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Journal of Toxicology and Environmental Health Part A

ISSN: 1528-7394 (Print) 1087-2620 (Online) Journal homepage: https://www.tandfonline.com/loi/uteh20

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To cite this article: Shih-Houng Young , Victor Robinson , Mark Barger , Michael Whitmer , Dale Porter , David Frazer & Vincent Castranova (2003) Exposure to Particulate 1→3-β-Glucans Induces Greater Pulmonary Toxicity Than Soluble 1→3-β-Glucans in Rats, Journal of Toxicology and Environmental Health Part A, 66:1, 25-38, DOI: 10.1080/15287390306462

To link to this article: https://doi.org/10.1080/15287390306462

Published online: 07 Jan 2011.

Journal of Toxicology and Environmental Health, Part A, 66:25–38, 2003

1528-7394/03 $12.00 + .00
DOI: 10.1080/15287390390155732

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EXPOSURE TO PARTICULATE 1→3-β-GLUCANS INDUCES GREATER PULMONARY TOXICITY THAN SOLUBLE 1→3-β-GLUCANS IN RATS

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1→3-β-Glucans, derived from the inner cell wall of yeasts and fungi, are commonly found in indoor air dust samples and have been implicated in organic dust toxic syndrome. In a previous study, it was reported that 1→3-β-glucan (zymosan A) induced acute pulmonary inflammation in rats. This study investigates which form of 1→3-β-glucans, particulate or soluble, is more potent in inducing pulmonary inflammation. Zymosan A was suspended in 0.25 N NaOH for 30 min, neutralized, dialyzed for 2 d using deionized water, and particulate and soluble fractions were collected. Male Sprague-Dawley rats were exposed via intratracheal instillation to NaOH-soluble or NaOH-insoluble zymosan A. At 18 h postexposure, various indicators of pulmonary response were monitored, including indicators of lung damage, such as serum albumin concentration and lactate dehydrogenase (LDH) activity in acellular bronchoalveolar lavage fluid. Inflammation was characterized by an increase in lavageable polymorphonuclear leukocytes (PMN). Pulmonary irritation (breathing frequency increase) and oxidant production (nitric oxide and chemiluminescence, CL) were also monitored. Exposure to the particulate form of NaOH-treated zymosan produced a significant increase in all these indicators. In contrast, rats exposed to the NaOH-soluble fraction were not markedly affected except for LDH, PMN, and CL. However, these increases were significantly less than with exposure to NaOH-insoluble zymosan. Therefore, results demonstrate that particulate zymosan A is more potent in inducing pulmonary inflammation and damage in rats than the soluble form of this β-glucan.

1→3-β-Glucans derived from the inner cell wall of yeasts and fungi are commonly found in indoor air dust samples and have been implicated in organic dust toxic syndrome. In a previous study, it was reported that 1→3-β-glucan (zymosan A) induced acute pulmonary inflammation in rats (Young et al., 2001). This study investigates which form of 1→3-β-glucans, particulate or soluble, is more potent in inducing pulmonary inflammation and damage.

1→3-β-Glucans are polymers of glucose with a molecular mass in the range of 10^4 to 10^7 Da. 1→3-β-Glucans are usually water insoluble, except

Received 11 January 2002; sent for revision 11 February 2002; accepted 21 March 2002.

This work was performed while S.-H. Young held a National Research Council–NIOSH Research Associateship.

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highly branched 1→6-linked 1→3-β-glucans (Whistler, 1973), and the particulate form of 1→3-β-glucans is generally the form found in environmental samples. For research purposes, some laboratories have developed protocols based on high pH (Fogelmark et al., 1992), in order to increase the solubility in water. However, such studies have ignored the possible alternation of biological activity of treated 1→3-β-glucans due to changes in physical form. High-pH treatment often results in two fractions, a soluble and a particulate fraction. This study examines the pulmonary inflammatory potential of these two fractions.

