Monoclonal Antibodies to T-2 Toxin

In Vitro Neutralization of Protein Synthesis Inhibition and Protection of Rats Against Lethal Toxemia

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Abstract

A murine monoclonal antibody (15H6) against the trichothecene mycotoxin T-2 was capable of neutralizing the in vitro protein synthesis inhibitory effect of T-2 toxin in human B lymphoblastoid cultures. It was further shown that 15H6 given to rats (250 mg/kg) 30 min before or 15 min after a lethal dose (1 mg/kg) of T-2 toxin conferred 100% survival. A lower dose of 15H6 (125 mg/kg), given 15 min after the lethal dose of T-2 toxin, protected 25% of the rats. An increased time to death and 45% survival was seen in rats given the full dose of 15H6 antibody 60 min after lethal toxin. These data are the first demonstration of effective prophylaxis and therapy for T-2 toxemia.

Introduction

The trichothecene mycotoxin T-2, a metabolite of fungi found on grain and grain products, causes severe intoxication of humans and livestock throughout the world (for review, see references 1 and 2). It has recently been suggested that T-2 toxin, as the major component of "yellow rain," may have been used as a biological-chemical warfare agent in Southeast Asia and Afghanistan (3, 4). T-2 toxin is a potent inhibitor of protein synthesis (5, 6) and causes a highly fatal disorder in man known as alimentary toxic aleukia, a syndrome that involves the gastrointestinal tract, hematopoietic system, and major autonomic functions (7, 8). Symptoms of acute intoxication include vomiting, diarrhea, multiple hemorrhages, necrotic angina, and bone marrow exhaustion. The mortality rate in T-2 intoxication has been estimated to be over 60%, due primarily to irreversible changes in the cardiorespiratory system (7). In experimental animals, cardiovascular responses to T-2 toxin administration include tachyarrhythmias, hypotension, and shock (9–13).

Although T-2 toxemia is a highly fatal disorder and has been associated with epidemic outbreaks in many countries around the world, no specific therapy has been established. Recently, a murine monoclonal antibody has been prepared that exhibits specificity for both T-2 toxin and its major metabolite, HT-2 toxin, but does not bind to other closely related trichothecenes (14). Previous studies have indicated that antibodies can neutralize in vitro toxic effects and provide in vivo protection against drugs (15–18) and certain low molecular weight toxins (19–23). We now report that monoclonal anti-T-2 antibody can neutralize the in vitro protein synthesis inhibition of T-2 toxin and protect rats against lethal T-2 toxemia.

Methods

In vitro studies. Protein synthesis inhibition was studied essentially as described earlier (14). USU-9-113-IIA7, a human B lymphoblastoid cell line isolated in our laboratory from a human spleen, was maintained in vitro in RPMI 1640 medium supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 1% nonessential amino acids, 50 μg/ml gentamicin, and 10% fetal bovine serum (HyClone Laboratories, Sterile Systems, Inc., Logan, UT). The affinity-purified mouse monoclonal anti-T-2 antibody (15H6)[1] used in this study was described earlier (14). The neutralizing effect of anti-T-2 was measured by preincubation of various concentrations of T-2 toxin with various concentrations of anti-T-2 antibody for 30 min at room temperature. This was followed by the addition of aliquots of the antibody-toxin mixtures to flat-bottomed microtiter plates containing 1.0×10^5 cells in 100 μl of leucine-free RPMI 1640 medium plus supplements. The cells were then pulsed with 0.5 μCi [^{3}H]leucine (40–60 Ci/mmol, Amersham Corp., Arlington Heights, IL). After 16–18-h incubation in a humidified 6% CO_2-94% air atmosphere at 37°C the cells were extracted with 5% trichloroacetic acid, harvested on glass fibers, and counted in aqueous scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA).

In vivo studies. Male Sprague-Dawley rats (270–300 g) were anesthetized with halothane (2% in oxygen) and both femoral arteries cannulated with PE-50 tubing filled with heparinized 0.9% NaCl (sterile, pyrogen free, 100 U/ml). The tubing was then tunneled under the skin of the back to emerge at the back of the neck where both lines were secured by a spring wire (24). The rats were then allowed to recover from surgery over 24–36 h in their home cages with food and water ad lib.

