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Kazem Ahmadi, Majid Riazipour

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SHORT PAPER

Effects of T-2 Toxin on Cytokine Production by Mice Peritoneal Macrophages and Lymph Node T-Cells

Kazem Ahmadi*, Majid Riazipour

Research Center of Molecular Biology, Baqiyatallah University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: T-2 toxin is a mycotoxin of type A trichothecenes produced by several fungal genera such as *Fusarium* species. Mycotoxins can affect both cell mediated and humoral immune compartments. **Objective:** The purpose of this study was to investigate the effect of T-2 toxin on cytokine production by mouse peritoneal macrophages and lymph node T cells. **Methods:** Mouse peritoneal macrophages and lymph node T cells were isolated and treated with different concentrations of T-2 toxin and incubated at 37°C and 5% CO₂ in air for 48 hours. Cell free media were removed and used for cytokine assay by an ELISA method. **Results:** T-2 toxin significantly reduced IL-1 β release in a concentration dependent manner ($p < 0.005$, $p < 0.001$). Interleukin-12 and TNF- α production were significantly increased in response to 0.001ng/ml, 0.01ng/ml and 0.1ng/ml of T-2 toxin ($p < 0.001$). However, T-2 toxin at higher concentrations ranging from 1ng/ml to 100ng/ml, reduced both IL-12 ($p < 0.001$) and TNF- α production ($p < 0.005$, $p < 0.05$). The effects of T-2 toxin on lymph node T cells showed that IL-4 and IL-10 release was decreased in a concentration dependent manner (all with $p < 0.01$). T-2 toxin at concentrations between 1ng/ml and 100ng/ml reduced the release of both IL-2 and IFN- γ ($p < 0.05$, $p < 0.001$). **Conclusion:** The results suggest that T-2 toxin at low concentrations can highly induce secretion of IL-12, TNF- α , IFN- γ and IL-2 and it may be used as a positive immunomodulator in the human model.

Keywords: T-2 toxin, IL-1 β , IL-12, TNF- α , IL-2, IFN- γ

INTRODUCTION

T-2 toxin a mycotoxins of type A trichothecenes produced by several fungal genera including *Fusarium* species and at some levels of exposure, these toxins typically act as

*Corresponding author: Dr. Kazem Ahmadi, Department of Immunology, Research Center of Molecular Biology, Baqiyatallah University of Medical Sciences, Tehran, Iran. Tel/ Fax: (+) 98 21 26127258, e-mail: kazahmadi@yahoo.com

immunosuppressive agents and can increase disease susceptibility. However, depending on the dose, timing and route of exposure, mycotoxins can also be immunostimulatory. They can affect both cell mediated and humoral immune compartments. They are extremely toxic to leukocytes and other rapidly dividing cells. Since these mycotoxins can be acquired via food or air, they have the potential to cause human and animal immune dysfunction and disease (1). It was also shown that, trichothecenes modulated kinetics of IL-2, IL-4, IL-6 production (1) and suppressed reovirus-induced IFN- γ elevation in bronchial alveolar lavage fluid, but enhanced production of IL-6 (2,3). Therefore, the purpose of this study was to elucidate the effect of T-2 toxin on cytokine profile from mouse peritoneal macrophages and lymph node T cells. Special attention is focused on Th₁ and Th₂ cytokine profiles and the possible relation of T-2 toxin to cytokine release by macrophages.

MATERIALS AND METHODS

Eight-week old BALB/c mice were anesthetized and peritoneal cells were extracted and plated out as the source of macrophages as previously described (4). To obtain a rich source of T cell, lymph nodes were removed and kept on ice at 2-8⁰C prior to washing at 4⁰C for three times (1500g for five minutes) with cold RPMI 1640. These cells were then passed through a syringe containing special sponge absorbing non-T cells. It was assumed that, by this method, from 75-90% of the achieved cells were T-cells (5), and contamination of T-cells by other cells in terms of IFN- γ , IL-2, IL-4, and IL-10 production was unlikely. The cells were then re-suspended in RPMI 1640 supplemented with 10% FCS (Fetal Calf Serum- Gibco Co., USA), 50 μ g/ml streptomycin and 50U/ml penicillin (Gibco Co., USA).

A sample of cell suspension was mixed with an equal volume of 0.4% (W/V) Trypan blue (Merck, Germany) in PBS and incubated for 10 minutes. The cells failing to exclude the dye were counted and expressed as the percentage of the total cells present (5). The peritoneal cells and lymph node T cells were finally plated out at 1x10⁶cells/well or 1x10⁴ cells/well in 24 or 96-well plates, respectively (Nunclon DELTA, Denmark) as previously described (4). Then the cells were incubated at 37⁰C in an atmosphere of 5% CO₂ in air for 48h (6) in RPMI 1640, containing 10% FCS, 50 μ g/ml streptomycin and 50U/ml penicillin. To the plates containing lymph node T cells, 2.5 μ g/ml concanavalin A was added in the presence (test group) or absence (control group) of 0.001 to 100ng/ml of T-2 toxin dissolved in ethanol (Sigma Co, USA).

