Effects of interferon-α and γ on development of LAK activity from mononuclear cells in breast cancer patients

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Abstract : We examined the effect of recombinant IFN-α and IFN-γ on induction of LAK cells from peripheral blood mononuclear cells (PBMNCs) in 7 pre-operative breast cancer patients and 4 healthy volunteers. Significant LAK activity was developed from PBMNCs of pre-operative breast cancer patients and healthy volunteers after incubation for 4 days with IL-2 (presence of IL-2 vs. absence of IL-2). Incubation of PBMNCs of pre-operative breast cancer patients with 1000 U/ml of IFN-α for 4 days suppressed the LAK activity significantly (P<0.05). By contrast, incubation of PBMNCs of pre-operative patients with 1000 U/ml of IFN-γ for 4 days increased the LAK activity significantly (P<0.05). Significan t cytotoxicity against MCF-7 cells (estrogen receptor positive human breast cancer cell line) was developed from PBMNCs of pre-operative breast cancer patients at 20:1and 40:1E/T ratios after incubation for 4days with IL-2 (absence of IL-2vs. 20:1or40:1, P<0.05, P<0.05), whereas PBMNCs of healthy volunteers did not. Stimulation of LAK cells with IFN-γ produced a significant augmentation of cytotoxic activity against MCF-7 (P<0.05), while IFN-α suppressed the cytotoxicity significantly (P<0.05). These findings suggested that combined stimulation by IFN-γ and IL-2 might be a reasonable treatment for breast cancer patients. J. Med. Invest. 45 : 71-75, 1998

Key words : *breast cancer, IL-2, IFN-α, IFN-γ, LAK*

INTRODUCTION

The cellular responses that follow the administration of interferons (IFNs) in vivo are very complex and difficult to unravel. Under some experimental conditions, IFNs stimulate different kinds of cells and cause up-regulation of expression of antigens and production of cytokines (1-7). Moreover, IFN-α and IFN-γ, administered simultaneously, act synergistically (8-10).This results in very elaborate and intricately related actions between carcinoma cells, endothelium, lymphocytes, and macrophages, which ultimately may lead to the destruction of tumor cells. IFN-α and IFN-γ augment LAK activity developed from PBMNCs of healthy volunteers (11-14). LAK cells alone, IL-2alone, or LAK cells plus IL-2 are used for cancer treatment and achieve regression of cancer (15, 16). It is the purpose of this study to draw an outline of the cellular events in breast cancer patients treated with IFN-α/IFN-γ and IL-2.

Received for publication March 1, 1998 ; accepted July 21, 1998.

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MATERIALS AND METHODS

Materials

The subjects were 7 pre-operative patients with resectable primary breast cancer and 4 healthy volunteers. Cancer cells of 6 of 7 pre-operative patients were positive in the estrogen receptor.

Reagents

Recombinant IL-2 was prepared by Takeda Pharmaceutical Co. (Osaka, Japan), and had a specific activity of 3.5×10^4 mU/mg as assayed using IL-2-dependent murine NKC 3 cells (17).

Cell Line

Human Burkitt lymphoma cells (Daudi) and estrogen receptor positive human breast cancer cells

(MCF-7) were maintained as stationary suspension cultures in RPMI 1640 medium supplemented with 10% heat-inactivated FBS and gentamicin at 37℃ in a humidified atmosphere of 5% CO_2 in air. MCF-7 cells were kindly provided by Otsuka Pharmaceutical Co. (Japan).

Isolation and culture of PBMNCs

MNCs were separated from the peripheral blood by discontinuous gradient centrifugation in lymphocyte separation medium (18, 19).

