Immunotoxicity of Mycotoxins[1]

RAGHUBIR P. SHARMA
Toxicology Program
Department of Animal, Dairy and Veterinary Sciences
Utah State University
Logan 84322-5600

ABSTRACT

The immune system is primarily responsible for defense against invading organisms. The effects of several mycotoxins on the immune responses have been investigated; however, most data concern laboratory animals. In some instances, farm animals and cells derived from livestock species have been employed to evaluate the immunotoxicity of mycotoxins. Immune responses are highly variable, and cost considerations usually preclude the use of dairy cattle as experimental models. Immunosuppression caused by aflatoxin B_1 has been demonstrated in various livestock species (e.g., turkeys, chickens, and pigs) and also in laboratory animals (mice, guinea pigs, and rabbits). The response of bovine lymphocytes to aflatoxin in vitro is similar to that of other laboratory animals. Trichothecenes are potent immunosuppressive agents that directly affect immune cells and also modify immune responses as a consequence of tissue damage elsewhere. Sheep and calves treated with fusarium T-2 toxin develop leukopenia and decreased functioning of peripheral lymphocytes. Immunosuppressive effects of ochratoxin A, rubratoxin B, and patulin have been reported. Citrinin produced lymphopenia but stimulated responses against antigens. Antibodies against mycotoxins conjugated to proteins have been produced and are useful for analytical purposes.

(**Key words**: immunotoxicity, mycotoxins)

Abbreviation key: **AFB_1** = aflatoxin B_1.

INTRODUCTION

The immune system is an important defensive mechanism against invading parasitic organisms or foreign cells. The system is highly evolved in mammals. In general, its complexity correlates with the evolutionary level of various animal species. In higher organisms, the system consists of specialized cells found throughout the body; these cells are localized in large quantities in certain organs, such as the thymus, spleen, and lymph nodes. Cells of the immune system and cells of the hemopoietic system originate in bone marrow. The bone marrow stem cells differentiate to perform specialized functions.

Immunotoxicology is a relatively new discipline, although the allergic responses to various chemicals have long been recognized. Chemicals, including mycotoxins, can either suppress or stimulate the immune system (39, 40). Immunosuppression likely decreases resistance to a variety of infectious diseases and may even predispose the host to the expression and dispersion of cancerous cells. Stimulation of the immune system is not always desirable because it may lead to hypersensitivity (allergic) reactions. The mycotoxin-induced immunotoxicity has been described earlier (39, 40).

Complexities of the Immune System

The immune system of mammals is highly complex, and various cells of this system interact with one another to produce the desired effect. Lymphocytes and macrophages are cellular units of the immune system. The two major forms of lymphocytes, T cells and B cells, differentiate in the thymus and fetal liver, respectively. The T cells are involved in cell-mediated immune responses, such as delayed hypersensitivity reaction and immune

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surveillance against foreign or altered cells. Several subpopulations of T cells exist: cytotoxic T cells, helper T cells, and suppressor T cells. The B cells are primarily involved in the production of a variety of antibodies; however, these cells are influenced by T cells. Some lymphocytes are natural killer cells that generally require no priming or proliferation to exert their effects. The T cells interact with one another or with other cells of the immune system via a variety of soluble factors called cytokines. In some instances, direct cell-cell interactions are necessary.

Other types of cells, such as macrophages, are derived from monocytes and are present in various body cavities, such as pulmonary alveoli and peritoneum; they are also found in the lymph nodes and liver. Macrophages are phagocytes that concentrate antigens and confer specific immunologic responses to various T or B cells. Other peripheral leukocytes are often involved in various immunopathologic mechanisms.

The immune system interacts with other systems and is profoundly influenced by the central nervous system, both directly via innervation of lymphatic organs and indirectly via neuroendocrine influence (2). The cells of the immune system produce factors that influence the nervous system. Hormones, such as somatotropin and thymosin (thymic maturation factor), stimulate the immune responses, whereas steroids, including sex hormones, generally suppress the immune responses.

Mechanisms Involved in Immunotoxicity

Little information exists on how mycotoxins produce immunotoxicity. Some mycotoxins, such as aflatoxin B_1 (AFB_1) and fusarium T-2 toxin inhibit protein synthesis and cell proliferation. This inhibition may not be the primary mechanism involved in their immunotoxic effects; both have selective effects on various subpopulations of lymphocytes. Several mycotoxins are cytotoxic to lymphocytes in vitro, perhaps because of their effects on membranes (including those involving lymphocytic receptors) or interference with macromolecular synthesis and function. Cytochalasins are highly cytotoxic and act on cytokinesis (perhaps by binding to the filamentous actin), but their immunotoxic potential has not been ascertained.

