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Composition of Extracts of Airborne Grain Dusts: Lectins and Lymphocyte Mitogens

by Stephen A. Olenchock,* Daniel M. Lewis,* and Judith C. Mull*

Airborne grain dusts are heterogeneous materials that can elicit acute and chronic respiratory pathophysiology in exposed workers. Previous characterizations of the dusts include the identification of viable microbial contaminants, mycotoxins, and endotoxins. We provide information on the lectin-like activity of grain dust extracts and its possible biological relationship. Hemagglutination of erythrocytes and immunochemical modulation by antibody to specific lectins showed the presence of these substances in extracts of airborne dusts from barley, corn, and rye. Proliferation of normal rat splenic lymphocytes *in vitro* provided evidence for direct biological effects on the cells of the immune system. These data expand the knowledge of the composition of grain dusts (extracts), and suggest possible mechanisms that may contribute to respiratory disease in grain workers.

Introduction

Respiratory pathophysiology associated with grain dusts is reported for grain storage workers worldwide. The potential adverse respiratory effects attributed to the inhalation of airborne gain dusts include acute reactions such as grain fever syndrome (*1*), allergy (*2*), and asthma (*3,4*). Chronic effects such as hypersensitivity pneumonitis (*5*) and chronic bronchitis (*6*) are reported as well. Airborne grain dusts are heterogeneous in nature and may contain multiple contaminants (*7,8*) that have the potential to elicit pulmonary reactivity in exposed workers.

Characterizations of grain dusts reveal the presence of contamination by bacteria and fungi (*9–15*) and their associated toxic substances, such as endotoxins (*9,16,17*) and the mycotoxins, aflatoxin (*18–22*), and secalonic acid D (*23,24*). Additionally, grain dusts may contain mites (*25*), quartz (*26*), and fumigants or pesticides (*27,28*). In an effort to characterize grain dusts further, the elemental compositions of a group of airborne and settled dusts were reported (*29*) as were the results of preliminary chemical analyses (*30,31*). Thus, the nature of the material within airborne grain dusts is of interest to many areas of scientific research. Inhalation of these and other, as yet unidentified, constituents in airborne grain dusts may have profound biological consequences.

It is the purpose of this report to characterize further grain dusts in terms of constituents that may be active immunologically. Specifically, the presence of lectins and lymphocyte mitogens is reported for aqueous extracts of a group of airborne grain dusts.

*Immunology Section, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, WV 26505.

Materials and Methods

Grain Extracts

Airborne grain dusts from barley, corn, durum wheat, oat, rye, and spring wheat were collected in active port grain terminals which were located in the Duluth-Superior areas of the United States (*16*). Aqueous extracts (10% w/v) were prepared for each dust by mixing with sterile, pyrogen-free water (Travenol Laboratories, Inc., Morton Grove, IL) for 24 hr at 4°C. The extracts were clarified by centrifugation at 1000*g*, filtered through 0.45-μm pore size filters, and lyophilized. The resulting residues were weighed and redissolved at various concentrations in sterile, pyrogen-free saline (0.15 M NaCl; Travenol Laboratories, Inc.). After dialysis against saline, aliquots of the dissolved solids were stored at −70°C until used.

Endotoxin Analyses

Quantification of gram-negative bacterial endotoxin concentrations of the extracts was performed in duplicate by a spectrophotometric modification of the Limulus amebocyte lysate gel test (Pyrostat; Millipore Corp., Bedford, MA). Sterile, nonpyrogenic plastic ware was used throughout these assays. The results were analyzed by linear regression, compared to a standard cure, and reported in terms of nanograms of

United States reference endotoxin per milligram of lyophilized sample.

Protein Analyses

The protein concentrates of the extracts were determined by the method of Lowry. Bovine serum albumin was used as a standard.

Lectin Determinations

Various dilutions (neat to 1:2048 in phosphate-buffered saline, pH 6.8) of the grain dust stock solutions were analyzed for the presence of lectin-like activity by studying the agglutination of sheep erythrocytes by the grain dust extracts (*32*). Twofold dilutions of 25 μL of the extracts were made in microtiter plates. Specific antisera against known lectins were obtained commercially (Sigma Chemical Co., St. Louis, MO, and United States Biochemical Corp., Cleveland, OH) and diluted 1:8 in phosphate-buffered saline (PBS). Aliquots (25 μL) of the diluted antisera were added to each dilution of extract, followed by the addition of 25 μL of sheep erythrocytes (2% v/v in PBS). After mixing, the plates were kept at room temperature until the cells had settled. The agglutination patterns were determined visually and reported as the last dilution of stock solution which caused agglutination. In order to enhance the sensitivity of the agglutination reaction, the experimental design was so altered that the lectins were reacted first with the sheep erythrocytes and incubated for 60 min at room temperature. After centrifugation, the cells were washed three times with PBS. Original volume was returned by the addition of PBS, and the antilectin antisera were added. Agglutination was observed and the results were reported as before.

