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# Effects of T-2 mycotoxin ingestion on selected resistance mechanisms and on experimentally induced aspergillosis in rabbits

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# Effects of T-2 mycotoxin ingestion on selected resistance mechanisms and on experimentally induced aspergillosis in rabbits

by

Kayleen Ann Niyo

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# GENERAL INTRODUCTION

T-2 mycotoxin is a trichothecene secondary metabolite produced primarily by Fusarium species growing on cereal grains in the temperate climatic zones of North America, Europe, and Asia,[1,2] and is associated with a wide variety of human and animal intoxications.[3] The known biological effects include severe skin irritation, vomiting, diarrhea, feed refusal, inhibition of DNA, RNA, and protein synthesis, and damage to hematopoietic and lymphoid cells.[4] The documented results of this mycotoxicosis have been reduced growth rates, immunosuppression, and deaths of domestic animals[5,6] and poultry,[7] as well as immunosuppression and deaths of humans.[1,8,9] Using improved methods of chemical detection, T-2 toxin has been demonstrated to occur in foods and feeds and ingestion of these materials by man or other animals could ultimately lower resistance to disease.[10]

Aspergillosis is a disease of avian species, domestic animals, and humans and is caused primarily by Aspergillus fumigatus Fresenius.[11] Although the disease is most common in avian species with severe annual economic losses to the poultry industry in the United States, pulmonary aspergillosis and mycotic abortion in cattle and guttural pouch mycosis in horses are important forms of aspergillosis in domestic animals.[12,13] In humans, aspergillosis is manifested in three ways: allergic, noninvasive, and invasive.[11] In the immunocompromised individual, opportunistic A. fumigatus is able to colonize the lungs, invade blood vasculature, cause infarcts and be disseminated throughout the body.

The widespread occurrence of T-2 toxin-producing fungi in foods and feed and the ubiquitous and pathogenic nature of A. fumigatus, suggest that harmful interactions may occur in animals, humans, and poultry. This study was undertaken to test T-2 toxin in vitro and in vivo for effects on the phagocytic and cell-mediated immune response to A. fumigatus and on other physical parameters in the rabbit. Natural routes of exposure, i.e., oral T-2 dosages and aerosolization of A. fumigatus conidia, were utilized.

This dissertation consists of two manuscripts to be submitted to The American Journal of Veterinary Research. A general summary and discussion follows the second manuscript. Literature cited in the dissertation introduction, literature review, general summary, and discussion appear at the end of the dissertation.

The Ph.D. candidate, Kayleen Ann Niyo, was the principal investigator for each study.

# LITERATURE REVIEW

Trichothecene mycotoxins The trichothecenes are a chemically related group of fungal secondary metabolites comprising the largest group of mycotoxins.[4,14] The name trichothecene[15] was based on the origin of the first compound isolated, trichothecin, identified as a result of screening cultures of Trichothecium roseum for metabolites with antifungal activity.[16,17] More than 68 naturally occurring trichothecene derivatives have been isolated and characterized as a result of the quest for antifungal and antileukemic agents, antibiotics, cytotoxins, phytotoxins, and animal toxins.[18]

All of the fungi known to produce trichothecenes are members of the Fungi Imperfecti, with teleomorphic stages, when known, belonging to the Class Ascomycetes, Pyrenomycetes, Orders Hypocreales or Xylariales.[19] This suggests a close natural taxonomic relationship of these fungi that produce trichothecene mycotoxins. These toxic metabolites are produced by 8 of the 9 species of Fusarium recognized by Snyder and Hansen.[20] These species are plant pathogens that infect primarily cereal grains in the temperate climatic zones of North America, Europe, and Asia[1,2] and are associated with animal and human intoxications throughout the world. Trichothecenes have been isolated from species of Trichothecium, Trichoderma, Myrothecium, Stachybotrys, Cephalosporium, Cylindrocarpon, Verticimonosporium, and Calonectria (teleomorph of F. nivale and F. rigidiusculum).[18]

Fungal biosynthesis of the trichothecenes begins with mevalonate, derived from three molecules of acetyl coenzyme A. Mevalonate is

metabolized by the usual pathway of lipid biosynthesis to isopentenyl-, geranyl-, and farnesyl-pyrophosphates.[19] The latter intermediates are then converted into the basic trichothecene molecule. The resulting family of secondary metabolites (trichothecenes) are known generally as sesquiterpenes and are characterized by the tetracyclic 12,13-epoxy-trichothec-9-ene nucleus (scirpene).

Natural trichothecenes are neutral lipid-like materials that are soluble in polar organic solvents.[19] They are chemically stable to moderate variations in temperature, exposure to light, air, and pH, although they are hydrolyzed by strong alkalis and rearranged by strong acids.

Naturally occurring trichothecenes are divided into four groups (A, B, C, or D) according to their structural features.[18] Important members of Group A include: T-2 toxin, HT-2 toxin, diacetoxyscirpenol, and neosolaniol; Group B: nivalenol, deoxynivalenol, trichothecin; Group C: verrucarin A, roridin A; and Group D: crotocin.

T-2 mycotoxin T-2 mycotoxin [3α-hydroxy-4β,15-diacetoxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene] is the best known and most widely studied toxin of the trichothecenes.[21] The known producers of T-2 toxin and its related compounds are Fusarium sporotrichioides Sherb. [F. tricinctum (Corda) Sacc.], F. poae (Peck) Wollenw., F. semitectum Berk. and Rav., F. sambucinum Fuckel, F. equiseti (Corda) Sacc. sensu Gordon, while possible producers are F. graminearum Schwabe, F. oxysporum Schlecht. emend. Snyd. and Hans., F. lateritium Nees, F. acuminatum Ell. and Ev. sensu Gordon, F. culmorum

(W. G. Smith) Sacc., and F. moniliforme Sheldon.[22] Natural production of the toxin by the fungus tends to occur in grain overwintered in the field and in high-moisture grains that have been improperly stored following a wet, cold harvest season. These grains are a hazard to humans and animals when ingested.

Analytic detection of T-2 in foodstuffs, tissues, and fluids has been reviewed recently.[10,18] Of the biological assays, the skin test is the most simple and reliable.[23] Physical methods of analysis include thin-layer chromatography (detection limit of 100 ng), reverse-phase high-performance liquid chromatography (detection limit of 1 μg), gas chromatography (detection limit of 10 ng), and gas chromatographic mass spectrometric analysis (sensitivity of 5 ppb).[18] Immunochemical methods of analysis have recently been developed for T-2 toxin. An antibody (Ab) to the toxin[24] has been used in a radioimmunoassay (RIA) for quantification of T-2 toxin in milk and urine[25] and in corn and wheat[26] (sensitive to 0.5 to 2.5 ppb). Antibody has also been used to detect T-2 in an enzyme-linked immunosorbent assay (ELISA) (detection limit of 2.5 pg).[27] However, the specificity of the Ab precludes their use for detecting the many unconjugated and glucuronide conjugated metabolites of T-2 toxin. Diagnosis of intoxications caused by T-2 toxin may be complicated by the fact that often multiple toxins are present in contaminated foodstuffs.

Historically, there have been several major disease outbreaks of fungal origin that are suspected to be T-2 or trichothecene-related. In 1891, Woronin described a "staggering grain toxicosis" that occurred

in the Ussuri district in eastern Siberia.[28] This syndrome was manifested by nausea, vomiting, vertigo, and visual symptoms in humans who consumed toxic millet and barley infected with F. roseum and caused feed refusal in farm animals.

Alimentary toxic aleukia (ATA) was a serious human intoxication that occurred in 1942-47 when over 10% of the population in the Orenburg district near Siberia were killed by consuming field overwintered toxic millet, barley, and wheat.[8,9] Joffe, in retrospective studies,[1] concluded that the fatalities were due to ingestion of T-2 toxin produced in these overwintered grains by F. sporotrichioides and F. poae.

Bean-hull poisoning of horses in Hokkaido, Japan, caused 10-15% fatalities within 2-3 days after ingestion and the bean hulls were found to be contaminated by F. sporotrichioides, the major T-2 toxin producer.[3,22] In Japan, Akakabi-byo (red mold disease or scab) of wheat, barley, oats, rye, and rice that may affect greater than one-third of the national production, is probably caused by several mycotoxins, including T-2 toxin.[22]

In 1972, Hsu et al.[5] were the first to identify T-2 toxin in extracts of moldy corn that caused death of 5 of 30 dairy cattle in Wisconsin. Petrie et al.[6] identified T-2 as the cause of a similar hemorrhagic syndrome in dairy cattle in Britain. Fusariotoxicosis in poultry is also attributable to T-2 toxin.[29]

More recently, allegations have been made that T-2 toxin was used as a chemical warfare agent in southeast Asia.[30,31] Others attributed

the "yellow rain" to pollen or bee feces.[32] Reports have confirmed the presence of T-2 toxin and polyethylene glycol, probably used as an emulsifier, in the yellow rain samples.[31] Samples of blood, urine, and feces of Iranian soldiers subjected to a gas attack during the Iran-Iraq War contained detectable T-2 toxin, as well as other trichothecenes.[33]

Biochemically, the mechanism of action for T-2 toxin is not fully understood. The toxin is thought to be an amphipathic molecule and to interact initially with the outer phospholipid bilayer of the cell.[34-36] The possible binding of T-2 to protein receptors on the cell membrane may interfere with signal transfer and thus with DNA, RNA, and protein synthesis.[37-39] T-2 is characterized by its ability to inhibit protein chain initiation in intact ribosomes.[18,40] Both resting and mitogen-stimulated murine lymphocytes were affected by T-2, with resting cells requiring a longer period to demonstrate the effect than actively dividing cells.[37] At greater dosages, T-2 may lyse cell membranes with <5.5 A lesions;[41] lysis may result by formation of free radicals.[42] Most studies have shown that proliferative cells[37] and cells containing many free polysomes[43] (hematopoietic, lymphoid, intestinal crypt, and bursa of Fabricius) are more susceptible to T-2 toxin[38,43-49] than are parenchymal tissue cells (liver and kidney) that have no proliferative undifferentiated cells and few free polysomes.

The effects of T-2 toxin on mitochondrial respiration are inconsistent in <u>in vitro</u> studies. Inhibition of oxygen consumption at sites

I and III were noted in two studies using high concentrations of T-2 toxin.[50,51]

Studies using radiolabeled T-2 toxin indicated that the toxin is rapidly metabolized by the liver and eliminated into the intestinal tract through the biliary excretion system primarily as glucuronide-metabolite conjugates.[45,52-54] Prior to the present two studies reported herein, pathological changes had not been seen in the liver.[46]

Results from studies on the mutagenic and carcinogenic activity of T-2 toxin are contradictory.[18] Evidence of single strand breaks in DNA,[55] lymphocyte and fibroblast chromosomal structural aberrations,[56] and tumors[57] suggest the need for further study in this area. Teratogenic effects have been known to occur, and T-2 readily crosses the placenta.[58]

The major effect of T-2 toxin is on hematopoietic and lymphoid cells with a resultant immunosuppressive effect. In vitro studies have demonstrated that T-2 toxin decreases chemotactic migration of neutrophils[52,59] and phagocytosis by alveolar macrophages (AM),[60-62] increases skin graft rejection time,[63] inhibits mitogen-induced blastogenesis of human lymphocytes without mutagenic activity,[59,64,65] inhibits platelet function,[66] and is cytotoxic to lymphocytes.[38,46,48,49,67-70]

Several in vivo studies with T-2 toxin have produced variable results depending on the species of experimental animal, and the route, amount, and duration of toxin administration.[44,45,49,59,67,71-73] A few investigators have examined the in vivo chronic effects of T-2

toxin on cell mediated resistance to an infectious disease.[74] T-2 toxin decreased resistance to mycobacterial infection in mice,[75] increased mortality in chickens challenged with Salmonella spp.,[76] increased susceptibility to herpes simplex virus in mice,[77] and increased mortality in mice due to listeriosis.[47,78] Few researchers have investigated the interaction of mycotoxins with mycotic disease.

Aspergillosis The genus name Aspergillus was first used by Micheli in his "Nova Plantarum Genera" of 1729 to denote 9 fungal species.[11] Presently, about 600 species have been described in the genus Aspergillus.[11] They are among the most common fungi of many environments throughout the world. However, only about 8 species are consistently involved in infectious disease, with A. fumigatus being the most common pathogen.[11,79]

Aspergillus fumigatus Fres., is a member of the Fungi Imperfecti, Form Order Moniliales, and Form Family Moniliaceae. The teleomorphic state of A. fumigatus is not known, but the closely related species A. fischeri has been placed in the Class Ascomycetes, Subclass Plectomycetidae, Order Eurotiales, Family Eurotiaceae, and Genus Sartorya as S. fischeri.[80]

Aspergillus fumigatus grows rapidly on Sabouraud dextrose agar incubated at 30°C and can be identified on the basis of its colony characteristics and microscopic morphology.[80,81] It is thermotolerant and grows at temperatures >45°C.

In 1815, Mayer and Emmert first described a fungal infection in the lungs of a jay (Corvus glandarius).[11] Fresenius introduced the

term "aspergillosis" in describing a fungal infection in the air sac of a bustard (Otis tardaga) from the Frankfort Zoo. He named the fungus isolate Aspergillus fumigatus.[11] Human Aspergillus pneumomycosis was first described by Sluyter in 1847.[11] The classic pathology paper by Virchow in 1856 accurately described A. fumigatus as the etiologic agent in human aspergillosis.[11]

Aspergillosis is usually manifested as a pulmonary disease and is recognized as having three categories: (1) allergic aspergillosis, (2) noninvasive aspergillosis, and (3) invasive aspergillosis.[11]

Allergic aspergillosis has been defined recently as having two forms and its frequency is established.[11] The first form occurs in atopic individuals manifested as either asthma, a Type I hypersensitivity, or as allergic bronchopulmonary aspergillosis (ABA), a Type I and III (Arthus) hypersensitivity. Asthma involves only the airways, possesses an eosinophilia, and is IgE mediated. There is no precipitating Ab (IgG) produced. ABA causes an Arthus reaction in the endothelium and IgG is present in the serum. The second form of allergic aspergillosis occurs in nonatopic individuals and is known as extrinsic allergic alveolitis involving both Type III and Type IV hypersensitivity. This disease affects the lung parenchyma rather than the airways. Several species of Aspergillus, including A. clavatus, A. flavus, and A. fumigatus, may be causative agents. Persons, such as farmers, miners, and grain workers, who are exposed to dusty, moldy inhalants can develop the disease, i.e., farmer's lung (actinomycetes and A. fumigatus) and malt worker's disease (A. clavatus).[82] Type IV

hypersensitivity is considered to be involved because a delayed type hypersensitivity can be elicited with a subcutaneous injection of Ag. Continued exposure to the organism(s) can lead to chronic and irreversible fibrosis in the lung.

Noninvasive aspergillosis (aspergilloma) results from colonization of a preexisting cavity present in the lung caused by the other diseases such as tuberculosis or sarcoidosis.[11,79] Conidia are inhaled, germinate, and grow to form a "fungus ball." Conidiophores and conidia may be produced within the cavity. The fungus usually remains contained within the cavity and does not invade blood vessels.

Invasive aspergillosis may be either chronic necrotizing or disseminated disease.[11] Chronic necrotizing aspergillosis is a less severe form of invasive aspergillosis lasting one to six months. This form does not invade blood vessels, but causes local pulmonary necrosis. Diabetics, persons with connective tissue disorders, chronic lung disease, inactive tuberculosis, malnourished individuals, or individuals on low dosages of corticosteroids or other immunosuppressants are possible candidates for this form of invasive aspergillosis. Cavitation can be seen radiographically.

Disseminated aspergillosis is considered a disease brought about by medical practices where immunosuppressive drugs are used or where the patient is immunocompromised by other factors.[11,79] The patient is placed at risk by any disease or treatment that depresses the phagocytic cell (macrophage and neutrophil) numbers or functions, or the cell mediated immune response of the lymphocytes, primarily the T

lymphocytes and their lymphokines [macrophage inhibition factor (MIF), macrophage chemotactic factor (MCF), interleukin 2 (IL-2), leukocyte inhibition factor (LIF), leukocyte chemotactic factor (LCF), and others]. In disseminated pulmonary aspergillosis the organism invades blood vasculature, causes infarcts, and becomes disseminated throughout the body. The causative organism may be found in most organs, with the brain and gastrointestinal tract being favored, but also liver, kidney, and spleen are likely sites for isolation of the organism.

The major group of individuals at risk for disseminated pulmonary aspergillosis are those with acute or chronic myelogenous lymphocytic leukemia.[79] The resulting neutropenia decreases the efficient destruction of A. fumigatus hyphae by neutrophils.[83] Individuals with lymphomas and other solid tumors are also prone to develop disseminated aspergillosis, indicating the importance of lymphocyte-directed cell mediated immunity in combating invasion by A. fumigatus. The increased use of antibiotics, glucocorticosteroids, and immuno- and myelosuppressive drugs in chemotherapy and transplantation patients in recent years has also caused a dramatic increase in disseminated pulmonary aspergillosis second only to candidiosis as a cause of death.[11,79,84]

Aspergillus lung disease can occur in humans regardless of host immune status, either in its allergic or noninvasive forms, or occasionally as invasive aspergillosis.[85,86] However, the manifestation of the disease is usually linked to the immune status of the host.[86]

Because Ab may be absent in these immunosuppressed patients, the ID test is unreliable as a means of immunodiagnosis. Other more sensitive assays, i.e., primary binding tests such as solid-phase radioimmunoassay (SP-RIA),[87] ELISA,[88] and countercurrent immunoelectrophoresis[89] have been used experimentally. Immunodiagnosis has also been accomplished by the ELISA or RIA detection of galactomannan antigenemia.[90]

There are several major problems encountered in serodiagnosis of aspergillosis. Aspergillus antigens are complex mixtures of protein and polysaccharide components, making it difficult to obtain comparable preparations even under controlled conditions within one laboratory.[79] Shared antigenicity among Aspergillus species and other microorganisms can influence serodiagnosis.[79] Cross-reactivity has been found among various species of Aspergillus. Shared antigens also have been found among Aspergillus, other fungi, mycobacteria, bacteria, house dust mite, and house dust preparations. Cross-reactivity is thought to be a result of the polysaccharide antigens.