Mycotoxins can indirectly influence the immunologic functions. Some of the compounds are neurotoxic or cause other organ pathology, and these compounds may activate the endocrine mechanisms (38). The stress-induced release of corticosteroids inhibits immune functions. Fusarium T-2 toxin, which acts via such mechanisms, is discussed later.

Mycotoxins or their metabolites in mammals may be highly reactive and may destroy tissues. The immune system probably responds to altered proteins or to other biological molecules formed by binding with reactive chemicals, although no experimental evidence exists of this mechanism involving mycotoxins. Antibodies against mycotoxins conjugated with proteins have been produced and are utilized in analytical immunoassays.

The influence of exogenous chemicals on immune responses may be highly variable and may increase, decrease, or fail to affect the response, depending on the testing protocol and dose (41).

MYCOTOXINS AND LIVESTOCK

The ubiquitous nature of fungi that produce mycotoxins implies that their occurrence in feeds cannot be overlooked. Several mycotoxins are biologically very reactive and inhibit protein synthesis or cell multiplication. Because immune responses depend largely on the macromolecular synthesis and cellular proliferation, not surprisingly, many of the mycotoxins are immunotoxic. The immunotoxic potential of mycotoxins such as AFB_1 and T-2 toxin has been extensively studied, but little similar information exists about other mycotoxins.

Much of the information concerning the immunotoxic potential of mycotoxins has been obtained in laboratory animals only. Few studies have involved large domestic animals, including dairy cattle. Dairy cattle or other large animals are expensive, and immune responses are highly variable in outbred stocks. However, peripheral lymphocytes or cells from the major lymphatic organs can be used to study immunotoxic effects in vitro. This paper emphasizes information from livestock or materials derived from large animals. Some examples refer to laboratory animals, especially mice, the species of choice for im-

TABLE 1. Selected examples of aflatoxin B_1 immunotoxicity in livestock species and mice after oral treatments.

Species	Effects reported[1]	Reference
Bovine	Suppressed mitogen-induced stimulation of peripheral lymphocytes[2]	(28)
Swine	Decreased lymphocyte response to mitogens, inhibited macrophage migration, and decreased DTH and antibody titer to SRBC	(19)
	Resulted in swine clinical disease after pathogenic challenge	(25)
Chicken	Decreased antibody formation against SRBC	(48)
	Increased mortality with *Salmonella*	(3)
	Impaired phagocytic and bactericidal activity of heterophils	(7)
	Decreased antibody formation	(15)
	Reduced phagocytic activity of RES cells	(24)
Turkey	Reduced acquired resistance to *Pasteurella multocida*	(29)
	Resulted in thymic involution	(30)
Mice	Decreased lymphocytic response mitogens, decreased antibody response to SRBC, and impaired DTH	(34, 35)
	Decreased DNA, RNA, and protein synthesis in cultured lymphocytes	(33)

[1]DTH = Delayed type hypersensitivity; RES = reticular endothelial system; SRBC = sheep red blood cells.

[2]Response after in vitro exposure of lymphocytes.

munotoxicology because the immune system of mice has been well studied.

Immunotoxic Effects of Selected Mycotoxins

Many episodes of mycotoxin poisoning in livestock species resulted in the death of animals from infectious organisms. This suspected chemically induced immunosuppression was later examined in experimental studies of target species. Tables 1 to 3 show selected examples of mycotoxin effects on immune functions detected in various species.

The most widely studied immunotoxic agent is AFB_1, which was consistently immunosuppressive in various animal models, although not in all experiments. The immune responses mediated by T cells appear to be more sensitive to AFB_1, although both helper T cells or suppressor T cells can be affected, depending on the challenge dose of the mycotoxin (16). As indicated in Table 1, peripheral bovine lymphocytes are susceptible to AFB_1 (33). In most experiments, a suppression of antibody formation occurred, generally against T-dependent antigens like sheep red blood cells.

Interest has increased in immune suppression induced by T-2, a mycotoxin that also inhibited protein synthesis. In toxic doses, T-2 produced necrosis of lymphatic organs in most of the species evaluated (Table 2). In lymphocyte cultures, T-2 inhibited blastogenesis (44); however, T-2 may also include indirect mechanisms involving the hypothalamic-pituitary-adrenal axis (47). Oral exposure of mice to this mycotoxin produced inflammation of the gut mucosa, leading to a systematic endotoxemia and triggering a stresslike response that increased concentrations of glucocorticoids. However, indirect effects on immune system explain only part of the immunotoxic effects of T-2.