Mitogenic Activity for Lymphocytes

The lymphocyte blast transformation assay was used to determine the mitogenic activity of the grain dust extracts. Spleens from normal, healthy, and specific pathogen-free Lewis rats were excised, minced, and prepared into single cell suspensions in Hank's Balanced Salt Solution (HBSS; GIBCO, Grand Island, NY). The suspensions were layered over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) and centrifuged at 400*g* for 15 min. Mononuclear cells were recovered from the interface. The cells were washed three times in HBSS and the cell concentration was adjusted appropriately in RPMI-1640 medium (GIBCO) which contained 10% (v/v) fetal bovine serum (GIBCO) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). The RPMI–serum–antibiotics mixture is designated as "complete medium." In some experiments, adherent cells were removed by incubation of the mononuclear cell preparation in complete medium for 2 hr at 37°C in plastic petri dishes. The nonadherent cells were washed off gently and then washed once and resuspended to the appropriate final concentrations.

Dilutions of known lymphocyte mitogens, phytohemagglutinin (PHA; Wellcome Reagents Limited, Bechenham, England) and Concanavalin A (Con A; Pharmacia Fine Chemicals), or grain dust extracts were prepared in complete medium, and 100 μL were dispensed in each well of microculture plates. To each well, 100 μL of the appropriate cell suspension were added, and the cells were incubated at 37°C for 96 hr in the presence of 5% CO_2. Tritiated thymidine (specific activity, 2 Ci/mM; New England Nuclear, Boston, MA) was added to each well (1 μCi/well) and the incubation continued for an additional 18 hr. The cells were harvested with a multichannel cell harvester (BRANDEL, Gaithersburg, MD), and the incorporation of tritiated thymidine was determined by scintillation counting. The results are expressed as the mean value of four replicates, and each experiment was repeated at least three times.

Results

Agglutination of Sheep Erythrocytes

In the first series of experiments to determine the presence of lectin-like activity in the grain dust extracts, the diluted extracts were added first and then followed by the addition of the antisera. The sheep erythrocytes were added last. Additional wells contained extract and sheep erythrocytes only. The extract of airborne barley dust agglutinated the erythrocytes at an extract dilution of 1:4 in the absence of antibody to the lectins. No other extract caused agglutination. The addition of specific antibody to the lectin *Dolichos biflorus* (horse gram) reduced the agglutination titer to 1:2. Antibody to *Glycine max* (soybean) and *Ricinus communis* (castor bean) each reduced the agglutination titer to neat.

The second series of experiments were designed to enhance the sensitivity of the agglutination reaction by reacting the extracts first with the sheep erythrocytes for 60 min. The specific antibody was then added, and the agglutination was observed. Table 1 shows the results of these studies. As before, only barley dust extract agglutinated the erythrocytes in the absence of antibody. The agglutination reaction was enhanced as evidenced by the greater number of agglutinations that were observed for barley dust extract. Additionally, extracts of corn and rye dusts caused agglutinations that were observable in the presence of certain antibodies. Table 1 also describes the sources and major specificities of the lectins.

Lymphocyte Reactivity

Figure 1 illustrates the mitogenic response of rat mononuclear cells to increasing concentrations of grain dust extracts. Each grain dust extract induced rat spleen cells to proliferate, and the amount of thymidine uptake was a function of the concentration of extract. For each extract except for corn dust extract, the maximum proliferation was obtained at the concentrations that were tested. Additionally, the amount of thymidine

Table 1. Agglutination of sheep erythrocytes by aqueous extracts of airborne grain dusts with and without enhancement by specific antibody to known lectins.

Antibody to lectin	Lectin source[a]	Major lectin specificity[a]	Extracts[b]		
			Barley	Corn	Rye
None			1:4		
Castor bean I	Castor bean	D-Galatose	—	—	—
Griffonia simplicifolia	*Bandeiraea simplicifolia*	*N*-Acetyl-D-galactosamine	Neat	—	—
Lentil bean A + B	Lentil	α-D-Mannose	—	—	—
Wheat germ	Wheat	Di-*N*-acetylchitobiose	—	—	1.8
Concanavalin A	Jack bean	α-D-Mannose	—	—	—
Bauhinia purpurea	*Bauhinia purpurea*	*N*-Acetyl-D-galactosamine	—	—	—
Gorse seed I	Gorse	L-Fucose	1:2	—	—
Phaseolus vulgaris	Kidney bean	Erythroagglutinin	1:4	1:16	—
Dolichos biflorus	Horse gram	*N*-Acetyl-D-galactosamine	1:8	Neat	—
Tetragonolobus purpureas	Asparagus pea, winged pea	α-L-Fucose	1:4	—	—
Glycine max	Soybean	*N*-Acetyl-D-galactosamine	1:2	—	—
Ricinus communis	Castor bean	D-Galactose	1:2	—	—

[a] Data of Sharon and Lis (*32*) and manufacturers' catalogs.