Conformation is an important factor determining the biological activity of glucans. The native conformation of 1→3-β-glucan is thought to be a triple-helix conformation (Bluhm & Sarko, 1976; Deslandes et al., 1980; Kashiwagi et al., 1981; Chuah et al., 1983). The conversion between different conformations can be mediated by different chemical or physical treatments (Yadomae & Ohno, 1996). Therefore, it was necessary to consider the effect of different methods used for solublization. Four major types of sample treatments are commonly found for solublization of particulate 1→3-β-glucans. They are heating, solublization in acid or base, or use of organic solvents. In addition to solublizing particulate 1→3-β-glucans, each treatment method modifies the properties of 1→3-β-glucans to some degree. Heating reduces the molecular weight of 1→3-β-glucans, and this reduces their immunological stimulating ability (Adachi et al., 1990). Oxidative degradation by acid has been reported with 1→3-β-glucans (Nono et al., 1991). Formic acid degradation of glucan is a useful method to prepare low-molecular-weight 1→3-β-glucans (Ohno et al., 1995). Linear glucans, such as curdlan (no branches) and pachyman (few branches), were easily degraded to oligosaccharides by treatment for 20 min at 100 °C with 90% formic acid. However, the highly branched glucans were resistant to degradation (Ohno et al., 1995). A weak alkaline solution normally will not degrade 1→3-β-glucans. The (1→6) branched glucans are more stable compared to unbranched glucans (Whistler & BeMiller, 1958). However, a strong alkaline solution will change the conformation of glucans from a helix to a random coil. This change in conformation will also affect biological activities of these glucans. Use of organic solvents, such as 8 *M* urea and dimethyl sulfoxide, is one method for preparing different conformers of glucans. Glucans lyophilized from organic solvent can have a very different biological activity than the parent glucans (Maeda et al., 1988; Stone & Clarke, 1992). The effect of NaOH alone can be removed by neutralization and dialysis, which provide a relatively easy way to remove any artifact induced by NaOH. Therefore, a 30-min 0.25 *N* NaOH treatment was chosen to solublize 1→3-β-glucans in the present study.

The purpose of this investigation was to compare the pulmonary inflammation and damage after exposure to the particulate or soluble form of 1→3-β-glucans. Such a comparison is important because although the particulate form of 1→3-β-glucans is found in nature, many laboratories have reported the effects of solubilized 1→3-β-glucans in the literature. Animals were exposed to particulate or soluble zymosan via intratracheal (IT) instillation. At 18 h postexposure, various indicators of pulmonary responses were monitored, including indicators of lung

damage [serum albumin concentration and lactate dehydrogenase (LDH) activity in acellular bronchoalveolar lavage fluid] and inflammation [lavageable polymorphonuclear leukocytes (PMN)], pulmonary irritation (breathing frequency increase), and oxidant production [nitric oxide and chemiluminescence (CL)].

MATERIALS AND METHODS

Preparation of Soluble and Particulate Zymosan A Samples

Zymosan A, from baker's yeast, was obtained from Sigma Chemical Company (St. Louis, MO). The majority of particles were between 2 and 5 μm in diameter, which is in the range of respirable particles for rats. The particle size distribution in suspension was about the same as reported in a previous inhalation study (Robinson et al., 1996). Zymosan (30 mg) was suspended in 1 ml of 0.25 *N* NaOH at room temperature for 30 min, followed by neutralization with 3 *N* HCl to pH 6. The resulting solution was dialyzed using deionized water (Milli-Q plus, Millipore, Cranberry Twp., PA) for 2 d at 4 °C. The dialysis membrane used was Spectra/Por 3 (Spectrum, Laguna Hills, CA) with a molecular mass cutoff at 3500 Da. After dialysis, the solution was centrifuged at 800×g for 10 min. The supernatant was adjusted for osmotic balance by adding an adequate amount of saline to the solution and then filtered through a 0.2-μm cellulose filter. The particulate fraction was suspended in phosphate-buffered saline (PBS), pH 7.3, for IT instillation.

Estimation of the concentrations of the NaOH-soluble zymosan preparations was done in separate but parallel experiments. Zymosan (30 mg) was treated with NaOH as already described. After dialysis, the retained NaOH–zymosan suspension was centrifuged at 800×g for 10 min, and particulate and soluble fractions of zymosan collected. The particulate fraction was placed in an oven (160 °C) for 2 h. The average weight collected in the particulate fraction after heating was 20.0±0.3 mg from 30 mg of starting material (of 5 experiments). The starting material by itself will lose 3.9±0.14 mg from 30 mg after 2 h of heating (of 5 experiments). Therefore, using back calculation, the estimated weight loss after NaOH treatment was 7 mg from 30 mg of starting material. The concentration of NaOH-soluble zymosan was calculated by assuming that this weight loss from starting zymosan was dissolved in the solution. The dosage of soluble zymosan administered by IT instillation was calculated to be 1.6 mg/kg body weight and was similar to that for the insoluble fraction, 1.9 mg/kg body weight.

Endotoxin Measurement

To exclude the possibility that pulmonary responses were due to contamination with LPS, aliquots of the NaOH–zymosan suspension used for IT instillation were centrifuged at 800×g for 10 min, and the supernates were analyzed for endotoxin content using the *Limulus* amebocyte lysate (LAL) assay (BioWhittaker kinetic-QCL assay, BioWhittaker, Walkersville, MD). The analysis was conducted according to the manufacturer's instructions.