Ascites fluid that contained 15H6 or an unrelated monoclonal antibody of the same isotype (IgG1) was precipitated with 50% saturated $(NH_4)_2SO_4$ and the precipitate dialyzed into phosphate-buffered saline, pH 7.4. The concentration of anti-T-2 antibody was determined by enzyme immunoassay using affinity-purified 15H6 as standard. The total protein content was measured by the Lowry et al. (25) method, and the control antibodies were adjusted to the same total protein concentration as the anti-T-2 antibodies. Systemic injections of 15H6 or control antibodies were given through an arterial cannula in volumes of 1 ml/100 g body weight or less over a 15-min period. T-2 toxin (Sigma Chemical Co., St. Louis, MO) was also administered through the arterial cannula as a 10% ethanol/normal saline solution (1 ml) at a dosage previously calculated to be close to the lethal dose (LD_{100}) (1 mg/kg, 15). To document that the animals were toxemic, several physiologic parameters that change after T-2 toxin administration were followed in each experiment (13).

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1. *Abbreviations used in this paper:* 15H6, mouse monoclonal anti-T-2 antibody, LD, lethal dose.

One arterial line was connected to a pressure transducer (RP 1500i, Narco Bio-Systems, a Healthdyne Co., Houston, TX) for blood pressure (mean and pulse pressure) and heart rate recording (Narco-Trace 80 computerized dynograph); the second femoral cannula served for systemic injection. These hemodynamic responses were recorded up to 6 h after T-2 administration and at 24 h in the surviving rats.

Statistical analysis. Data in text and figures are mean ± standard error for the indicated number of rats in each group. Survival ratio was evaluated by the Fisher exact probability test; hemodynamic responses were examined by analysis of variance with repeated measured design (26). Differences were presumed significant at $P < 0.05$.

Results

Effect of anti-T-2 antibody on in vitro protein synthesis inhibition by T-2 toxin. To assess whether anti-T-2 could neutralize the toxic effect of T-2 toxin in vitro, various concentrations of toxin were incubated with various concentrations of antibody for 30 min at room temperature, then the mixtures assayed for inhibition of protein synthesis by the standard method. The data shown in Fig. 1 demonstrate a dose-responsive neutralizing effect of monoclonal anti-T-2 antibody. Although a threefold molar excess of anti-T-2 toxin was required to completely neutralize a 100% inhibitory dose of T-2 toxin in vitro (6.7×10^{-8} M antibody; 2.1×10^{-8} M T-2), significant neutralization was seen with equimolar concentrations. The failure of an isotype-matched control antibody to neutralize the inhibitory effect on protein synthesis suggests that the effect was due to a true antigen-antibody interaction. Furthermore, the 15H6 antibody failed to neutralize the protein synthesis inhibitory effects of two related trichothecenes, diacetoxyscirpenol and monoacetoxyscirpenol (Fig. 1 *B*), a finding consistent with the known binding specificity of this antibody (14).

Effect of anti-T-2 antibody on rat survival. Injection of 1 mg/kg T-2 toxin, either alone, or with a control immunoglobulin preparation that contained no antibodies against T-2 toxin, resulted in high (90–100%) mortality as previously observed (13). When rats were pretreated with 15H6 antibody 30 min before T-2 administration, none of the rats died throughout the experimental period (Fig. 2). However, no differences were observed between the antibody-treated and untreated rats with regard to their blood pressure and heart rate responses to T-2 toxin; heart rate of both groups was significantly elevated as previously shown, while arterial pressure (elevated somewhat by the plasma injection) showed the same course of return to baseline levels (Fig. 3). When two different doses of the 15H6 antibodies were administered 15 min after T-2 toxin, the higher dose (250 mg/kg) provided 100% protection, while none of the control rats survived 24 h (Fig. 4); the lower dose (125 mg/kg) also provided substantial protection, although the survival rate was significantly lower than the full dose of the antibody (25%). Injection of the full dose of the antibody 15 min after T-2 toxin did not prevent the increase in heart rate, although significant attenuation of the tachycardia was seen at 2 h after T-2 toxin injection (data not shown). It is also noteworthy that in the surviving rats where hemodynamic recordings were obtained ($n = 4$) the mean arterial pressure was 99±7 mmHg, pulse pressure was 47±13 mmHg, and heart rate was 431±30 beats/min; these physiologic variables indicate a normal cardiovascular status at this time.