[b] Stock concentrations of extracts used: 10 mg/mL, barley; 25 mg/mL for corn and rye. Not shown were durum wheat (25 mg/mL), oat (25 mg/mL), and spring wheat (10 mg/mL) which were negative for each test. Data shown as greatest dilution of stock solution that showed agglutination.

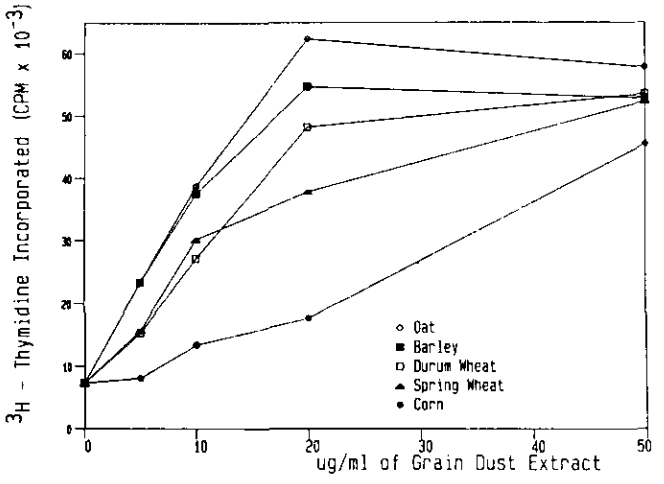

FIGURE 1. Dose–response curves for mitogenic activity of aqueous extracts of airborne grain dusts for rat splenic mononuclear cells as measured by tritiated thymidine incorporation.

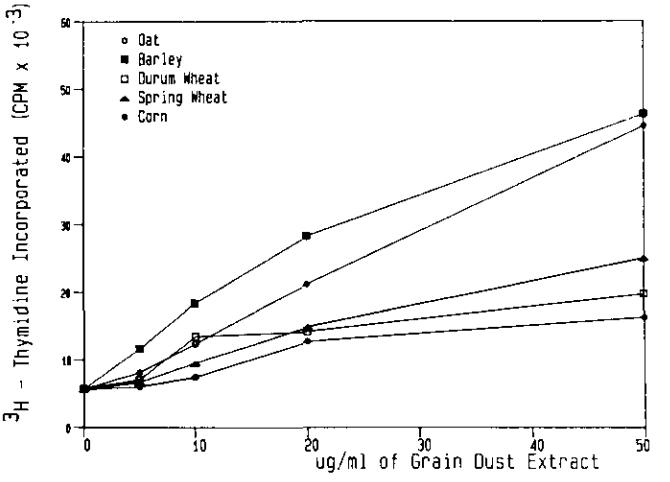

FIGURE 2. Dose–response curves for mitogenic activity of aqueous extracts of airborne grain dusts for nonadherent rat splenic mononuclear cells as measured by tritiated thymidine incorporation.

uptake appeared to be related to the type of grain dust. These responses were seen when the cells were cultured at a relatively high cell density (6×10^6 cells/mL of medium). Little or no proliferation was detected when cells were cultured at lower densities. Positive control response curves to the known mitogens, PHA and Con A, were included in each assay, and the maximum responses to these agents were 196,961 cpm and 345,962 cpm, respectively.

In order to begin to define the type of responsive cell, adherent spleen cells were removed, and the nonadherent lymphocytes were cultured with the extracts as before. Dose–response curves were generated for each extract at several cell densities, and the results for the mitogenic responses at a density of 6×10^6 cells/mL of medium are shown on Figure 2. It is apparent from the curves that grain dust extracts can induce splenic lymphocytes to proliferate in a dose-dependent manner. The curves, however, show that the maximum proliferation is shifted to the right for all the extracts when compared to Figure 1. In these experiments, the maximum responses for the control mitogens, PHA and Con A, were 350,272 cpm and 172,043 cpm, respectively.

