Toxicity of Mycotoxins for the Rat Pulmonary Macrophage in Vitro

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The presence of mycotoxins in grains is well documented. Workers in grain handling occupations are commonly exposed to grain dust aerosols. Work in our laboratory has shown that T-2 toxin is highly toxic to rat alveolar macrophages in vitro, causing loss of viability, release of radiolabeled chromium, inhibition of macromolecular synthesis, inhibition of phagocytosis, and inhibition of macrophage activation. Similarly, patulin caused a significant release of radiolabeled chromium, decrease in ATP levels, significant inhibition of protein and RNA synthesis, and inhibition of phagocytosis. The data show that both T-2 toxin and patulin are highly toxic to rat alveolar macrophages in vitro. The data further suggest that the presence of these mycotoxins in airborne respirable dust might present a hazard to exposed workers.

Introduction

Several previous studies have shown that exposure to grain dust can lead to various respiratory disorders (1,2), yet much remains to be learned of the role of various grain dust components in pulmonary disease. Grain dust is a heterogeneous mixture that is often contaminated by silica, bacterial endotoxins, insects, mites, mammalian debris, various chemical additives such as pesticides and herbicides, and fungi and their metabolites. Mycotoxins are fungal metabolites. Contamination of various grain products with mycotoxins has been well documented (3–5), and recently aflatoxin has been shown to occur in the respiratory fraction of airborne corn dust (6,7). More recently, Hayes et al. (8) demonstrated that mortality for total cancer and respiratory cancer in aflatoxin-exposed peanut oil press workers in the Netherlands was higher than expected based on standardized mortality ratio (SMR) analysis.

T-2 toxin is a product of several Fusarium species. Fusarium species are common contaminants of such field crops as corn, wheat, and oats (5,9). Toxic metabolites of Fusarium were responsible for a human disease outbreak (alimentary toxic aleukia) and have been implicated as the causative agent in many animal intoxica-

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tions (10). Trichothecenes such as T-2 toxin are cytostatic for unstimulated and mitogen-stimulated lymphocytes (11), fibroblasts (12), neoplastic cells such as HeLa cells (13), and transformed intestinal cells (14). In addition, Lefarge-Frayssinet et al. (15) have shown that T-2 toxin induces severe damage to rat splenic cells in vitro. T-2 toxin induces the depletion of murine thymus (16), inhibits the synthesis of anti-sheep red blood cell antibodies, and prolongs the period required for skin graft rejection (17). It strongly inhibits protein and DNA synthesis (18).

Patulin is a polyketide lactone mycotoxin produced by several common species of Aspergillus and Penicillium. It has been reported to be common in moldy corn silage (19) and in naturally rotted apples (20). The initial interest in patulin was for its antimicrobial properties, but subsequent work has shown it to be toxic in experimental animals (21,22), carcinogenic in rats (23), mutagenic in yeast (24), and teratogenic in chick embryos (25). Patulin was also shown to be the etiologic agent in an accidental epidemic of feed poisoning in Japan resulting in the mass death of 118 dairy cows in 1952 (26). Patulin induces single-strand and double-strand breaks in HeLa DNA (27) and causes a high percentage of polyploid cells in human leukocyte cultures (28). More recent studies have demonstrated that patulin inhibits transcription in RNA synthesis in a cell-free system from rat liver (29), inhibits translation of protein syntheses in rabbit reticulocyte lysates (30), and inhibits synthesis of r-RNA, t-RNA, and probably m-RNA in Saccharomyces cerevisiae (31).
The purpose of this investigation was to study the toxicity of T-2 toxin and patulin in rat alveolar macrophages in vitro. Pulmonary alveolar macrophages perform several important functions in the lung including phagocytosis of living and nonliving foreign particles, regulation of T-lymphocyte proliferation, provision of T-helper activity for antibody production, and production of mediators of cellular immunity (32). Thus, cytotoxic damage to alveolar macrophages could lead to serious pulmonary and/or systemic damage.

