LFA-1)/ICAM-1 interaction between cytotoxic effector cells and ICAM-1$^+$ target tumor cells. The cytotoxic activity of lymphokine-activated killer (LAK) cells incubated with spent media from ICAM-1$^+$ tumor cells, especially interferon-γ-stimulated tumor cells, was significantly decreased as compared with that of LAK cells treated with fresh culture medium without ICAM-1 antigen. Treatment of LAK cells with spent media from ICAM-1$^-$ tumor cells did not cause a significant decrease of the cytolytic activity towards ICAM-1$^+$ tumor cells. These findings suggest that shedding of ICAM-1 antigen could be involved in binding of LFA-1 to LAK cells, resulting in reduced cytolytic activity.

Key words: ICAM-1 — Shedding ICAM-1 — LAK cells — Malignant disease

The intercellular adhesion molecule-1 (ICAM-1) antigen has been reported to be a member of the immunoglobulin supergene family with five domain structures[1] and was subsequently established to be the counter-receptor for leukocyte function-associated antigen-1 (LFA-1). The LFA-1/ICAM-1 interaction is important in a number of leukocyte adhesion activities including the conjugate formation between cytotoxic T lymphocytes (CTL) and their targets[2–4] and natural killer (NK)- or lymphokine-activated killer (LAK)-mediated cytolysis.[3–5] However, active growth of tumor cells was observed in metastatic sites, in spite of greatly increased expression of ICAM-1 antigen on tumor cells and also induction of lymphoid cells with surface markers of $CD8^+CD11b^-$, $CD8^+CD28^+$ and $CD8^+S6F1^+$,[6,7] which are regarded as killer cells.[8–10] In addition, the ICAM-1 molecule has been suggested to play a role in the progression of metastasis in malignant melanoma[11] and other cancers.[7,12] These findings posed a particular dilemma in the evaluation of real ICAM-1 expression on tumor cells. Recently, circulating ICAM-1, which may be shed from the primary or metastatic tumor, has been detected in sera.[13,14] Thus, it seems likely that shedding ICAM-1 antigen may be related to escape mechanisms of the tumor from the immune system in cancer patients. However, it has remained unclear whether or not LFA-1 on lymphoid cells has affinity for shedding ICAM-1 from tumor. This study was undertaken to examine whether shedding ICAM-1 molecule can bind to LFA-1 on activated lymphoid cells, resulting in lower cell-mediated cytotoxicity.

The peripheral blood lymphocytes (PBL) were isolated from buffy coats of patients with gastric carcinoma by centrifugation over Ficoll-Hypaque density gradients as described.[15–17] LAK cells were generated in the culture of PBL for 10 days in complete medium with 100 U/ml of recombinant interleukin 2 (rIL 2, Shionogi Co. Ltd., Osaka) in 5% CO_2 in air at 37°C as described previously.[15–17] A Burkitt's lymphoma cell line, Daudi, was obtained from the Japanese Cancer Research Resources Bank, Tokyo. Gallbladder carcinoma cell line G-415 used for the experiments was established in our laboratory.[18,19] The gastric cancer cell lines SC-1[19] and SC-3 were cloned and have been cultured continuously in our laboratory. These cell lines have been maintained in RPMI-1640 (GIBCO, Grand Island, NY) medium supplemented with 10% fetal calf serum (M.A. Bioproducts, Walkersville, MD), 20 m*M* N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES; Wako Junyaku Co. Ltd., Tokyo) and 100 μg/ml kanamycin (complete medium). LAK cell activities were tested against NK-resistant Daudi and G-415 cells, using a standard 4 h ^{51}Cr-release assay.[3,4,6,15–17] To determine shedding ICAM-1 antigen from tumor cells, spent media were collected from culture flasks (Falcon, 3013, Mountain View, CA) in which approximately 2×10^6 tumor cells had been cultured with or without 100 U/ml of interferon-gamma (IFNγ, Toray Co. Ltd., Tokyo) for 7 days. The LAK cells were mixed and incubated with spent media from each tumor culture flask for 2 h at 37°C. In the control experiment, the LAK cells were also incubated with fresh complete medium. The labeled target

