Effects of interferon-\(\alpha\) and \(\gamma\) on development of LAK activity from mononuclear cells in breast cancer patients

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Abstract: We examined the effect of recombinant IFN-\(\alpha\) and IFN-\(\gamma\) on induction of LAK cells from peripheral blood mononuclear cells (PBMCs) in 7 pre-operative breast cancer patients and 4 healthy volunteers. Significant LAK activity was developed from PBMCs 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 PBMCs of pre-operative breast cancer patients with 1000 U/ml of IFN-\(\alpha\) for 4 days suppressed the LAK activity significantly (P<0.05). By contrast, incubation of PBMCs of pre-operative patients with 1000 U/ml of IFN-\(\gamma\) for 4 days increased the LAK activity significantly (P<0.05). Significant cytotoxicity against MCF-7 cells (estrogen receptor positive human breast cancer cell line) was developed from PBMCs of 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. 20:1or40:1, P<0.05, P<0.05), whereas PBMCs of healthy volunteers did not. Stimulation of LAK cells with IFN-\(\gamma\) produced a significant augmentation of cytotoxic activity against MCF-7 (P<0.05), while IFN-\(\alpha\) suppressed the cytotoxicity significantly (P<0.05). These findings suggested that combined stimulation by IFN-\(\gamma\) 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-\(\alpha\), IFN-\(\gamma\), 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-\(\alpha\) and IFN-\(\gamma\), 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-\(\alpha\) and IFN-\(\gamma\) augment LAK activity developed from PBMCs 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-\(\alpha\)/IFN-\(\gamma\) and IL-2.

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 3.5x10^4 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

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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°C under 5% CO₂ 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 (1x10⁶) 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 x 10⁶) 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:

\[
\text{% cytotoxicity} = \frac{\text{experimental cpm - spontaneous cpm}}{\text{total cpm - spontaneous cpm}} \times 100
\]

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 x 10⁶) or MCF-7 (1 x 10⁶) after lysis with 2N 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-7 by IL-2 from PBMNCs

Significant cytotoxicity against MCF-7 was exhibited...
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).

**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 lympho-
cytes in breast cancer patients to develop cytotoxicity
against breast cancer cell. As previously reported
(24), PBMCs from breast cancer patients in the
present study developed significant LAK activity. More-
over, PBMCs separated from pre-operative breast
breast cancer patients developed significant cytotoxicity
against breast cancer cells. Biological
characteristics of breast cancer cells may induce
the immunological activation of PBMCs in pre-operative
breast cancer patients as previously reported (26).
The efficient LAK activity might improve the prog-
nosis and survival of cancer patients (27, 28). Breast
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
breast cancer patients might be the activated state of
lymphocytes. IFN-γ augments LAK activity from
PBMCs of breast cancer patients (23). In our study,
IFN-γ augmented not only LAK activity from PBMCs
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.

REFERENCES

2. Webb DS, Mostowski HS, Gerrard TL: Cytokine-induced enhancement of ICAM-1 expression results in increased vulnerability of tumor cells to
monocyte-mediated lysis. J Immunol146: 3682-
3686, 1991
3. Aversa GG, Hall BM Cell surface markers of
4. Makgoba MW, Sanders ME, Shaw S: The
CD 2-LFA-3 and LFA-1-ICAM pathways: re-
levance to T-cell recognition. Immunol Today 10:
417-422, 1989
5. Buckle AM, Hogg N: Human memory T cells
express intercellular adhesion molecule-1 which
be increased by interleukin 2 and interferon-γ.
6. Griffiths CEM, Voorhees JJ, Nickoloff BJ:
Charac-terization of intercellular adhesion
molecule-1 and HLA-DR expression in normal
and inflamed skin : modulation by recombiant
γ interferon and tumor necrosis factor. J Am
Acad Dermatol 20: 617-629, 1989
7. Prober JS: Cytokine-mediated activation of vas-
8. Ozzello L, Habif DV, DeRosa CM, Cantell k:
Treatment of human breast cancer xenografts
using natural interferons-α and-γ injected singly
or in combination. J Interferon Res 8: 679-690,
1988
JKV, Cummings KB, Borden EC : Synergistic
antiproliferative effects of human recombinant
α 54- or βser-interferon with γ-interferon on
human cell lines of various histogenesis. Cancer
10. Ozzello L, Habif DV, DeRosa CM: Antiproliferative
effects of natural interferon β alone and in com-
bined with natural interferon γ on human
breast carcinomas in nude mice. Breast Cancer
Res Treat 16: 89-96, 1990
Generation of activated killer (AK) cells by
recombinant interleukin-2 (IL-2) in collabora-
tion with interferon-γ (IFN-γ). J Immunol 134:
3124-3129, 1985
Cancer 37: 787-793, 1986
FH : Long term growth of lymphokine-activated
killer (LAK) cells : role of anti-CD 3, β-IL-1,
interferon-γ and β. J Immunol 138: 2728-2733,
1987
effects of recombinant interferon α, β, and