Animals

The animals used in these experiments were male Sprague-Dawley rats [Hla:(SD) CVF] weighing 200–300 g (approximately 10 wk old at arrival) obtained from Hilltop Labs (Scottdale, PA). The animals were housed in a AAALAC-accredited, specific-pathogen-free, environmentally controlled facility. The rats were monitored to be free of endogenous viral pathogens, parasites, mycoplasms, *Helicobacter*, and CAR *Bacillus*. Rats were acclimated for at least 5 d before use, and were housed in ventilated cages that were provided HEPA-filtered air, with Alpha-Dri virgin cellulose chips and hardwood Beta-chips used as bedding. The rats were maintained on ProLab 3500 diet and tap water, both of which were provided ad libitum.

Intratracheal Instillation

Rats were anesthetized with an intraperitoneal injection of 0.6 ml of 1% (w/v) sodium methohexital (Eli Lilly Co., Indianapolis, IN) and IT instilled with either NaOH-soluble (1.6 mg/kg body weight) or insoluble (1.9 mg/kg body weight) zymosan. Control animals were instilled with an equivalent volume of PBS. Each experimental group included at least 5 rats. Pulmonary responses to 1→3-β-glucans were measured 18 h post-IT instillation.

Breathing Rate Measurement

Breathing frequencies were determined using a flow plethysmograph that has been previously described in detail (Frazer et al., 1997). Instillation of dust particles that are classified as pulmonary irritants has been shown to cause an increase in respiratory rate (Robinson et al., 1997; Porter et al., 1999). Breathing frequencies were determined immediately prior to the IT exposures and at 18 h post-IT instillation.

Bronchoalveolar Lavage and Biochemical Assay of Bronchoalveolar Lavage Fluid

Rats were euthanized with sodium pentobarbital (≥100 mg/kg body weight, ip; Butler, Columbus, OH). The trachea was cannulated, and the lungs were lavaged with ice-cold Ca^{2+}/Mg^{2+}-free PBS (145 m*M* NaCl, 5 m*M* KCl, 1.9 m*M* NaH_2PO_4, 9.35 m*M* Na_2HPO_4; pH 7.4) at a volume of 6 ml for the first lavage and 8 ml for subsequent lavages. A total of 80 ml of bronchoalveolar lavage fluid was collected from each rat and centrifuged at 800×g for 10 min at 4 °C. The acellular supernatant from the first lavage (first BAL fluid) was saved for analysis of albumin concentration and LDH activity. Supernatants from other lavages were decanted and discarded. All cell pellets from an individual rat were combined as bronchoalveolar lavage (BAL) cells, resuspended in 1 ml HEPES-buffered solution (145 m*M* NaCl, 5 m*M* KCl, 10 m*M* *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid, 1 m*M* $CaCl_2$, and 5.5 m*M* D-glucose; pH 7.4) and placed on ice. Aliquots of cell suspensions from each rat were taken for determination of total cell and differential cell counts using a Coulter Multisizer II

and AccuComp software (Coulter Electronics, Hialeah, FL). The remaining BAL cells were used to determine alveolar macrophage (AM) chemiluminescence, and production of nitric oxide.

The albumin concentration in the first acellular BAL fluid was used to evaluate the permeability of the alveolar–capillary barrier. The activity of LDH, a cytosolic enzyme, in the acellular first BAL fluid was assayed to evaluate cytotoxic cell damage. The albumin concentration and LDH activity were measured using an automated Cobas Fara II Analyzer (Roche Diagnostic Systems, Montclair, NJ). The albumin concentration was determined colorimetrically at 628 nm based on albumin binding to bromcresol green (albumin BCG diagnostic kit, Sigma Chemical Company, St. Louis, MO) and expressed as milligrams per milliliter BAL fluid. LDH activity was measured by the formation of NADH and expressed as units per liter BAL fluid using Roche Diagnostic reagents and procedures (Roche Diagnostic Systems).

Alveolar Macrophage Chemiluminescence

The alveolar macrophage (AM) chemiluminescence (CL) assay was conducted in a 0.25-ml reaction mixture of HEPES-buffered solution. Resting AM CL was determined by incubating 0.25×10^6 AMs cells at 37 °C for 20 min, then adding 0.008 mg% (w/v) luminol (Sigma Chemical Company, St. Louis, MO), followed by the measurement of CL for 15 min. To determine the responses of AM to zymosan, the reaction mixture was modified to include 0.5 mg unopsonized zymosan (Sigma Chemical Company, St. Louis, MO), which was added immediately prior to the measurement of CL. Measurement of AM CL was conducted with an automated luminometer (Berthold Autolumat LB 953, Wallace, Inc., Gaithersburg, MD) at 390–620 nm for 15 min, and the integral of counts per minute (cpm) versus time was calculated. Resting CL was obtained from cpm of AM in the presence of luminol. Particle-stimulated CL was calculated as the cpm in the presence of the zymosan minus the cpm in the resting assay. The NO-dependent CL was determined by adding 1 m*M* *N*-nitro-L-arginine methyl ester HCl (L-NAME, Sigma Chemical Company, St. Louis, MO) to the cells prior to the 20-min preincubation. NO-dependent CL was calculated as the difference between zymosan-stimulated CL measurements in the absence or presence of L-NAME.