cells (5×10^3 cells) were mixed with effector cells at ratios (E/T ratios) of 5:1, 10:1 and 20:1, and were distributed to each well in a final volume of 200 μl in U-bottomed 96-well Nunc microplates (No. 163320, Roskilde, Denmark) in quadruplicate. After incubation, the plates were centrifuged and cytolysis was evaluated by counting 0.1 ml of supernatant in a gamma-counter. Specific lysis was expressed according to the following formula: % specific lysis = 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release). The maximum releasable counts were determined by repeated freezing and thawing of the labeled cells, and amounted to 80–90% of the total reactivity incorporated into the cells. Spontaneous release determined from Daudi or G-415 cells incubated in the cultured medium was always 5% or less of the maximum release in 4 h. For FACS analysis, the tumor cells were incubated with monoclonal antibody, anti-ICAM-1 (84H10 clone, Cosmo Bio Co. Ltd., Tokyo) for 30 min at 4°C. The cells were washed with phosphate-buffered saline containing 0.02 m*M* sodium azide and 1% bovine serum albumin (Sigma, St. Louis, MO), and incubated with FITC-labeled goat anti-mouse IgG antibody (Biomeda, Foster City, CA) for 30 min at 4°C as described previously.[19] After two additional washes, the labeled cell samples were analyzed by flow cytometry on a FACScan (Becton Dickinson, Mountain View, CA).

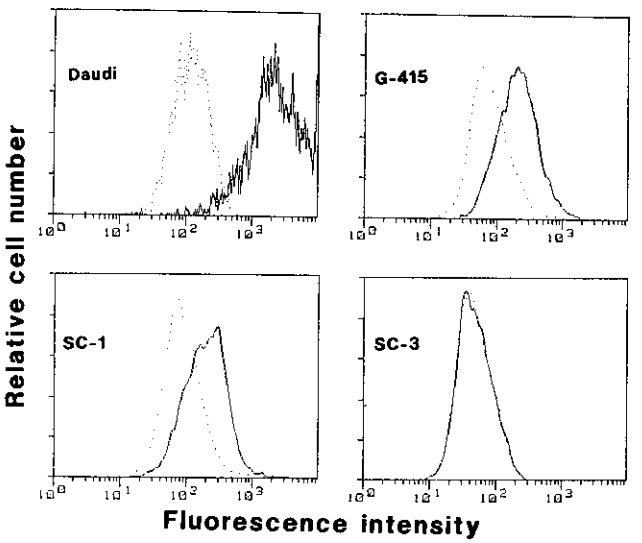

Fig. 1. FACS analysis of expression of the ICAM-1 molecule on tumor cell lines. The net percentages of positive cells were 94.1% for Daudi, 87.2% for G-415, 69.6% for SC-1 and 0% for SC-3 cells. Background (second step alone) for the control is superimposed as a dotted line in each panel. Fluorescence distributions were analyzed by FACScan using $\log_{10}$ unit.

FACS profiles of the tumor cells immunofluorescence-stained with anti-ICAM-1 antibody are shown in Fig. 1. Daudi, SC-1 and G-415 cells were highly reactive to anti-ICAM-1. However, the antibody failed to react to SC-3 cells. ICAM-1 antigen could not be induced on cultured SC-3 cells even by IFNγ stimulation. Thus, ICAM-1 antigen is expressed on the cell surface of Daudi, SC-1 and G-415 cells but not that of SC-3 cells.

