T-2 Toxin-Induced Thymic Apoptosis Appears to Be Independent of Endogenous TNF- α and Glucocorticoid *in Vivo* in Mice

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Abstract

We investigated whether endogenous glucocorticoid and/or tumor necrosis factor- α (TNF- α) are involved in T-2 toxin (T-2)-induced apoptosis by using adrenalectomized mice and anti-TNF- α antibody-injected mice. The magnitude of DNA fragmentation found in T-2-treated-adrenalectomized mice was similar to sham-operated T-2-injected group. Agarose gel electrophoresis showed that thymocytes from mice treated with T-2 alone and those from mice treated with T-2 together with anti-TNF- α antibody exhibited a similar pattern of DNA fragmentation. LPS-induced DNA fragmentation was markedly inhibited by the anti-TNF- α antibody. Taken together, T-2-induced thymic apoptosis appears to be independent of endogenous TNF- α and glucocorticoid *in vivo* in mice.

Key words : T-2 toxin, thymocyte, apoptosis, tumor necrosis factor- α , glucocorticoid

Introduction

T-2 toxin (T-2) is a trichothecene mycotoxin principally produced by *Fusarium* species. T-2 has been detected in a great number of field crops, and causes several outbreaks of mycotoxicosis in human and animals.¹⁾ Although it is known that T-2 exhibits profound immunosuppressive effects, the operating mechanism of the toxicity remains unclear. Recently, we indicated that apoptosis appeared to be involved in the thymic atrophy induced by T-2.^{2,3)} We show here that T-2-induced thymic apoptosis appears to be independent of endogenous tumor necrosis factor- α (TNF- α) and glucocorticoid *in vivo* in mice.

Materials and Methods

T-2 was prepared and purified in the laboratory of Dr. Yoshizawa, Kagawa University. The purity of these mycotoxins was over 98% by gas-liquid chromatography. Cycloheximide (CHX) and dimethylsulfoxide (DMSO) were purchased from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Lipopolysaccharide (LPS, *E. coli* O111: B4) was purchased from Sigma Chemical Co. (St. Louis, MO). Rat monoclonal anti-mouse TNF- α antibody was purchased from Upstate Biotechnology Inc. (Lake Placid, NY). Female BALB/c mice and

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adrenalectomized female BALB/c mice of 4-weeks of age were purchased from Japan SLC Inc. (Hamamatsu, Japan). T-2 and DMSO (166 μ l/kg: vehicle control) were injected intraperitoneally (ip) into female BALB/c and adrenalectomized mice of 34 ± 2 days old. DNA fragmentation in the thymocytes and percentage of thymic apoptotic cells, hallmarks of apoptosis, were detected by agarose gel electrophoresis and diphenylamine colorimetric method, respectively.^{4,5)}

Results and Discussion

The magnitude of the DNA fragmentation found in T-2-treated-adrenalectomized mice was similar to sham-operated T-2 injected group (Fig. 1). Thus, it is suggested that T-2-induced thymic apoptosis occurs through a process which is independent of the glucocorticoid pathway. Next, we examined whether endogenous TNF is involved in the T-2-triggered apoptosis by administering a monoclonal antibody to TNF- α (3 mg/kg, ip) into animals along with T-2. As shown in Fig. 2, agarose gel electrophoresis showed that

Fig. 1 Effect of Adrenalectomy on Thymic Apoptosis by T-2.

[A]: Agarose gel electrophoretic patterns of individual murine thymic DNA isolated either from vehicle-treated adrenalectomized mice, or from adrenalectomized mice treated with T-2 toxin, or from vehicle-treated sham-operated mice, or from sham-operated mice treated with T-2 toxin. (+): T-2 toxin treated; (-): vehicle treated. Molecular size markers are shown on the left. [B]: DNA fragmentation of the thymus from mice used in [A] was quantitated by the diphenylamine method. Values shown are means $\pm$ SEM. Results are representative of three separate experiments.