Injection of the anti-T-2 toxin antibodies (250 mg/kg) as late as 60 min after T-2 toxin administration (Fig. 5) also provided significant protection; 50% survival in the control and antibody-treated rats were 5 and 17 h, respectively (over threefold increased survival time). Whereas 45% of the 15H6-treated rats survived, none of the control rats in this study survived beyond 12 h. Injection of the 15H6 antibodies 60 min after T-2 toxin administration attenuated the blood pressure response at 1 h after the treatment, but other hemodynamic indices were not significantly affected by the antibody administration (data not shown). In three antibody-treated rats where adequate recordings were obtained at 24 h, all the cardiovascular variables were normal (MAP = 114±8, pulse pressure = 47±10 mmHg, and heart rate = 394±25 beats/min).

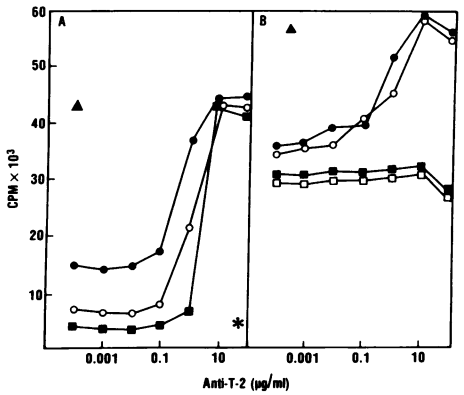

Figure 1. Protective effect of anti-T-2 antibody in vitro. (*A*) Neutralization of the protein synthesis inhibitory effect of T-2 toxin on human B lymphoblastoid cells in vitro by anti-T-2 antibody. The levels of T-2 toxin were: 2.5 ng/ml (●); 5.0 ng/ml (○); 10 ng/ml (■). Each point is the mean of six replicates from one representative experiment. The symbol (▲) indicates protein synthesis in the presence of 15H6, but without T-2 toxin. The effect of 100 μg/ml of an isotype-matched monoclonal antibody against an unrelated hapten is indicated by the symbol (•). (*B*) Specificity of T-2 toxin neutralization by 15H6 antibody. The following compounds were tested: T-2, 2 ng/ml (●); HT-2, 7.5 ng/ml (○); diacetoxyscirpenol, 3.5 ng/ml (■); and monoacetoxyscirpenol, 9.8 ng/ml (□). Each point is the mean of three replicates from a representative experiment. The symbol (▲) indicates protein synthesis in the presence of 15H6, but without T-2 toxin.

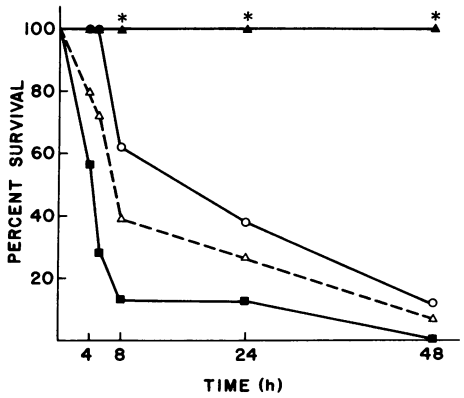

Figure 2. Effect of monoclonal anti-T-2 antibody pretreatment on survival of rats treated with T-2 toxin. 30 min before the intravenous infusion of a lethal dose of T-2 toxin (1 mg/kg), rats were treated with 15H6 monoclonal antibody (▲, $n = 7$), the same amount of an isotype-matched control monoclonal antibody (■, $n = 7$), or normal saline (○, $n = 8$). Asterisks denote significant survival ($P < 0.05$, Fisher Exact Probability Test) of 15H6 monoclonal antibody-treated rats vs. the combined control rats (△, $n = 15$).