Materials and Methods

Mycotoxins

T-2 toxin (3-hydroxy-4,15-diacetoxy-8[3-methylbutyryloxy]-12, 13-epoxy-Δ8-trichothecene) was purchased from Calbiochem, La Jolla, CA, and dissolved in 100% dimethyl sulfoxide (DMSO, Pierce Chemical Co., Rockford, IL). Substocks were prepared by performing 10-fold dilutions in 10% DMSO and the final DMSO concentration in all cultures was 0.1%.

Patulin was obtained from Aldrich Chemical Company (Milwaukee, WI), and fresh stock solutions were prepared daily in 100% DMSO for each experiment (33). Appropriate solvent controls were included in all experiments.

Alveolar Macrophage Isolation and Culture

Alveolar macrophages were harvested from male Long-Evans hooded rats by tracheal lavage (33–35). The cells were routinely tested for viability, cell purity, and esterase (34). Cells were incubated at 37°C in 5% CO2 in Debecco's medium 199 containing 10% fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin) unless indicated otherwise.

Viability, Cell Number, and Viability Index Assay

The toxicity of T-2 toxin was studied using the methods described by Waters et al. (36,37). Percent viability was determined by the trypan blue exclusion technique with a hemocytometer. Cell number was defined as the number of intact cells in treated cultures expressed as percent of control. Viability index was defined as the product of the viability ratio and cell number ratio. Arcsine transformation and linear regression calculations were done by computer.

Determination of Mean Cell Volume

Mean cell volume was determined by Coulter Counter (Model ZB) equipped with a Channelyzer. In experiments with T-2 toxin where exposures were 6 and 18 hr, cells were removed from the plates with trypsin (34). Patulin treatments were < 2 hr and were done with cells suspended in Hank's balanced salt solution (33).

Chromium Release Assay

Freshly isolated AM were incubated with [51Cr]-sodium chromate for 45 min, washed three times, and resuspended in medium 199. Aliquots (0.1 mL) of labeled cells, along with 0.1 mL of medium 199 containing the mycotoxin or solvent control, were incubated for the desired interval. After incubation, 0.1 mL of supernatant (550µL) was transferred to a clean tube and counted in a gamma scintillation counter. Total release was obtained by lysing the cells with Triton X-100. The percent chromium release equals the experimental release value divided by the total release value. Both values were corrected for spontaneous release (33).

Adenosine Triphosphate Determination

ATP levels in treated and untreated monolayer cultures were determined by the luciferin-luciferase assay. At the time of treatment, the medium was removed by aspiration and replaced with fresh medium containing mycotoxin. Sodium iodoacetate was used as a positive control. ATP was extracted directly from the cells without removing the cells from the plates (33). ATP determinations were done in a darkened laboratory with an ATP photometer using dark-adapted scintillation vials (33).

Macromolecular Synthesis

For protein-synthesis studies AM monolayers were incubated in Minimum Essential Medium (MEM) with

Table 1. Characteristics of purified alveolar macrophages from Long-Evans hooded rats.

|                  | Percentage of purified AM positive |
|------------------|------------------------------------|
|                  | Cell volumeb | Esterasec | Phagocytosisd |
| Preadhered       | 111.9 ± 46.3 | 98.6 ± 1.7 | — |
| Cultured (20 hr) | 1279.5 ± 102.9 | 98.7 ± 2.4 | 92.3 ± 2.09 |

*Percentage viability as determined by trypan blue exclusion.

bCell volume values as determined by a Coulter Model ZB electronic cell counter interfaced with a Channelyzer II Cell-sizing attachment. Cell volume units are in cubic micrometers.

cCells staining positive for nonspecific esterase.

dCells actively phagocytizing zymosan particles.

*Values represent the mean ± standard deviation.
incubated

**FIGURE**

were washed, washed, L-glutamine liquid

| Concentration, M | MCV, μm² | 6 hr* | 18 hr* |
|------------------|----------|-------|--------|
| Control          | 1278.4 ± 18.3 | 1286.2 ± 101.5 |
| 10⁻⁹             | 1281.7 ± 21.2 | 1182.9 ± 138.5 |
| 10⁻⁸             | 1279.5 ± 50.6 | 1107.1 ± 98.0 |
| 10⁻⁷             | 1264.6 ± 23.8 | 1002.3 ± 26.5 |
| 10⁻⁶             | 1259.0 ± 21.0 | ND |
| 10⁻⁵             | 1244.1 ± 19.9 | ND |

*Values shown represent the mean ± standard deviation.