Fig. 2 Effect of Anti-TNF- α Antibody on T-2-induced Thymic Apoptosis *in vivo*.

[A]: Agarose gel electrophoretic patterns of individual murine thymic DNA either from vehicle-treated mouse (lane 1), T-2 toxin-treated mice (lanes 2, 3, 4), mice injected ip with T-2 toxin along with 3 mg/kg of anti-TNF- α (tumor necrosis factor- α) antibody (ip, 5 min after T-2 toxin injection) (lanes 5, 6, 7), mice injected ip with 3 mg/kg LPS (lipopolysaccharide) alone (lanes 8, 9, 10), mice injected ip with LPS (3 mg/kg) along with 3 mg/kg anti-TNF- α antibody (ip, 5 min after LPS injection) (lanes 11, 12, 13), or mice injected ip with same amount of anti-TNF- α antibody alone as another control (lanes 14, 15, 16). [B]: DNA fragmentation of the thymus from mice in [A] was quantitated by the diphenylamine method. Values shown are means $\pm$ SEM. * $p < 0.01$ vs. LPS-injected group. Results are representative of three separate experiments.

thymocytes from mice treated with T-2 alone (lanes 2, 3, 4) and those from mice treated with T-2 together with anti-TNF- α antibody (lanes 5, 6, 7) exhibited a similar pattern of DNA fragmentation. Lipopolysaccharide (LPS, 3 mg/kg, ip; positive control)-induced DNA fragmentation (lanes 11, 12, 13) was inhibited by the anti-TNF- α antibody (lanes 14, 15, 16). Thus, the endogenous TNF does not seem to be involved in the pathway leading to the thymic apoptosis induced by T-2 *in vivo*. CHX inhibits apoptosis by impairing *de novo* protein synthesis.⁶⁾ To examine whether T-2 toxin-induced apoptosis was dependent on *de novo* protein synthesis, a group of mice was injected with CHX (15 mg/kg) 5 min after ip injection of T-2 toxin, and the apoptotic phenomenon in terms of the DNA fragmentation was quantitated. As shown in Fig. 3, T-2 toxin alone induced clear DNA fragmentation

Fig. 3 Effect of Cycloheximide on T-2 Toxin-induced Thymocyte Apoptosis *in vivo* at 6 hr. Groups of mice, three mice per group, were injected ip with vehicle (control), or CHX (cycloheximide, 15 mg/kg), or T-2 toxin (1.75 mg/kg), or T-2 toxin along with CHX (T-2+CHX). Percentage of DNA fragmentation in each thymus was assessed by the diphenylamine colorimetric method. Values shows are means $\pm$ SEM. * $p < 0.01$ vs. T-2 toxin-injected group.

which was significantly inhibited by CHX.

In this study, our significant finding is that neither endogenous glucocorticoid nor TNF- α are the causal factors of T-2 toxin-induced thymic apoptosis (Figs. 1 and 2). This was supported by *in vivo* experiments using adrenalectomized mice and anti-TNF- α antibody treatment. Our results are consistent with those of others,⁷⁾ in that calcium channel blocker-induced thymic apoptosis in rats was independent of endogenous glucocorticoids. By contrast, it was reported that both glucocorticoid and TNF- α were required in bacterial LPS-induced thymic apoptosis.^{8,9)} Furthermore, in sharp contrast to our presently reported T-2 toxin-activated apoptosis (Fig. 3), administration of anti-TNF- α antibody in mice completely blocked *E. coli*-induced thymocyte apoptosis.¹⁰⁾

The mechanism of thymic atrophy production by T-2 toxin exposure is poorly understood. Here, we found that a TNF- α -independent pathway of apoptosis may be involved in T-2 toxin induced thymic atrophy. We have also shown that the apoptotic process in the present system might be independent of glucocorticoid. Thus, further studies are needed to answer an question as to whether T-2 toxin exposure in our experimental system also targets lymphocyte progenitors in addition to double positive thymocytes.

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