Mammalian pulmonary aspergillosis occurs quite frequently in cattle.[12,13] The condition is manifested by bronchitis and peribronchitis arising from focal degenerative changes of bronchial epithelium with subsequent sloughing of infected cells. Inflammatory cells may fill the alveoli, and nodules of infected cells occur scattered throughout the lung. A severe bronchitis may ensue with the formation of a mycelial mat along portions of the bronchial wall and, with continued tissue reaction, the smaller bronchi may become plugged.[91] A

specific type of lesion known as an "asteroid body" was originally described in bovine aspergillosis.[92] Asteroid bodies consist of a small portion of fungal hyphae surrounded by eosinophilic clubs. These bodies possessed a fibrous capsule and were surrounded by lymphocytes, neutrophils, and histiocytes.[13,79]

As mentioned earlier, A. fumigatus readily invades blood vessels and thrombosis is a frequent occurrence in acute cases of bovine pulmonary aspergillosis. This phenomenon may be important in the pathogenesis of bovine mycotic abortion, the most economically important aspect of aspergillosis of cattle. Annual losses in the state of South Dakota due to this disease have been estimated at $175,000.[93] It is economically important in foreign countries also.[94] Evidence of the interrelationship of the pathogenesis of pulmonary aspergillosis and mycotic abortion is that both conditions often occur in the same animal;[91,95] however, experimental production of mycotic abortion has not been achieved with A. fumigatus given to animals by the pulmonary route.[12,13]

Pulmonary aspergillosis or mycotic abortion has been described in other species such as the horse and pig, but are of infrequent or rare occurrence.[12,13]

Guttural pouch mycosis is a common disease in horses that is caused primarily by A. fumigatus and A. nidulans conidia produced in bedding or feed.[13] Infections form a diptheritic membrane within the guttural pouch and may spread to major blood vessels and nerves at the

base of the skull, causing symptoms such as ocular defects and facial paralysis.

Aspergillosis is a common and economically important disease in avian species[13,96] with economic losses estimated to be approximately $50 million annually to the turkey industry in the United States. Infections occur in poultry without any apparent preexisting decreased resistance of the host.

The research literature is voluminous relating to aspergillosis in humans having defects in cell mediated immunity,[86] and these phenomena occur in mammalian aspergillosis as well.[97] However, few researchers have investigated the interaction of mycotic disease with naturally consumed immuno- and myelosuppressive agents such as mycotoxins.[98-100] Two studies have shown that use of the trichothecene, diacetoxy-scirpenol, enhanced the course of experimental candidosis[98] and cryptococcosis in mice.[99] Because both Aspergillus and mycotoxins are omnipresent in the environment and in foodstuffs, the potential for detrimental interaction in animals and humans exists.

# SECTION I. EFFECTS OF T-2 MYCOTOXIN INGESTION ON PHAGOCYTOSIS OF *ASPERGILLUS FUMIGATUS* CONIDIA BY RABBIT ALVEOLAR MACROPHAGES AND ON HEMATOLOGIC, SERUM BIOCHEMICAL, AND PATHOLOGIC CHANGES IN RABBITS

# Effects of T-2 Mycotoxin Ingestion on Phagocytosis of *Aspergillus fumigatus* Conidia by Rabbit Alveolar Macrophages and on Hematologic, Serum Biochemical, and Pathologic Changes in Rabbits

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## SUMMARY

Rabbits were given T-2 mycotoxin orally at 0, 0.25, 0.5, and 0.75 mg/kg/d for 3 weeks. Deaths (4 of 5 rabbits) occurred in only the 0.75 mg/kg/d group. Alveolar macrophages (AM) were harvested on Day 22 and used for *in vitro* phagocytosis of killed *Aspergillus fumigatus* Fres. conidia. The cultures included sera from either untreated or T-2 treated rabbits. Phagocytosis was significantly reduced ($p<0.01$) in cultures using serum from 0.5 mg/kg/d T-2 treated rabbits and AM from either untreated or T-2 treated rabbits. There was little reduction in phagocytosis when AM from T-2 treated rabbits and normal serum were used. Ingestion of 0.5 mg/kg/d T-2 toxin significantly reduced ($p<0.05$) weight gains, serum alkaline phosphatase (ALP), serum sorbitol dehydrogenase (SDH), and serum bacteriostasis. T-2 toxin at 0.75 mg/kg/d significantly reduced ($p<0.05$) packed cell volume (PCV), total WBC and differential leukocyte counts except for neutrophil counts which declined, but not significantly ($0.05<p<0.10$). Significant changes were not detected in alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), or complement. Histopathologic changes consisting of centrolobular hepatocellular swelling, mild portal and periportal fibrosis and lymphocyte necrosis within secondary lymphoid tissues occurred in most T-2 treated rabbits. Thymic atrophy, bile duct reduplication, and lymphocyte depletion of secondary lymphoid tissues occurred in the 0.75 mg/kg/d group. The severity of lymphoid depletion in secondary lymphoid tissues occurred

in the following order: appendix > sacculus rotundus > ileal Peyer's patch > lymph nodes and spleen.

This study provides additional evidence that at these oral dosages of T-2 toxin, rabbits could be immunosuppressed as evidenced by reduced AM phagocytosis and histopathologic changes in lymphoid tissues, and that these dosages caused reductions in weight gains, certain hematologic parameters, and serum ALP and SDH levels.

# INTRODUCTION

The trichothecenes are the major toxic secondary metabolites that are produced by several Fusarium spp.[1,2] Most research with trichothecenes has been done with T-2 toxin, diacetoxyscirpenol, and deoxynivalenol, primarily using cattle, swine, and poultry. The effects of these toxins have been grouped into four categories[2] as 1) feed refusal, 2) dermal necrosis, 3) gastrointestinal effects, and 4) coagulopathy.

Another important effect of T-2 toxin is that of immunosuppression. Turkeys were more susceptible to T-2 toxin than chickens and this toxin caused a decrease in the size of lymphoid organs.[3] Depletion of thymic cortical lymphocytes occurred in turkeys fed 10 ppm dietary T-2 toxin. T-2 toxin has been demonstrated to affect various immune phenomena including the functional capacity of phagocytic cells, especially neutrophils.[4] In vitro studies with T-2 toxin have demonstrated inhibition of chemotaxis and phagocytosis by rat leukocytes,[5] and Buening et al.[6] found a depression of chemotaxis by neutrophils from orally dosed cattle. Bactericidal activity by neutrophils was depressed in monkeys dosed orally with T-2 toxin.[7]

Phagocytosis has been decreased by other mycotoxins, such as aflatoxins. Richard and Thurston[8] demonstrated reduced phagocytosis by AM from rabbits fed various dosages of aflatoxin for 3 weeks and the reduction was greatest when serum from treated rabbits was incorporated in the tissue culture assay system. Aflatoxin inhibited phagocytosis of latex particles and uptake and incorporation of labeled leucine and

uridine by rat liver macrophages.[9] Because T-2 toxin has been shown to depress several immune phenomena, we investigated the possible interference of this compound with phagocytosis by AM from rabbits fed T-2 toxin for 21 days.

## MATERIALS AND METHODS

Animals Twenty New Zealand white female rabbits (Small Stock Industries, P.O. Box 157, Pearidge, AR) each weighing approximately 2 kg were used in this study. They were housed in individual cages and given food (Laboratory Rabbit Diet #0533, Teklad, Winfield, IA 52659) and water ad libitum.

T-2 toxin Crystalline T-2 toxin was prepared from extracts of white corn-meal inoculated with Fusarium sporotrichioides Sherb. NRRL 3299 [F. tricinctum (Corda) Sacc.] according to the method of Burmeister.[10] Purity was determined to be 97% by thin-layer chromatographic and gas chromatographic-mass spectral analyses conducted in another laboratory (C.J. Mirocha, University of Minnesota, St. Paul, MN).

T-2 toxin was dissolved in acetone at concentrations that would yield 0.1 ml solution of each daily dosage. This amount of solution was placed in No. 5 gelatin capsules to provide daily dosages for one week. Acetone was allowed to evaporate before assembling the capsule. Capsules were stored at $4^{o}C$.

Conidial suspension/serum solution Aspergillus fumigatus Fres. conidia were harvested as described by Richard et al.[11] and killed by ethylene oxide sterilization. A suspension of conidia in Medium 199 (M199) was added to serum at a 2:1 ratio yielding a final concentration of $2.5 \times 10^{6}$ conidia/ml.

Experimental design The rabbits were randomly assigned to 4 groups of 5 rabbits each according to dosages of T-2 toxin and

observed daily throughout the study. Dosage groups included 0, 0.25, 0.5, and 0.75 mg/kg/d of T-2 toxin. Each rabbit was given either an empty capsule (for the 0 dosage group) or a capsule containing T-2 toxin daily for 21 days with a balling gun. Rabbits were weighed weekly and dosages of T-2 toxin were adjusted each week according to weight changes. A six ml sample of peripheral blood was obtained weekly from each rabbit. Serum was separated by centrifugation, removed, and stored at -70°C. On Day 22 of the study, all surviving rabbits were euthanatized, and after removal of the lungs, were necropsied. Alveolar macrophages were obtained in the following manner. The upper trachea was clamped and transected craniad to the clamp. After removal of the heart and lungs, the bronchus to the right lung was doubly clamped and transected. The right lung was removed, perfused with 2% glutaraldehyde in 0.1 M sodium cacodylate, and used for microscopy. The left lung was lavaged twice with 30 ml of Hanks balanced salt solution (BSS) to obtain AM for the phagocytosis study.[8]

Phagocytosis assay Macrophages from each rabbit were tested for viability by exclusion of 0.125% aqueous Trypan blue. After refrigerated centrifugation for 20 min at 1500 rpm, the macrophages were resuspended in 40 ml of BSS, adjusted to the density of a #5 McFarland nephelometer tube, and 0.2 ml of the suspension was placed in each of the 8 wells of a tissue culture slide (Lab-Tek Products, Division of Miles Labs., Inc., Naperville, IL). Macrophages were allowed to attach for 20 min at 37°C, rinsed in M199, and 0.2 ml of the appropriate conidial suspension/serum solution (Table 1) was placed in

Table 1. Combinations of alveolar macrophages and conidial suspension/serum solutions used in phagocytosis study

| Macrophages from control* or T-2 treated rabbits† | + | M199 conidial suspension + serum from control‡ or T-2 treated rabbits |
|---|---|---|
| Control rabbits | + | Control rabbits |
| Control rabbits | + | 0.25 mg T-2/kg/d |
| Control rabbits | + | 0.5 mg T-2/kg/d |
| 0.25 mg T-2/kg/d | + | Control rabbits |
| 0.5 mg T-2/kg/d | + | Control rabbits |
| 0.25 mg T-2/kg/d | + | 0.25 mg T-2/kg/d |
| 0.5 mg T-2/kg/d | + | 0.5 mg T-2/kg/d |

*Pooled macrophages from control rabbits.

†Given T-2 toxin orally.

‡ Pooled serum from control rabbits.

each well and incubated at 37°C for 1 h. The cultures were rinsed and fixed in absolute methanol for 15 min. The plastic culture chambers were removed from the glass slides and cells were stained with Diff Quik (American Scientific Products, Div. of American Hospital Supply Co., McGaw Park, IL). Approximately 200 macrophages/rabbit (>60/well in 3 wells) were examined with a light microscope and the number of conidia ingested by each macrophage was recorded.

Hematologic and serum biochemical determinations Packed cell volume (PCV), white blood cell (WBC) counts (using a Coulter counter [Coulter Counter, Coulter Electronics, Hialeah, FL]) and differential counts were determined on each blood sample. Serum alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), alanine amino transferase (ALT), aspartate amino transferase (AST), and blood urea nitrogen (BUN) were determined using a centrifical analyzer (Rotachem IIa centrifical analyser, Travenol Labs, Inc., Deerfield, IL).

Complement and bacteriostasis determinations Complement was titrated on all serum samples, using a 50% hemolytic end point.[12] Serum bacteriostasis of a strain of Escherichia coli was measured using the method of Thurston et al.[13] Rabbit serum was used at a dilution of 1:4 in phosphate buffered saline solution (pH 7.4).

Histopathology Portions of kidney, liver, thymus, heart, adrenal, spleen, pancreas, Peyer's patch, mesenteric and jejunal lymph nodes, sacculus rotundus, appendix, and gastrointestinal tissues were fixed in buffered 10% formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin (H&E) stain and examined by

light microscopy. The right lung was perfused with 2% glutaraldehyde in 0.1 M sodium cacodylate, and processed as other tissues. Tissues stained with Gomori's one-step trichrome and periodic acid-Schiff's were used for evaluating hepatic fibrosis and glycogen, respectively.

Statistical analysis Group means were compared by standard Student's t-test. Time trends were measured and tested by linear regression analysis.

# RESULTS

Clinical observations Severe physical effects of T-2 toxin were observed in the rabbits of the 0.75 mg/kg/d group. After 2 to 3 days of dosages, these rabbits became lethargic, were apparently inappetent, amount of feces decreased markedly, and the fecal pellets were small and moist. They had excessive salivation 2 to 4 days before death. Fur around the mouth, on the neck, and front limbs became wet and tan-stained with saliva.

Three of the 5 rabbits in this group died 8 to 14 days after dosing began, and one was killed *in extremis* on Day 15. No further dosages were given to the fifth rabbit after Day 18 because that group could not be used in the phagocytosis study.

The final 24 hours before death, the rabbits appeared weak and felt cool. The difference in rate of body weight gain between the control and the 2 greatest dosage groups was significant ($p<0.05$) (Fig. 1). Rabbits given lesser dosages appeared healthy.

Phagocytosis assay The Trypan blue exclusion test indicated that 81% of the macrophages from control rabbits, 72% from the 0.25 mg/kg/d group, and 57% from the 0.5 mg/kg/d group were viable. The number of conidia phagocytized by the macrophages from any dosage group was decreased 52% ($p<0.01$) when serum from the 0.5 mg/kg/d group was used in the phagocytosis assay (Table 2). Nonsignificant differences in phagocytosis attributed to the macrophages from T-2 treated rabbits were noted (Table 2). Similar results were obtained when the data were analyzed for the number of macrophages ingesting conidia and/or the

Figure 1. Mean body weights (g) of rabbits (5/dosage group) given T-2 toxin orally. The 0.75 mg/kg/d group had 5,4,2, and 1 rabbits at 0-3 weeks, respectively. The 2 highest dosage groups were different from control ($p<0.05$)

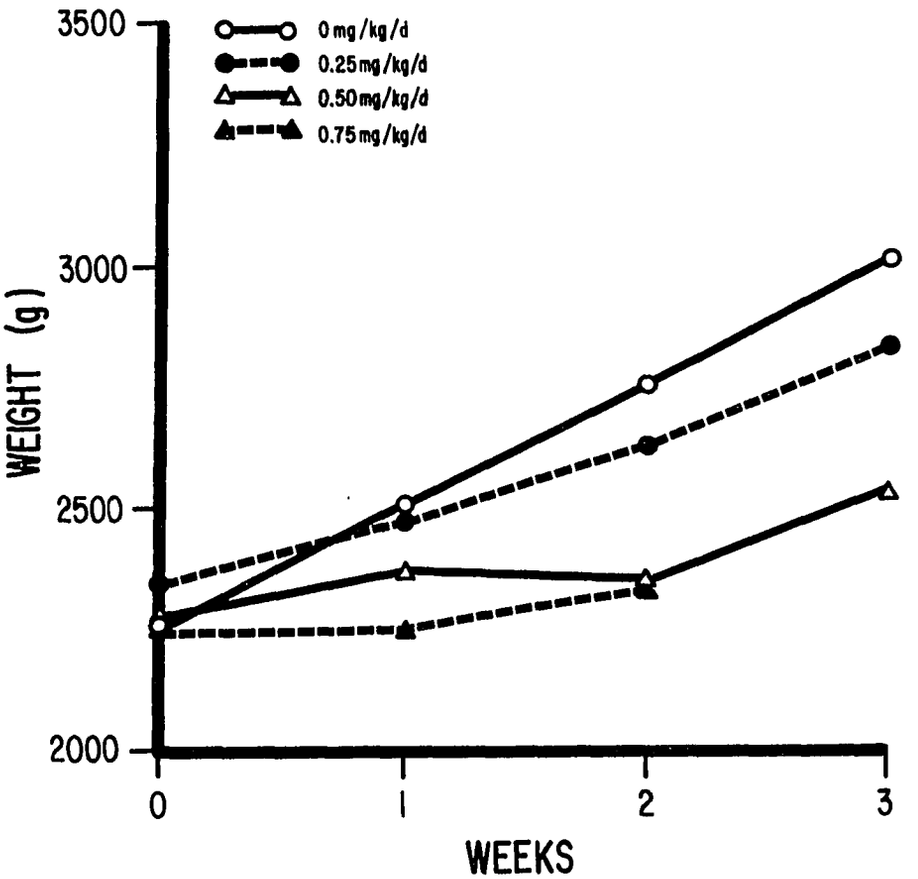

0 mg/kg/d
0.25 mg/kg/d
0.50 mg/kg/d
0.75 mg/kg/d
3500
3000
2500
2000
WEIGHT (g)
0
1
2
3
WEEKS

Table 2. Mean number of conidia ingested in one hour by rabbit alveolar macrophages (MØ)

| MØ from rabbit dosage groups (mg T-2/kg/d) | Serum from rabbit dosage groups (mg T-2/kg/d) | | |
|---|---|---|---|
| | 0.0 | 0.25 | 0.5 |
| 0.0 | 2.4 | 1.5 | 1.1 |
| 0.25 | 2.4 | 1.6 | ND* |
| 0.5 | 2.1 | ND | 1.1 |
| | 2.3† (0.12) | 1.5 (0.9) | 1.1‡ (0.11) |

*Not Determined.

†Mean, figure in parentheses is Standard Error.

‡ $p<0.01$.

number of conidia ingested/macrophage. The phagocytosis assay was not determined with AM from the 0.75 mg/kg/d group because of the high mortality rate.

Hematologic and serum biochemical changes Hematologic changes were most severe in the 0.75 mg/kg/d group. The weekly means for PCV, WBC, and absolute lymphocyte numbers declined during the 21 day period. The declines, as measured by linear regression, were statistically significant ($p<0.05$). After 3 weeks, PCV values (Fig. 2) had decreased from 32% to 25%. The total WBC count (Fig. 3) decreased from 4.6 to 1.4 x $10^9$/L and the absolute number of lymphocytes (Fig. 4) decreased from 3.1 to 0.5 x $10^9$/L. Neutrophil numbers (Fig. 5) declined (approaching significance, $p<0.09$) only in the 0.75 mg/kg/d group from 1.3 to 0.3 x $10^9$/L. Rabbits in this group had a marked increase in nucleated erythrocytes (NRBC) in peripheral blood after the first week of treatment. The surviving rabbit had 288 NRBC/100 WBC. Polychromasia, poikilocytosis, anisocytosis, echinocytosis, acanthocytosis, and basophilic stipling were common findings. No changes were noted in other blood cell types. Changes in other dosage groups were not significant.

The reductions in serum ALP (Table 3) and SDH (Table 4) concentrations were statistically significant ($p<0.05$) in the 0.5 mg/kg/d group by the end of the first week. There were no significant changes in ALT, AST, and BUN levels.

