MODULATION OF NK ACTIVITY BY THYMIC HORMONES: IN VITRO EFFECTS OF THYMOSTIMULIN

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Plastic-adherent depleted or not depleted peripheral mononuclear blood cells (PMBC) from healthy donors showed enhanced lytic activity against ⁵¹Cr-labelled K562 target cells when exposed to thymostimulin (TS), 1 µg ml⁻¹, for 3 h, washed and incubated in TS-free medium before testing for natural killer (NK) cytotoxicity. No modification of NK cell activity was seen when effector cells were treated with placebo (splenic extract). The NK boosting activity of TS was lost when effector cells were treated for 3 h immediately before the performance of the cytotoxic test or when this thymic extract was added directly to the mixture of effector and target cells during the lytic phase of ⁵¹Cr release assay.

Key words: Thymostimulin, Natural killer, Thymic hormones.

INTRODUCTION

Several sets of experimental and clinical data have firmly established the critical role that the endocrine thymus plays in the normal phenotypic and functional development of T-cells. Furthermore, thymic hormones have been shown to exert an anti-tumor activity, in vivo, in animal models and to improve the course of viral disease in immunodeficient patients. However, it is by no means clear how these T-cell maturation processes are regulated and how the above mentioned in vivo activities are effected. Much research has recently been carried out on the modulation exerted by thymic hormones on lymphokine production and on natural killer (NK) cell function. Indeed, various thymic preparations have been shown to regulate the synthesis of lymphocyte inhibiting factor (LIF), migration inhibiting factor (MIF), interleukin-3 (IL-3), interleukin-2 (IL-2) and interferon-γ (IFN-γ). The effects of these last two molecules on the processes which lead to the phenotypic and functional maturation of 'precursors' into immunocompetent T-cells are well known, and may explain, at least in part, some of the differentiating effects of thymic hormones of which we are already aware. In addition, both IL-2 and IFN-γ modulate cytotoxic cell systems, including the NK cell system. An increased in vivo lytic activity may help to explain the antitumor effects which thymic hormones have been seen to exert in experimental models. It should, however, be noted that thymic hormones have also been shown to exert a modulating effect on NK cell activity which does not appear to be mediated by either IL-2 or IFN-γ. This therefore suggests that thymic hormones may regulate NK cell function by acting directly on these cells. In our present, ongoing study, we have therefore investigated the in vitro effect of thymostimulin (TS) on the NK cell function of healthy donors and the experimental conditions which affect the TS activity on this immune function.

MATERIALS AND METHODS

Reagents

Thymostimulin (TS) was kindly provided by the Serono Pharmaceutical Company, Rome, Italy. TS is a soluble extract of calf thymus originally isolated by Falchetti et al. The methods for extraction and partial purification have already been described elsewhere. A splenic preparation (SE), extracted and partially purified using the same methods employed for the TS preparation, was also kindly supplied by the Serono Company.
Heparinized venous blood (mixed 3:1 with phosphate-buffered saline, PBS) was separated by Ficoll-Hypaque density centrifugation. Mononuclear cells, recovered at the ring interface, were washed three times with PBS and then resuspended in RPMI 1640 medium (Grand Island Biological Co.) containing 24 mM Hepes buffer, 2 mM l-glutamine and gentamycin (50 mg ml⁻¹). This medium will henceforth be referred to as complete medium. Plastic-adherent cells were removed by incubating PMBC at a concentration of 15-20 × 10⁶ cells ml⁻¹ in complete medium supplemented with fetal calf serum, FCS, (30%) in 250 cm² plastic culture flasks for 30-45 min at 37°C in 5% CO₂ atmosphere. Non-adherent cells were removed by gently washing the plastic surface three times with warm complete medium, the cells were then washed three times in PBS and finally resuspended in complete medium.

Spontaneous cell-mediated cytotoxicity (SCMC)

The human myeloid cell line K562 was used to detect SCMC, and the test was performed as previously described. 37 In all, 2-5 × 10⁶ target cells were labelled by incubation with 100-200 μCi ⁵¹Cr as sodium chromate (Sorin, Saluggia, Italy) in a final volume of 0.5 ml complete medium supplemented with 10% FCS for 45 min in a water bath at 37°C. Labelled target cells were mixed in a 96-well round-bottom microplate with various concentrations of effector cells. Mixtures of effector and target cells were then incubated at 37°C for 4 h. The percent specific lysis was computed using the formula:

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%\ lysis = \frac{\text{test cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}} \times 100.
\]

Treatment of PMBC and K562 target cells with TS

TS treatment of effector (PMBC-depleted or not, plastic-adherent cells) and target (K562) cells, either singly or in combination, was always performed in FCS-free medium. Indeed, results of preliminary experiments have shown that the NK cell modulating activity of TS is completely absent when effector cells are treated in a medium containing FCS. This is probably due to the presence of proteases in the serum which degrade the TS to an inactive compound.

Statistical methods

The Scheffe test was used in each single experiment to analyse the differences in cytotoxicity levels observed in the groups treated with TS with respect to the nontreated group. This analysis was repeated for each E:T ratio. Each individual donor whose PMBC were cytotoxicity tested in vitro was considered either responsive or non-responsive to TS, depending on the statistical significance of the NK cell activity increase in E:T ratios: a significant increase in at least two out of three of the ratios resulted in the classification 'responsive'.

The Wilcoxon matched-pairs signed-ranks test was used to analyse the cumulative results for each set of experiments.

RESULTS

Effects of various concentrations of TS on NK activity of human PMBC, reproducibility of these effects and their relation to baseline NK cell levels

PMBC from 28 healthy donors were incubated for 3 h in the presence or absence of TS (0, 0.1, 1, 10 μg ml⁻¹) then washed and incubated for a further 12-15 h in TS-free complete medium containing 10% FCS, before testing against K562 target cells. This range of concentrations was chosen based on previous data which showed enhancement of E-rosetting and of phytohaemagglutinin (PHA)-induced blastogenesis by PMBC from active Hodgkin's disease patients. The results (Fig. 1) show that TS slightly but significantly enhanced the NK activity of normal PMBC in a dose-dependent manner. Of the overall 28 subjects tested, eight showed a significant increase in cytotoxic levels in all three or at least in two of the three effector to target cell (E:T) ratios assayed at the TS dose of 1 μg ml⁻¹. In addition, in 14% of the individuals tested, the NK activity of TS (1 μg ml⁻¹)-treated PMBC was statistically significantly higher than that of untreated PMBC at only one E:T ratio. Concordant though statistically non-significant increases in lytic levels were observed at the other two E:T ratios tested. No significant changes in NK cell activity were observed at this TS dose in the remaining 13 subjects tested.

In order to determine the TS effect of doses close to 1 μg ml⁻¹, a narrow range of concentrations (0.5, 0.75, 1 and 2 μg ml⁻¹) of this thymic preparation were assayed. In some instances, a significant increase in NK activity was observed over the whole range of TS doses covered, whilst in other cases a significant enhancement was obtained at only one of the doses tested (data not shown). Since the most