Primary BAL Cell Culture

The BAL cells from each rat were suspended in RPMI-1640 culture medium (RPMI, Sigma Chemical Co.) containing 2 m*M* glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin, and 10% heat-inactivated fetal bovine serum. Aliquots of cell suspensions from each rat, adjusted to 0.25×10^6 AM, were added to each well of a 24-well tissue culture plate. AMs were allowed to adhere to the plastic culture plate for 120 min in a humidified incubator (37 °C and 5% CO_2). The nonadherent BAL cells were then removed by rinsing the monolayers three times with RPMI media. These AM-enriched preparations were incubated (37 °C and 5% CO_2) in fresh RPMI medium for 24 h. The

AM-conditioned media were collected, and the supernates were immediately analyzed for nitric oxide, or stored at −70 °C for later analyses.

Effect of In Vivo Zymosan Exposure on Ex Vivo LPS-Induced Nitric Oxide Production by AM

Ex vivo LPS-induced nitric oxide production was assessed as a measurement of the ability of AMs to respond to bacterial (LPS) treatment. The effect of pretreatment with zymosan in vivo on LPS-induced nitric oxide production was studied by culturing the BAL cells from zymosan-exposed or control rats. These BAL cells were cultured for 120 min, then rinsed twice to obtain AM-enriched adherent cells. These AM-enriched cells were then treated with 10 μg/ml LPS for an additional 24 h. The nitric oxide production was measured by the Griess reaction.

Determination of Nitric Oxide

Nitric oxide was determined as nitrite (NO_2^-) with Griess reagent as described by Green et al. (1982). Specifically, AM-conditioned media (100 μl) were collected, mixed with 100 μl of Griess reagent (0.5% sulfanilamide, 0.05% naphthylethylenediamine dihydrochloride, 2.5% H_3PO_4), and analyzed spectrophotometrically at 546 nm. Nitrite concentrations were determined using a standard curve prepared from sodium nitrite with a linear range from 1.6 to 206 μ*M*. Three replicates were done for each sample in the experiment.

Statistical Analysis

All data were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using SigmaStat v2.0. software (Jandel Corporation, San Rafael, CA). Significance was set at $p < .05$. For multiple comparison, a one-way analysis of variance (ANOVA) for comparing several treatment groups with one control was used.

RESULTS

Endotoxin

The endotoxin content of zymosan suspensions was measured by the LAL assay. Values for soluble zymosan ranged from 254 to 388 EU/ml, while endotoxin for particulate zymosan ranged from 8 to 89 EU/ml. Therefore, the amount of endotoxin that rats received ranged from 2 to 97 EU for NaOH-soluble and NaOH-insoluble zymosan samples. This dose is equivalent to 0.2 to 9.7 ng endotoxin. Previous studies from our laboratory indicate that such levels of endotoxin did not affect breathing rate or cause significant PMN infiltration in rats (Young et al., 2001).

Lung Damage

First BAL Fluid Albumin Concentration The albumin concentration was measured in first BAL fluid as an indicator of damage to the alveolar blood–gas

