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Short Report

Exogenous Recombinant Human IL-12 Augments MHC Class I Antigen Expression on Human Cancer Cells in vitro

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Department of Respiratory Oncology and Molecular Medicine, Division of Cancer Control, [2]Department of Nuclear Medicine and Radiology, Division of Brain Sciences, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980-8575, and [1]The First Department of Oral and Maxillo-Facial Surgery, Tohoku University School of Dentistry, Sendai 980-8575

SUZUKI, S., UMEZU, Y., SAIJO, Y., SATOH, G., ABE, Y., SATOH, K. and NUKIWA, T. *Exogenous Recombinant Human IL-12 Augments MHC Class I Antigen Expression on Human Cancer Cells in vitro.* Tohoku J. Exp. Med., 1998, **185** (3), 223-226 —— We investigated whether expressions of MHC class I and class II antigens relevant to tumor antigen presentation were changed on human tumor cells cultured with or without recombinant human IL-12(rhIL-12). We showed that the expression of MHC class I antigen on UTC-8, 28-1Cl and SBC-3 cells was augmented when these cancer cells were cultured with rhIL-12. The expression of class II antigen was slightly raised on UTC-8 and 28-1Cl cells by rhIL-12, but not enhanced on SBC-3 cells. These results suggest that rhIL-12 may provide possible enhancement of immunologic tumor recognition, and cytotoxic activity of lymphocytes against tumors through the enhanced expression of MHC class I antigen. ———— rhIL-12; MHC class I antigen; cancer cells

IL-12 was discovered as a potent cytokine which activates natural killer cells (Kobayashi et al. 1989) and matured cytotoxic T lymphocytes (Stern et al. 1990). Biological activities of IL-12 provide series of immunologic modulations (Kobayashi et al. 1989; Chan et al. 1991; Chouaib et al. 1994). As a result, IL-12 showed surprising anti-tumor activity in both in vitro and in vivo, syngeneic mouse-tumor systems (Brunda et al. 1993). However, it has not yet been reported

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whether IL-12 directly affects the expression of cell surface antigens on cancer cells.

With these as backgrounds, we investigated whether expressions of MHC class I antigen and MHC class II antigen relevant to tumor antigen presentation (Goldberg and Rock 1992; Kovacsovics and Rock 1995) were changed on a cell lines of well differentiated oral squamous cell carcinoma cells, and two histologically different lung cancer cell lines when rhIL-12 was added in the culture medium. The expression of MHC class I antigen on UTC-8 was gradually enhanced from day 1 through day 5 in culture with rhIL-12 (100 units/ml) and reached to plateau at day 5 (Fig. 1). Remarkable enhancement of MHC class I antigen on cell surfaces of 28-1Cl and SBC-3 cells was also observed when cultured with rhIL-12 (100 units/ml) (Figs. 2-a, b and c). The mean fluorescence intensities were shifted from 803 to 7025 on UTC-8 cells, from 453 to 971 on 28-1Cl cells, and from 54.2 to 355 on SBC-3 cells.

In contrast, the expression of MHC class II antigen was only slightly raised on cell surfaces of UTC-8 cells and 28-1Cl cells cultured with rhIL-12 (100 units/

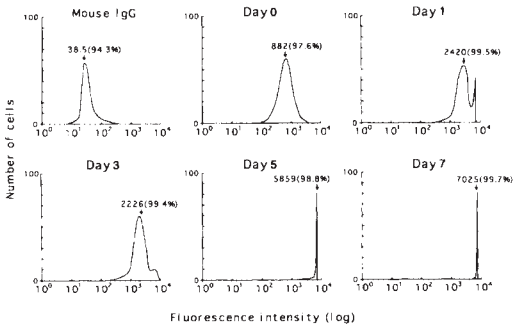

Fig. 1. Effect of exogenous rhIL-12 on time course of expression of MHC class I antigen in UTC-8 cells.
UTC-8 cells (3×10^5), a cell line from human oral squamous cell carcinoma, were maintained in RPMI1640 supplemented with 10% FCS. UTC-8 cells were cultured with 100 U/ml of rhIL-12 protein (1 unit is defined as 1.7×10^{-2} pg of rhIL-12 protein/ml of medium. Batch number 2853-93, Genetics Institute Inc., Boston, MA, USA) for day 0 to day 7. Immunologic staining was performed as follows: UTC-8 cells (3×10^5 cells) were prepared and washed twice with cold-PBS. Antibody to human MHC class I antigen (Bioline, Torino, Italy), belonging to IgG_{2a}, was added as a first antibody into the cell pellets, and incubated at 4°C for 60 minutes. Cells were washed twice with cold-PBS, and goat anti-mouse IgG (L+H) conjugated with FITC (Cedar Lane, Ontario, Canada) was added as a second antibody into cell pellets. After incubation at 4°C for 60 minutes in dark, cells were washed twice with cold-PBS. The fluorescence intensity was analyzed by FACSort (Becton Dickinson, San Jose, CA, USA). Mouse IgG was used as negative control. x-axis denotes the fluorescence intensity, and y-axis is for number of cells. Percentages in parentheses indicate the ratios of MHC class I antigen positive or negative cells to all gated cells counted. Increase of non-specific mouse immunoglobulin bindings was observed on cancer cells cultured with rhIL-12. Note that fluorescence intensity shifted to the right in the incubation with rhIL-12. The steep peak shown in the right edge indicates that the MHC class I antigen was augmented to the cell surface on some cells.