Mitogenic Potential

The mitogenic potential for each airborne grain dust extract was determined from the responses observed in Figure 1. Table 2 shows that the aqueous extract of barley dust was the most mitogenic for rat spleen cells, in that a concentration of 6.5 μg/mL was required to stimulate 50% of maximum proliferation. The extract of corn dust was the least active, as it required 26.0 μg/mL to achieve 50% of the maximum response. Also shown on Table 2 are the concentrations of endotoxins and protein in the extracts. There is no readily apparent

Table 2. Mitogenic potential of airborne grain dust extracts and comparison to endotoxin and protein contents.

Dust extract	Mitogenic potential, μg/mL[a]	Endotoxin, ng/mg[b]	Protein, μg/mg[b]
Corn	26.0	10600	11.5
Durum wheat	9.5	8500	29.2
Spring wheat	9.2	11500	19.5
Oat	7.6	1700	29.9
Barley	6.5	10100	37.8

[a] Concentration of extract which stimulates 50% maximum proliferation of rat lymphocytes.
[b] Protein and endotoxin levels/mg lyophilized sample.

association between mitogenic potential and either endotoxin or protein content for the extracts.

Discussion

Exposures to airborne dusts from grain occur in a variety of agricultural industries. From the farm to the storage, transport, and processing areas, a large workforce worldwide is exposed to the effects of inhaled grain dusts (*29*). Deleterious effects of the dusts on lung function are well documented, and some investigators suggest that airborne grain dusts should not be regarded merely as nuisance dusts (*33*). Recognition of the medical importance of grain dusts has prompted a number of studies that investigated grain dusts in terms of specific components, such as viable microorganisms, toxic metabolites, applicants and fumigants, and insects and mites. Our laboratories have been interested in the mechanisms of pulmonary reactions to airborne grain dusts. In that regard, we are interested in those agents which are active immunologically (*34*). This report, therefore, expands our studies by demonstrating the presence of lectins in the airborne grain dusts and by showing the potential for aqueous extracts of the dusts to be mitogenic for lymphocytes (*35*).

The agglutination of erythrocytes by extracts of grain dusts was shown previously with phenol extracts but not with water or saline extracts (*36*). Our studies show that, of the six dusts tested, only the aqueous extract of airborne barley dust agglutinated sheep erythrocytes directly. With enhancement by the addition of specific antibody to known lectins, however, we demonstrated agglutination by aqueous extracts of airborne corn and rye dusts as well. Of interest, rye dust extract agglutinated sheep erythrocytes in the presence of antibody to wheat germ, while the same reactivity was not observed with extracts of either durum or spring wheats. The lack of detection of any lectins in the extracts of durum wheat, oat, or spring wheat may be related to the concentration of the lectins in our extracts. Perhaps also, the correct lectins were either not studied for those grain dusts, or the antibodies were not of the correct specificity to aid in their detection.

Wheat germ agglutinin has been shown to stimulate human peripheral lymphocytes to undergo blast transformation *in vitro* (*37,38*). Similarly, rice was shown to contain lymphocyte-stimulating activity (*39*), and lectin-like constituents were found in certain seeds and cereal grains (*40*). We reported previously in preliminary fashion that grain dust extracts have a direct effect on cells of the immune system (*35*). This report presents our findings in detail.

Aqueous extracts of each of the five airborne grain dusts that we tested can induce rat spleen cells to proliferate in a dose-dependent fashion. When the cell preparations were enriched for nonadherent cells, lymphocytes, proliferation was likewise demonstrated, although the dose–response curves were shifted to the right. In each experiment, the response that was induced by grain dust extracts was always less than that which was induced by PHA or Con A. In addition, maximum stimulation of spleen cells by the grain dust extracts was observed only when the cells were cultured at a high cell density. These observations together suggest that lymphocytes, and perhaps only a subpopulation of lymphocytes, are the cell type which is activated directly by the extracts. However, it should be considered that all of the adherent cells may not have been removed by the procedure which we used, and adherent cells may be required for enhancement of the proliferative response. The mitogenic potential of each dust extract was related to the type of grain and not to the content of endotoxins or protein in the extracts. Previous studies have demonstrated also that airborne grain dusts differ in their relative potency for activation of the human complement system as well (*16*). This differential toxicity for humoral, and now cellular, responses suggests that some grain dusts may be more irritating than others to the immune system *in vivo*.

In conclusion, we demonstrated the presence of lectins in aqueous extracts of airborne dusts of barley, corn, and rye. Extracts of durum wheat, oat, or spring wheat did not contain detectable lectins, although those three extracts as well as those from barley and corn dusts induced rat lymphocytes to proliferate *in vitro*. Lymphocyte mitogens were present, therefore, in the grain dust extracts, and may contribute to a lymphocyte-mediated reactivity *in vivo* after inhalation of airborne grain dusts.

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Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

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