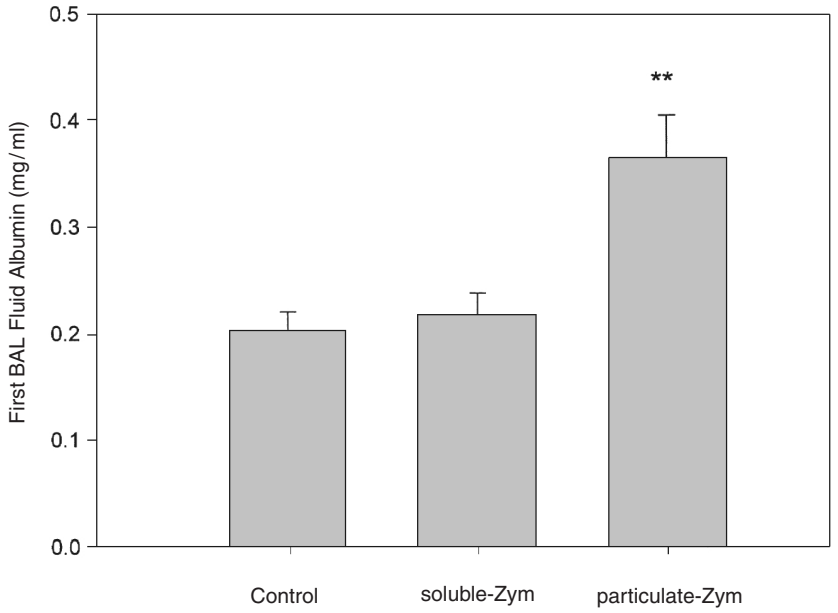

FIGURE 1. First BAL fluid albumin concentration. Particulate zymosan induced greater damage to the alveolar air/blood barrier than soluble zymosan. Values are means ± SEM of five rats per exposure group. Double asterisk indicates a significant increase versus both control and soluble zymosan levels ($p < .05$).

barrier. Figure 1 shows that exposure to soluble zymosan did not affect the albumin concentration in the BAL fluid compared to the control. However, particulate zymosan induced a 1.7-fold increase in albumin concentration versus control, which was significantly higher than that induced by soluble zymosan or the control level.

First BAL Fluid Lactate Dehydrogenase (LDH) Activity Pulmonary cell damage resulting from zymosan exposure was evaluated by measuring LDH activity in the acellular first BAL fluid. Figure 2 shows that exposure of rats to soluble zymosan induced a significant 1.8-fold increase in LDH activity versus control. However, exposure to particulate zymosan induced a 2.5-fold increase in LDH activity, which was significantly higher than control.

Inflammation

Pulmonary inflammation was evaluated by monitoring infiltration as determined by the number of polymorphonuclear leukocytes (PMN) harvested by BAL. Figure 3 shows that exposure to soluble or particulate zymosan both induced a significantly higher PMN infiltration than control. However, particulate zymosan induced a 10.5-fold increase in PMN number versus control, which was significantly higher than that induced by soluble zymosan (2.2-fold).

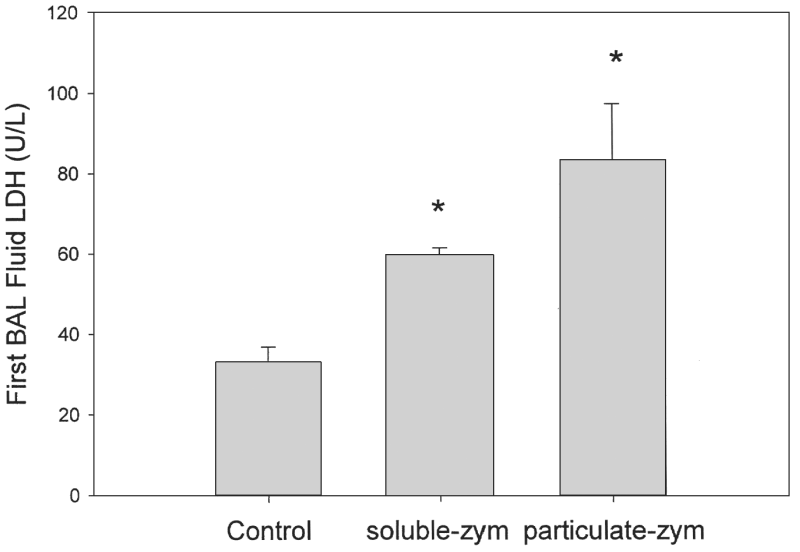

FIGURE 2. First BAL fluid LDH activity. Particulate and soluble zymosan induced greater cytotoxicity compared to the control. Values are means ± SEM of five rats per exposure group. Asterisk indicates a significant increase versus the control level ($p < .05$).