Complement and bacteriostatic effects Bacteriostatic activity (Fig. 6) of serum from rabbits in the 0.5 and 0.75 mg/kg/d groups was

Figure 2. Mean packed cell volume (%) from rabbits (5/dosage group) given T-2 toxin orally. The 0.75 mg/kg/d group had 5,4,2, and 1 rabbits at 0-3 weeks, respectively, and was different from control ($p<0.05$)

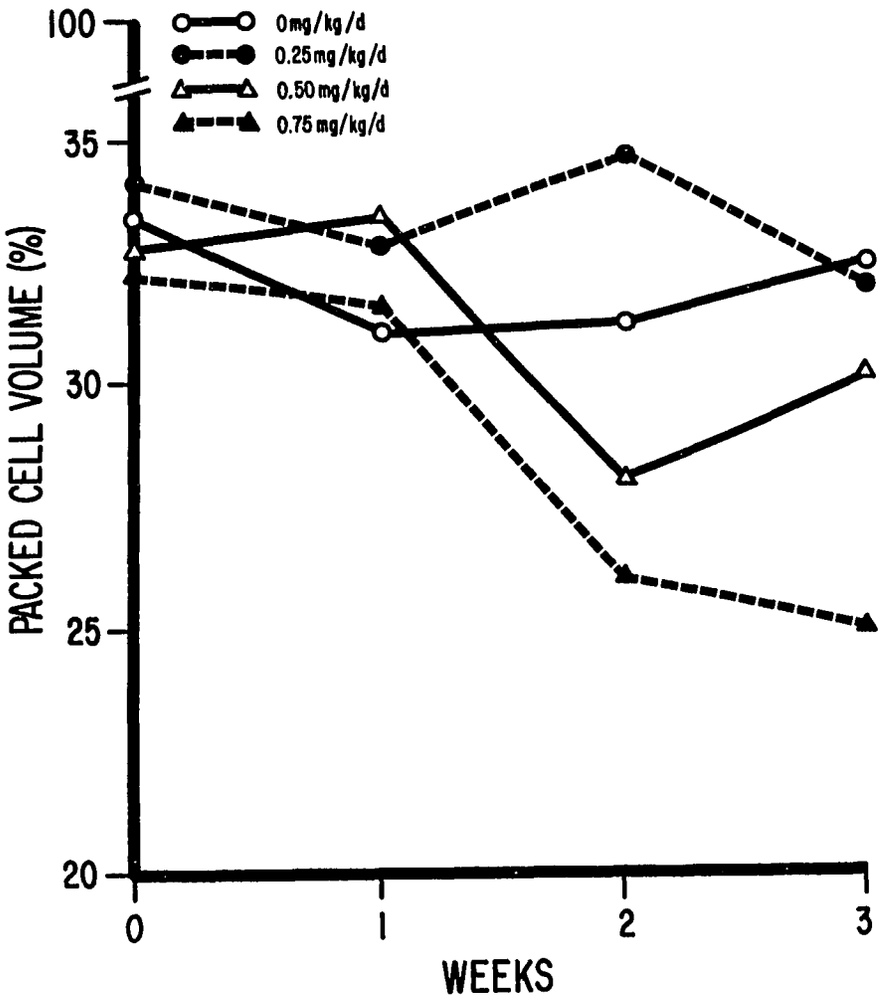

0 mg/kg/d
0.25 mg/kg/d
0.50 mg/kg/d
0.75 mg/kg/d
100
35
30
25
20
PACKED CELL VOLUME (%)
0
1
2
3
WEEKS

Figure 3. Mean total white blood cell counts (N/µl) from rabbits (5/dosage group) given T-2 toxin orally. The 0.75 mg/kg/d group had 5,4,2, and 1 rabbits at 0-3 weeks, respectively, and was different from control ($p<0.05$)

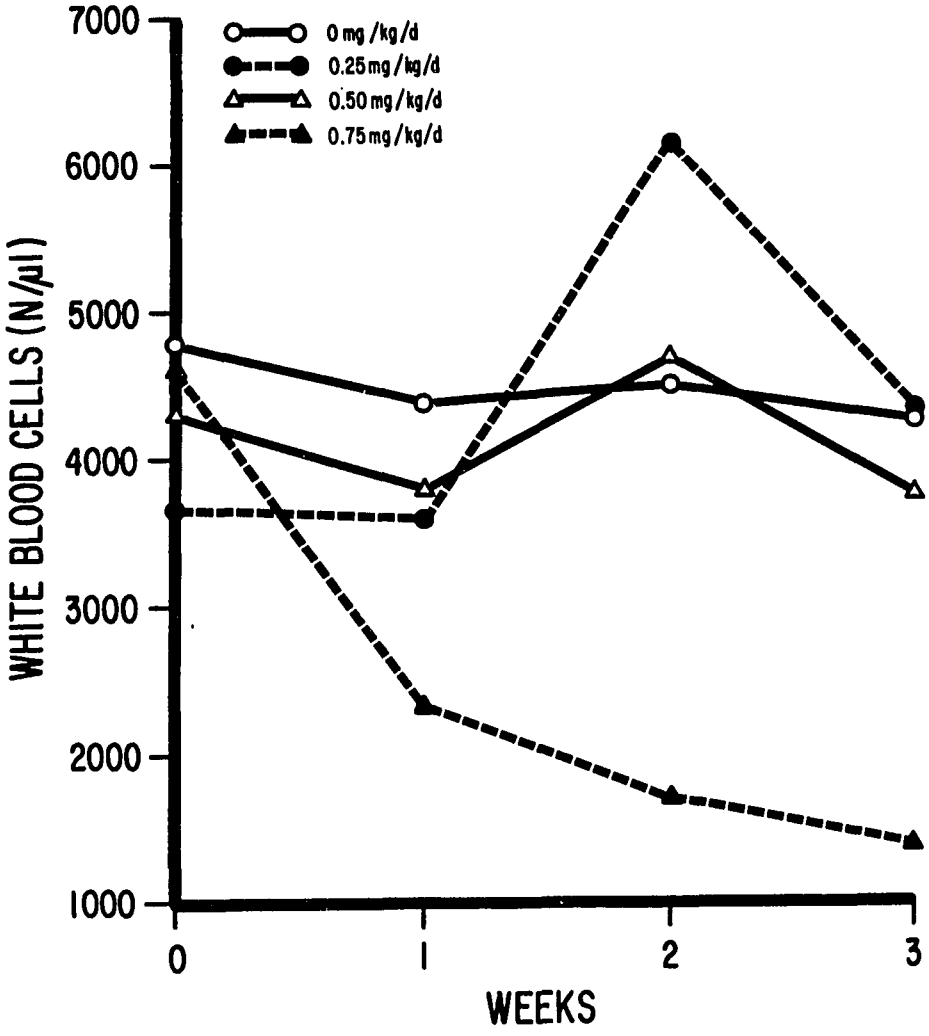

7000
6000
5000
4000
3000
2000
1000
WHITE BLOOD CELLS (N/µl)
0 mg/kg/d
0.25 mg/kg/d
0.50 mg/kg/d
0.75 mg/kg/d
0
1
2
3
WEEKS

Figure 4. Mean lymphocytes (N/μl) from rabbits (5/dosage group) given T-2 toxin orally. The 0.75 mg/kg/d group had 5,4,2, and 1 rabbits at 0-3 weeks, respectively, and was different from control ($p<0.05$)

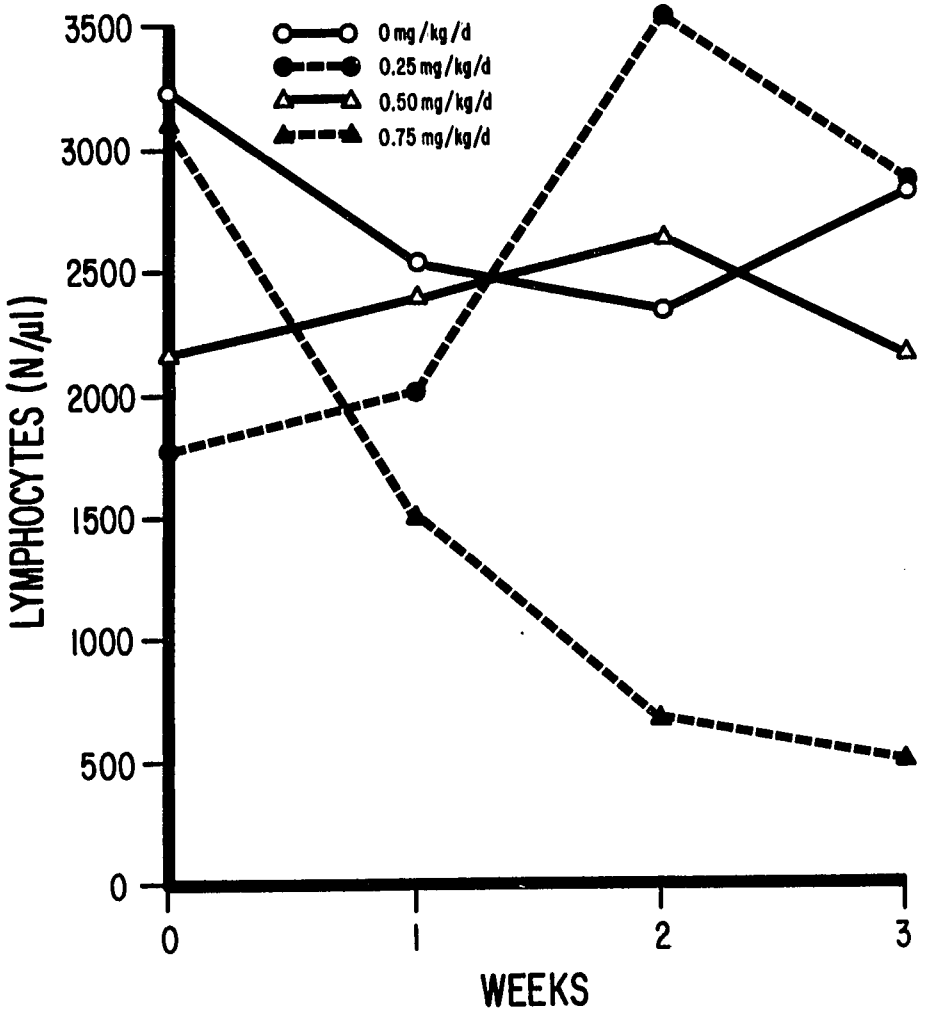

0 mg/kg/d
0.25 mg/kg/d
0.50 mg/kg/d
0.75 mg/kg/d
LYMPHOCYTES (N/µl)
3500
3000
2500
2000
1500
1000
500
0
0
1
2
3
WEEKS

Figure 5. Mean neutrophils (N/μl) from rabbits (5/dosage group) given T-2 toxin orally. The 0.75 mg/kg/d group had 5,4,2, and 1 rabbits at 0-3 weeks, respectively, and was different from control ($p<0.09$)

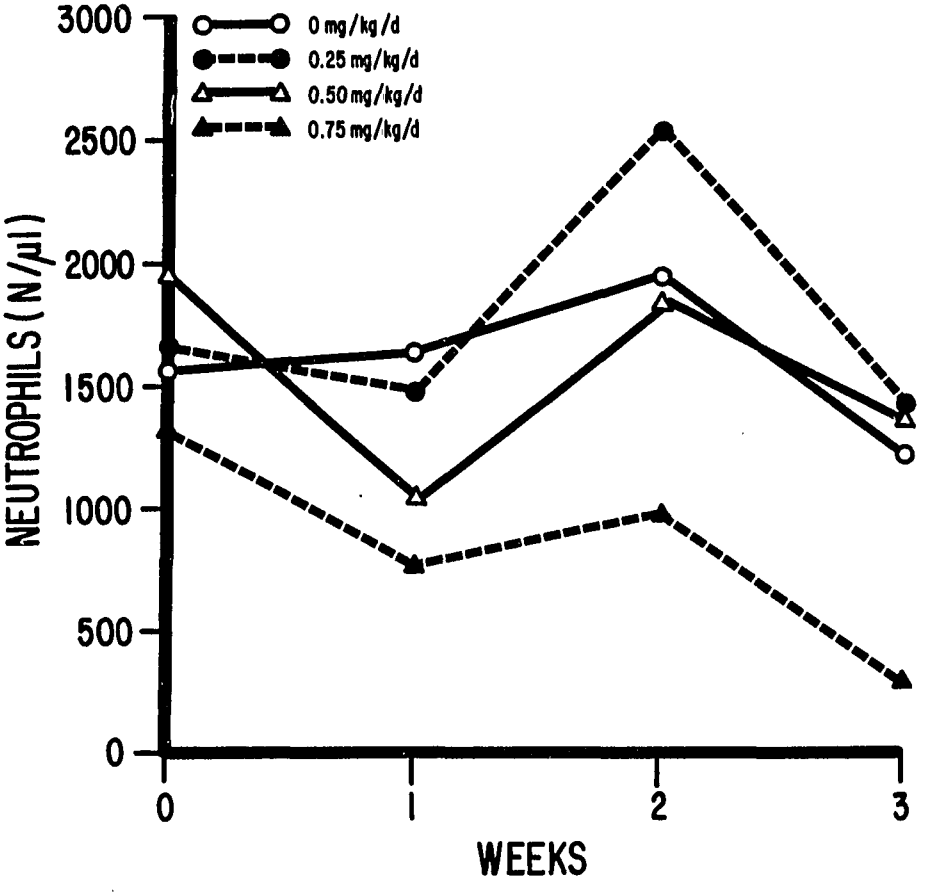

3000
2500
2000
1500
1000
500
0
NEUTROPHILS (N/µl)
0 mg/kg/d
0.25 mg/kg/d
0.50 mg/kg/d
0.75 mg/kg/d
0
1
2
3
WEEKS

Table 3. Mean serum alkaline phosphatase (ALP) concentrations (IU/L) from rabbits given T-2 toxin orally for 21 days

| Week | Dosage Group (mg/kg/d) | | | |
|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 0.75 |
| 0 | 89 | 81 | 82 | 99 |
| 1 | 91 | 65 | 55 | 53 |
| 2 | 94 | 67 | 45 | 38 |
| 3 | 94 | 57 | 43 | 44* |
| | 2.1† (2.3) | -7.6‡(2.3) | -12.3‡(2.3) | |

*Data from one surviving rabbit not treated for last 4 days of experiment.

†Coefficient of linear regression, figure in parentheses is Standard Error.

‡$p<0.05$.

Table 4. Mean serum sorbitol dehydrogenase (SDH) concentrations (IU/L) from rabbits given T-2 toxin orally for 21 days

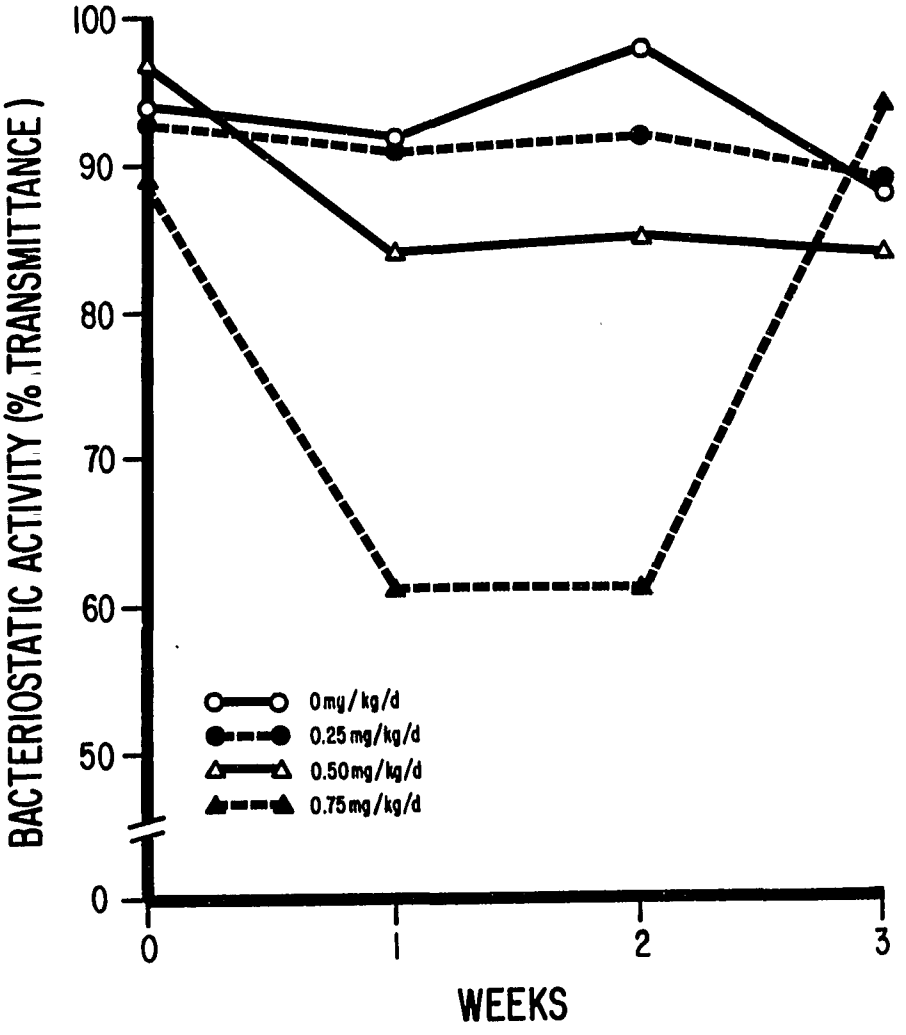
| Week | Dosage Group (mg/kg/d) | | | |
|---|---|---|---|---|
| | 0 | 0.25 | 0.5 | 0.75 |
| 0 | 32 | 29 | 25 | 27 |
| 1 | 29 | 20 | 16 | 28 |
| 2 | 27 | 17 | 13 | 23 |
| 3 | 25 | 18 | 17 | 21* |
| | -0.7†(1.0) | -3.2‡(1.0) | -4.3‡(1.0) | |

*Data from one surviving rabbit not treated for last 4 days of experiment.

†Coefficient of linear regression, figure in parentheses is Standard Error.

‡$p<0.05$.

Figure 6. Bacteriostatic activity (% transmittance at 540nm for 120m) of serum from rabbits (5/dosage group) given T-2 toxin orally. The 0.75 mg/kg/d group had 5,4,2, and 1 rabbits at 0-3 weeks, respectively. The 2 highest dosage groups were different from control ($p<0.05$)


100
90
80
70
60
50
0
BACTERIOSTATIC ACTIVITY (% TRANSMITTANCE)
0
1
2
3
WEEKS
0 mg/kg/d
0.25 mg/kg/d
0.50 mg/kg/d
0.75 mg/kg/d

significantly reduced ($p<0.05$ and $p<0.01$, respectively) by the end of the first week. There was no significant change in the 0.25 mg/kg/d group. T-2 toxin at the dosages given had no effect on complement activity.

Gross lesions Mucosal petechiation and erosions were observed in the stomachs of 2 rabbits given 0.75 mg of T-2 toxin/kg. Gastric ulcers were seen in one rabbit in this group. Three of the 5 rabbits in the 0.5 mg/kg/d group had hyperemic mucosal areas in their stomachs. Gross lesions were not found in either the 0.25 mg/kg/d or the control group.

