Interferon Producing Activity of Lymphnode and Peripheral Blood Lymphocytes in Cancer Patients

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KONN, M., SASAKI, M., YAMANAKA, Y., MORITA, T. and ONO, K. Interferon Producing Activity of Lymphnode and Peripheral Blood Lymphocytes in Cancer Patients. Tohoku J. exp. Med., 1987, 152 (3), 231-235 — The IFN producing activity of RLNL and PBL was studied in cancer patients using K562, PHA-P and OK-432 as inducers. In response to K562, IFN production was significantly greater in the PBL than in the RLNL, and the titer was about equal in the PBL and RLNL when OK-432 was used as an inducer. On the contrary, the amount of IFN produced by PHA-P was significantly higher in the RLNL than in the PBL. There was no definite relationship recognized between tumor progression and IFN productivity in the cases studied. interferon ; stomach cancer ; colo-rectal cancer ; lymphnode lymphocytes ; peripheral blood lymphocytes

Unquestionably, the regional lymphnode lymphocytes (RLNL) may be involved immunologically in the host response to neoplasma. However, the role of the RLNL in neoplasia and in metastasis is still controversial. Thus, in order to evaluate the immunological function of the RLNL, we investigated the interferon (IFN) producing activity of RLNL and peripheral blood lymphocytes (PBL) using various inducers in cancer patients.

MATERIALS AND METHODS

Subjects

The subjects studied consisted of 16 stomach and 8 colorectal cancer patients of 17 males and 7 females ranging in age from 26 to 76 years.

IFN inducers

OK-432, a product of Streptococcus pyogenes (Su strain, Chugai Pharmaceutical Co., Tokyo), phytohemagglutinin-p (PHA-P, Difco laboratories, Michigan, IL, USA), and K562, a cultured cell line derived from human erythroleukemia, were used as the inducers.

Separation of lymphocytes

Lymphocytes were separated from peripheral blood and regional lymphnode by a Ficoll-Isopaque centrifugation method. The right gastro-epiploic lymphnode of stomach
cancer and superior rectal lymphnode of colo-rectal cancer, both grossly metastasis negative, were selected for the experiments.

**IFN induction**

Cell cultures were performed at the following concentration: a, $5 \times 10^6$ lymphocytes/ml + 0.1 KE/ml of OK-432; b, $2 \times 10^6$ lymphocytes/ml + 60 μg/ml of PHA-P; c, $1 \times 10^5$ lymphocytes/ml + $2.5 \times 10^5$ K562/ml. One KE (Klinische Einheit) of OK-432 corresponds to 0.1 mg of dried streptococci.

**Determination of IFN titer**

The determination of IFN titers was done by the method of 50% plaque reduction of vesicular stomatitis virus (VSV) using human amnion derived WISH cells. One unit of IFN in this experiment was that amount required to reduce the number of PFU per well by 50% compared to the control.

**IFN typing experiments**

Determination of IFN type was performed by neutralization method using anti-IFN-α and anti-IFN-γ (Paesel Co., Frankfurt, FRG).

**Detection of mycoplasma contamination**

Mycoplasma contamination was examined in K562 by using Micotrim-TM (Hana Biologies, Inc., Berkeley, CA, USA) and shown to be negative.

**RESULTS**

**Antigenicity of OK-432, PHA-P and K562 induced IFN** (Table 1.)

The antiviral activity induced by PHA-P was almost completely neutralized by treatment with anti-IFN-γ, while K562 induced IFN by anti-IFN-α. On the other hand, OK-432 induced IFN was neutralized only partly by treatment with anti-IFN-α and anti-IFN-γ.

**Table 1. Antigenicity of OK-432, PHA-P and K562 induced interferon**

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Residual IFN activity (U/ml) after treatment with</th>
<th>Anti-IFN-α</th>
<th>Anti-IFN-γ</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>140</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Anti-IFN-α</td>
<td>150</td>
<td>120</td>
</tr>
<tr>
<td>OK-432</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>360</td>
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</tr>
<tr>
<td>PHA-P</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>230</td>
<td>200</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>170</td>
<td>20</td>
</tr>
<tr>
<td>K-562</td>
<td></td>
<td>&lt;10</td>
<td>290</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>280</td>
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</tbody>
</table>

For IFN neutralization test, small aliquots of IFN (100–200 μl) were mixed with appropriate concentration of anti-IFN-α and γ, and incubated at room temperature for 60 min. Following incubation, the residual IFN in each aliquots was assayed.
Comparison of IFN productivity of the RLNL and PBL in cancer patients

In response to OK-432, the amount of IFN induced by PBL was $232 \pm 161$ units/ml, while that of RLNL was $173 \pm 122$ units/ml (Fig. 1a). When K562 was used, the average amount of IFN was $237 \pm 147$ units/ml in PBL, while that of RLNL was $100 \pm 64$ units/ml, evidently in favor of the PBL with statistically significant difference ($p < 0.01$, Fig. 1b). In contrast, when PHA-P was used as an inducer, the amount of IFN of PBL and RLNL averaged $54 \pm 60$ units/ml and $119 \pm 64$ units/ml, respectively and the latter was significantly higher as compared with the former ($p < 0.05$, Fig. 1c).

Comparison of IFN productivity in different clinical stages

Relationship between clinical stages and IFN productivity was studied by dividing cancer patients into 4 stages. When OK-432 was used, both PBL and RLNL showed about even IFN production in each stages (Fig. 2a). In response to K562 and PHA-P, although relatively high titers of IFN were yielded in stage I and IV in some cases, there was generally no definite correlation between clinical stages and IFN productivity (Figs. 2b, 2c).

DISCUSSION

In the present study, the IFN productivity was compared in PBL and RLNL of cancer patients, and the former produced about 2-3 times more than did the
latter in response to K562. On the other hand, the amount of IFN in use of PHA-P was significantly greater in the RLNL than the PBL. Thus, it was suggested that both peripheral blood and lymphnodes were lymphatic organs directed to the production of IFN-α and IFN-γ, respectively. These results were considered to indicate the difference in the distribution of natural killer (NK) cells and T cell subsets between RLNL and PBL (Epstein et al. 1971; Timonen et al. 1980). Furthermore, it was demonstrated that there was no definite correlation between clinical stages and IFN productivity, and sufficient preservation of IFN-γ productivity was noted even in cases of advanced stages. This result may reflect the existence of immune reaction against tumor, and high productivity of IFN-γ in RLNL may be of extreme advantage to the adjuvant immunotherapy.

Fig. 2. IFN productivity of PBL and RLNL in different stages in response to OK-432 (a), K562 (b) and PHA-P (c).

○, colo-rectal cancer; ●, stomach cancer.
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Acknowledgments

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References