Assay of LAK activities and cytotoxicity against MCF-7

LAK activity and cytotoxicity against MCF-7 were assayed by measuring ^{51}Cr release using a method described previously (18, 19). Briefly, PBMNCs were incubated with or without serial concentrations of IFN-α, IFN-γ or IL-2 (400U/ml) for 4 days in RPMI 1640 medium supplemented with 5% FBS and gentamicin at 37℃ under 5% CO_2 in humidified air. Unless otherwise noted, 400 U/ml of IL-2 was used for development of LAK activity in PBMNCs ; we confirmed that these concentrations of IL-2 were optimal for development of LAK activity from PBMNCs. There was no significant difference between the number of cells after four days of culture with or without IFN-α, IFN-γ, or IL-2. The cytotoxicity of cultured PBMNCs against ^{51}Cr-labeled Daudi cells ($1x10^4$) was measured at a 10 : 1 effector : target (E/T) cell ratio in triplicate cultures. And the cytotoxicity of cultured PBMNCs against ^{51}Cr-labeled MCF-7 (1×10^4) was measured at 10 : 1, 20 : 1, and 40 : 1 E/T cell ratios in triplicate cultures. Coculture of effector cells and target cells was terminated after 4h, and the radioactivity of the supernatants (0.1ml per well), separated by brief centrifugation at 65 x g was determined using a gamma counter. The percent cytotoxicity was calculated as follows :

$$\% \text{ cytotoxicity} = \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}} \%$$

Spontaneous cpm was about 10% (range : 7 to 13%) of total cpm. Total cpm was determined from the radioactivity in the supernatant of Daudi cells (1×10^4) or MCF-7 (1×10^4) after lysis with 2 *N* HCl.

Statistical Analysis

The significance of differences between groups was determined using the paired or non paired *t*-test for 2 corresponding groups.

RESULTS

Effect of IFN-α on development of LAK activity by IL-2 from PBMNCs

Significant LAK activity was developed from PBMNCs of pre-operative breast cancer patients and healthy volunteers after incubation for 4 days with IL-2 (presence of IL-2 vs. absence of IL-2, $p< 0.01$ (data not shown)). Stimulation with 1000U/ml of IFN-α significantly suppressed the LAK activity of PBMNCs from pre-operative breast cancer patients by IL-2 ($P<0.05$) (Figure 1). But stimulation with IFN-α showed no change in the LAK activity of PBMNCs from healthy volunteers.

Effect of IFN-γ on development of LAK activity by IL-2 from PBMNCs

Stimulation with 1000 U/ml of IFN-γ significantly augmented LAK activity of PBMNCs from pre-operative breast cancer patients by IL-2 ($P<0.05$) (Figure2). On the other hand, stimulation with IFN-γ did not change the LAK activity developed from PBMNCs of healthy volunteers.

Development of cytotoxicity against MCF-7by IL-2 from PBMNCs

Significant cytotoxicity against MCF-7 was exhibited

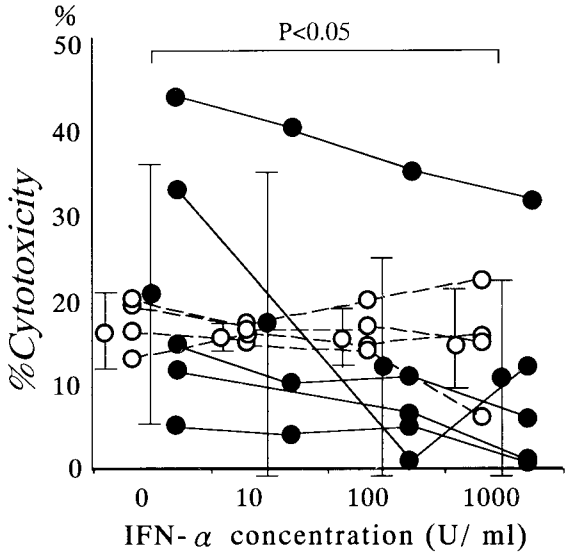

Fig.1. Effect of IFN-α on development of LAK activity by IL-2 from PBMNCs.
PBMNCs (10^5) from 4 healthy volunteers (○) and 5 pre-operative breast cancer patients (●) were incubated for 4 days with IL-2 (400U/ml) and serial concentrations of IFN-α varying from 0 to 1000U/ml in 96-well plastic plates. % cytotoxicity was determined as described in the text. The bars represent the mean percentage of cytotoxicity against an allogeneic cell line of Daudi cells at a 10 : 1 effector : target ratio.