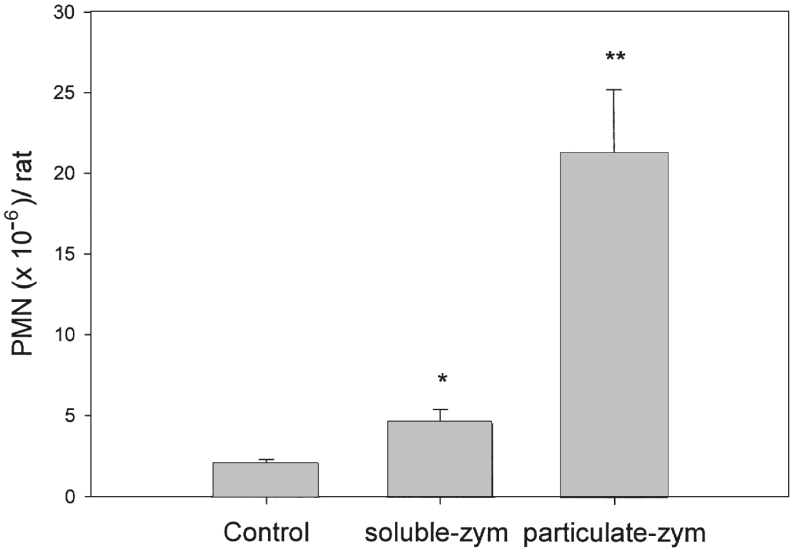

FIGURE 3. BAL PMN yield. Particulate zymosan induced a greater increase in PMN infiltration than soluble zymosan at 18 h post-IT instillation. Asterisk indicates a significant increase versus control ($p < .05$). Double asterisk indicates a significant increase versus both control and soluble zymosan ($p < .05$).

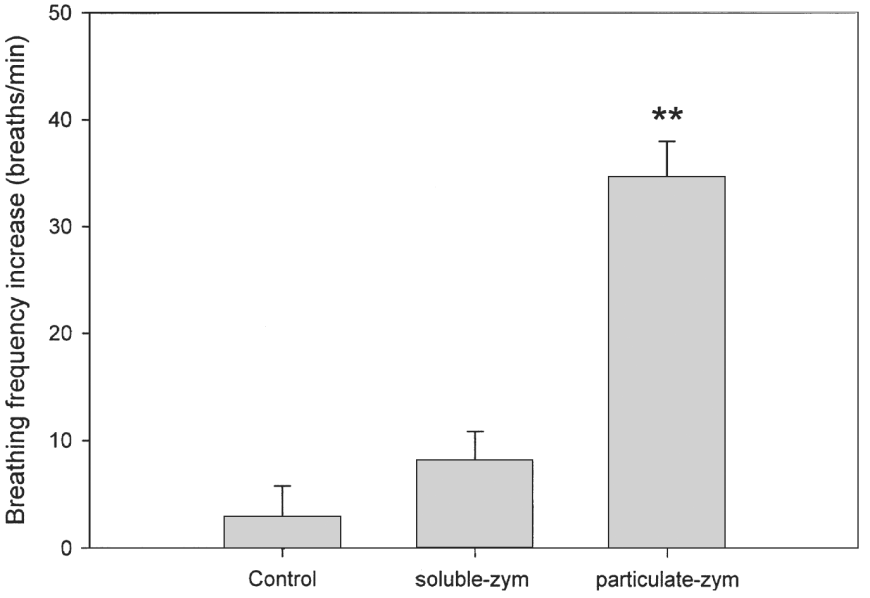

FIGURE 4. Breathing frequency increase. Results were expressed as the net difference between the 18 h post-IT and pre-IT instillation breathing frequency. Particulate zymosan induced a greater increase in breathing frequency than soluble zymosan at 18 h post-IT instillation. Double asterisk indicates a significant increase above the pre-IT instillation level and soluble zymosan levels, as well as control levels ($p < .05$).

Pulmonary Irritation

The breathing frequency increase was defined as the net difference between the 18 h post-IT and pre-IT instillation breathing frequency. The increased breathing frequencies post-IT instillation have been shown to have a direct correlation (correlation coefficient, $r = .97$) with zymosan dosage (Young et al., 2001). In this experiment, soluble zymosan did not affect breathing frequency compared to control rats (Figure 4). However, instillation of particulate zymosan was associated with a significantly higher increase in the breathing frequency compared to the control and soluble zymosan group. The particulate zymosan-induced an increase in breathing frequency 4.3-fold higher than that with soluble zymosan.

Oxidant Production

Nitric Oxide Production Nitric oxide is one of the cellular oxidants produced by AM after zymosan or LPS stimulation. No detectable nitric oxide was produced under basal conditions by AM from either control or soluble zymosan-treated rats (Figure 5). IT exposure to particulate zymosan produced 64.9 μ*M* of basal (0 μg LPS) nitric oxide production. In vivo soluble or particulate zymosan treatment followed by ex vivo LPS stimulation of AM resulted in