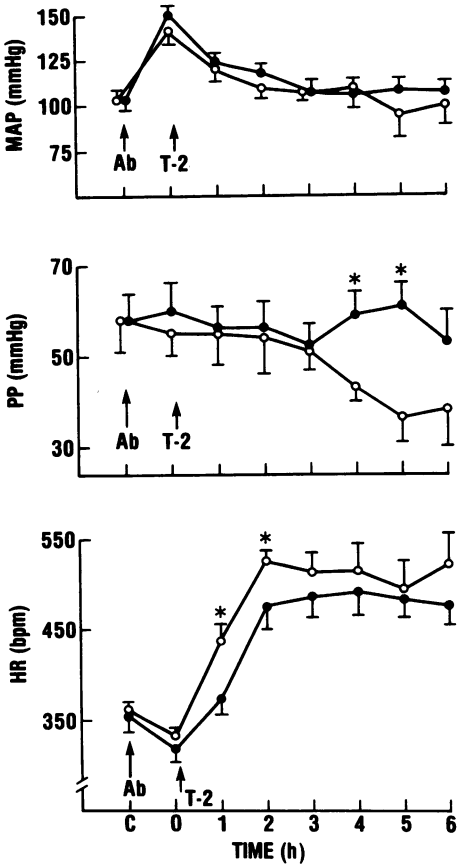

Figure 3. Selected cardiovascular parameters in rats given 15H6 (●, n = 7) or control (○, n = 7) monoclonal antibody at 30 min before T-2 toxin. MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate (beats per minute). Asterisks denote statistical significance at the $P < 0.05$ level.

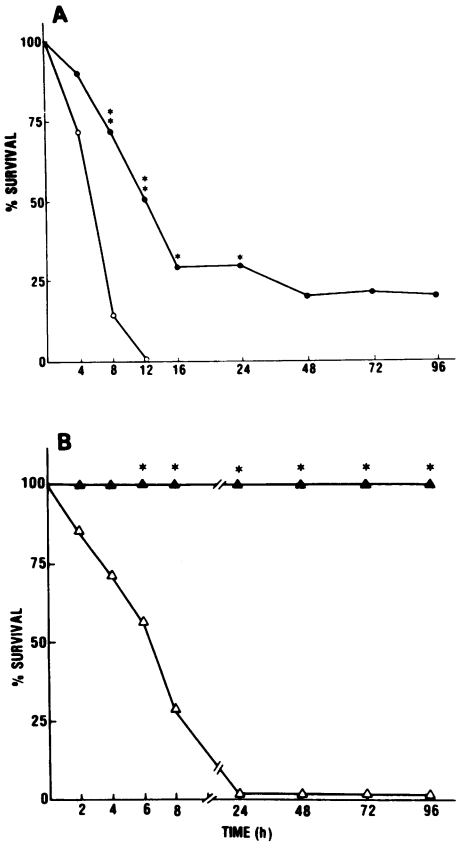

Figure 4. Effect of monoclonal anti-T-2 antibody therapy on survival of rats given T-2 toxin. (*A*) Groups of rats treated with 125 mg/kg 15H6 monoclonal antibody (●, n = 10) or an equal concentration of control monoclonal antibody (○, n = 14) 15 min after T-2 toxin (1 mg/kg) administration. (*B*) The same type of experiment with different groups of rats given 250 mg/kg of 15H6 (▲, n = 6) or control (△, n = 7) antibody. Asterisks denote statistical significance.

Discussion

No prophylaxis or therapy is currently available for lethal T-2 intoxication. In this study we have demonstrated for the first time that protein synthesis inhibition, the molecular mechanism of T-2 toxicity (5, 6), can be neutralized in vitro by a T-2 toxin-specific murine monoclonal antibody, and that this same antibody can be used in vivo for prophylaxis and therapy of rats exposed to a lethal dose of T-2 toxin.