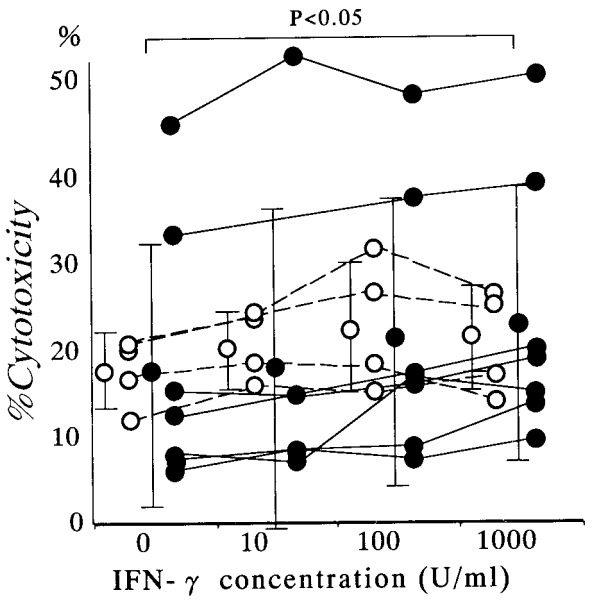

Fig. 2 . Effect of stimulation with IFN-γ on development of LAK activity by IL-2 from PBMNCs.
PBMNCs (10^5) from 4 healthy volunteers (○) and 7 pre-operative breast cancer patients (●) were incubated for 4 days with IL-2 and serial concentrations of IFN-γ varying from 0 to 1000 U/ml in 96-well plastic plates before LAK assay. % cytotoxicity was determined as described in the text. The bars represent the mean percentage of cytotoxicity against an allogeneic cell line of Daudi cells at a 10 : 1 effector : target ratio.

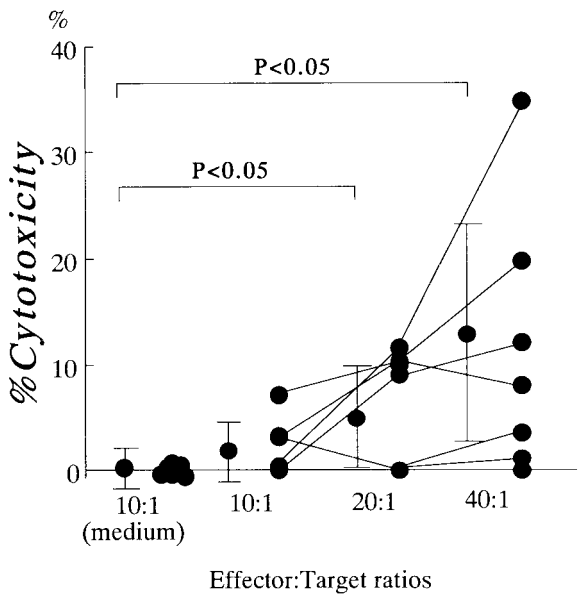

Fig. 3 . Development of cytotoxicity against MCF-7 by IL-2 from PBMNCs. Various numbers (1 x 10^5, 2 x 10^5, 4 x 10^5) of freshly separated PBMNCs from 7 pre-operative breast cancer patients were incubated for 4 days with IL-2 in 96-well plastic plates before assay. Then the % cytotoxicity was determined. Results represent the mean±SD.

by PBMNCs from pre-operative breast cancer patients at 20 : 1 and 40 : 1 E/T ratios after incubation for 4 days with IL-2 (absence of IL-2 vs. 20 : 1 or 40 : 1, P<0.05, P<0.05) (Figure 3). PBMNCs of healthy volunteers did not develop cytotoxicity against MCF-7 after incubation for 4 days with IL-2 at serial E/T ratios (data not shown).

Effect of stimulation with IFN-α or IFN-γ on development of cytotoxicity against MCF-7 from PBMNCs of pre-operative breast cancer patients

Stimulation with 1000 U/ml of IFN-γ significantly augmented cytotoxicity against MCF-7 developed from PBMNCs from pre-operative breast cancer patients by IL-2 at a 40 : 1 E/T ratio (P<0.05) (Figure 4).