Histologic lesions Microscopic lesions were confined to liver, thymus, and to the secondary lymphoid tissues of the T-2 treated groups (Table 5).

In the 0.25 mg/kg/d group, lesions were limited to the liver, ileal Peyer's patches, appendix, and sacculus rotundus. Hepatic lesions consisting of centrolobular hepatocellular swelling (Fig. 7) and mild portal and periportal fibrosis occurred in 4 of the 5 rabbits. Swollen hepatocytes were positive for glycogen. Changes in the appendix, sacculus rotundus, and ileal Peyer's patches were characterized by scattered areas of lymphocyte necrosis in all 5 rabbits. Lymphocyte necrosis was centered around the germinal centers and was less prominent in the dome area of sacculus rotundus and appendix. Mild lymphoid depletion was noted in 2 rabbits.

Lesions in the 0.5 mg/kg/d group were essentially similar to those in the 0.25 mg/kg/d group but were slightly more severe (Fig. 8).

Table 5. Frequency of occurrence and location of major microscopic lesions in rabbits given T-2 toxin orally for 21 days

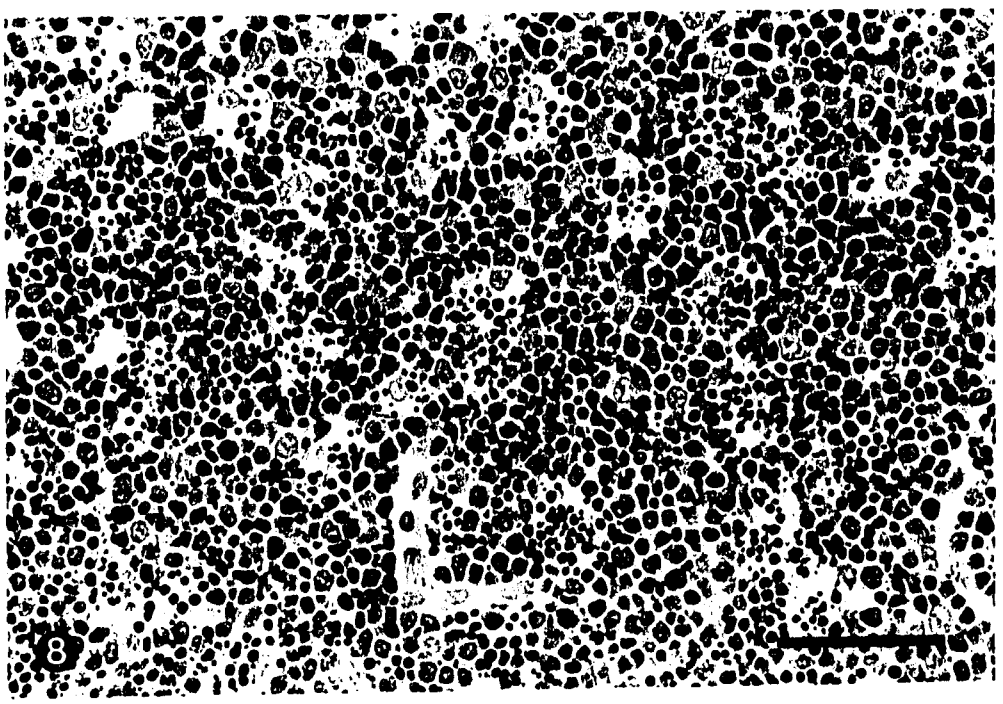
| Organ/ Tissue | Lesions | Dosage Group (mg/kg/d) 0.0 | 0.25 | 0.5 | 0.75 |
|---|---|---|---|---|---|
| Liver: | Hepatocellular swelling | 0(5)* | 4(5) | 4(5) | 3(4)† |
| | Portal fibrosis | 0(5) | 4(5) | 4(5) | 3(4) |
| | Bile duct proliferation | 0(5) | 0(5) | 0(5) | 3(4) |
| Appendix: | Lymphocyte necrosis | 0(5) | 5(5) | 5(5) | 3(4) |
| | Lymphoid depletion | 0(5) | 2(5) | 2(5) | 3(4) |
| Sacculus rotundus: | Lymphocyte necrosis | 0(5) | 5(5) | 5(5) | 4(4) |
| | Lymphoid depletion | 0(5) | 2(5) | 2(5) | 3(4) |
| Ileal Peyer's patches: | Lymphocyte necrosis | 0(5) | 5(5) | 5(5) | 3(4) |
| | Lymphoid depletion | 0(5) | 2(5) | 2(5) | 2(4) |
| Thymus: | Lymphocyte necrosis | 0(5) | 0(5) | 0(5) | 2(4) |
| | Lymphoid depletion | 0(5) | 0(5) | 0(5) | 2(4) |
| Mesenteric: lymph nodes: | Lymphocyte necrosis | 0(5) | 1(5) | 1(5) | 2(4) |
| | Lymphoid depletion | 0(5) | 0(5) | 0(5) | 2(4) |
| Spleen: | Lymphocyte necrosis | 0(5) | 0(5) | 0(5) | 3(4) |
| | lymphoid depletion | 0(5) | 0(5) | 0(5) | 3(4) |

*No. of rabbits with lesions (No. of rabbits examined).

†Three of 5 rabbits died on Days 8, 11, and 14, respectively, and one rabbit was killed in extremis on Day 15. No further dosages were given to the fifth rabbit because that group could not be used in the phagocytosis study. It was not necropsied on Day 22.

Figure 7. Centrolobular hepatocellular swelling from a rabbit given T-2 toxin orally at 0.25 mg/kg/d for 3 weeks. H&E stain; Bar = 40 μm

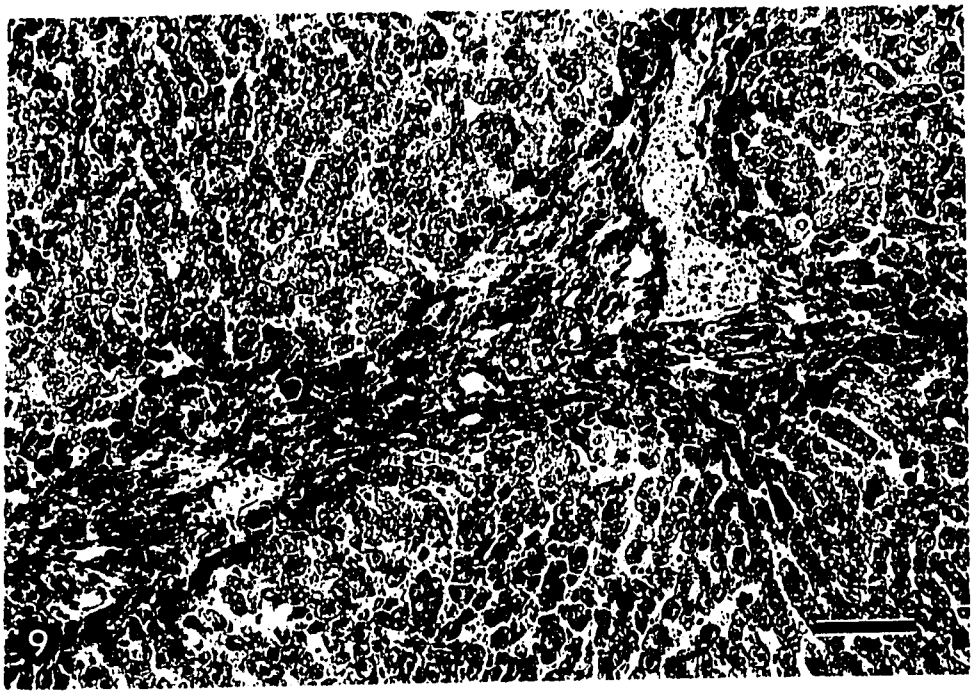
Figure 8. Moderate lymphocyte necrosis in appendix from a rabbit given T-2 toxin orally at 0.5 mg/kg/d for 3 weeks. H&E stain; Bar = 50 μm



Microscopic lesions in the 0.75 mg/kg/day group were more severe and more extensive than those of the other groups. Liver lesions consisted of centrolobular hepatocellular swelling, mild to moderate portal and periportal fibrosis (Fig. 9), and mild bile duct reduplication. Lymphocyte necrosis was a prominent change in ileal Peyer's patches, appendix, sacculus rotundus, mesenteric lymph nodes, and spleen. Increased numbers of macrophages and degenerate granular leukocytes were present in areas of lymphocyte necrosis within the appendix and sacculus rotundus. Lymphoid depletion in mesenteric lymph nodes was particularly evident within germinal centers and paracortical zones while splenic lymphoid depletion was centered around periarteriolar sheaths in 3 rabbits. Thymic lesions were observed in 2 rabbits. They consisted of severe lymphoid depletion (Fig. 10) and scattered foci of lymphocyte necrosis. Other lesions observed in this group included moderate goblet cell hyperplasia in mucosal epithelium of appendix and sacculus rotundus, hyperkeratosis and parakeratosis of esophageal mucosa, focal hemorrhages in gastric mucosa, and gastric ulcers in one rabbit.

Figure 9. Portal and periportal fibrosis in liver of a rabbit given T-2 toxin orally at 0.75 mg/kg/d for 3 weeks. Gomori's one-step trichrome; Bar = 100 μm

Figure 10. Severe lymphoid depletion of thymic cortex from rabbit given T-2 toxin orally at 0.75 mg/kg/d for 3 weeks. H&E stain; Bar = 100 μm


9


10

## DISCUSSION

The trypan blue exclusion test indicated that the effect of ingested T-2 toxin on AM viability is dose dependent. This reduction in phagocytic capacity could decrease the effectiveness of the primary nonimmunologic and immunologic defense mechanisms in the pulmonary system. Although phagocytosis was reduced in AM in the presence of homologous serum from rabbits given 0.5 mg T-2 toxin /kg/d, these macrophages were capable of normal phagocytosis when serum from control rabbits was used in the culture system. Others have shown a reduction in AM viability when T-2 toxin was added directly to an *in vitro* culture system using rat AM.[14,15]

The depression of *in vitro* phagocytosis in this study could be due to one or more of the following factors: (1) a metabolite(s) of T-2 toxin remaining in the serum of treated rabbits; (2) inhibition of production or action of serum factors such as opsonins, monokines, or lymphokines. In another study, conjugated metabolites of T-2 toxin were detected in primarily plasma and the intestinal tract of swine given tritium-labeled T-2 toxin intravascularly.[16]

Immunologic phagocytosis is enhanced by 2 major opsonins, immunoglobulins (primarily IgM, $IgG_1$, and $IgG_3$ in humans) and complement component C3b.[17,18] Others have found decreased IgM and IgG levels in monkeys[7] and mice[19,20] and decreased IgM and IgA levels in calves given T-2 toxin.[21] The decrease in serum bactericidal activity in the present study may indicate a similar effect on Ig, because the bactericidal reaction was found to be $2.5\text{-}10 \times 10^3$ times more sensitive

than the agglutination reaction for Ab detection.[22,23] Therefore, opsonization by Ig may have been affected in the present study. Because complement activity was not affected by T-2 ingestion in this study, opsonization could have occurred but at reduced efficiency, utilizing complement and the C3b receptors on the macrophage leading to the Ab-independent alternate pathway mechanism of killing.

If one or more of the factors discussed above are operable in reducing phagocytosis, as assayed in this study, the functional immune capacity of the remaining viable macrophages in an animal consuming appropriate amounts of T-2 toxin also may be adversely affected.[24,25] This could limit interleukin 1 (IL-1) production by macrophages, IL-2 production by T cells, T and B cell proliferation, and functions of macrophages and polymorphonuclear leukocytes. Absolute numbers of lymphocytes also were decreased in this study and the effect could be similar. If other macrophages in the body are similarly affected by T-2 ingestion, cell mediated immunity (CMI) could be impaired.

Our preliminary experiments showed that dosages of 2 mg/kg/d caused death of rabbits within 24-48 h. Others have found that high doses of T-2 toxin caused shock and subsequent death in other species of animals.[26,27,28] In the present study, 0.75 mg/kg/d produced marked physical deterioration within the first week, with the tan, saliva-stained fur as the first clinical sign before the rabbits died. Because rabbits are coprophagic, reingestion of T-2 toxin and its metabolites that can occur in feces[29] may have caused irritation of the mouth resulting in excessive salivation and staining of the fur.

The mechanism of action for T-2 toxin is not clear. The toxin is thought to be an amphipathic molecule and to interact initially with the outer phospholipid bilayer of the cell.[30,31,32] The possible binding of T-2 to receptors on the cell membrane, may interfere with signal transfer and thus with DNA, RNA, and protein synthesis[33,34,35,36] and ultimate reduction of the immune response.[6] Both resting and mitogen stimulated murine lymphocytes were affected by T-2, with resting cells requiring a longer time frame than actively dividing cells.[33] The effect of long term ingestion of T-2 toxin on the lymphoid cells and tissues noted in the present study is consistent with these hypotheses. At greater dosages, T-2 may lyse cell membranes with formation of <5.5 Å lesions.[37] Concurrently, free radicals that are formed may potentiate the direct effect of T-2 toxin on the membrane.[38]

Cells that contain numerous free polysomes (cells that regenerate from special proliferating undifferentiated germinal cells and/or blast cells; i.e., hematopoietic, lymphoid, intestinal crypt, and bursa of Fabricius) apparently are more susceptible to T-2 toxin than cells with few free polysomes (parenchymal tissues such as liver and kidney, that have no proliferative undifferentiated cells).[39] These effects are evident in the present study with striking dosage-related histopathologic lesions in all lymphoid and gastrointestinal (GI) tissues examined. Similar effects were demonstrated in these tissues in other studies.[16,19,20,34,39,40,41] The relative severity of germinal center lesions in secondary lymphoid tissues was: appendix >

sacculus rotundus > ileal Peyer's patch> lymph nodes and spleen. This reflects the known proliferation rates of lymphoid cells in these organs.[42]

Leukopenia, increases in nucleated erythrocytes, changes in erythrocyte morphology, and decreased PCV in rabbits of the 0.75 mg/kg/d group are indicative of the possible amphipathic nature of T-2 toxin and its effect on proliferative cell types and the bone marrow endothelium.[41,43,44,45] The 22% decrease in PCV could be due to GI hemorrhage or to suppression of hematopoiesis.[39,41,46,47,48]

Studies using radiolabeled T-2 toxin indicate that the toxin is rapidly metabolized by the liver and eliminated into the intestinal tract through the biliary excretion system primarily as glucuronide metabolite conjugates.[16,26,29,49] Centrolobular hepatocellular swelling and vacuolation, hepatic portal and periportal fibrosis, and bile duct reduplication in the present study provide evidence of the process. It is possible that the centrolobular hepatocellular swelling could have been a reflection of hypoxic change caused by anemia because some rabbits were anemic as evidenced by decreased PCV. Decreases in serum ALP concentrations may be due to reduced ALP synthesis in the liver and intestinal tract,[32] or this decrease may reflect the amphipathic nature of T-2 whereby the toxin causes membrane alterations without cell lysis and interferes with release of the enzyme from the cell.[50] Similar mechanisms may explain the reduced SDH serum concentrations. However, SDH is specific for the liver.

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## SECTION II. EVALUATION OF PATHOLOGIC, HEMATOLOGIC, SEROLOGIC, AND MYCOLOGIC CHANGES IN RABBITS GIVEN T-2 MYCOTOXIN ORALLY AND EXPOSED TO AEROSOLS OF ASPERGILLUS FUMIGATUS FRESENIUS CONIDIA

# Evaluation of Pathologic, Hematologic, Serologic, and Mycologic Changes in Rabbits Given T-2 Mycotoxin Orally and Exposed to Aerosols of *Aspergillus fumigatus* Fresenius Conidia

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## SUMMARY

The influence of immunosuppression in rabbits by T-2 mycotoxin on the fungal disease, aspergillosis, was investigated. Rabbits were given 0.5 mg T-2 toxin /kg/d orally for 17 or 28 days (Groups 1 and 3) and/or exposed to aerosols of Aspergillus fumigatus Fres. conidia from Days 7 through 16. One-half of each group were necropsied on Day 17 and the remaining animals were necropsied on Day 28. Three rabbits from Group 1 and one from Group 3 died. Changes caused by T-2 toxin included leukopenia, anemia, and increased numbers and morphologic changes in nucleated erythrocytes by Day 21, followed by a regenerative response. Serum alkaline phosphatase (ALP), serum sorbitol dehydrogenase (SDH), and antibody (Ab) response to A. fumigatus, as measured by indirect hemagglutination (IHA), were decreased by T-2 toxin ingestion. Rabbits with aspergillosis had leukocytosis and an increased Ab response (IHA) to A. fumigatus. Histopathologic changes consisting of centrolobular hepatocellular swelling, portal and periportal fibrosis, and lymphocyte necrosis and/or depletion within secondary lymphoid tissue occurred in most T-2 treated rabbits. Normal defense mechanisms to A. fumigatus infection were compromised by T-2 treatment, as evidenced by the severity and extent of lung lesions, greater number of hyphal elements observed, and greater numbers of A. fumigatus colony forming units (CFU) isolated from Group 3 rabbits.

# INTRODUCTION

T-2 mycotoxin is a trichothecene secondary metabolite produced by Fusarium species growing primarily on cereal grains in the temperate climatic zones of North America, Europe, and Asia.[1,2] This toxin has been associated with mycotoxicoses characterized by immunosuppression, such as fatal alimentary toxic aleukia in humans,[1,3,4] Akakabi-byo (red mold) disease in humans and animals in Japan and bean hull poisoning in horses in Japan,[5,6,7] fusariotoxicosis in poultry,[8] and moldy corn toxicosis in cattle.[9,10]

T-2 toxin is an amphipathic molecule that binds to the cell membrane, possibly via receptors,[11-14] and interferes with DNA,[15] RNA,[16] and protein synthesis.[14,17-21] At higher dosages, it may lyse cell membranes with <5.5 Å lesions[22] or possibly cause interaction with free radicals that are formed.[23] Proliferative cells[14] and cells containing many free polysomes[24] (hematopoietic, lymphoid, intestinal crypt, and bursa of Fabricius) are more susceptible to T-2 toxin than nonproliferative cells or cells containing few free polysomes.[19,24-30]

In vitro studies have demonstrated that T-2 decreases chemotactic migration of neutrophils,[31,32] and phagocytosis by AM,[33-35] increases skin graft rejection time,[36] inhibits mitogen-induced blastogenesis of human lymphocytes without mutagenic activity,[31,37,38] inhibits platelet function,[39] and is cytotoxic to lymphocytes.[19,27,29,30,32,40,41] Several in vivo studies with T-2 toxin have produced varying results depending on the species of experimental animal, and the route, amount, and duration of toxin administration.[25,26,30-32,40,42-46] A few

studies have examined the *in vivo* chronic effects of T-2 toxin on cell mediated resistance to an infectious disease.[47] T-2 toxin decreased resistance to mycobacterial infection in mice,[48] increased mortality in chickens challenged with *Salmonella* spp.,[49] increased susceptibility to herpes simplex virus in mice,[50] and increased mortality in mice due to listeriosis.[28,51]

*Aspergillus fumigatus* causes aspergillosis in avian species,[52] in nonimmunocompromised, nonleukopenic individuals,[53,54] and is a major cause of morbidity and mortality in humans with defects in cell-mediated immunity.[55] Few researchers have investigated the interaction of mycotoxins with mycotic disease.[56] Previously, we demonstrated the suppressive effect of T-2 toxin ingestion by rabbits on *in vitro* AM phagocytosis of *A. fumigatus* conidia by their alveolar macrophages (AM).[33] An unknown serum factor appeared to limit phagocytosis by the AM. Because the AM is the first line of cellular defense in aspergillosis, we tested *in vivo* effects of T-2 toxin given orally to rabbits subsequently challenged with aerosols of *A. fumigatus* conidia.