Table I. Effect of Spent Media from Cultured ICAM-1$^+$ or ICAM-1$^-$ Tumor Cells on LAK Cell Activity

Group	Spent media from	Target cells	Specific ^{51}Cr release (%)		
			20:1[a]	10:1	5:1
1	None[b]	Daudi	30.9±0.8[c]	20.9±0.3	11.8±0.3
2	Daudi	Daudi	24.7±0.6	15.9±0.5	8.3±0.6
3	IFNγ-treated Daudi	Daudi	20.8±0.6	14.3±0.2	8.1±0.3
4	IFNγ-treated SC-1	Daudi	9.6±0.6	6.3±0.8	3.8±0.6
5	SC-3	Daudi	28.3±1.4	20.2±0.6	12.6±0.4
6	None	G-415	20.9±0.8	14.9±0.4	9.6±0.2
7	G-415	G-415	13.5±0.5	9.4±0.5	6.2±0.2
8	IFNγ-treated G-415	G-415	7.5±0.4	5.4±0.3	3.2±0.2
9	IFNγ-treated SC-1	G-415	10.7±0.5	7.1±0.3	3.4±0.2
10	SC-3	G-415	18.5±0.6	12.3±0.4	8.8±0.3

a) Effector cell:target cell ratios.
b) Fresh complete medium.
c) Mea±SE in quadruplicate cultures.

Table I shows the effect of spent media from tumor cell cultures on LAK cell activities. The LAK cells from a cancer patient showed a substantial increase in cytolytic activities against NK-resistant Daudi or G-415 cells at increased E/T ratios (Groups 1 and 6). The LAK cells (Groups 2 and 7) incubated with spent media from cultured Daudi or G-415 cells showed significant reduction of the cytolytic activity as compared with those at the corresponding E/T ratio from Groups 1 and 6 ($P<0.01$). Treatment of the LAK cells with spent media from ICAM-1^+ tumor cells cultured together with IFNγ (Groups 3, 4, 8 and 9) brought about an even more significant decrease in cytolytic activities against Daudi or G-415 cells ($P<0.01$ for Group 3; $P<0.001$ for Groups 4, 8 and 9). However, the LAK cells incubated with spent media from cultured ICAM-1^- SC-3 cells (Groups 5 and 10) exhibited no significant decrease of cytolytic activity against Daudi or G-415 cells as compared with those of Groups 1 and 6, respectively.

It is well known that the membrane surface antigens of tumors have been detected in culture media of actively growing cell lines *in vitro*[20] and in body fluids of humans and animals with various malignant tumors.[21] The antigens detected are differentiation antigens, tumor-associated antigens or ICAM-1 antigen. Extensive shedding of these antigens reduced the immunogenicity of the original tumor cells[20] or absorbed circulating IgM antibodies thus neutralizing their anti-tumor effects.[21] Some soluble tumor antigens may induce suppressor cells capable of suppressing the activity of cytotoxic cells against the tumor.[22] However, the immunological effect of shedding ICAM-1 antigen from tumors has remained unknown. We previously found that ICAM-1 antigen on gastric carcinoma was preferentially expressed in metastatic gastric carcinoma cells from pleural and peritoneal effusions, and their carcinoma cells maintained *in vitro*.[7] Tsujisaki *et al.*[13] showed that shedding ICAM-1 antigen could be detected in spent media of cultured carcinoma cells, and the level of the antigen in spent media of IFNγ-treated cells was much higher than in those of non-treated cells. As shown in Fig. 1, we have established a new variant gastric carcinoma cell line, SC-3, which does not express the ICAM-1 antigen. Thus, SC-3 cells could be used in the present experiment as a negative control for ICAM-1 antigen. It has also been reported that LFA-1 and ICAM-1 molecules are involved in target tumor cell lysis, as anti-LFA-1 and/or anti ICAM-1 antibodies inhibited the cytolytic activity of LAK cells.[3,4] In the present study, LAK cells treated with spent media, especially from IFNγ-stimulated ICAM-1^+ tumor cells, showed a significant decrease of cytolytic activity as compared with those treated with spent media from ICAM-1^- SC-3 tumor cells or fresh complete medium without clearly containing ICAM-1 antigen. This inhibition was reproducible. Judging from these results, it seemed likely that shedding ICAM-1 antigen could be involved in binding of LFA-1 to LAK cells, *in vitro* at least.