Values are significantly different from controls.

Not determined.

L-glutamine but lacking leucine (33,35). The cells were incubated with mycotoxin and 1 μCi/mL [³H]-leucine. After the desired incubation interval the culture plates were chilled on ice, the medium removed and the cells washed, and the monolayers solubilized. TCA-precipitable material was collected on glass-fiber filters, washed, placed in scintillation vials, and counted by liquid scintillation (33,35).

For RNA-synthesis studies, the treated and untreated AM cultures were incubated with [³H]-uridine. After incubation the cultures were chilled on ice, rinsed, and solubilized. The solubilized material was counted by liquid scintillation (33,35).

**Phagocytosis Studies**

Experiments with T-2 toxin employed [³H]-Staphylococcus aureus (35). In brief, AM monolayers were prepared in flat-bottomed glass vials and incubated for 48 hr. The medium was removed and replaced with medium containing T-2 toxin. After 6 hr incubation, the culture medium was removed, a suspension containing labeled S. aureus cells was added, and the cultures incubated an additional hour. After this second incubation, the monolayers were rinsed and solubilized and the solubilized suspensions collected and counted by liquid scintillation.

Studies with patulin were done with sheep erythrocytes labeled with [⁶⁵Cr]-sodium chromate and coated with specific antibody. AM monolayers were prepared in Linbro tissue culture plates and exposed to the mycotoxins. After exposure, the culture medium was re-

| Concentration, M | CPM* | % release | CPM* | % release |
|------------------|------|-----------|------|-----------|
| Control          | 1986.3 ± 168.5 | — | 6473.1 ± 949.1 | — |
| 10⁻⁹             | 2252.0 ± 201.1 | 0.95 | 6575.0 ± 529.0 | 0.4 |
| 10⁻⁸             | 2280.1 ± 404.0 | 1.06 | 10202.8 ± 250.1 | 14.1 |
| 10⁻⁷             | 2390.6 ± 291.6 | 1.40 | 14077.8 ± 259.4 | 28.6 |
| 10⁻⁶             | 2783.1 ± 450.1 | 2.85 | 17856.5 ± 918.3 | 41.3 |
| 10⁻⁵             | 2813.9 ± 293.3 | 3.00 | 18758.7 ± 483.3 | 46.4 |

*Counts per minute; average of 5 replicate tubes ± standard deviation.

b% release = (treated–control)/(total–control). Total release determined by lysing the cells with Triton X-100.

Values are significantly different from control, based on raw data in CPM, p < 0.05.
moved and 0.5 mL of fresh medium containing $2 \times 10^7$ $[^{51}Cr]$-labeled sheep red blood cells was added (38). The monolayers were then incubated 1 hr. Next, the medium was removed and replaced with fresh medium containing various concentrations of T-2 toxin and $[^{14}C]$-glucosamine and incubated for 6 hr. The monolayers were then rinsed, solubilized, and counted by liquid scintillation. Activation was expressed as the stimulation index relative to untreated controls (35).

**Activation of Alveolar Macrophage by Lipopolysaccharide**

AM monolayers were prepared and incubated in 1 mL medium 199 containing 10 µg/mL lipopolysaccharide (LPS). After 15 hr incubation with LPS, the medium was removed and replaced with fresh medium containing various concentrations of T-2 toxin and $[^{14}C]$-glucosamine and incubated for 6 hr. The monolayers were then rinsed, solubilized, and counted by liquid scintillation. Activation was expressed as the stimulation index relative to untreated controls (35).

**Lymphokine Activation of Alveolar Macrophages**

AM activation with lymphokines was assayed as has been described for LPS activation. Briefly, crude lymphokine preparations were made from lymphocytes isolated from rat spleens and tested for their ability to stimulate incorporation of $[^{14}C]$-glucosamine. The details of this procedure have been described elsewhere (35).

