The effect of E-N-L-trimethyllysine (TML) on the humoral and cellular immune response

G. Elek, I. Lang, B. Szende and K. Lapis

First Institute of Pathology and Experimental Cancer Research and Second Department of Medicine, Semmelweis University Medical School, Budapest, Hungary

Abstract

E-N-L-Trimethyllysine glutamate (TML) influences the humoral and cellular immune response of mice. Chronic pre- and post-treatment (100 mg/kg/day, 5 times) transitionally increased the anti-SRBC haemagglutinin titre of female CBA mice. After 400 r whole body irradiation, TML treatment accelerated the normalization of the haemagglutinin level. TML treatment prolonged the life-span of BDF1 hybrid mice that had first been immunized and then inoculated with L1210 cells. TML diminished the delayed type hypersensitivity reaction in vivo of irradiated and non-irradiated CBA female mice and dose-dependently decreased the spontaneous (SLMC) and antibody-dependent (ADCC) cytotoxicity of healthy human lymphocytes, in vitro. As a low molecular weight immunomodulant, TML may also be considered as a therapeutic tool.

Introduction

E-N-L-Trimethyllysine (TML) is a cell proliferation promoting agent [1-3] and causes blast transformation of human lymphocytes in vitro [4, 5].

The present work has been carried out in order to investigate the possible immunomodulating effect of this compound.

Materials and methods

Chemicals. Endotoxin was prepared from E. coli by the method of Westphal [6]. A single dose of 25 μg/mouse was used.

Levamisole (Decaris, Gedeon Richter Chemical Works, Budapest, Hungary) was injected at 3 mg/kg body weight.

100 mg/kg body weight TML-glutamate (Gedeon Richter Chemical Works, Budapest, Hungary) was administered as a single dose.

L-Lysine was the product of Reanal (Budapest, Hungary).

Animals. Three-month-old inbred CBA females and BDF1, hybrid females were used.

Irradiation. 400 r whole body irradiation of mice was performed according to Frölen et al. [7].

Humoral immune responses were measured after intraperitoneal administration of 5 × 10⁶ sheep red blood cells (SRBC). Blood samples were taken from the orbital vein 3 times a week [8] and the haemagglutinin titre was determined [9]. The mercaptoethanol resistant antibody titre was also measured [10].

Each experimental group consisted of 10 animals.

The in vivo cellular immune response was measured according to Miller et al. [11]. As a sensitizing dose 10⁷ SRBC/mouse were injected into the right rear footpad of the mice [12]. 10⁸ SRBC were injected 3, 6, 9, 13 and 20 days later into the left rear footpad of 5-5 mice. The amount of footpad swelling was determined 24 and 48 h after the second injection.

Immunity against L1210 tumour was investigated according to the method of Spreafico et al. [13].

The in vitro cellular immune response was studied in antibody-dependent cellular cytotoxicity (ADCC) and spontaneous lymphocyte mediated cytotoxicity (SLMC) systems using human peripheral blood lymphocytes as effector cells. Lymphocytes were separated from the venous blood of 6 healthy volunteers by the method of Büyum [14]. Phagocytic cells were removed by carbonyl iron treatment. SLMC was assessed on ⁵¹Cr-labelled K-562 targets, while ADCC was simultaneously determined in a xenogeneic CRBC test system and in an allogeneic assay using rabbit anti-K-562 coated K-562 target cells. The effector:target cell ratio was 50:1 in the K-562 assays and 20:1 in the CRBC system. Incubation time was 4 h. Details of the cytotoxicity assays are described elsewhere [15].

TML was incubated together with target and effector cells during the whole incubation period in a concentration range of 200-800 μg/ml. In this concentration range TML was not toxic to target cells (unchanged spontaneous release) and did not influence the viability of effector cells either (trypan blue exclusion).

Statistical significance was evaluated by Student's "t"-test.

Results

1. The effect of pre- and post-treatment with TML on the humoral immune response

The titres of the E. coli endotoxin-treated group were the highest. The titres of the TML as well as the levamisole pre-treated groups showed a significant increase (p < 0.01) on the 5th-7th
days after the administration of the antigen (Fig. 1).

The titres of the animals repeatedly post-treated with TML did not reach the control values until the 8th day, but exceeded those on the 9th–17th days. Single TML post-treatment was ineffective. The mercaptoethanol-resistant titre showed alterations similar to the total titre (Fig. 2).

2. The effect of TML treatment on the restoration of the humoral immune response in irradiated mice

The titres of irradiated mice – both treated with TML or levamisole or untreated – were significantly lower than those of the non-irradiated controls between the 3rd and 13th days. However, on the 17th day, the titres of the TML as well as of the levamisole-treated and irradiated groups reached the normal control values and were significantly higher when compared with that of the irradiated but untreated animals. The alterations of the mercaptoethanol-resistant titres followed those of the total titre (Fig. 3).

3. The effect of repeated TML treatment on the cellular immune response of irradiated and normal mice

Irradiation caused a delay of 3 days in reaching the maximum footpad swelling, but by then the maximum value of the irradiated group exceeded that of the control group.

Irradiation and levamisole treatment on the 3rd day resulted in a significantly higher value than irradiation alone. The footpad swelling of