## MATERIALS AND METHODS

Animals Thirty-six New Zealand white female rabbits (Small Stock Industries, P.O. Box 157, Pearidge, AR) weighing 2.5 to 3 kg were used in this study. They were housed in individual cages and given food (Laboratory Rabbit Diet #0533, Teklad, Winfield, IA 52659) and water ad libitum.

T-2 toxin Crystalline T-2 toxin was prepared from extracts of white corn-meal inoculated with Fusarium sporotrichioides Sherb. NRRL 3299 [F. tricinctum (Corda) Sacc.] according to the method of Burmeister.[57] Purity was determined to be 97% by thin-layer chromatographic and gas chromatographic-mass spectral analyses conducted in another laboratory (C.J. Mirocha, University of Minnesota, St. Paul, MN).

T-2 toxin was dissolved in acetone at concentrations that would yield 0.1 ml solution of each daily dosage. This amount of solution was placed in No. 5 gelatin capsules to provide daily dosages for one week. Acetone was allowed to evaporate before assembling the capsule. Capsules were stored at 4°C.

Inoculum Aspergillus fumigatus Fres., strain 0073, was used for the production of conidia and preparation of antigen for double immunodiffusion (ID) tests. This strain was a National Animal Disease Center isolate from the gastric contents of an aborted bovine fetus.[58] Aspergillus fumigatus var ellipticus (VE) was used to prepare antigen for the indirect hemagglutination test (IHA).[59] This isolate was obtained from a human patient at Cook County Hospital, IL. Methods of

growth, collection, and determination of conidial viability have been previously reported.[60]

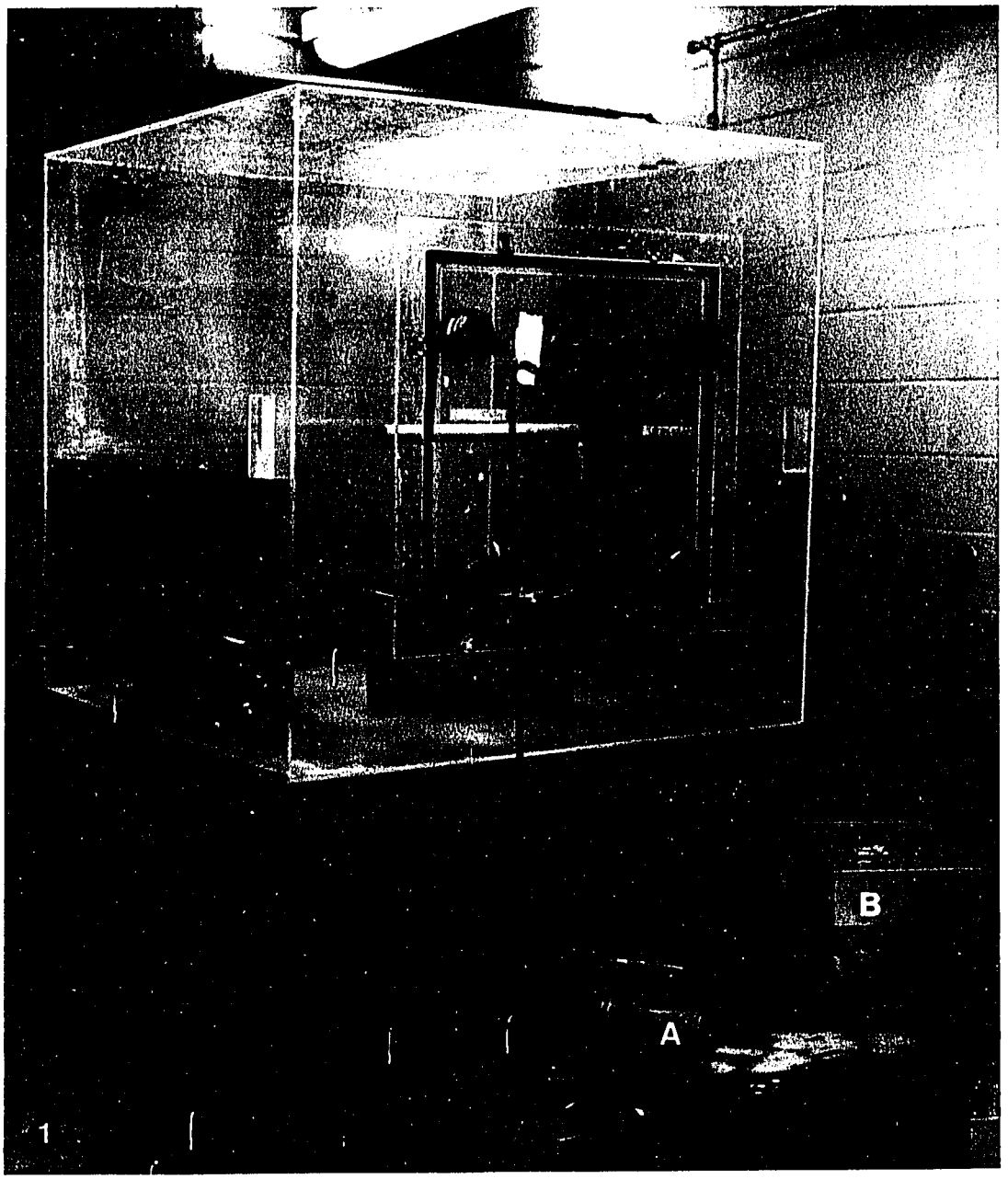
Experimental design The rabbits were randomly assigned to 6 groups of 6 rabbits each (Table 1). Groups 1A and B and 3A and B received 0.5 mg/kg/d of T-2 toxin for the duration of the experiment. The toxin in capsules was given daily with a balling gun. Rabbits were weighed weekly and dosages were adjusted each week according to weight changes. Groups 2A and B and 3A and B were exposed to aerosols of A. fumigatus conidia for 1/2 hour daily from Days 7 through 16. Groups 1A, 2A, and 3A were bled 6 and 24 h after time of aerosolization (Days 16 and 17) and euthanatized on Day 17. Blood and selected tissues were subjected to plate counts to determine viable colony forming units (CFU) of A. fumigatus. The remaining animals (Groups 1B, 2B and 3B) were euthanatized on Day 28.

Aerosol exposure Rabbits from Groups 2 and 3 were confined in individual wire mesh cages and placed in a clear, plexiglass, 1 $m^3$, aerosol chamber (Fig. 1) designed to hold 12 cages/aerosolization. Conidia (100 mg/15min) were aerosolized into the chamber for 30 min exposures each day for 10 consecutive days using an air pump capable of displacing 12 L of air/min. The concentration of conidia within the chamber was recorded once a minute with a particle counter (Royco Particle Monitor Model 218, Royco Instruments, Inc., Menlo Park, CA) set to exclude particles <2 μm in diameter. The level of conidia in the chamber was maintained at >1 X $10^5/m^3$/min. From Days 7 to 16, all

Table 1. Experimental design for study of the interaction of T-2 mycotoxin ingestion by rabbits exposed to *Aspergillus fumigatus* conidia

| Treatment Groups | No. in Group | T-2 toxin given (Days) | Aerosol exposure (Days) | Bled for culture (Days) | Day Killed |
|---|---|---|---|---|---|
| 1 *T-2 only* | | | | | |
| 1A | 6 | 1-16 | ND | 16 and 17 | 17 |
| 1B | 6 | 1-27 | ND | ND | 28 |
| 2 *A. fumigatus conidia only* | | | | | |
| 2A | 6 | ND | 7-16 | 16 and 17 | 17 |
| 2B | 6 | ND | 7-16 | ND | 28 |
| 3 *T-2 + A. fumigatus conidia* | | | | | |
| 3A | 6 | 1-16 | 7-16 | 16 and 17 | 17 |
| 3B | 6 | 1-27 | 7-16 | ND | 28 |

Figure 1. Chamber (1 $m^3$) for exposure of rabbits to aerosols of Aspergillus fumigatus conidia. Air pumps (A) capable of displacing 12 L of air/min. Particle counter (B) to determine concentration of conidia/m volume of air.


B
A
1

rabbits were housed in a room that was positive in pressure and adjacent to the aerosol room.

Personnel protection from aerosol Aerosolization was conducted in a room with controlled and filtered airflow. Human exposure to A. fumigatus was minimized during handling of rabbits and aerosolization by use of full-face safety masks equipped with 0.8 μm filters. Personnel wore coveralls that were autoclaved after use and showers were taken before donning normal laboratory clothing and exiting the facility.

Hematologic and serum biochemical determinations A six ml peripheral blood sample was obtained weekly from each rabbit. Serum was separated by centrifugation, collected, and stored at -70°C. Packed cell volume (PCV), white blood cell (WBC) counts (using a Coulter counter [Coulter Counter, Coulter Electronics, Hialeah, FL]), and differential counts were determined on each blood sample. Serum alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), alanine amino transferase (ALT), aspartate amino transferase (AST), and blood urea nitrogen (BUN) were determined using a centrifical analyzer (Rotachem IIa Centrifical Analyzer, Travenol Labs, Inc., Deerfield, IL). Complement was titrated on all serum samples, using a 50% hemolytic end point.[61]

Serology Antibody (Ab) to A. fumigatus was measured by IHA and ID in all sera collected weekly, and from rabbits killed on Day 27. ID tests were done on microscope slides using 0.6% agarose gel. Aspergillosis fumigatus strain 0073 was grown on neopeptone dyalysate

medium, filtered, dialyzed, and lyophilized.[62] The antigen was used at a concentration of 20 mg/ml. The IHA test was done as previously described,[59] at a dilution of 1:16. Sheep red blood cells (SRBC) were sensitized with antigen made from cultures of VE because it produces a better erythrocyte sensitizing antigen than strain 0073.[59]

Fungal isolation from tissues and blood One ml of blood per animal collected aseptically was plated on Sabouraud's dextrose agar plates. Selected tissue samples (lung, ileal Peyer's patch, and sacculus rotundus) were collected aseptically during necropsy. Tissues were weighed in sterile petri plates, placed into sterile TenBroeck grinders with sufficient sterile saline solution to produce at 1:10 (w/v) concentration. Tenfold dilutions of ground tissues in sterile saline were plated on Sabouraud's dextrose agar in triplicate (1 ml/plate) to determine the average viable CFU of A. fumigatus in the rabbit tissues.

Histopathology Tissue samples from kidney, liver, thymus, heart, adrenal, spleen, pancreas, ileal Peyer's patch, mesenteric and jejunal lymph nodes, sacculus rotundus, appendix, and gastrointestinal tissues were fixed in buffered 10% formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin stain, and examined by light microscopy. Lungs were perfused with 2% glutaraldehyde in 0.1 M sodium cacodylate, and processed as other tissues. Special stains used included Gridley's and Gomori's methenamine silver (GMS) for demonstration of fungi and Gomori's one-step trichrome for evaluation of hepatic fibrosis.

Statistical analysis Group means were compared by standard Student's t-test. Time trends were measured and tested by linear regression analysis.

# RESULTS

Clinical observations Rabbits that died had excessive salivation 4 to 8 days before death. The fur around the mouth, on the neck, and front limbs was wet and tan-stained. These rabbits became lethargic and were apparently inappetent. The amount of feces decreased markedly, and the fecal pellets were small and moist. Three rabbits in Group 1 died after 11, 12, and 15 days of T-2 dosages. One rabbit in Group 3 died on Day 11 (after 11 days of T-2 dosage and 5 days of aerosolization with A. fumigatus conidia).

Body weight (Fig. 2) decreased in Groups 1 and 3 and the rate of weight gain from Group 2 was significant ($p<0.01$).

Hematologic and serum biochemical changes. For most hematologic parameters (Table 2, Figs 3-6), Groups 1 and 3 had similar trends that were different from Group 2 as determined by linear regression. The consistent hematologic trends in rabbits receiving T-2 (Groups 1 and 3) were leukopenia, decreased PCV, and increased nucleated erythrocytes by Day 21, followed by a regenerative response by Day 28 (with the exception of lymphocytes in Group 1). Group 2 rabbits had a significant increase ($p<0.01$) in PCV. Significant increases ($<0.05$) were seen in WBC, neutrophils, and segmented neutrophils of Group 2 rabbits. Significant decreases ($p<0.01$) in lymphocytes counts and eosinophil counts were seen in Group 1 and 3, respectively. Leukocyte counts in Group 1 rabbits decreased ($p<0.05$).

Sixteen of 24 rabbits in Groups 1 and 3 had significant increases ($p<0.05$) in nucleated erythrocytes (NRBC) (Fig. 7), ranging from 9 to

Figure 2. Mean body weights (g) of rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3). Increase in rate of gain for Group 2 was significant ($p<0.01$)

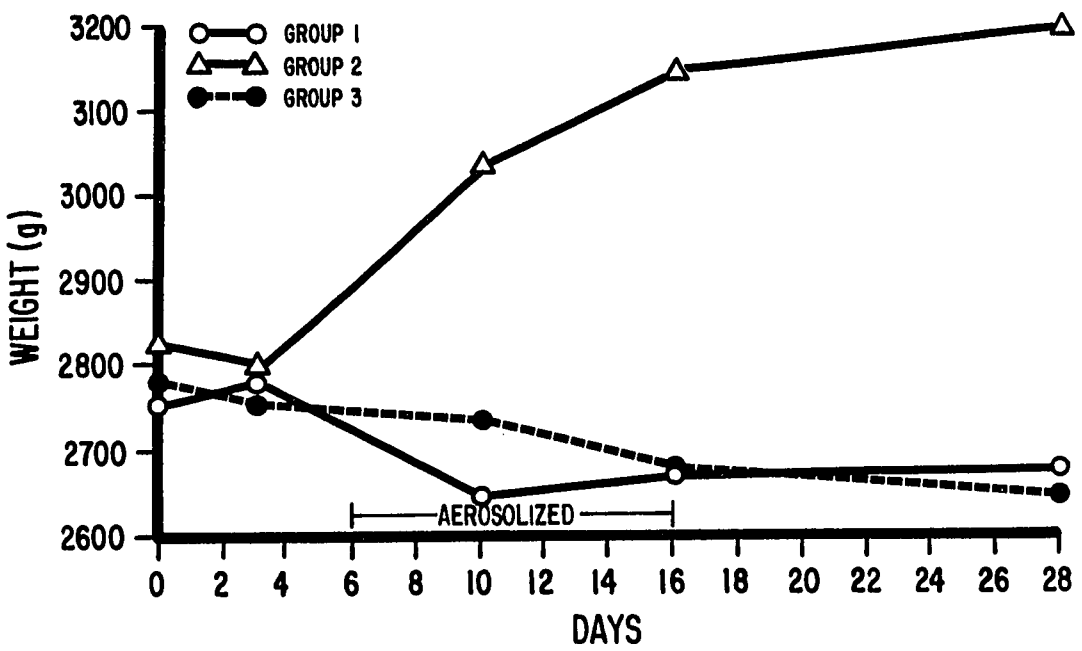

GROUP 1
GROUP 2
GROUP 3
WEIGHT (g)
3200
3100
3000
2900
2800
2700
2600
AEROSOLIZED
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
DAYS

Table 2. Sequential changes in body weights and hematologic values of rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3)*

| | Group | Days 0 | 6 | 14 | 21 | 28 |
|---|---|---|---|---|---|---|
| Body weight (g) | 1 | 2754 ± 52 | 2778 ± 58 | 2640 ± 77 | 2667 ± 99 | 2675 ± 140 |
| | 2 | 2823 ± 43 | 2798 ± 46 | 3035 ± 63 | 3144 ± 73 | 3195 ± 146 |
| | 3 | 2782 ± 49 | 2755 ± 85 | 2737 ± 86 | 2678 ± 103 | 2644 ± 121 |
| PCV (%) | 1 | 32.3 ± 0.7 | 33.6 ± 0.6 | 31.3 ± 0.9 | 28.4 ± 1.6 | 34.6 ± 2.6 |
| | 2 | 32.3 ± 1.0 | 33.9 ± 0.5 | 34.4 ± 0.4 | 34.8 ± 1.4 | 38.0 ± 1.3 |
| | 3 | 32.3 ± 1.3 | 33.4 ± 1.0 | 30.7 ± 1.2 | 31.1 ± 1.5 | 32.8 ± 3.0 |
| WBC (N/µl) | 1 | 5181 ± 294 | 4818 ± 424 | 5711 ± 1196 | 2927 ± 256 | 4175 ± 1181 |
| | 2 | 5571 ± 599 | 5756 ± 648 | 6980 ± 409 | 7713 ± 1520 | 7184 ± 1174 |
| | 3 | 4739 ± 248 | 4123 ± 409 | 3648 ± 670 | 2789 ± 639 | 5363 ± 789 |
| Lymphocytes (N/µl) | 1 | 3243 ± 168 | 3240 ± 322 | 2975 ± 464 | 2037 ± 345 | 1996 ± 457 |
| | 2 | 3268 ± 246 | 3554 ± 320 | 4101 ± 405 | 4525 ± 470 | 3533 ± 1195 |
| | 3 | 2666 ± 221 | 2786 ± 261 | 2537 ± 417 | 2185 ± 409 | 3138 ± 481 |
| Neutrophils (N/µl) | 1 | 1767 ± 180 | 1350 ± 167 | 2970 ± 872 | 892 ± 321 | 2332 ± 833 |
| | 2 | 2223 ± 375 | 2078 ± 431 | 2705 ± 283 | 3610 ± 1379 | 3433 ± 566 |
| | 3 | 1851 ± 113 | 1201 ± 206 | 1186 ± 272 | 686 ± 242 | 2388 ± 716 |
| Segmented neutrophils (N/µl) | 1 | 1699 ± 168 | 1289 ± 154 | 2934 ± 864 | 878 ± 322 | 2319 ± 829 |
| | 2 | 2162 ± 358 | 1852 ± 387 | 2600 ± 279 | 3587 ± 1383 | 3414 ± 566 |
| | 3 | 1809 ± 116 | 1141 ± 187 | 1167 ± 276 | 676 ± 244 | 2330 ± 720 |
| Banded neutrophils (N/µl) | 1 | 67 ± 32 | 61 ± 28 | 35 ± 14 | 14 ± 14 | 13 ± 13 |
| | 2 | 61 ± 20 | 225 ± 97 | 105 ± 36 | 23 ± 16 | 19 ± 13 |
| | 3 | 42 ± 16 | 60 ± 27 | 19 ± 7 | 10 ± 9 | 8 ± 8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Monocytes (N/µl) | 1 | 171 ± 38 | 131 ± 36 | 119 ± 61 | 12 ± 7 | 17 ± 8 |
| | 2 | 191 ± 60 | 133 ± 37 | 112 ± 38 | 20 ± 13 | 12 ± 12 |
| | 3 | 161 ± 28 | 93 ± 37 | 27 ± 13 | 29 ± 24 | 21 ± 21 |
| Eosinophils (N/µl) | 1 | 23 ± 14 | 45 ± 22 | 5 ± 5 | 0 | 0 |
| | 2 | 26 ± 11 | 116 ± 79 | 12 ± 8 | 6 ± 6 | 0 |
| | 3 | 27 ± 8 | 21 ± 9 | 0 | 0 | 0 |
| Nucleated erythrocytes/ 100 WBC | 1 | 1 | 7 ± 3 | 51 ± 44 | 17 ± 5 | 45 ± 45 |
| | 2 | 0 | 2 ± 1 | 4 ± 1 | 3 ± 1 | 3 ± 2 |
| | 3 | 0 | 8 ± 4 | 55 ± 23 | 122 ± 64 | 15 ± 11 |

*Through Day 14, there were a minimum of 10, 12, and 11 rabbits in groups 1, 2, and 3, respectively. On Days 21 and 28, there were 5, 6, and 6 rabbits in groups 1, 2, and 3, respectively.