The cell free media of both the above-mentioned cells were subsequently removed from each well, and centrifuged at 13000g for 10 minutes at room temperature. Finally, the supernatants were transferred to clean tubes and stored at -70⁰C until analysed for cytokines assay. All mouse cytokine ELISA kits were purchased from Bender Med System Company, USA.

Statistical Analysis. The data were expressed as the mean \pm S.E.M. An analysis of variance (ANOVA) was used to determinate the differences between the control and test wells.

RESULTS AND DISCUSSION

In this study, T-2 toxin reduced IL-1 β , IL-4 and IL-10 production in a dose dependent manner indicating its suppressive effect on macrophage and lymph node Th₂ cell function

(Table 1). It is of interest to note that, Jaradat et al. (7) in supporting our view, demonstrated that modulation of the immune system is one of the major effects of T-2 toxin in animals and humans. Thus, acute exposure of animals or humans to T-2 toxin results in severe damage to actively dividing cells in tissues such as bone marrow, lymph nodes, spleen, thymus and intestinal mucosa (8). T-2 toxin, depending on dosage, had different effects on cytokine production. At higher concentrations it reduced the release of IL-12, TNF- α , IFN- γ , and IL-2, but enhanced them at lower concentrations used. This is in agreement with an earlier study which reported that the exposure to T-2 toxin either enhanced or suppressed B and T lymphocyte mitogen proliferation in a dose dependent manner (9). Related to the above mentioned effect, Pestka et al. (10) demonstrated that low doses of T-2 toxin enhanced the production of IL-1, but at high concentrations, it caused suppression of phagocytosis and lymphocyte proliferation (11). Therefore, it is not surprising if T-2 toxin, depending on various doses, time of administration and cell origin could induce different effects on immune cell function (6, 12-14).

Table 1. Effect of different concentrations of T-2 Toxin on cytokine production by peritoneal macrophages and lymph node T-cells

T-2 Toxin $\mu\text{g/ml}$	IL-1 β Pg/ml	IL-12 Pg/ml	TNF- α Pg/ml	IFN- γ Pg/ml	IL-2 Pg/ml	IL-4 Pg/ml	IL-10 Pg/ml
Control	150	180	80	42	56	62	84
0.001	130***	215****	140****	54****	60ns	55**	80ns
0.01	110***	225****	152****	48*	60ns	53***	72ns
0.1	80****	237****	172****	46	58ns	45****	64****
1	30****	170****	72*	38*	52*	40****	60****
10	29****	160****	70***	28****	46****	32****	48****
100	30****	144****	68***	25****	32****	24****	34****

Level of significant: ****p<0.001, ***p<0.005, **p<0.01, *p<0.05

In contrast to our results in which T-2 toxin reduced IL-1 β production by peritoneal macrophages, Fu et al. reported a significant increase in IL-1 β and IL-6 in supernatants of chondrocytes cultured for 24h with T-2 toxin at 8ng/ml after phorbol ester PMA stimulation(6). These differences between our findings and those of Fu et al. (6), in term of cytokine production could be due to the source of the cells used.

As leukocytes are primary targets for trichothecene mycotoxins (15,16), demonstrating the effect of T-2 toxin on cytokine production by macrophages could help in understanding the mechanism by which mycotoxin compromises immune system. In addition, Zhou et al. (17) reported that trichothecene induces pro-inflammatory cytokine and chemokine expression in mononuclear phagocytes via a mechanism known as the ribotoxic stress response that involves activation of multiple intracellular signalling cascades (17). In contrast to our data in which IL-1 β decreased in response to T-2 toxin, Pestak and Zhou (18), showed that Deoxynivalenol toxin (the other member of trichothecene family) enhanced IL-1 β , IL-6 and TNF- α release by pre-exposed macrophages. Therefore, the differences in the results could be due to the fact that in our study T-2 toxin at concentrations ranging from 0.001ng/ml to 100ng/ml was added to the culture in the absence of LPS, while Pestka and Zhou (18) used murine RAW 264.7 macrophage cell line or peritoneal murine macrophages primed with a TLR4 agonist, LPS, at 250ng/ml. Therefore, the LPS pre-activated macrophages produced an increased amount of cytokines.

Based on our results and those of others (19-22) it can be concluded that T-2 toxin has immuno-modulatory effects on cytokine production and further investigations are recommended to elucidate the underlying molecular mechanisms.

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