Some studies have concerned the immunotoxicity of other trichothecenes, including those that are macrocyclic. Selected examples are listed in Table 2. Macrocyclic trichothecenes, even those that are very toxic, did not uniformly affect immune responses (20, 22).

The immunotoxicity of most other mycotoxins has not been evaluated. The studies concerning miscellaneous compounds are listed in Table 3. Ochratoxin A and patulin may have immunosuppressive effects. The lympholytic effect of oral doses of ochratoxin A was largely limited to the gut-associated lymphatic tissue (42, 43) and often required doses that were systemically toxic. Patulin inhibited DNA synthesis in peripheral lymphocytes; these effects were mitigated by cysteine, which suggested that sulfhydryl binding was involved in patulin-induced toxicity (11). The response to citrinin was marked by immunostimulation (36), but its effects were reversible and appeared to be related to the nephrotoxic potential of this mycotoxin (32).

TABLE 2. Influence of various trichothecenes on immune responses in various animal species.

Species	Trichothecene[1]	Effects[2]	Reference
Cattle	T-2	Reduced neutrophil function and reduced lymphocyte blastogenesis	(23)
		Decreased response of lymphocytes to PHA	(5)
		Resulted in necrosis in lymphoid organs and tissues	(1)
Sheep	T-2	Resulted in lymphopenia and leukopenia	(14)
Swine	T-2	Resulted in necrosis in B-cell regions of lymphoid tissues	(1)
	T-2 (inhalation)	Decreased lymphocytic proliferation with mitogens	(27)
	T-2 (topical)	Resulted in transient alteration of immune responses	(26)
	T-2	Decreased leukocyte count and antigen-induced lymphocyte transformation	(31)
		Resulted in massive lymphocytic necrosis	(49)
	DAS[3]	Resulted in mild and inconsistent leukopenia and necrosis of germinal centers in mesenteric lymph nodes and splenic white pulp	(50)
Chicken	T-2	Increased mortality to pathogenic bacterial challenge	(4)
		Resulted lymphopenia and lymphatic necrosis	(17, 18)
Turkey	T-2	Resulted in lymphatic necrosis	(37)
Mice	T-2	Decreased antibody formation	(46)
		Interfered with in vitro mitogen-induced blastogenesis	(44)
	Macrocyclic trichothecenes	Resulted in inconsistent effects, not well correlated with acute toxicity	(20, 21, 22)

[1]Exposures were oral or dietary, except as indicated.

[2]PHA = Phytohemagglutinin.

[3]DAS = Diacetoxyscirpenol.

Rubratoxin B may also suppress the immune system (45).

Various mycotoxins appear to have considerable immunotoxic potential, depending on the level of exposure. Only the mycotoxins that can occur in relatively large amounts in feed [e.g., AFB_1 and trichothecenes (T-2 or zearalenone)] and can also have a toxicity potential are of practical significance in livestock feeding. The effects of long-term low level feeding of mycotoxins have not been well characterized. However, because immunotoxic potential of chemicals can be evaluated on cells from target animal species or even from humans (10), more studies may be conducted in immune cells derived from large animals. However, these evaluations will probably be limited to direct effects of mycotoxins

TABLE 3. Immunotoxic effects of miscellaneous mycotoxins.

Mycotoxin	Species	Effects[1]	Reference
Ochratoxin A	Swine	Resulted in necrosis of gut-associated lymph nodes	(43)
	Dog	Resulted in necrotic lymph nodes in mesentery	(42)
	Chicken	Resulted in leukopenia and impaired phagocytosistosis by heterophils	(8, 9)
	Turkey	Resulted in leukocytopenia, heterocytopenia, and thymic atrophy	(6)
Patulin	Mice	Increased resistance to *Candida albicans* and decreased concentrations of circulating immunoglobulins	(12)
	Rabbits	Decreased serum immunoglobulins and resulted in reduced blastogenesis of lymphocytes and reduced chemiluminescence of peritoneal leukocytes	(12, 13)
Citrinin	Mice	Resulted in transient stimulation of lymphoproliferative responses, increased antibody against SRBC	(36)
Rubratoxin B	Mice	Resulted in leukopenia and decreased lymphoproliferation	(45)

[1]SRBC = Sheep red blood cells.

on immunocompetent cells or to a comparison of the relative sensitivity of cells from different species.

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