Figure 3. Mean packed cell volume (%) from rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3). Increase for Group 2 was significant ($p<0.01$)

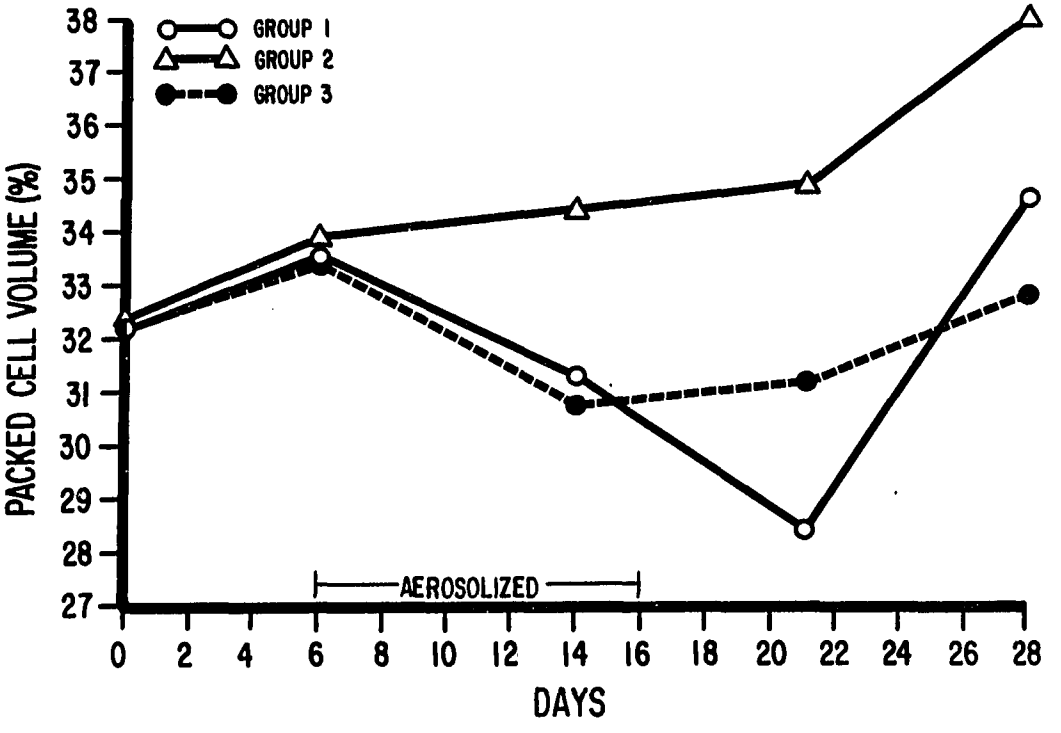

GROUP 1
GROUP 2
GROUP 3
PACKED CELL VOLUME (%)
38
37
36
35
34
33
32
31
30
29
28
27
AEROSOLIZED
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
DAYS

Figure 4. Mean total white blood cell counts (N/μl) from rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3). Decrease for Group 1 and increase for Group 2 were significant ($p<0.05$)

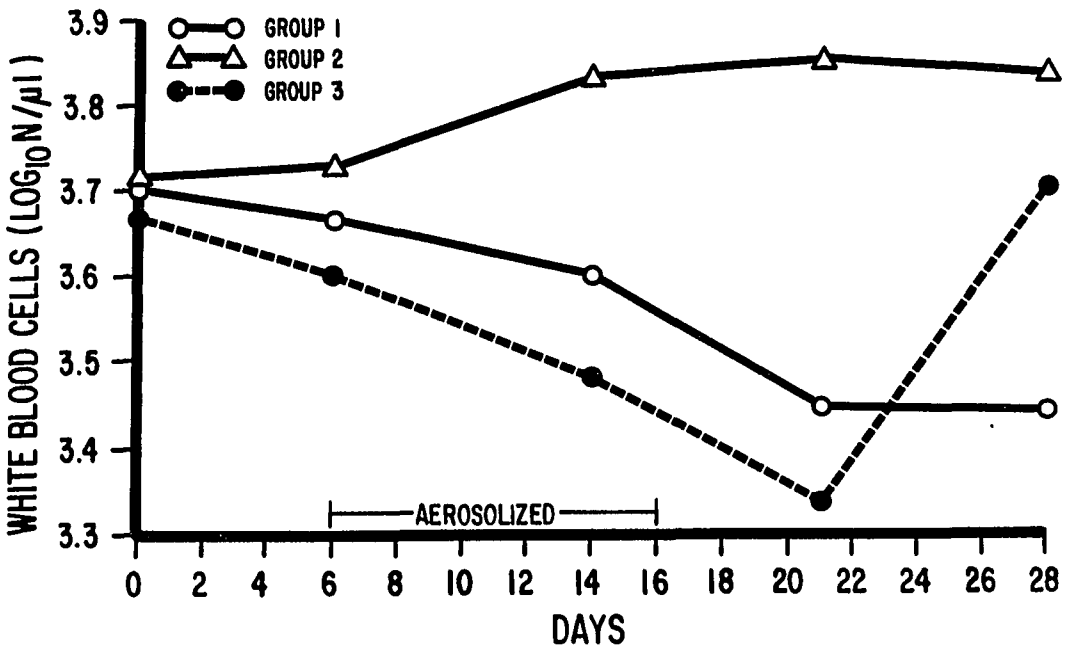

GROUP 1
GROUP 2
GROUP 3
WHITE BLOOD CELLS (LOG10N/µl)
3.9
3.8
3.7
3.6
3.5
3.4
3.3
AEROSOLIZED
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DAYS

Figure 5. Mean lymphocytes (N/μl) from rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3). Decrease for Group 1 was significant ($p<0.01$)

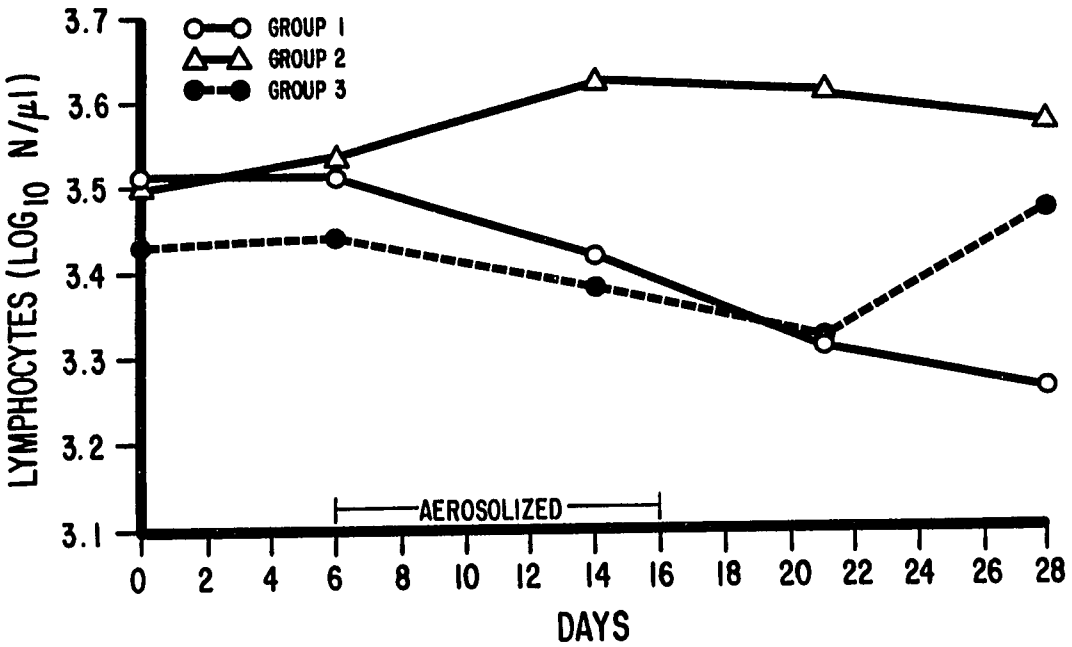

GROUP 1
GROUP 2
GROUP 3
LYMPHOCYTES (LOG10 N/μl)
3.7
3.6
3.5
3.4
3.3
3.2
3.1
AEROSOLIZED
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
DAYS

Figure 6. Mean neutrophils (N/μl) from rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3). Increase for Group 2 was significant ($p<0.05$)

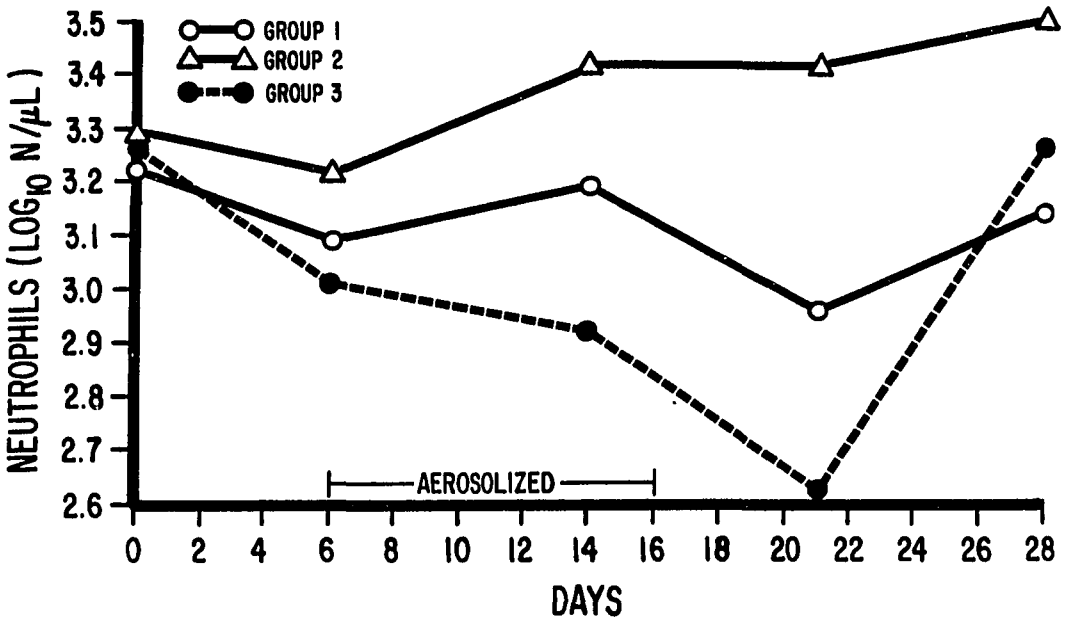
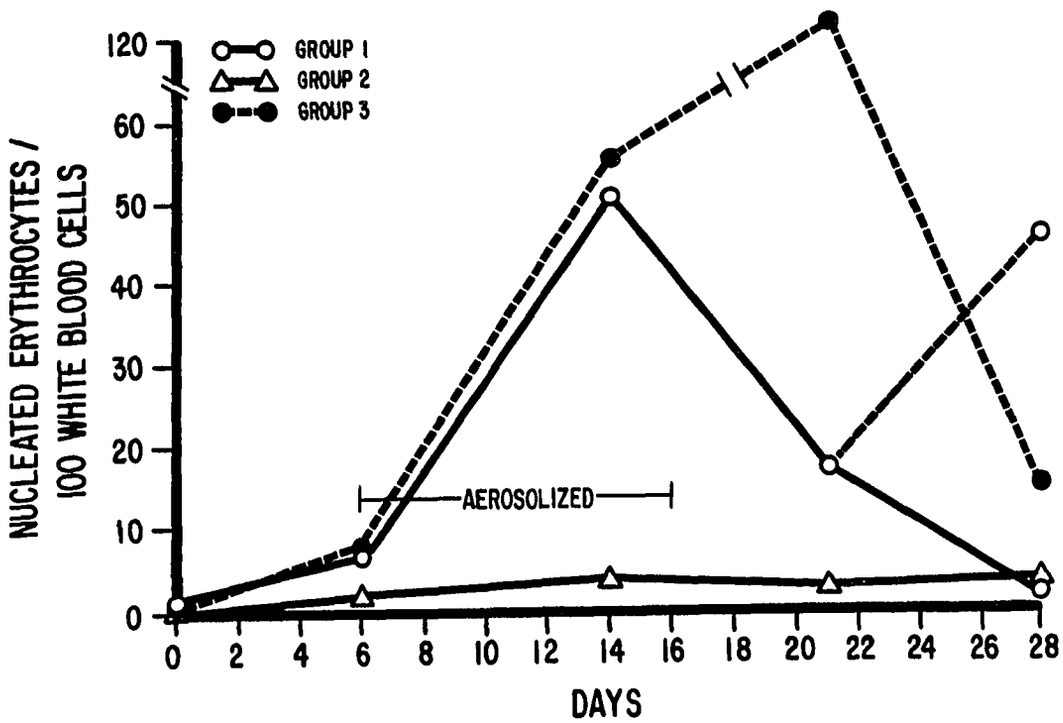

GROUP 1
GROUP 2
GROUP 3
NEUTROPHILS (LOG10 N/μL)
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
AEROSOLIZED
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
DAYS

Figure 7. Mean nucleated erythrocytes/100 WBC from rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3). Increases for Groups 1 and 3 were significant ($p<0.05$). (High mean for Group 1 on Day 28 due to 224 NRBC/100 WBC in one rabbit.)


GROUP 1
GROUP 2
GROUP 3
NUCLEATED ERYTHROCYTES / 100 WHITE BLOOD CELLS
120
60
50
40
30
20
10
0
AEROSOLIZED
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DAYS

446 NRBC/100 WBC. Polychromasia, poikilocytosis, anisocytosis, echinocytosis, acanthocytosis, and basophilic stipling were common findings. No changes were noted in other blood cell types.

The reductions in serum ALP and SDH concentrations (Table 3) in Groups 1 and 3 were significant ($p<0.01$). There were no significant changes in ALT, AST, and BUN levels.

Serology There was no Ab response to A. fumigatus as measured by IHA (Table 4) in Group 1 rabbits. In Group 2, 5 of 6 rabbits had Ab on Day 17, and 6 of 6 were positive for Ab on Days 21 and 28. In Group 3, only 1 of 5, 2 of 6, and 1 of 6 rabbits were positive on Days 17, 21, and 28, respectively. Only 2 rabbits had precipitating Ab (ID test) (one rabbit each, in Groups 2 and 3) on Day 28.

Neither ingestion of T-2 toxin nor aerosolization with A. fumigatus conidia appeared to have an effect on serum complement activity of the rabbit.

Fungal isolation from tissues and blood The number of A. fumigatus CFU/g lung tissue (Table 5) was negligible in Group 1 rabbits on Days 17 and 28. Group 3 had approximately 80% more CFU/g lung tissue than Group 2. Both Groups 2 and 3 had a 99% reduction in the number of CFU in lung tissue from Day 17 to 28. On Day 17, there were few CFU in ileal Peyer's patch and sacculus rotundus tissues of most rabbits in Groups 2 and 3, but by Day 28 no A. fumigatus was recovered. Aspergillus fumigatus was isolated from the blood of only 4 rabbits (2 in each of the exposed groups) at 6 h post aerosolization and was not isolated at 24 h.

Table 3. Mean serum alkaline phosphatase (ALP) and sorbitol dehydrogenase (SDH) concentrations (IU/L) from rabbits* given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3)

| Day | Group 1 | | Group 2 | | Group 3 | |
|---|---|---|---|---|---|---|
| | ALP | SDH | ALP | SDH | ALP | SDH |
| 0 | 79 | 317 | 65 | 293 | 87 | 396 |
| 6 | 45 | 234 | 72 | 254 | 48 | 195 |
| 14 | 31 | 136 | 58 | 226 | 28 | 133 |
| 17 | 41 | 99 | 54 | 189 | 20 | 100 |
| 21 | 34 | 145 | 56 | 243 | 26 | 172 |
| 28 | 54 | 211 | 55 | 248 | 31 | 147 |
| | 50†‡ (3.6) | 214‡ (13.9) | 62 (2.5) | 247 (8.4) | 46‡ (4.0) | 192‡ (14.2) |

*Through Day 17, there were a minimum of 10, 12, and 11 rabbits in Groups 1, 2, and 3, respectively. On Days 21 and 28, there were 5, 6, and 6 rabbits in Groups 1, 2, and 3, respectively.

†Mean, figure in parentheses is standard error.

‡$p<0.01$.

Table 4. Antibody response to A. fumigatus (measured by Indirect Hemagglutination) of sera from rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3)

| Group (Treatment) | Days | | |
|---|---|---|---|
| | 17 | 21 | 28 |
| 1 (T-2 toxin) | 0(4)* | 0(5) | 0(5) |
| 2 (A. fumigatus) | 5(6) | 6(6) | 6(6) |
| 3 (Both) | 1(5) | 2(6) | 1(6) |

*No. of rabbits with positive response (No. of rabbits tested).