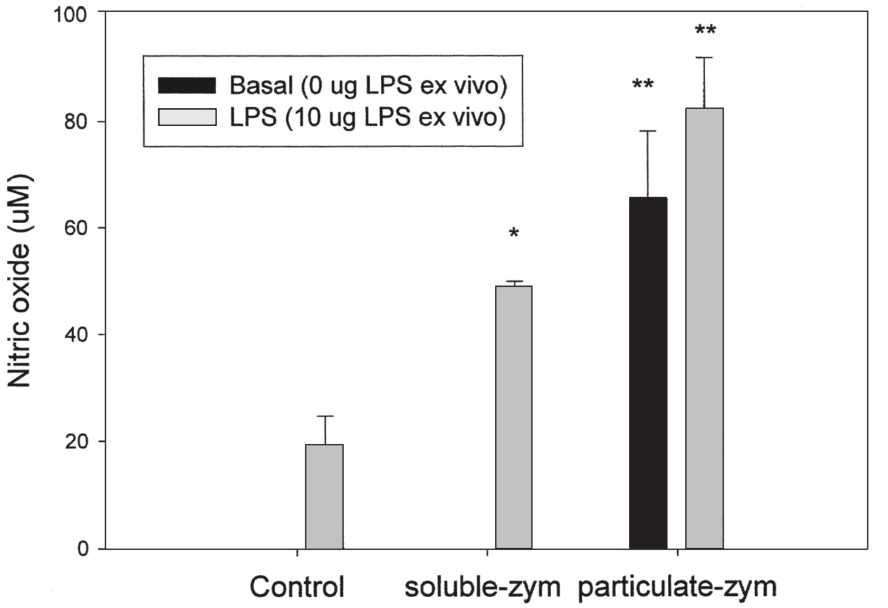

FIGURE 5. AM nitric oxide production. Particulate zymosan induced a greater increase in nitric oxide production from AM than soluble zymosan. Rats were exposed to PBS (control), soluble zymosan, or particulate zymosan by IT instillation for 18 h. AM were obtained by BAL and cultured for 24 h. The supernatant was collected for nitric oxide level determination. Values are means ± SEM of five rats per exposure group. Asterisk indicates a significant increase versus the respective (basal or LPS) control level ($p < .05$). Double asterisk indicates a significant increase above the respective control and soluble zymosan levels ($p < .05$).

a significantly higher nitric oxide production than control. A 2.6-fold increase in nitric oxide production over control was observed in the soluble zymosan group, while particulate zymosan induced a 4.4-fold increase in nitric oxide production by LPS-stimulated AM over control. In Figure 5, note that although the basal level (0 μg LPS) of nitric oxide induced by soluble zymosan was the same as that for control, when stimulated ex vivo with LPS (10 μg LPS), AM from the soluble zymosan group generated significantly higher nitric oxide than control.

Chemiluminescence Chemiluminescence (CL) generated by AM was monitored to assess the activation of AMs after IT exposure. For resting AM CL, there is no difference between CL from AM isolated from control and soluble zymosan exposed rats (Figure 6). However, in vivo exposure to particulate zymosan induced a twofold increase of resting AM CL compared to controls. For ex vivo particle-stimulated AM CL, soluble zymosan induced a 3.2-fold increase of AM CL above the control, while particulate zymosan induced a 8.6-fold increase of AM CL. For the NO-dependent ex vivo particle-stimulated AM CL, soluble zymosan induced a 4-fold increase of AM CL above control, while particulate zymosan induced a 9.7-fold increase of NO-dependent AM

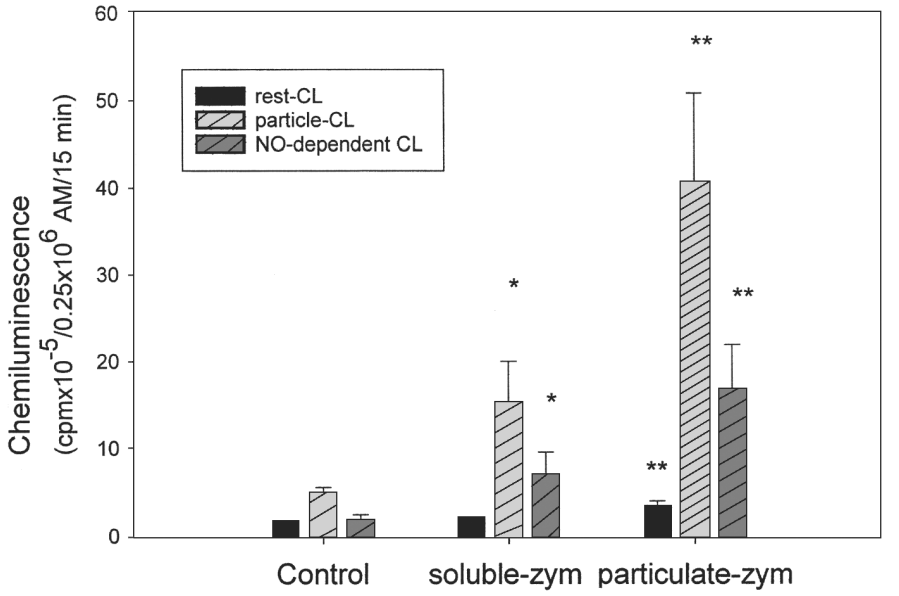

FIGURE 6. AM chemiluminescence. Particulate zymosan induced a greater increase in chemiluminescence than the soluble zymosan at 18 h post-IT instillation. Values are means ± SEM of five rats per exposure group. Asterisk indicates a significant difference versus the respective (rest-CL, particle-CL, or NO-dependent CL) control levels ($p < .05$). Double asterisk indicates a significant increase versus both the respective control and soluble zymosan levels ($p < .05$).