**Results**

**Studies with T-2 Toxin**

The cellular characteristics of isolated and cultured rat alveolar macrophages are presented in Table 1. The data indicate that the cultured cells are a viable and nearly homogeneous population of AM.

The effect of T-2 toxin on viability, cell number, and viability index is presented in Figure 1 (A-C, respectively). According to Waters et al. (36,37), the arsine transformation is appropriate for dose–response studies and viability index provides a better indication of cytotoxicity than either percent viability or the cell number ratio when cell lysis is rapid. T-2 toxin produced clear cytotoxic effects by all three parameters. The ED$_{50}$ for viability index was $8.9 \times 10^{-8}$ M after 20 hr treatment.

The effect of T-2 toxin on mean cell volume (MCV) after 6 and 18 hr exposure is shown in Table 2. MCV values were not significantly different from control values after 6 hr exposure at any concentration $< 1 \times 10^{-5}$ M. On the other hand, $1 \times 10^{-5}$ M T-2 toxin resulted in a significant decrease in mean cell volume after 18 hr exposure. Chromium release data for AM treated with T-2 toxin for 6 and 18 hr are shown in Table 3. Although there was a trend toward increasing chromium release with increasing T-2 toxin concentration at 6 hr, there was no significant difference between treated and control cultures at any dose $< 1 \times 10^{-5}$ M T-2 toxin. On the other hand, there was a significant increase in chromium release in cultures treated for 18 hr at $1 \times 10^{-5}$ M T-2 toxin. Thus, the effects of T-2 toxin were both dose- and time-dependent. ATP levels in cultures treated with T-2 toxin showed a slight but not significant ($p > 0.05$) elevation in ATP (35).

Figure 2 illustrates the effect of T-2 toxin on synthesis of protein (Fig. 2A) and RNA (Fig. 2B) in cultured rat AM. The results indicate that control cultures incorporated labeled precursors at a nearly linear rate, whereas incorporation of label into macromolecules was reduced in the presence of T-2 toxin. The inhibitory effect was most remarkable with respect to protein synthesis. For example, leucine incorporation ceased immediately in cultures containing $1 \times 10^{-7}$ M T-2 toxin and terminated after 2 hr incubation in cultures con-
taining \(1 \times 10^{-6}\) M T-2. On the other hand, RNA synthesis was terminated after 4 hr at \(1 \times 10^{-7}\) M T-2 toxin.

The results of phagocytosis studies with \(^{3}H\)-S. aureus indicate that the phagocytic activity was significantly inhibited at \(1 \times 10^{-7}\) M but not at \(1 \times 10^{-8}\) M T-2 toxin (Table 4). In two identical experiments the phagocytic activity of AM cultures treated with \(1 \times 10^{-7}\) M T-2 toxin was reduced to 83.5 and 82.1% of control cultures.

The effect of T-2 toxin on AM macrophage activation was determined by incubating AM with either LPS (10 \(\mu\)g/mL) or lymphokines generated by mitogen-stimulated rat lymphocytes. LPS markedly enhanced \[^{14}C\]-glucosamine uptake. However, both \(1 \times 10^{-9}\) M and \(1 \times 10^{-7}\) M T-2 toxin caused significant inhibition of \[^{14}C\]-glucosamine uptake in LPS-stimulated cultures (Table 5). Similarly, lymphokines derived from phytohemagglutinin (PHA)-stimulated rat lymphocyte cultures promoted \[^{14}C\]-glucosamine uptake. The addition of T-2 toxin at \(1 \times 10^{-8}\) or \(1 \times 10^{-7}\) M concentrations inhibited \[^{14}C\]-glucosamine uptake by AM stimulated with lymphokines (Table 5). An identical experiment, performed with Concanavalin-A-stimulated rat lymphocyte cultures gave similar results (data not shown).

### Studies with Patulin

In contrast to the effect of T-2 toxin on MCV, patulin caused a significant increase in mean cell volume after 2 hr (but not 1 hr) exposure at \(1 \times 10^{-6}\) M. Chromium release from alveolar macrophages following exposure to patulin was both time- and concentration-dependent. Treatment of these cells at \(\geq 1.5 \times 10^{-4}\) M caused significant chromium release within 30 min (Table 6). Adenosine triphosphate (ATP) concentrations in AM monolayer cultures were markedly inhibited within 1 hr at concentrations of \(> 5 \times 10^{-6}\) M patulin (Table 7).