Table 5. Mean number of colony forming units (CFU) of Aspergillus fumigatus/g in tissues of rabbits given T-2 toxin orally for 17 or 28 days (Groups 2 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3)

| Day | Group | No. of Rabbits | Tissue | | |
|---|---|---|---|---|---|
| | | | Lung | Peyer's Patch | Sacculus Rotundus |
| 17 | 1A | 4 | 0.4 x 10 | 0 | 0 |
| 17 | 2A | 6 | 168 x 10 | 2.3 | 6.8 |
| 17 | 3A | 5 | 776 x 10 | 0.4 | 5.1 |
| 28 | 1B | 5 | 0.1 x 10 | 0 | 0 |
| 28 | 2B | 6 | 1.1 x 10 | 0 | 0 |
| 28 | 3B | 6 | 6.0 x 10 | 0 | 0 |

Gross lesions Lung lesions were observed in all rabbits exposed to A. fumigatus conidia (Groups 2 and 3). They consisted of multiple, firm, tan to grey, nodules widely distributed throughout the lung parenchyma. Subpleural nodules tended to be raised above the lung parenchyma. The thymus appeared atrophic in 2 rabbits that were treated with T-2 toxin (Groups 1 and 3). Two rabbits that died (Group 1A) and 2 rabbits that were killed (Group 3A) had congested gastric mucosa and focal mucosal erosions. Gross lesions were not observed in other body organs and tissues examined.

Microscopic lesions

Lung Microscopic lung lesions were present in all rabbits that had been aerosolized with A. fumigatus conidia (Groups 2 and 3) (Table 6). They consisted of multiple, often coalescing, granulomas or pyogranulomas composed of masses of epithelioid macrophages, neutrophils, lymphocytes, plasma cells, and few multinucleated giant cells. These lesions were most prominent within terminal bronchioles and associated alveoli. Embedded within some of these granulomas were short, radiating and branching hyphae that were often surrounded by a mantle of acidophilic homogeneous precipitate. Small clusters of epithelioid macrophages and alveolar macrophages containing one or several A. fumigatus conidia were scattered within alveolar spaces at the periphery of the granulomas. Type II pneumocyte hyperplasia and squamous metaplasia were frequently seen in areas of granuloma formation. Lung lesions in non-T-2 treated rabbits, aerosolized with A.

Table 6. Frequency of occurrence and location of major microscopic lesions in rabbits given T-2 toxin orally for 17 or 28 days (Groups 1 and 3) and/or rabbits exposed to aerosols of A. fumigatus conidia on Days 7 through 16 (Groups 2 and 3)

| Organ/ Tissue | Necropsied on Day | Group | Lesion | Rabbits |
|---|---|---|---|---|
| Lung: | 17 | 1A | NSL* | |
| | | 2A | Medium granulomas | 6(6)† |
| | | | Hyphae and conidia | 6(6) |
| | | | Increased lymphocytes and plasma cells | 6(6) |
| | | | Increased neutrophils | 6(6) |
| | | 3A | Large pyogranulomas | 5(5) |
| | | | Masses of hyphae and conidia | 5(5) |
| | | | Decreased inflammatory reaction | 5(5) |
| Lung: | 28 | 1B | NSL | |
| | | 2B | Small granulomas | 6(6) |
| | | | Few hyphae and conidia | 2(6) |
| | | | Increased lymphocytes and plasma cells | 6(6) |
| | | | Decreased neutrophils | 6(6) |
| | | 3B | Medium pyogranulomas | 3(6) |
| | | | Hyphae and conidia | 3(6) |
| | | | Decreased inflammatory reaction | 6(6) |

| | | | | |
|---|---|---|---|---|
| Liver: | 17 | 1A | Centrolobular hepatocellular degeneration<br>Mild portal fibrosis | 2(4)<br>1(4) |
| | | 2A | NSL | |
| | | 3A | Centrolobular hepatocellular degeneration<br>Mild portal fibrosis<br>Bile duct reduplication | 1(5)<br>3(5)<br>2(5) |
| Liver: | 28 | 1B | Centrolobular hepatocellular degeneration<br>Mild/moderate portal fibrosis<br>Bile duct reduplication | 3(5)<br>4(5)<br>1(5) |
| | | 2B | NSL | |
| | | 3B | Centrolobular hepatocellular degeneration<br>Mild/Moderate portal fibrosis<br>Bile duct reduplication | 2(6)<br>5(6)<br>5(6) |
| Appendix/ Sacculus rotundus: | 17 | 1A | Mild lymphoid necrosis/depletion | 4(4) |
| | | 2A | NSL | |
| | | 3A | Moderate/severe lymphocyte necrosis/depletion<br>Hyphae or conidia<br>Focal granulomas | 5(5)<br>4(5)<br>4(5) |
| Appendix/ Sacculus rotundus: | 28 | 1B | Mild/moderate lymphocyte necrosis/depletion | 5(5) |
| | | 2B | NSL | |
| | | 3B | Moderate/severe lymphocyte necrosis/depletion | 6(6) |

*No significant lesions.

†No. of rabbits with lesions (No. of rabbits examined).

Table 6 continued

| Organ/ Tissue | Necropsied on Day | Group | Lesion | Rabbits |
|---|---|---|---|---|
| Ileal Peyer's Patch: | 17 | 1A | Mild/moderate lymphocyte necrosis/depletion | 4(4) |
| | | 2A | NSL | |
| | | 3A | Mild/moderate lymphocyte necrosis/depletion | 5(5) |
| Ileal Peyer's Patch: | 28 | 1B | Mild/moderate lymphocyte necrosis | 3(5) |
| | | 2B | NSL | |
| | | 3B | Mild/moderate lymphocyte necrosis | 5(6) |
| Mesenteric/ Jejunal Lymph Nodes | 17 | 1A | Mild lymphocyte necrosis/depletion | 2(4) |
| | | 2A | NSL | |
| | | 3A | Severe lymphocyte necrosis/depletion | 2(5) |
| Mesenteric/ Jejunal Lymph Nodes | 28 | 1B | NSL | |
| | | 2B | NSL | |
| | | 3B | Mild lymphocyte necrosis | 2(6) |

| | | | | |
|---|---|---|---|---|
| Spleen | 17 | 1A | Nucleated erythrocytes in red pulp | 4(4) |
| | | 2A | NSL | |
| | | 3A | Mild/moderate lymphocyte necrosis/depletion<br>Nucleated erythrocytes in red pulp | 2(5)<br>5(5) |
| Spleen | 28 | 1B | Nucleated erythrocytes in red pulp | 5(5) |
| | | 2B | NSL | |
| | | 3B | Nucleated erythrocytes in red pulp | 6(6) |
| Thymus | 17 | 1A | Severe lymphoid depletion | 1(4) |
| | | 2A | NSL | |
| | | 3A | Severe lymphoid depletion | 1(5) |
| Thymus | 28 | 1B | Mild lymphoid depletion | 1(5) |
| | | 2B | NSL | |
| | | 3B | NSL | |

fumigatus conidia and killed on Day 17 (Groups 2A) had larger lung granulomas than those rabbits killed on Day 28 (Group 2B). While neutrophils constituted a significant cellular component of the larger granulomas in Group 2A (Fig. 8), lymphocytes and plasma cells predominated in the lesions of Group 2B (Fig. 9). Additionally, fewer fungal hyphae and conidia were observed in lung lesions of Group 2B than those of Group 2A.

Lung lesions in Groups 3A and 3B (T-2 treated and aerosolized with A. fumigatus conidia) appeared to be more severe and more extensive than those of Groups 2A and 2B. In Group 3A, large areas of the lung parenchyma had been replaced by irregular masses of granulomatous or pyogranulomatous exudate. Although the lesions appeared to be centered within alveoli, terminal bronchioles, and bronchi, some granulomas had obliterated all normal parenchymal structures as they expanded outward. In some areas, the granulomas had breached mucosal walls of several bronchioles and tended to project into their lumens. In other areas, bronchiolar lumina were filled with necrotic, amorphous material. The centers of many granulomas were necrotic and most contained tangled masses of hyphal elements and conidia surrounded by radiating acidophilic clubs (Fig. 10). Type II pneumocyte hyperplasia and hyperplasia of bronchiolar epithelium were prominent in many areas of the lung. Macrophages containing 2 to 10 conidia were frequently found in alveoli adjacent to the granulomas.

Lung lesions in Group 3B were essentially similar to those in Group 3A rabbits. However, the granulomas tended to be smaller and

Figure 8. Medium-sized granuloma in lung of a rabbit exposed to aerosols of A. fumigatus conidia on Days 7 through 16 and necropsied on Day 17 (Group 2A). Neutrophils constitute a significant cellular component of the lesion. H&E stain; Bar = 50 μm

Figure 9. Small granuloma in lung of a rabbit exposed to aerosols of A. fumigatus conidia on Days 7 through 16 and necropsied on Day 28 (Group 2B). Lymphocytes and plasma cells predominate in these lesions. H&E stain; Bar = 50 μm

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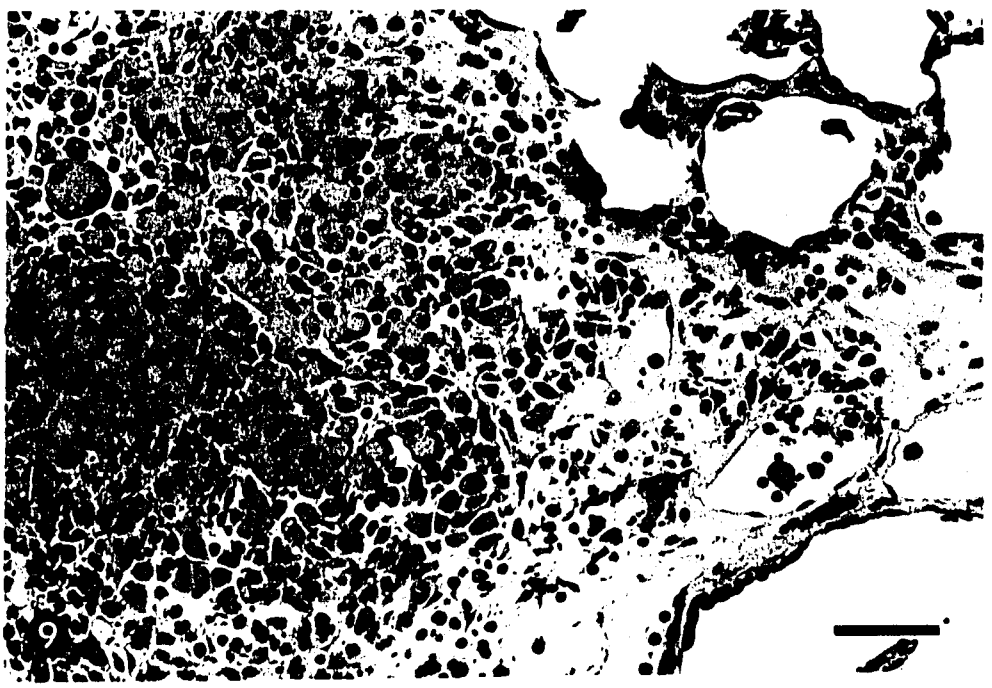

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less numerous in Group 3B (Fig. 11) than in Group 3A rabbits. Hyphae and conidia were frequently found in the granulomas but they were less numerous in Group 3B than in Group 3A.

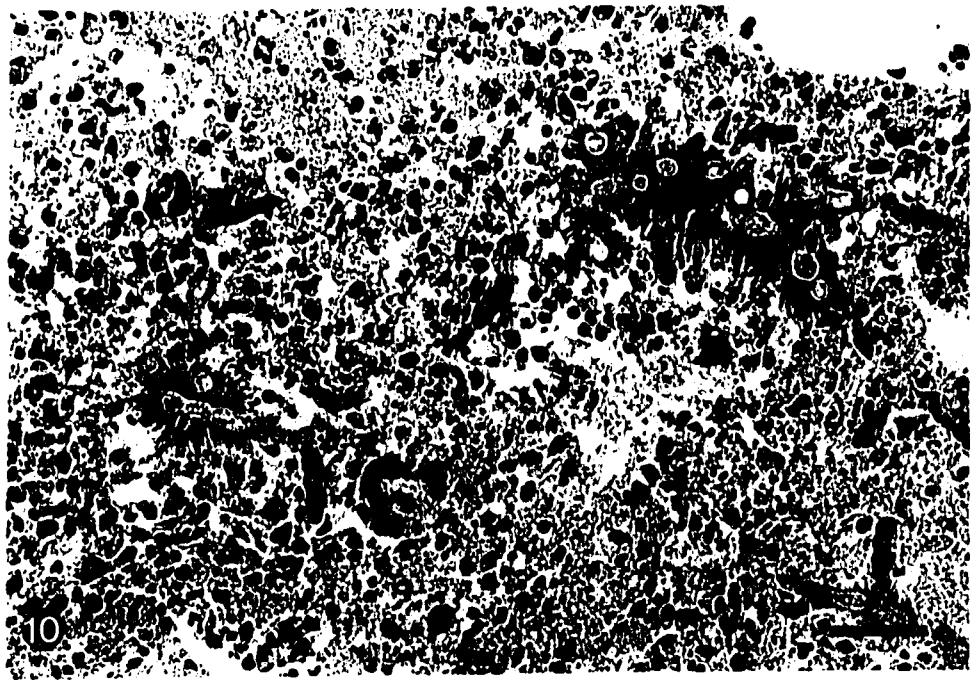
Liver Hepatic lesions consisting of centrolobular hepatocellular degeneration (Fig. 12) and mild portal fibrosis (Fig. 13) were found in approximately one-half of the rabbits in Groups 1A and 3A. Rabbits treated with T-2 toxin and killed at Day 28 (Groups 1B and 3B), had more severe hepatic lesions consisting of centrolobular hepatocellular degeneration, mild to moderate portal and periportal fibrosis, and bile duct proliferation. There were no significant hepatic lesions in Groups 2A and 2B rabbits.

Appendix and sacculus rotundus Changes in the appendix and sacculus rotundus of T-2 treated rabbits (Groups 1 and 3) ranged from mild to severe lymphocyte necrosis within the dome, corona, and lymphoid follicles. Debris-laden macrophages were frequently found in areas of lymphocyte necrosis. Small focal granulomas (Fig. 14) were found in the appendix and/or sacculus rotundus of 4 rabbits in Group 3A. Some of these granulomas contained variable numbers of fungal hyphae and conidia (Fig. 15).

Ileal Peyer's patch There was mild to moderate lymphocyte necrosis and/or lymphoid depletion in nearly all T-2 treated rabbits (Groups 1 and 3). Degenerate macrophages and neutrophils were present in areas of lymphocyte necrosis. These changes were most prominent in Group 3A rabbits.

Figure 10. Large pyogranuloma in lung of a rabbit given T-2 toxin orally for 17 days, exposed to aerosols of A. fumigatus conidia on Days 7 through 16, and necropsied on Day 17 (Group 3A). Center of granuloma was necrotic and contained tangled masses of hyphae and conidia surrounded by radiating acidophilic clubs. H&E stain; Bar = 25 μm

Figure 11. Medium-sized pyogranuloma in lung of a rabbit given T-2 toxin orally for 28 days, exposed to aerosols of A. fumigatus conidia on Days 7 through 16, and necropsied on Day 28 (Group 3B). H&E stain; Bar = 50 μm



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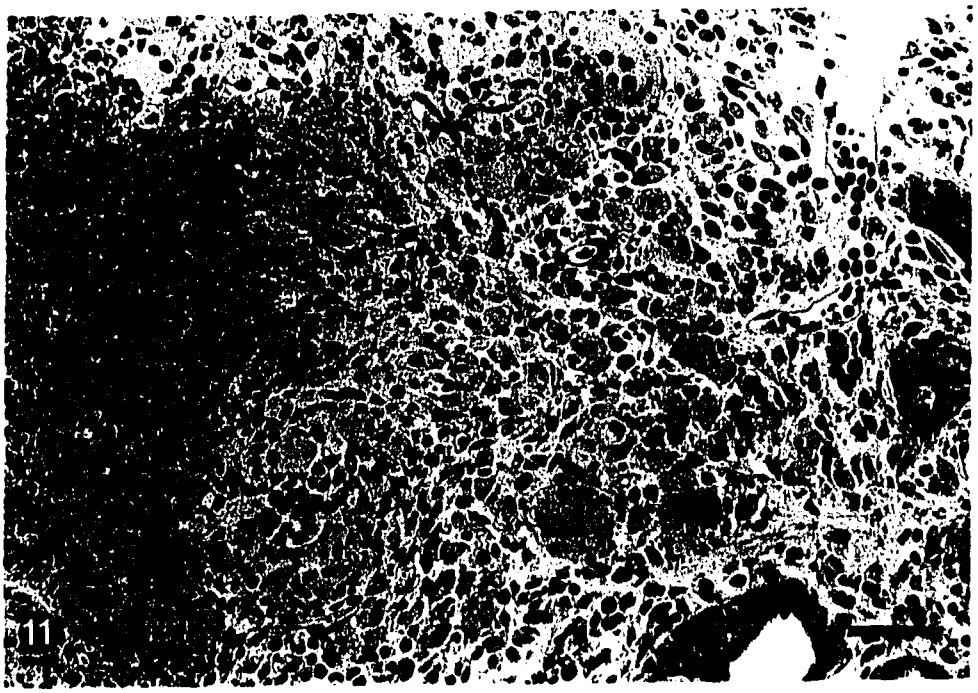

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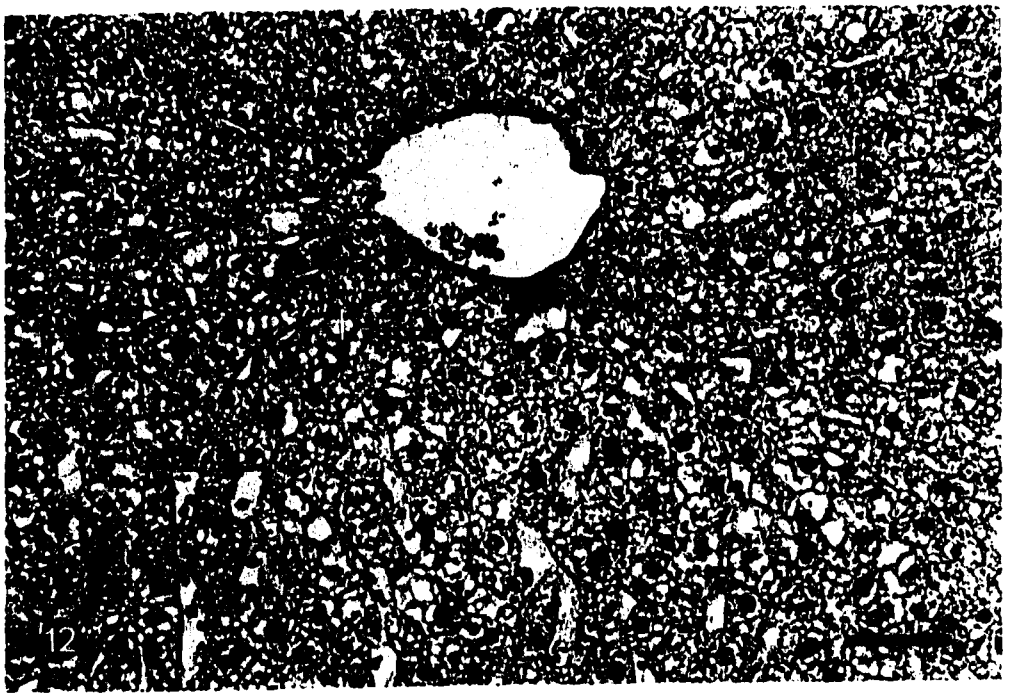
Figure 12. Centrolobular hepatocellular degeneration in a rabbit given T-2 toxin orally for 28 days, exposed to aerosols of A. fumigatus conidia on Days 7 through 16, and necropsied on Day 28 (Group 3B). Bar = 50 μm