CL above control. Again the potency of particulate zymosan was significantly greater than the soluble fraction. In Figure 6, note that although in vivo exposure to soluble zymosan did not increase resting AM CL above control, it did significantly increase AM CL after ex vivo stimulation with particles.

DISCUSSION

Many investigators have used solubilized 1→3-β-glucans in their studies as a matter of experimental convenience even though the particulate form is the one found in nature. This has led to confusion as to the pulmonary inflammatory potency of this fungal product. For example, Rylander (1993) reported that 1→3-β-glucans (soluble form) produced little pulmonary inflammation, while inhalation of particulate zymosan was reported as inflammatory (Robinson et al., 1996). In addition, the immunologic effects of 1→3-β-glucans have been associated with exposure to the water-insoluble, particulate form of 1→3-β-glucans (Williams, 1997). In this experiment, the pulmonary responses to in vivo exposure to the soluble and particulate fraction of a known inflammatory 1→3-β-glucan, zymosan were evaluated. The results show that exposure to the particulate form of NaOH-treated zymosan induced a significant increase in lung damage (BAL fluid albumin levels and LDH activity), inflammation

(yield of PMN in BAL), pulmonary irritation (increase in breathing frequency), and AM activity (nitric oxide production and CL). In contrast, rats exposed to the NaOH-soluble fraction did not respond, except for LDH, PMN, and CL. In addition, these increases in the soluble zymosan group were substantially smaller than with exposure to NaOH-insoluble zymosan. The results demonstrate that particulate zymosan is more potent in inducing pulmonary inflammation and damage in rats than the soluble form of this β-glucan. Therefore, the particulate form of β-glucan is more appropriate in evaluating the role of this fungal product in diseases, such as organic dust toxic syndrome.

Data from the present study indicate that in vivo exposure to β-glucan increased ex vivo production of nitric oxide and generation of chemiluminescence from BAL cells. This oxidant production has been attributed to alveolar macrophages rather than neutrophils for the following reasons.

1. Nitric oxide production was measured from adherent BAL cells in culture. Adherence has been shown to enrich the preparation in alveolar macrophages by preferentially removing neutrophils (Blackford et al., 1994). In addition, alveolar macrophages have been found to be three to four times more potent producers of nitric oxide than neutrophils (L. J. Huffman and D. W. Porter, personal communications).
2. Chemiluminescence was stimulated with unopsonized zymosan in this study. Reports indicate the neutrophils do not response to unopsonized particles (Allen, 1977; Hill et al., 1977).

Therefore, in this study, ex vivo production of nitric oxide and generation of chemiluminescence from BAL cells are indications of the activity of alveolar macrophages (Castranova et al., 1987).

In vivo exposure to soluble zymosan was associated with the ability to prime AM to produce a significantly higher response to an ex vivo stimulant. This priming of AM was observed for LPS-induced nitric oxide production and particle-stimulated chemiluminescence assays. Priming of AM after IT exposure to particulate zymosan was also noted for the ex vivo particle-stimulated CL and NO-dependent CL assay.

The determination of 1→3-β-glucan concentration in an environmental or experimental sample is a challenging problem. Only a few assays are able to detect 1→3-β-glucan, but each one has some limitations. The LAL assay is commonly used to evaluate gram-negative bacterial endotoxin. Endotoxin activates a specific enzymatic pathway in the LAL assay through factor C (Iwanaga, 1993). However, 1→3-β-glucan can also produce a positive response to the LAL assay through factor G (Aketagawa et al., 1994). LAL kits from different manufacturers have different sensitivities to 1→3-β-glucan (Roslansky, 1990; Roslansky & Novitsky, 1991). LAL kits from BioWhittaker have been modified to reduce the response from nonendotoxin, LAL-reactive material, such as glucans. In our study, a gravimetric method was employed to calculate the concentration of soluble zymosan in solution. The results from LAL (Bio Whittaker)

measurements also indicate that 1→3-β-glucan was present in the soluble zymosan fraction.

In summary, data indicate that particulate zymosan is more potent in inducing pulmonary damage, inflammation, irritation, and activation of alveolar macrophages than is soluble zymosan. Therefore, it would seem better to study the biological effects of 1→3-β-glucan in their most natural state, the particulate form.

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