PRIMING EFFECT OF INTERFERONS AND INTERLEUKIN 2 ON ENDOGENOUS PRODUCTION OF TUMOR NECROSIS FACTOR IN MICE

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The effects of interferons (IFNs) and interleukin 2 (IL 2) on endogenous production of tumor necrosis factor (TNF) were investigated in mice. Production of serum TNF was triggered by iv injection of OK-432 and tested by in vitro cytotoxicity assay. Injection of recombinant IFN-α with OK-432 or of IFN-β, recombinant IFN-β, recombinant IFN-α A/D or recombinant IL 2 six hours before OK-432 enhanced TNF production about 10-fold, which indicated priming actions of these compounds in TNF production. These findings suggest that these compounds could also be used as priming agents for endogenous production of TNF in cancer patients.

Key words: Interferons — Interleukin 2 — Tumor necrosis factor

Tumor necrosis factor (TNF),*4 an endogenous factor with tumor-selective cytotoxicity secreted by macrophages, was first found by Carswell et al.1) as a circulating factor with tumor necrosis activity that appears in the serum of BCG-sensitized mice after their treatment with lipopolysaccharide (LPS). So far, no compound is known that can be used in place of BCG or other Gram-positive bacteria for priming endogenous production of TNF in cancer patients.

Two steps are required for endogenous production of TNF: a priming step (e.g. BCG-sensitization) and a triggering step (e.g. LPS treatment). Previously, we reported that secondary immunological responses recollected by a corresponding antigen could act as a primer in BCG-sensitized mice2) and that lymphokines secreted by activated T lymphocytes may be implicated in the mechanism.

The present work was undertaken to ascertain whether lymphokines and cytokines, which may be endogenous biological response modifiers (BRMs), are capable of priming the endogenous production of TNF. Murine interferons of various types (i.e. natural IFN-α/β,3) recombinant IFN-β,4) and recombinant IFN-α A/D5); specific activities, 2.4 × 10⁶, 3.0 × 10¹, and 8.3 × 10⁷ U/mg, respectively) were supplied by Toray Industries Inc. (Tokyo). Recombinant human hybrid interferon, recombinant IFN-α A/D (Bgl),6) was supplied by Nippon Roche K.K. (Tokyo). Recombinant human IL 2 (5 × 10⁷ U/mg)7) was a gift from Ajinomoto Co. (Yokohama). Endogenous production of TNF was triggered by iv injection of OK-432, which was supplied by Chugai Pharmaceutical Co. (Tokyo). OK-432 has a triggering action when injected locally into human tumors8) and it triggers serum TNF production in the same way as LPS when injected iv.2,9) Male C3H/He mice of 7 weeks old or more from Shizuoka Experimental Animal Farm (Shizuoka) were primed with test samples and given an iv injection of OK-432 (3 KE per mouse). They were exsanguinated 2 hr later, and their serum was separated and stored at -80º until use. Serum TNF activity was measured by in vitro cytotoxicity...
assay with L-929 cells as a target. Units of activity were calculated as the dilution factor of the serum allowing survival of half the L-929 cells with a human recombinant TNF preparation donated by Asahi Chemical Ind. (Tokyo) as a standard.

As shown in Fig. 1, the TNF activity triggered by iv injection of OK-432 alone was 18 U/ml. After iv injection of recombinant IFN-\( \gamma \) (100 U/mouse) with OK-432, TNF production was significantly enhanced to 160 U/ml (\( P<0.001 \)). This priming effect was not observed with other types of interferon (IFN-\( \alpha/\beta \), recombinant IFN-\( \beta \) and recombinant IFN-\( \alpha \) A/D) (Fig. 1). However, when these interferons were injected iv 6 hr before OK-432 they showed priming effects (Fig. 2). In this case, TNF activity was 30 U/ml with OK-432 alone and 420 U/ml with OK-432 after pretreatment with IFN-\( \alpha/\beta \) or recombinant IFN-\( \beta \) (both 100 U per mouse), or recombinant IFN-\( \alpha \) A/D (20 U per mouse). In addition, iv pretreatment with recombinant IL 2 (100 U per mouse) had a priming effect, increasing TNF activity to 200 U/ml from 20 U/ml (Fig. 2). Treatment with recombinant IL 2 simultaneously with OK-432 had no effect on TNF triggering by OK-432 (data not shown). No detectable TNF activity (less than 1 U/ml) was observed in mice treated iv with interferons or recombinant IL 2 alone. In addition, although the in vitro synergistic cytotoxic action of IFN-\( \gamma \) and TNF was reported, the effect of interferons or IL 2 possibly remaining in the test serum may be negligible in this study, because addition of interferons or IL 2 to the serum of mice given an injection of OK-432 (3 KE per mouse) alone did not enhance the in vitro cytotoxic activity of the serum (data not shown).
This is the first report that interferons and IL 2 have priming activity for endogenous production of TNF when administered systemically to normal animals. The active molecule(s) presented in this study remains to be defined and compared with murine TNF characterized previously.\textsuperscript{12,13} IFN-\(\gamma\) differs from other types of interferon and IL 2 in having an immediate priming action. Thus, its mechanism of action may be different; that is it may act directly whereas other interferons and IL 2 act indirectly on TNF-producing cells, probably macrophages. Details of these studies will be described elsewhere.

The priming activities of the above endogenous BRMs indicate that these BRMs in combination with a suitable TNF-triggering agent such as OK-432 may have clinical applications in cancer patients. In fact, we have done a clinical trial; in a woman with metastatic endometrial stromal sarcoma of the lung, endogenous TNF production was induced by human recombinant IFN-\(\gamma\) and OK-432 treatment at 13 U/ml in serum 2 hr after treatment, resulting in subsequent reduction of metastatic lesions. The results of this clinical trial will be reported in detail elsewhere.

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