Incorporation of \(^{3}H\)-precursors into protein and RNA was also strongly inhibited by patulin (Table 6). Inhibition was both time- and concentration-dependent for both classes of molecules but protein synthesis was sensitive to 10- to 100-fold lower concentrations of patulin than RNA synthesis at the same time interval. The dose producing 50% inhibition at 1 hr (ED\(_{50}\)) was estimated at \(1.6 \times 10^{-6}\) M and \(1.0 \times 10^{-6}\) M for \(^{3}H\)-leucine and \(^{3}H\)-uridine incorporation, i.e., protein and RNA synthesis, respectively.

Patulin strongly inhibited phagocytosis of \(^{5}C\)-sheep erythrocytes (Table 8) and there was significant inhibition of phagocytosis at \(> 5 \times 10^{-7}\) M patulin (\(p < 0.05\) (Table 9).

### Table 4. Effect of T-2 toxin on phagocytosis of \(^{3}H\)-Staphylococcus aureus by alveolar macrophages.*

| Concentration, M | CPM \(^{a}\) | % control | CPM \(^{b}\) | % control |
|------------------|-------------|-----------|-------------|-----------|
| Control          | 1643.8 ± 214.1 | 100       | 1868.1 ± 216.4 | 100       |
| \(10^{-8}\)      | 1635.1 ± 101.7 | 99.5      | 1846.6 ± 121.5 | 98.8      |
| \(10^{-7}\)      | 1367.4 ± 232.5 | 83.5      | 1533.3 ± 79.7  | 82.1      |

*Macrophages were treated 6 hr with T-2 toxin.

### Table 5. T-2 toxin inhibition of alveolar macrophage activation by phytohemagglutinin-generated lymphokines.

| Treatment\(^{a}\) | \[^{14}C\] Glucosamine incorporation | SI\(^{a}\) |
|------------------|------------------------------------|------------|
|                  | CPM\(^{b}\) |                        |            |
| Negative control | 70.8 ± 15.7 |                        |            |
| 10 \(\mu\)g/mL LPS | 1068.8 ± 166.3 | 23.8       |            |
| Control supernatant\(^{d}\) | 112.1 ± 8.6 | 1.6               |            |
| Reconstitution control\(^{e}\) | 135.4 ± 10.4 | 1.9               |            |
| 50 \(\mu\)g/mL PHA\(^{f}\) | 1308.9 ± 108.2 | 18.5         |            |
| 50 \(\mu\)g/mL + \(10^{-8}\) M T-2 | 688.5 ± 69.9\(^{x}\) | 9.7           |            |
| 50 \(\mu\)g/mL + \(10^{-7}\) M T-2 | 684.6 ± 20.4\(^{x}\) | 9.7           |            |
| 100 \(\mu\)g/mL PHA\(^{f}\) | 1559.0 ± 87.4 | 22.6         |            |
| 100 \(\mu\)g/mL + \(10^{-8}\) M | 473.9 ± 20.0\(^{x}\) | 6.7           |            |
| 100 \(\mu\)g/mL + \(10^{-7}\) M | 468.5 ± 89.2\(^{x}\) | 6.6           |            |

\(^{a}\)Macrophages were stimulated for 15 hr with lymphokines and then treated 6 hr with T-2 toxin.

\(^{b}\)Mean values ± standard deviation.

\(^{c}\)Stimulation index.

\(^{d}\)Supernatant from mitogen-free lymphocytes; no PHA added.

\(^{e}\)PHA was added to supernates of control lymphocyte cultures at the conclusion of the incubation period to control for the carryover of the mitogens into macrophage culture.

\(^{f}\)Supernatant from lymphocytes incubated with 50 or 100 \(\mu\)g/mL PHA; 10 \(\mu\)L of the lymphokine preparation was added to the macrophage culture.