The shedding ICAM-1 antigen or the antigen/LFA-1 binding to effector lymphoid cells, in particular may block host cell-mediated anti-tumor immunity. In fact, Ladisch *et al.*[23] have suggested that gangliosides shed by tumor cells extremely potent enhancers of tumor formation. Recently, Bernhard and Dippold[24] have proposed that shedding of ganglioside (GD3) may cause a local accumulation at the tumor site, with immunosuppressive function. Thus, significantly lower cytolytic activity of LAK cells treated with spent media containing shedding ICAM-1 antigen may have profound implications for down-regulation of the immune system (i.e., escape of the tumor from the immune system by the shedding of ICAM-1 antigen) and for enhancing the tumor metastatic capacity in cancer patients. The control of ICAM-1 antigen shedding, therefore, may be a potential means to achieve more effective anti-tumor immunity in tumor patients.

This study was supported by a Grant-in-Aid (No. 05670444) from the Ministry of Education, Science and Culture of Japan, and a Grant from the Foundation for Promotion of Cancer Research, Tokyo.

(Received August 18, 1993/Accepted November 2, 1993)

REFERENCES

1) Williams, A. F. and Barclay, A. N. The immunoglobulin superfamily — domains for cell surface recognition. *Ann. Rev. Immunol.*, **6**, 381–405 (1988).
2) 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 lymphocytes *in vitro*. *Cancer Immunol. Immunother.*, **31**, 19–27 (1990).
3) Ebihara, T., Koyama, S. and Fukao, K. Functional effector mechanisms of CTL and LAK cells on tumor-cytolytic activity. *Proc. Jpn. Cancer Assoc., 51st Annu. Meet.*, 255 (1992) (in Japanese).
4) Koyama, S. Metastasis of gastric carcinoma and molecules in the immunoglobulin superfamily. *Seitai No Kagaku*, **44**, 321–328 (1993) (in Japanese).
5) Timonen, T., Patarroyo, M. and Gahmberg, C. G. CD11a-c/CD18 and GP84(LB-2) adhesion molecules on human