Figure 13. Portal and periportal fibrosis in liver from a rabbit given T-2 toxin orally for 28 days, exposed to aerosols of A. fumigatus conidia on Days 7 through 16, and necropsied on Day 28 (Group 3B). H&E stain; Bar = 100 μm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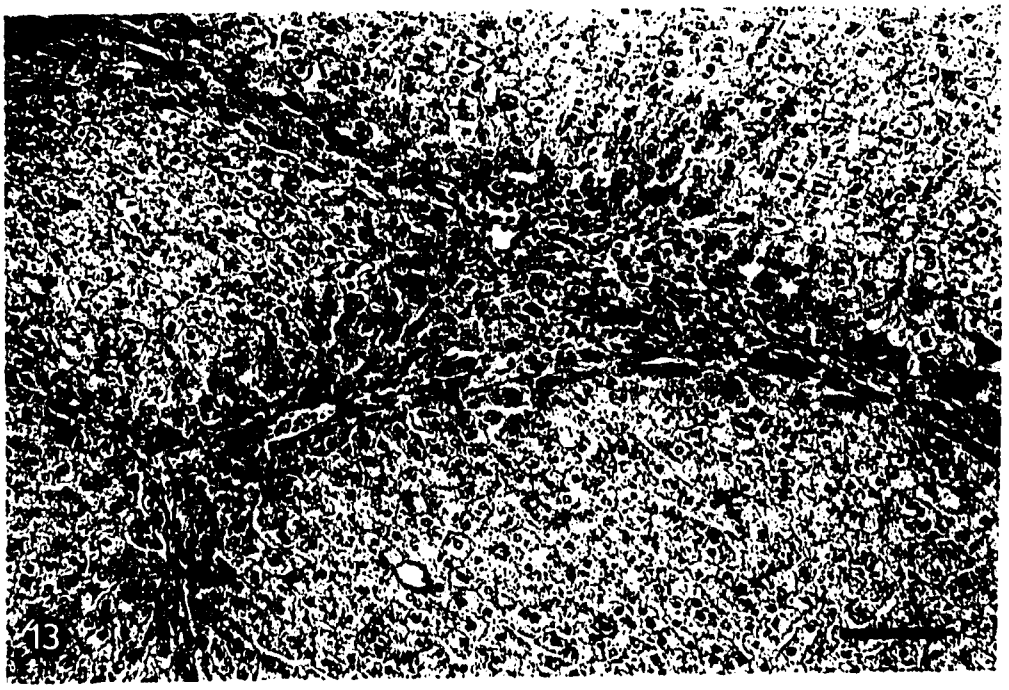
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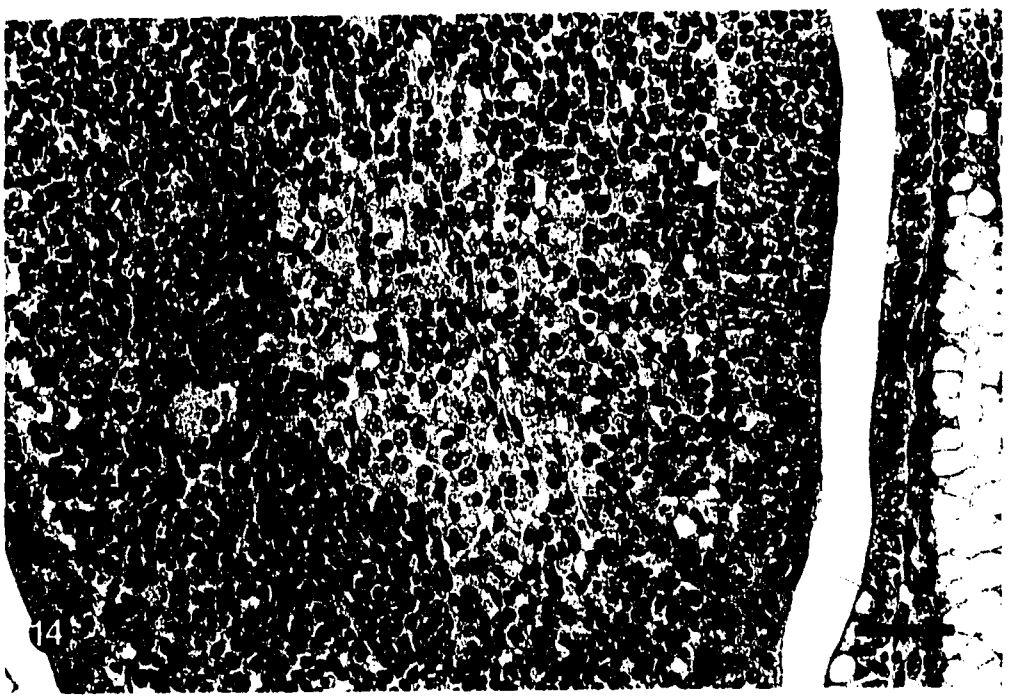
Figure 14. Focal collection of macrophages in sacculus rotundus of a rabbit given T-2 toxin orally for 17 days, exposed to aerosols of A. fumigatus conidia on Days 7 through 16, and necropsied on Day 17 (Group 3A). H&E stain; Bar = 50 μm

Figure 15. Aspergillus fumigatus hyphae in lesion in sacculus rotundus of a rabbit given T-2 toxin orally for 17 days, exposed to aerosols of A. fumigatus conidia on Days 7 through 16, and necropsied on Day 17 (Group 3A). H&E stain; Bar = 25 μm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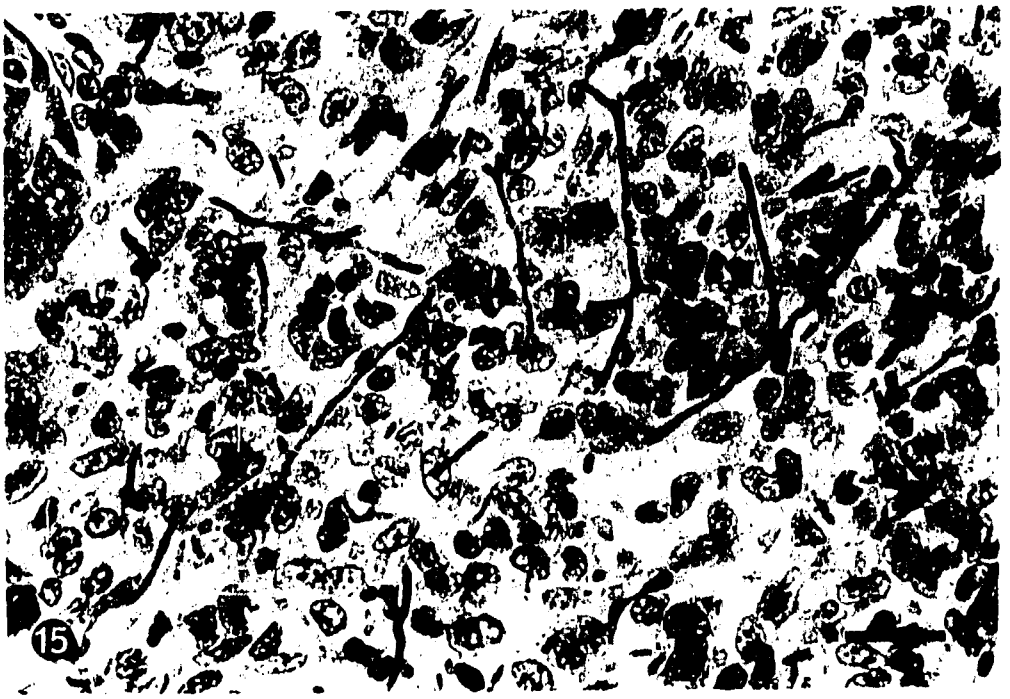
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Mesenteric/jejunal lymph nodes Lymph node changes consisting of mild to severe lymphocyte necrosis or lymphoid depletion were observed in only a few T-2 treated rabbits.

Spleen Lymphocyte necrosis and lymphoid depletion occurred in 2 rabbits from Group 3A. Nucleated erythrocyte precursors were numerous within the red pulp of all T-2 treated rabbits (Groups 1 and 3).

Thymus Severe lymphoid depletion was observed in the thymus of one rabbit from Group 1A and one rabbit from Group 3A.

Stomach Gastric mucosal hyperemia, hemorrhage, and superficial mucosal necrosis were observed in 2 rabbits from Group 3A and rabbits that died early (Group 1A).

# DISCUSSION

We previously demonstrated that ingestion of T-2 toxin by rabbits for 3 weeks caused a depression of *in vitro* phagocytosis by their AM due to a serum factor.[33] The AM were decreased in numbers and in ability to phagocytize *A. fumigatus* conidia. Another *in vitro* study has shown the requirement for a macromolecular serum component(s) necessary for full fungistatic capability of activated alveolar macrophages.[63]

The present *in vivo* study demonstrated that ingestion for 17 or 28 days of 0.5 mg T-2 toxin /kg/d by rabbits and challenge with aerosols of *A. fumigatus* conidia for 10 consecutive days decreased the cell mediated resistance and immune response necessary to combat aspergillosis. Changes included reduced AM efficiency, suppression of hematopoiesis (with resulting transient anemia and leukopenia), lymphocyte necrosis, and depleted lymphoid tissue.

The rabbit's defense mechanisms to *A. fumigatus* infection were compromised by T-2 treatment. This was reflected in the severity and extent of lung lesions in Group 3 rabbits. Numerous fungal hyphae and conidia were observed in Group 3 rabbits killed early (Group 3A) as well as in those killed late in the course of the experiment (Group 3B). Additionally, focal granulomas containing fungal elements were observed in the appendix and sacculus rotundus of these rabbits. These changes are evidence of a diminished capacity of the phagocytic cells in T-2 treated rabbits to destroy *A. fumigatus*. In contrast, the much smaller lung granulomas and the relatively fewer fungal elements

observed in Group 2B rabbits suggested a partial resolution of these lesions. These findings indicate that normal rabbits are capable of mounting effective host defenses against A. fumigatus infection. This is in agreement with other researchers.[64-66]

Considerably more CFU of A. fumigatus were isolated from T-2 treated rabbits (Group 3) than from rabbits that were not treated with the toxin. This was additional evidence for decreased phagocytic and killing ability of alveolar macrophages in the T-2 treated rabbits.

The diminished cell-mediated resistance to aspergillosis caused by T-2 toxin ingestion may be a reflection of the amphipathic effect of the toxin[11-13, 67] primarily on proliferating undifferentiated germinal cells and/or blast cells.[24] The toxin is thought to interact initially with the outer phospholipid bilayer of the cell, possibly binding to the receptors on the cell membrane, interfering with signal transfer, and causing decreased RNA, DNA, and protein synthesis.[14,19-21] At greater dosages, T-2 may lyse cell membranes with formation of <5.5 A lesions.[22] Concurrently, free radicals that are formed may potentiate the direct effect of T-2 toxin on the membrane.[23] A decrease in numbers of AM,[33,34] neutrophils, and other leukocytes and a decrease in synthesis of monokines or lymphokines would suppress the cellular response to the disease.

The Ab response of Group 3 rabbits to A. fumigatus, as measured by IHA, may be a result of lymphopenia. This could cause a decrease in T-helper cell numbers and/or the possible inability of B cells to differentiate into plasma cells with subsequent decrease in Ab

synthesis. Similar results were found in rabbits given immunosuppressive drugs.[68] Opsonization of the conidia by Ab is potentially reduced because only 24% of Group 3 rabbits produced antibodies to A. fumigatus. Complement activity in rabbits was not affected by T-2 ingestion in this study, nor in studies with guinea pigs[47] or monkeys.[40] Therefore, opsonization may still occur, but at reduced efficiency, utilizing complement and the C3b receptors on the AM resulting in the Ab-independent alternate pathway mechanism of killing conidia.

The initial decrease in all leukocyte numbers in Group 3 rabbits explains the decreased inflammatory response seen in tissue sections. This and other studies[29,69,70] have shown a resumption of hematopoiesis (with the exception of lymphocytes)[70] after 2 to 3 weeks of T-2 toxin ingestion. The mechanism of recovery is not known, but could be explained by hepatic biotransformation of T-2 toxin into metabolites[26,42,71,72] that do not affect hematopoiesis.[69] Interaction of other mycotoxins in natural T-2 mycotoxicoses also may influence hematopoiesis.

T-2 toxin is known to cause hypoplasia of bone marrow and splenic red pulp, followed by regeneration of hematopoietic cells after approximately 2 to 3 weeks of toxin consumption.[24,29,41,43,73] Dietary studies with T-2 toxin showed that the suppression was generally unrelated to undernutrition.[29,69] Marginal anemia, characterized in T-2 treated rabbits by the transient 5 to 12% decrease in PCV, is additional evidence of temporary hematopoietic suppression. T-2 toxin

was responsible for the morphologic changes and reduced percent of erythrocytes in peripheral blood in Group 1 and 3 rabbits. Increased nucleated erythrocytes, observed on Days 14 and 21 in Group 1 and 3 rabbits, suggest a regenerative anemia. This has been reported in other T-2 feeding studies.[29,69,74,75] Numerous erythrocyte precursors were present throughout the splenic red pulp in histologic sections of T-2 treated animals in this and other studies.[29,69]

The greater numbers of CFU in ileal Peyer's patch and sacculus rotundus in Group 2A rabbits were likely due to normal efficient phagocytosis of conidia by AM and transport via the mucociliary escalator to the digestive tract. Lymphocyte necrosis and/or depletion of primary and secondary lymphoid tissues evident in Groups 1 and 3 are indicative of the immunocytotoxic effects of T-2 toxin.[19,24,26-30,36] The relative severity of germinal center lesions in secondary lymphoid tissues examined in this study, as well as from the previous experiment,[33] was appendix > sacculus rotundus > ileal Peyer's patch > lymph node and spleen, which reflects the known proliferation rates of lymphoid cells in these organs.[76] The immunosuppression by T-2 toxin in Group 3 rabbits resulted in more severe lesions in these tissues, with hyphae and conidia readily apparent in appendix and sacculus rotundus of only Group 3A rabbits.

T-2 toxin is rapidly metabolized in the liver and is eliminated as glucuronide metabolite conjugates via the biliary excretion system.[26,42,71,72] Other T-2 toxin studies have shown that depletion of hepatic-reduced glutathione transferase and/or production of free

radicals causing lipid peroxidation in the liver could contribute to hepatotoxicity.[77] The hepatotoxic effect of T-2 toxin was apparent in the rabbits in this study, whereby centrolobular hepatocellular swelling and vacuolation, hepatic portal and periportal fibrosis, and bile duct reduplication were seen. The evidence for hepatotoxicity and decreases in serum ALP and SDH concentrations may be due to reduced synthesis in the liver.[13] Alternatively, these changes may be due to interference in enzyme release caused by membrane alterations without cell lysis.[78] It is possible that the centrolobular hepatocellular swelling could have been a reflection of hypoxic change caused by anemia because some rabbits were marginally anemic as evidenced by a decreased PCV.

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## GENERAL SUMMARY AND DISCUSSION

Few studies of the *in vitro* effects and *in vivo* chronic effects of T-2 toxin on cell mediated resistance to a variety of infectious diseases have been conducted.[47,74-78] Even fewer studies have been reported in which the interaction of mycotoxins with mycoses *in vitro*[100] or *in vivo*[98,99] was examined. The interaction of T-2 toxin and aspergillosis has not been investigated. In this study, the rabbit was used as a model to test *in vivo* and *in vitro* chronic effects of T-2 toxin on the phagocytic and pathologic response to *Aspergillus fumigatus*.

Results of the first study indicate that T-2 toxin given orally at 0.5 mg/kg/d for 21 days significantly reduced the *in vitro* phagocytic capacity of alveolar macrophages, apparently due to an unknown serum factor. Other physical parameters that were reduced at 0.5 mg/kg/d included weight gains, serum alkaline phosphatase, serum sorbitol dehydrogenase, and serum bacteriostasis. Oral dosages of 0.75 mg T-2 toxin/kg/d caused mortality (4 of 5 rabbits) and decreases in PCV, total WBC, and differential leukocyte counts. Histopathologic changes consisting of centrolobular hepatocellular swelling, mild portal and periportal fibrosis were found in most T-2 treated rabbits. Additionally, lymphocyte necrosis within secondary lymphoid tissue occurred in these rabbits. Thymic atrophy, bile duct reduplication, and lymphoid depletion of secondary lymphoid tissue occurred in the 0.75 mg/kg/d group. The changes observed in the liver have not been previously reported in other studies using T-2 toxin,[18,46] whereas lymphocyte

necrosis and depletion have been typical changes observed in T-2 intoxications.[38,46,48,49,67-70]

Based on results from the first study, we then tested the hypothesis that ingestion of 0.5 mg T-2 toxin/kg/d by rabbits for 17 or 28 days and exposure to aerosols of A. fumigatus conidia for 10 days would result in immunosuppression and reduced resistance to aspergillosis. The results of the second study proved this hypothesis to be correct.

In the second study, changes caused by T-2 toxin included leukopenia, anemia, and increased numbers and morphologic changes in nucleated erythrocytes by Day 21, followed by a regenerative response. Four of the rabbits given T-2 toxin died. Serum alkaline phosphatase and serum sorbitol dehydrogenase also were decreased by T-2 toxin ingestion. While aspergillosis in rabbits caused leukocytosis and an increased Ab response (IHA) to A. fumigatus, T-2 toxin ingestion caused a decrease in the Ab response of rabbits exposed to aerosols of A. fumigatus conidia. Normal pulmonary defense mechanisms to A. fumigatus infection were compromised by T-2 treatment, as evidenced by the severity and extent of lung lesions, greater numbers of hyphal elements observed in lesions, and greater numbers of A. fumigatus colony-forming units (CFU) isolated from these rabbits. Histopathologic changes consisting of centrolobular hepatocellular swelling, portal and periportal fibrosis, and lymphocyte necrosis and/or depletion within secondary lymphoid tissue occurred in most T-2 treated rabbits.

These studies have provided evidence that normal phagocytic and cell mediated immune defense mechanisms to A. fumigatus infection were

compromised by oral dosages of 0.5 mg T-2 toxin/kg/d. In addition, they have contributed to a better understanding of pathologic, hematologic, serologic, and mycologic changes in rabbits given T-2 mycotoxin orally and exposed to aerosols of A. fumigatus conidia.

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