\(^{x}\)Values are significantly different from controls at \(p < 0.005\).
### Table 6. \(^{51}\)Cr release following short-term exposure of alveolar macrophages to patulin.

| Treatment | CPM* | Probability* | % release* |
|-----------|------|--------------|------------|
| 30-min exposure |      |              |            |
| Spontaneous release | 1766.4 ± 52.7 | >0.5 | 0.4 |
| Total release | 12018.0 ± 163.5 | <0.0002 | 2.7 |
| 5 \(\times\) 10\(^{-8}\) M | 1811.6 ± 24.3 | <0.0001 | 7.8 |
| 1.5 \(\times\) 10\(^{-4}\) M | 2048.2 ± 87.0 |          |            |
| 5 \(\times\) 10\(^{-4}\) M | 2562.8 ± 57.3 |          |            |
| 60-min exposure |      |              |            |
| Spontaneous release | 2216.0 ± 60.2 | <0.05 | 0.5 |
| Total release | 12274.8 ± 453.6 | <0.0001 | 5.1 |
| 5 \(\times\) 10\(^{-8}\) M | 2273.4 ± 14.2 |          |            |
| 1.5 \(\times\) 10\(^{-4}\) M | 2783.2 ± 101.0 |          |            |
| 5 \(\times\) 10\(^{-4}\) M | 4092.4 ± 130.7 |          |            |
| 120-min exposure |      |              |            |
| Spontaneous release | 2330.6 ± 116.0 | >0.05 | 1.7 |
| Total release | 12319.4 ± 129.9 | <0.0001 | 4.4 |
| 1.5 \(\times\) 10\(^{-5}\) M | 2156.4 ± 108.4 |          |            |
| 5 \(\times\) 10\(^{-5}\) M | 3074.4 ± 76.7 |          |            |
| 1.5 \(\times\) 10\(^{-4}\) M | 4967.6 ± 138.1 |          |            |
| 5 \(\times\) 10\(^{-4}\) M | 6754.4 ± 57.3 |          |            |

*Counts per minute; average of 5 replicate tubes ± standard deviation.

\(^{a}\)One-tailed t test, 8 degrees of freedom

\(^{b}\)% release = (treated–spontaneous)/(total–spontaneous).

\(^{c}\)Untreated control cells.

\(^{*}\)Cells lysed with Triton X-100.

### Table 7. Effect of patulin on ATP levels in rat alveolar macrophage cultures.

| Concentration (M) | ATP, ng/mL | % of control |
|-------------------|------------|-------------|
| Medium control\(^{b}\) | 287.8 ± 26.2 | 100 |
| Solvent control\(^{b}\) | 323.5 ± 69.6 | 112.4 |
| 1.5 \(\times\) 10\(^{-4}\) M | 274.9 ± 43.2 | 95.5 |
| 5 \(\times\) 10\(^{-8}\) M | 253.7 ± 34.9 | 88.2 |
| 1.5 \(\times\) 10\(^{-4}\) M | 216.5 ± 21.7 | 75.2 |
| 5 \(\times\) 10\(^{-8}\) M | 180.2 ± 38.7 | 62.6 |
| 1.5 \(\times\) 10\(^{-4}\) M | 98.6 ± 10.5 | 34.6 |
| 5 \(\times\) 10\(^{-4}\) M | 33.9 ± 5.3 | 11.8 |
| Positive control\(^{b}\) | 67.6 ± 12.1 | 23.5 |

\(^{a}\)1 hr exposure at 37°C in 5% CO\(_2\).

\(^{b}\)20 \(\mu\)L of medium used in place of DMSO.

\(^{c}\)20 \(\mu\)L of DMSO; final concentration = 2.0% DMSO.

\(^{d}\)0.3 mM sodium iodoacetate.

\(^{*}\)Significant, one-tailed t test, \(p < 0.05\).

### Discussion

Viability determinations after exposure of AM to submicromolar concentrations of T-2 toxin for 20 hr demonstrated that the toxin was cytotoxic and that the effect of T-2 toxin on AM viability, cell number, and viability index was dose dependent. The viability index parameter developed by Waters et al. (36,37) at the Environmental Protection Agency to assess cytotoxicity of environmental compounds is useful when cell death proceeds via mechanisms that result in differing degrees of cell lysis.