Using a rapidly proliferating human B lymphoblastoid cell line as indicator, the anti-T-2 monoclonal antibody T-2 neutralized the protein synthesis inhibition of T-2 toxin in vitro in a specific and dose-dependent manner. It is difficult to correlate the molecular mode of action of T-2 toxin with observed physiologic changes and morbidity seen in animals and humans. It appears that inhibition of protein synthesis is nonselective at the tissue level, although the radiomimetic effect of T-2 toxin suggests that tissues with rapidly dividing cells are most susceptible (27). Rats exposed to lethal doses of T-2 toxin show cardiovascular changes consistent with the development of shock (9–13). Though we demonstrated a dose-dependent salutary effect of the 15H6 antibodies in poisoned rats, the cardiovascular changes observed in control rats were not significantly reversed in the antibody-treated rats during the first 6 h after exposure. It is possible that antibody alone, or complexes of antibody and T-2 toxin, might have been responsible for the persistent cardiovascular effects. However, we have previously observed car-

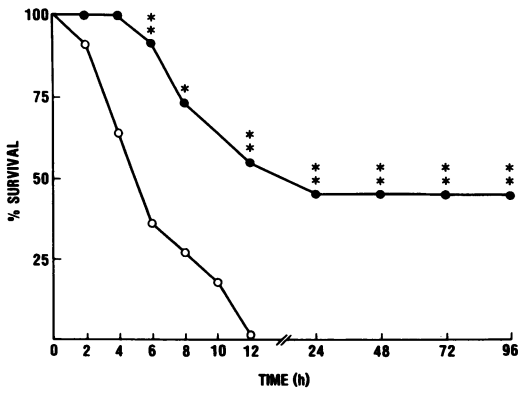

Figure 5. Effect of monoclonal anti-T-2 antibody therapy on survival of rats given T-2 toxin. Rats were treated with 250 mg/kg of 15H6 (●, n = 11) or control (○, n = 11) monoclonal antibody at 60 min after the infusion of 1 mg/kg T-2 toxin. Asterisks denote significant differences between groups (*$P < 0.05$; **$P < 0.01$).

diovascular changes in rats infused with T-2 toxin alone that closely parallel the changes seen in antibody plus toxin-treated rats (13). In the surviving rats, the cardiovascular changes had returned to baseline levels at 24 h. A more detailed analysis of the cardiovascular parameters will be required to determine the point where improvement occurs in 15H6 antibody-treated rats.

The dose of anti-T-2 antibody (250 mg/kg) that conferred complete protection when administered 30 min before or 15 min after an LD_{100} dose of T-2 toxin (1 mg/kg) was an amount approximately equimolar to the amount of toxin. It is interesting to note that this ratio is similar to that required for neutralization in vitro. It is possible that the anti-T-2 antibody establishes a concentration gradient that sequesters T-2 (or HT-2) toxin in the plasma where it can be conjugated for excretion. It was also interesting to note that the 15H6 antibody administered 60 min after T-2 toxin was protective. By 60 min the rats were prostrate and obviously suffering from the acute effects of T-2 intoxication, yet some of the animals were rescued with the antibody. This finding is consistent with the ability to repair protein synthesis in vitro by removing the T-2 toxin (unpublished results) and similar in effect, if not mechanism, to antidigoxin antibody therapy (15, 17) and antibody-mediated reversal of ouabain toxicity (28).

The levels of anti-T-2 that conferred protection were well above those attainable through active immunization. However, the 250 mg/kg passively administered to the rats in this study represents a physiologically acceptable dosage; 500 mg/kg of human immunoglobulin can be given to humans with no untoward effects (29). The size difference between antibody and low molecular weight toxins like T-2 requires that large amounts of antibody be administered to achieve equimolar concentrations, even though IgG antibodies are divalent. Indeed, antibodies may even be more effective against an LD_{100} dosage of a very potent toxin than against a less potent toxin since higher molar ratios of antibody to toxin can be achieved. The protein burden could be reduced somewhat by employing Fab fragments of the 15H6 antibody, and these fragments may also have superior pharmacokinetics as suggested by studies with other low molecular weight toxins and drugs (30).

Mouse monoclonal antibodies have been suggested for prophylaxis and therapy in humans (31), and early experimental evidence suggests that murine immunoglobulins, unlike most heterologous immunoglobulins, are very well tolerated in humans (32). A higher affinity anti-T-2 antibody would probably provide better therapeutic efficacy, and human monoclonal anti-T-2 antibodies should be considered as well.

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