large granular lymphocytes and their participation in natural killing. *J. Immunol.*, **141**, 1041–1046 (1988).
6) Ebihara, T. and Koyama, S. Functional and phenotypic characteristics of effusion-associated lymphoid cells cultured in the presence of either recombinant interleukin 2 or T-cell growth factor from malignant pleural and peritoneal effusions in patients with advanced carcinoma. *Tohoku J. Exp. Med.*, **162**, 49–63 (1990).
7) Koyama, S., Ebihara, T. and Fukao, K. Expression of intercellular adhesion molecule 1 (ICAM-1) during the development of invasion and/or metastasis of gastric carcinoma. *J. Cancer Res. Clin. Oncol.*, **118**, 609–614 (1992).
8) Clement, L. T., Dagg, H. D. and Landy, A. Characterization of human lymphocyte subpopulations: alloreactive cytotoxic T-lymphocyte precursor and effector cells are phenotypically distinct from Leu $2a^+$ suppressor cells. *J. Clin. Immunol.*, **131**, 2296–2300 (1984).
9) Koide, J. and Engelman, E. G. Differences in surface phenotype and mechanism of action between alloantigen-specific $CD8^+$ cytotoxic and suppressor T cell clones. *J. Immunol.*, **144**, 32–40 (1990).
10) Morimoto, C., Rudd, C. E., Letven, N. L. and Schlossman, S. F. A novel epitope of the LFA-1 antigen which can distinguish killer effector and suppressor cells in human CD8 cells. *Nature*, **330**, 479–482 (1987).
11) Notali, P., Nicotra, M. R., Cavaliere, R., Biotti, A., Romano, G., Temponi, M. and Ferrone, S. Differential expression of intercellular adhesion molecule 1 in primary and metastatic melanoma lesions. *Cancer Res.*, **50**, 1271–1278 (1990).
12) Tomita, Y., Nishiyama, T., Watanabe, H., Fujiwara, M. and Sato, S. Expression of intercellular adhesion molecule-1 (ICAM-1) on renal-cell cancer: possible significance in host immune responses. *Int. J. Cancer*, **46**, 1001–1006 (1990).
13) Tsujisaki, M., Imai, K., Hirata, H., Hanazawa, Y., Masuya, J., Nakano, T., Sugiyama, T., Matsui, M., Hinoda, Y. and Yachi, A. Detection of circulating intercellular adhesion molecule-1 antigen in malignant diseases. *Clin. Exp. Immunol.*, **85**, 3–8 (1991).
14) Harning, R., Mainolfi, E., Bystryn, J. C., Henn, M., Merluzzi, V. J. and Rothlein, R. Serum Levels of circulating intercellular adhesion molecule 1 in human malignant melanoma. *Cancer Res.*, **51**, 5001–5005 (1991).
15) Koyama, S., Ebihara, T., Fukao, K. and Osuga, T. Differential activation of lymphokine-activated killer cells with different surface phenotypes by cultivation with recombinant interleukin-2 or T-cell growth factor in gastric cancer patients. *Jpn. J. Cancer Res.*, **80**, 150–157 (1989).
16) Ebihara, T. and Koyama, S. Suppression by sorted $CD8^+CD11b^-$ cells from T-cell growth factor-activated peripheral blood lymphocytes on cytolytic activity against tumor in patients with gastric carcinoma. *Eur. J. Cancer*, **27**, 1654–1657 (1991).
17) Koyama, S. and Fukao, K. Phenotypic analysis of nylon-wool adherent suppressor cells which inhibit the effector process of tumor cell lysis by lymphokine-activated killer cells in patients with advanced gastric carcinoma. *J. Cancer Res. Clin. Oncol.*, **120** (1994), in press.
18) Koyama, S., Yoshioka, T., Mizushima, A., Kawakita, I., Fukutomi, H., Sakita, T., Kondo, I. and Kikuchi, M. Establishment of a cell line (G-415) from a human gallbladder carcinoma. *Gann*, **71**, 574–575 (1980).
19) Koyama, S., Mukai, R., Fukao, K., Arimura, H., Iwasaki, Y. and Osuga, T. Monoclonal antibody against human gallbladder carcinoma-associated antigen. *Cancer Res.*, **47**, 4667–4673 (1987).
20) Chiba, I., Jin, R., Hamada, J., Hosokawa, M., Takeichi, N. and Kobayashi, H. Growth-associated shedding of a tumor antigen (CE7) detected by a monoclonal antibody. *Cancer Res.*, **49**, 3972–3975 (1989).
21) Vinuela, J. E., Rodriguez, R., Gil, J., Dela Concha, E. G. and Subiza, J. L. Antigen shedding vs. development of natural suppressor cells as mechanism of tumor escape in mice bearing Ehrlich tumor. *Int. J. Cancer*, **47**, 86–91 (1991).
22) Takahashi, K., Ono, K., Hirabayashi, Y. and Taniguchi, M. Escape mechanisms of melanoma from immune system by soluble melanoma antigen. *J. Immunol.*, **140**, 3244–3248 (1988).
23) Ladisch, S., Kitada, S. and Hays, E. F. Gangliosides shed by tumor cells enhance tumor formation in mice. *J. Clin. Invest.*, **79**, 1879–1882 (1987).
24) Bernhard, H. and Dippold, W. Ganglioside GD3 shedding by cultured human malignant melanoma cells. *Proc. Am. Assoc. Cancer Res.*, **29**, 383 (1988).