SHORT COMMUNICATION

In vitro Toxicity of T-2 Mycotoxin in Mouse Lymphoid Cells

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The *in vitro* toxicity of T-2 toxin towards mouse lymphoid cells prepared from spleen, thymus, peritoneal lavage and bone marrow cells was studied. Bone marrow cells were more resistant to damage by T-2 toxin than thymus, spleen and peritoneal cell preparations.

INTRODUCTION

Degenerative and necrotic alterations are produced in the lymphoid tissues of several animal species by trichothecene mycotoxins (DeNicola *et al.*, 1978). In general, restoration of normal histologic features occurs after a single dose (Sato *et al.*, 1975; Lutsky *et al.*, 1978). Mice fed a diet containing T-2 toxin suffered lymphocyte depletion from the thymic cortex. In addition, depletion of thymus-dependent lymphoid populations of the periarteriolar sheaths in the spleen and paracortical regions of lymph nodes occurred (Hayes *et al.*, 1980). Decreases in B-cell dependent areas in the intestinal lamina propria, medullary cords of lymph nodes and spleen cords were also observed. In contrast, the bone marrow became hypocellular but, even upon continued feeding of T-2 toxin, regeneration and development of cellular marrow occurred (Hayes *et al.*, 1980). This raises the possibility of the existence of populations of haematological and lymphoid cells with varying susceptibility to the toxic effects of T-2 toxin. In this paper, we report the existence of resistant cells in the bone marrow of mice following *in vitro* exposure to T-2 toxin.

METHODS

Cells and culture conditions. Cells were maintained for the duration of the experiment in Eagle's modified minimal essential medium with glutamine (EMEM; Flow Laboratories) containing 2 ml 1 M-HEPES buffer and 1 ml antibiotic solution (5000 units penicillin, 500 µg streptomycin) per 100 ml medium. The cells were cultured in 12 × 75 mm polystyrene disposable culture tubes in an incubator at 37 °C in a 5% CO₂ atmosphere.

Preparation of mouse cell suspensions. C-57 black male mice, 4–6 weeks old, were used for all experiments. They were killed by CO₂ inhalation and flooded with 70% ethanol before dissection. The spleen and thymus were removed, and washed with EMEM. Spleen cells, thymocytes, bone marrow cells and peritoneal cells were prepared as described by Shiigi *et al.* (1980). Spleen cells and thymocytes were then prepared by passing the organs through a stainless steel grid into 2 ml EMEM per organ. Peritoneal cells were prepared by injecting 5 ml EMEM into the peritoneal cavity and gently massaging the peritoneal cavity for 2–3 min. The mice were then killed by cervical dislocation and the medium was retrieved by aspiration with a 5 ml syringe and a 20-gauge needle. Bone marrow cells were prepared from the leg bones as follows. The legs were separated at the thigh joint, taking care not to damage the epiphyses of the long bones, and placed in EMEM. The bones were then cleansed of adherent muscle tissue from the femur. The clean femur was separated from the tibia, the epiphyses removed with scissors and the end of the bone punctured with a 20-gauge needle. By the use of a syringe with a 25-gauge needle attached,

Abbreviation: EMEM, Eagle's modified minimal essential medium.

Fig. 1. Percentage survival of mouse thymocytes (a), peritoneal lavage (b), bone marrow (c) and spleen cells (d). In each case, 1×10^7 cells ml^{-1} were exposed to $400 \mu\text{g}$ T-2 toxin and the percentage survival calculated at hourly intervals. The mean and standard error of not less than five separate experiments are shown: ●, control cells; ○, T-2 treated cells.

the marrow was expelled by pushing the EMEM through the centre of the bone. The bone marrow was drawn in and out of the needle and syringe to obtain a single-cell suspension. All cell suspensions were then adjusted to 2×10^7 cells ml^{-1} .

Viability assay. Mycotoxin T-2 (Sigma) was dissolved (2 mg ml^{-1}) in dimethylsulphoxide (DMSO). Toxin dissolved in DMSO was added to $500 \mu\text{l}$ cells adjusted to 2×10^7 cells ml^{-1} . The amount of DMSO added to cell cultures did not exceed $25 \mu\text{l}$. Appropriate controls were also set up and the viability of the cell suspension was assessed at hourly intervals by removing an aliquot and mixing it with an equal volume of 0.2% Eosin Y solution prepared with culture medium. A percentage viability count was determined by counting in a Neubauer haemocytometer; four areas containing a total of not less than 400 cells were counted in each instance. All statistical analyses were done using a statistical package for F test analyses and linear regression, available for the Tektronix 4054 (Tektronix Inc., Beaverton, Ore., USA).

RESULTS AND DISCUSSION

Trichothecene mycotoxin T-2 causes widespread degenerative and necrotic changes in lymphoid cells of the spleen, thymus, lymph nodes and bone marrow. In spite of this toxicity, there is regeneration and repopulation of lymphoid tissue even upon prolonged exposure to T-2 toxin in feed (Hayes *et al.*, 1980). This repopulation phenomenon indicates either the existence of some haemopoietic cell line resistant to T-2 toxin and therefore capable of repopulating the various lymphoid organs, or the slow adaptation of haemopoietic cells to the toxic effects of T-2 toxin. Fig. 1 illustrates the percentage survival of various lymphoid cells upon exposure to a standard dose of $400 \mu\text{g}$ T-2 toxin per 1×10^7 cells ml^{-1} . Thymocytes (Fig. 1a) and cells collected by peritoneal lavage (Fig. 1b) are exquisitely sensitive to the effects of T-2 toxin, showing virtually no survival after 5 to 6 h exposure. Spleen cells (Fig. 1d) show an intermediate

pattern of survival and, while less sensitive than thymocytes, still show a rapid decline in viability. Bone marrow cells (Fig. 1c), on the other hand, proved to be quite resistant to T-2 toxin, showing only a small but significant decline in viability. A large proportion of the heterogeneous population of bone marrow cells was resistant to T-2 toxin and, even if incubated for 24 h (data not included) in the presence of the toxin, showed a 40–50% survival. While this resistant population awaits identification, we suggest that it is these cells which allow the lymphoid organs to be repopulated even upon prolonged exposure to T-2 toxin. Lower doses of toxin (i.e. as little as $10\text{ }\mu\text{g ml}^{-1}$) showed the same pattern but the time required for killing was longer. At these low doses there was no difference in viability between control and toxin treated bone marrow cells.

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