By contrast, stimulation with 1000 U/ml of IFN-α significantly suppressed cytotoxicity against MCF-7 developed from PBMNCs from pre-operative breast cancer patients by IL-2 at a 40 : 1 E/T ratio (P<0.05) (Figure 4).

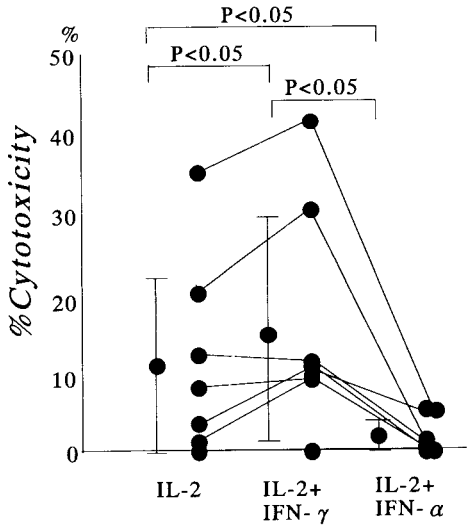

Fig. 4 . Effect of stimulation with IFN-γ and α on development of cytotoxicity by IL-2 from PBMNCs of pre-operative breast cancer patients against MCF-7.
PBMNCs (10^5) from 7 pre-operative breast cancer patients were incubated for 4 days with IL-2 and/or 1000U/ml of IFN-γ or 1000 U/ml of IFN-α in 96-well plastic plates before LAK assay. % cytotoxicity was determined as described in the text. The bars represent the mean percentage of cytotoxicity against an allogeneic cell line of MCF-7 cells at a 40 : 1 effector : target ratio.

DISCUSSION

In the present study, we examined whether IFN-α or IFN-γ augment LAK activity and cytotoxicity against MCF-7m of PBMNCs from pre-operative breast cancer patients and healthy volunteers by IL-2. PBMNCs from healthy volunteers developed significant LAK activity but these cells did not show significant cytotoxicity against MCF-7 at a 10 : 1, 20 : 1, or 40 : 1 E/T ratio. Peripheral blood lymphocytes migrate into tumor and may play a role as tumor infiltrating

lymphocytes (TIL) (20). Indeed, TIL in breast cancer may be important to immunological defense (21). And the roles of TIL may relate to improved prognosis and survival in breast cancer patients (22, 23). It is, therefore, important to activate peripheral lymphocytes in breast cancer patients to develop cytotoxicity against breast cancer cell. As previously reported (24), PBMNCs from breast cancer patients in the present study developed significant LAK activity. Moreover, PBMNCs separated from pre-operative breast cancer patients developed significant cytotoxicity against MCF-7, whereas PBMNCs from healthy volunteers did not. Cell-mediated immunity is suppressed to a much greater extent in some cancer patients (25). But PBMNCs in pre-operative breast cancer patients have been already activated to develop significant cytotoxicity against breast cancer cells. Biological characteristics of breast cancer cells may induce the immunological activation of PBMNCs in pre-operative breast cancer patients as previously reported (26). The efficient LAK activity might improve the prognosis and survival of cancer patients (27, 28). Breast cancer patients have been reported to have a better prognosis than patients with other malignancies (29, 30). One reason for the good prognosis of breast cancer patients might be the activated state of lymphocytes. IFN-γ augments LAK activity from PBMNCs of breast cancer patients (23). In our study, IFN-γ augmented not only LAK activity from PBMNCs of pre-operative patients but also cytotoxicity against breast cancer cells. In contrast, IFN-α significantly suppressed this activity. Although IFN-α and γ have antiproliferative effects on human breast cancer cells in culture (31-34), IFN-α suppressed LAK activity and cytotoxicity against MCF-7.

These results indicated that the induction of cytotoxicity from lymphocyte by a combination of IFN-γ and IL-2 may be helpful in designing more effective cancer immunotherapeutic protocols for breast cancer patients.

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