72. Synergistic Inhibition of the Growth of Adenocarcinoma 755 by the Combination of Interleukin-2 and Interferon- β

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Introduction. Interleukin-2 (IL-2) is a secretory product of activated helper T-lymphocytes. It has been demonstrated that IL-2 is essential not only for expansion of antigen-triggered T-lymphocytes and cytotoxic T cells, but also for the activation of natural killer (NK) cells, which have an inhibitory effect on tumor metastasis. Currently there is a great deal of interest in using IL-2 in clinical cancer therapy because large amounts of recombinant human IL-2 (rHIL-2) are available as a result of advances in the field of genetic engineering. Now it is possible for us to use sufficient quantities of rHIL-2 to conduct properly controlled clinical trials. In animal studies, it has been reported that rHIL-2 together with lymphokine activated killer (LAK) cells induces marked decrease in the numbers of established pulmonary metastasis of MCA-105 sarcoma cells in C57BL/6 mice although rHIL-2 alone shows little effect in this experimental system.¹⁾ We report here that the growth of adenocarcinoma 755 was markedly inhibited by a combination of rHIL-2 and recombinant murine interferon- β (rIFN- β) in C57BL/6 mice, though rHIL-2 or rIFN- β alone showed little effect on the tumor growth.

Materials and methods. Specific pathogen-free (SPF) male C57BL/6 mice were obtained from the Shizuoka Laboratory Animal Center (Hamamatsu). Male nu/nu mice (BALB/c background) were supplied by CLEA Japan. The mice housed under SPF conditions were used at the age of 6 (C57BL/6) and 10 (nu/nu) weeks at the beginning of the experiments. Adenocarcinoma 755, which is syngeneic with the C57BL/6 mouse, was used for this study. Lyophilized anti-asialo GM1 antiserum (Wako Pure Chemical Industries, Osaka) was reconstituted with balanced salt solution. As reported previously,²⁾ when 500 μ g of anti-asialo GM1 antibody was administered intravenously into the tail vein of C57BL/6 mice, the function of NK cells was suppressed. In this study we injected intravenously 1 mg of anti-asialo GM1 antibody on days 5 and 9. Lyophilized human recombinant interleukin-2 (rHIL-2) (specific activity, 1×10^7 units/mg of protein) was obtained from Biogen S.A. Company, Switzerland, and Shionogi & Co., Osaka. Mouse recombinant interferon- β (rIFN- β) (specific activity, 5.5×10^7 international units/mg of protein) was supplied by Toray Industries, Inc., Kamakura.

Groups of 8 (C57BL/6) or 4 (nude) mice were used. Adenocarcinoma 755 (5×10^5 cells/mouse) was implanted subcutaneously (s.c.) into the right thigh

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on day 0. The mice were given rHIL-2 (1×10^5 units/mouse) and rIFN- β (1×10^5 international units/mouse) 8 times by s.c. injection into the opposite side (left thigh) in the advanced tumor system (days 5–12). The growth of s.c.-implanted adenocarcinoma 755 was monitored by measurement of the perpendicular diameters with calipers, and tumor volume (mm^3) was calculated by the formula $1/2 \times (\text{major diameter in mm}) \times (\text{minor diameter in mm})^2$.

Results and discussion. rHIL-2 alone was found to have a weak inhibitory effect on the growth of s.c.-implanted adenocarcinoma 755, and rIFN- β showed no effect. However, the combination of rHIL-2 and rIFN- β showed a marked inhibitory effect on tumor growth (Fig. 1). On the 14th day after im-

Fig. 1. Inhibitory effect of rHIL-2 and/or rIFN- β on tumor growth (adenocarcinoma 755) in C57BL/6 mice. Arrows indicate the treatments of rHIL-2 and/or rIFN- β . Anti-asialo GM1 antibody was treated on days 5 and 9. Values represent the mean of 8 mice; the S.E.s of the mean were less than 35% of the mean values. Inhibition in combination groups on 12 through 22 days after transplant was significantly greater than that in rHIL-2 group ($P < 0.05$). —○—, untreated control; —△—, rHIL-2; —▽—, rIFN- β ; —△·—, rHIL-2+rIFN- β ; —□—, anti-asialo GM1 antibody; —■—, rHIL-2+rIFN- β +anti-asialo GM1 antibody.

plantation of tumor, the tumor volumes were 2202 ± 314 (S.E.), 1536 ± 235 (T/C = 68%), 2226 ± 190 (T/C = 101%), and $296 \pm 28 \text{ mm}^3$ (T/C = 13%) ($P < 0.001$, different from rHIL-2 alone) for untreated control, rHIL-2, rIFN- β , and the combination of rHIL-2 and rIFN- β , respectively. rHIL-2 and rIFN- β are both potent inducers of NK cell activity in mouse cytotoxic T-cell lines,^{(3), (4)} and synergistic induction of NK activity is obtained by the combination of IL-2 and IFN- β .⁽⁵⁾ Various recent reports suggest that NK cells play an important role in resistance against tumor development *in vivo*.^{(6), (7)} Anti-asialo GM1 antibody suppresses the NK activity.^{(2), (8)} However, anti-asialo GM1 antibody treatment did not influence the inhibitory effect of the combination of rHIL-2 and

rIFN- β on tumor growth. This suggests that inhibition of tumor growth is primarily mediated by a mechanism that is independent of augmentation of NK cells. Kuribayashi *et al.*⁹⁾ showed a spleen cell population exposed to IFN inducer had a markedly augmented ability to bind IL-2, suggesting that IFN causes an increased number and/or affinity of IL-2 receptors. To investigate the participation of T-cell in the potentiation of the inhibition of tumor growth, rHIL-2 and rIFN- β were injected s.c. into nude (nu/nu) mice with adenocarcinoma 755. The results show that the combination of rHIL-2 and rIFN- β had an additive, not synergistic, effect (Fig. 2) unlike that in C57BL/6 mice.

Fig. 2. Inhibitory effect of rHIL-2 and/or rIFN- β on tumor growth (adenocarcinoma 755) in nude mice. The S.E.s of the mean were less than 31% of the mean values. Inhibition in combination group on 11 through 15 days after transplant was significantly greater than that in rHIL-2 group ($P < 0.05$). —○—, untreated control; —△—, rHIL-2; —▽—, rIFN- β ; —▲—, rHIL-2 + rIFN- β .

Therefore, these results suggest that T-cell activation is important for the synergistic inhibitory effect of the combination of rHIL-2 and rIFN- β in C57BL/6 mice. This synergistic inhibition in normal mice and additive inhibition in nude mice of tumor growth by the combination of rHIL-2 and rIFN- β may offer a new possibility for the therapeutic application of these biological response modifiers.

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References

- 1) Rosenberg, S. A. *et al.* (1985): *Surgery*, **98**, 437-444.
- 2) Saijo, N. *et al.* (1984): *J. Cancer Res. Clin. Oncol.*, **107**, 157-163.
- 3) Kabelitz, D. *et al.* (1985): *Cell Immunol.*, **93**, 38-45.
- 4) Djeu, J. Y. *et al.* (1979): *J. Immunol.*, **122**, 175-181.
- 5) Brooks, C. G. *et al.* (1985): *ibid.*, **135**, 1145-1152.
- 6) Herberman, R. B., and Holden, H. T. (1978): *Adv. Cancer Res.*, **27**, 305-377.
- 7) Haller, O. *et al.* (1977): *Nature*, **270**, 609-611.
- 8) Habu, S. *et al.* (1981): *J. Immunol.*, **127**, 34-38.
- 9) Kuribayashi, K. *et al.* (1981): *ibid.*, **126**, 2321-2327.