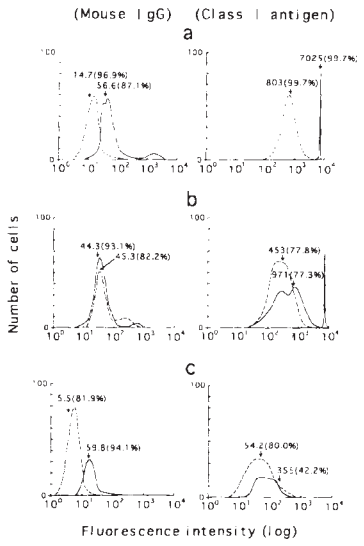

Fig. 2

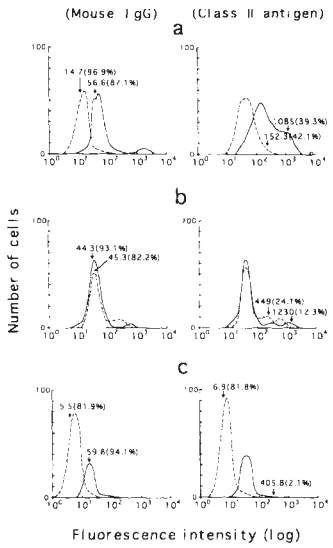

Fig. 3

Fig. 2. Effect of exogenous rhIL-12 on expression of MHC class I antigen in different human cancer cells. SBC-3 and 28-1Cl are cell lines from human small cell lung cancer and large cell lung cancer, respectively, and maintained as UTC-8 (Fig. 1). All of these cancer cells were cultured for 7 days with or without 100 U/ml of rhIL-12 protein. Each tumor cells (10^6 cells) were prepared and washed twice with cold-PBS. Immunologic staining was performed as in Fig. 1. (a) UTC-8 cells, (b) 28-1Cl cells, and (c) SBC-3 cells. Mouse IgG was used as negative control.
——, with IL-12; ------, without IL-12.

Fig. 3. Effect of exogenous rhIL-12 on expression of MHC class II antigen in different human cancer cell lines. Cell lines of UTC-8, 28-1C1, and SBC-3 were immunostained as is written in Fig. 1., except that the antibody used as first antibody was against human MHC class II antigen (Pharmingen, San Diego, CA, USA). (a) UTC-8 cells, (b) 28-1Cl cells, (c) SBC-3 cells.
Mouse IgE was ussed as negative contro.
——, with IL-12; ------, without IL-12.

ml), but no enhancement was observed on SBC-3 cells by rhIL-12 (Figs. 3-a, b and c).

The extent of the enhancement of MHC class I and class II antigen expression by rhIL-12 was different in three cancer cell lines examined. As a consequence of these experiments, we found that exogenous rhIL-12 markedly augmented the expression of MHC class I antigen on cancer cells. As there is a possibility that interferon-gamma (IFN-γ) may act as a enhancer of the MHC class I expression as is shown on murine sarcoma cell line, MCA 101 (Restifo et al. 1992), IFN-γ was measured along with culture period by ELISA (R and D Systems, Minneapolis, MN, USA). However, no IFN-γ was detected in all culture supernatant of UTC-8 and SBC-3 cells examined even when cultured with exogenous rhIL-12 (Table 1). These results suggest that IL-12 may provide the possible enhancement of immunologic tumor recognition and promote cytotoxic activity of lymphocytes against tumors through the enhanced expression of MHC class I antigen.

TABLE 1. *Detection of IFN-γ in the culture supernatants of cancer cell lines co-cultured with rhIL-12*

		Days cultured[a]						
		1	2	3	4	5	6	7
Concentration of IFN-γ (Pg/ml)								
	Control[b]	64	61	51	n.d.[c]	n.d.	n.d.	n.d.
	UTC-8	<10	<10	n.d.	<10	<10	<10	<10
	SBC-3	n.d.	n.d.	<10	n.d.	n.d.	n.d.	<10

[a] 100 units/ml of rhIL-12 was added to the culture medlum. Culture conditions were same as is described in Fig. 1.
[b] IFN-γ in the culture supernatants from IL-12 stimulated, normal human peripheral blood mononuclear cells (3×10^5 cells/3 ml/well).
[c] n.d., not done.

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