Mean cell volume values in cells treated with T-2 toxin for 6 hr were not significantly different from untreated cells but AM incubated 18 hr with T-2 toxin displayed a dose-dependent decrease in their cell volume values. Cultures containing 1 \(\times\) 10\(^{-7}\) M T-2 toxin were significantly different from control cultures after 18 hr exposure. Scanning electron microscopy revealed surface morphological alterations in the alveolar macrophage in vitro that reflect varying degrees of cell damage (34), and it is possible that the mean cell volume values reflect extensive cell fragmentation.

Chromium release studies were performed after 6 and 18 hr of incubation to study the effect of T-2 toxin on membrane integrity. At low concentrations known to inhibit protein and RNA synthesis, there was no effect on the amount of chromium released after 6 hr of exposure. However, with 1 \(\times\) 10\(^{-5}\) M T-2 toxin, a significant amount of chromium was released after 6 hr in comparison to control cultures. There was extensive leakage of chromium from cells treated for 18 hr at < 1 \(\times\) 10\(^{-8}\) M T-2 toxin. It is unlikely that membrane damage plays a significant role in cytotoxicity at low concentrations of T-2 toxin at < 6 hr, since sublethal concentrations of T-2 toxin significantly inhibited protein synthesis without significant chromium release.

The most remarkable effect of T-2 toxin on rat alveolar macrophage was on protein synthesis. Inhibition of protein synthesis occurred at an earlier time and with a lower concentration of T-2 toxin than RNA synthesis, viability index, mean cell volume changes, or chromium release. It is possible that these other effects are secondary to the inhibition of protein synthesis observed. Measurements of ATP levels in treated cells suggest that the inhibition of protein synthesis observed cannot be explained by depletion of ATP levels in the cells. Although there was a 90% decrease in ATP levels in the positive control (sodium iodoacetate), there was no decrease in cultures treated with T-2 toxin at concentrations which significantly inhibit protein synthesis (35).
Table 8. Inhibition of incorporation of $^{3}$H-leucine and $^{3}$H-uridine in alveolar macrophage cultures by patulin.

| Exposure, hr | Concentration, M | CPM* ($\bar{X} \pm SD$) | % of control |
|-------------|-----------------|--------------------------|-------------|
| 0.5 Control | 262.3 ± 37.8    | 100                      |
| 10–6        | 186.7 ± 30.7    | 71.2                     |
| 10–5        | 54.7 ± 12.4     | 20.8                     |
| 10–4        | 39.6 ± 10.5     | 15.1                     |

| Concentration, M | CPM* ($\bar{X} \pm SD$) | % of control |
|-----------------|--------------------------|-------------|
| 1.0 Control     | 583.4 ± 100.2            | 100         |
| 10–6            | 475.3 ± 74.1             | 81.5        |
| 10–5            | 77.5 ± 9.6               | 13.3        |
| 10–4            | 38.1 ± 10.9              | 6.5         |

| Concentration, M | CPM* ($\bar{X} \pm SD$) | % of control |
|-----------------|--------------------------|-------------|
| 2.0 Control     | 1634.2 ± 497.4           | 100         |
| 10–6            | 969.3 ± 368.4            | 59.3        |
| 10–5            | 125.7 ± 40.3             | 7.7         |
| 10–4            | 51.2 ± 9.4               | 3.1         |

| Concentration, M | CPM* ($\bar{X} \pm SD$) | % of control |
|-----------------|--------------------------|-------------|
| 4.0 Control     | 2262 ± 646.4             | 100         |
| 10–6            | 1203.6 ± 269.7           | 53.2        |
| 10–5            | 114.6 ± 23.9             | 5.1         |
| 10–4            | 45.0 ± 9.2               | 2.0         |

*Used to monitor protein synthesis.

Table 9. Effect of patulin on phagocytosis of $^{41}$CrShEA.a,b

| Concentration, M | CPM* | % of control |
|-----------------|------|-------------|
| Control         | 689.3 ± 119.7 | 100         |
| 5.0 x 10–7      | 506.9 ± 140.2 | 73.5        |
| 1.5 x 10–6      | 376.4 ± 76.2  | 54.6        |
| 5.0 x 10–6      | 265.7 ± 34.5  | 38.5        |
| 5.0 x 10–5      | 180.4 ± 26.2  | 26.2        |
| 1.5 x 10–4      | 114.4 ± 24.1  | 16.6        |

*aPatulin exposure 1 hr.

*b$^{41}$Cr-labeled antibody-coated sheep erythrocytes.

Values shown represent the mean ± standard deviation. The experiment was repeated twice with similar results.

Values are significantly different from the control, p < 0.05.

T-2 toxin had a significant effect on phagocytosis of serum-opsonized *S. aureus*. Massaro et al. (39) have shown that protein synthesis is not required and is depressed during phagocytosis. It is possible, however, that T-2 toxin inhibits phagocytosis by inhibiting the synthesis of proteins needed for the phagocytosis, without directly inhibiting the endocytosis process per se. Several proteins are known to be required for phagocytosis (40–42).

The activated AM is a critical component of the immune response. For example, activated macrophages demonstrate an increased capacity of phagocytosis and increased production of various monokines involved in regulation of both T- and B-cell function (43). Hammond and Dvorak (44) have observed that activated macrophages preferentially incorporate glucosamine into their membranes. Our results indicate that T-2 toxin significantly inhibited AM incorporation of labeled glucosamine (Table 5). Macrophage activation was further investigated by stimulating the cells with mitogen-generated lymphokines in the presence and absence of T-2 toxin. The results clearly demonstrate that macrophage activation is due to the lymphocyte mediators and not due to the mitogens. AM activation by LPS and the crude lymphokines, as assayed by glucosamine incorporation, is also significantly inhibited by T-2 toxin. By suppressing macrophage activation the cell becomes unable to function normally as an immunologically competent cell. The means by which T-2 toxin inhibits AM activation is probably due to the ability of the toxin to inhibit protein synthesis. Inhibition of macrophage monokines such as lymphocyte-activating factor (interleukin I), which are known to help regulate the immune response to foreign antigens, would be quite detrimental to the immunological state of an individual. Also, inhibition of phagocytosis would likely increase susceptibility to opportunistan infections. Since there is abundant evidence that macrophages in collaboration with T- and B-lymphocytes can destroy neoplastic cells in vivo (45,46), the possibility that exposure to T-2 toxin could lead to increased risk for cancer should be considered. In this context, Schoental et al. (47) have reported induction of tumors in the digestive tract and the brain in rats given T-2 toxin by intragastric administration.

Patulin is toxic to rat alveolar macrophages in vitro, causing an increase in mean cell volume, chromium release, decrease in cellular ATP, inhibition of protein and RNA synthesis, and inhibition of phagocytosis. Mean cell volume and chromium release reflect membrane transport properties. Our data suggest the possibility that patulin may have a direct effect on the membrane (33) because these effects were observed more quickly than one would predict as a secondary effect of inhibition of protein synthesis. In contrast, the effect of T-2 toxin on mean cell volume and chromium release was delayed and appeared to be secondary to the inhibition of protein synthesis. T-2 toxin is approximately two orders of magnitude more toxic to protein synthesis in these cells than...
patulin, yet T-2 toxin had no measurable effect on mean cell volume, chromium release or ATP levels after 6 hr exposure at levels of T-2 toxin which strongly inhibited protein synthesis. The decrease in mean cell volume observed after 18 hr exposure appeared to be the result of cell fragmentation. Thus the action of patulin in these cells is distinct from that of T-2 toxin. Patulin had a roughly comparable effect on phagocytosis to T-2 toxin if one considers both dose and exposure time, even though T-2 toxin was more toxic than patulin with respect to protein synthesis. Cells treated with $5 \times 10^{-7}$ M patulin for 1 hr had a similar phagocytic response to cells treated with $10^{-7}$ M T-2 toxin for 6 hr.

T-2 toxin and patulin were shown to inhibit several critical cellular functions in cultured alveolar macrophages. Therefore, inhalation of airborne grain dust or silage particulates contaminated with these mycotoxins could have deleterious effects on normal macrophage function and could pose a hazard to exposed workers.

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