EFFECTS OF VEGETABLE DUST EXTRACTS ON CELLULAR IMMUNE RESPONSES

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Abstract

In an attempt to better define the inflammatory and/or immunogenic potential of vegetable dusts we have assessed the ability of aqueous extracts of grain and cotton dusts to stimulate interleukin 1 (IL-1) production. IL-1 is a recently recognized mediator of inflammatory and immunologic reactions and could serve as an index of the inflammatory potential of these extracts. The results obtained indicate that both grain and cotton dust extract can stimulate IL-1 production but vary markedly in their ability to do so. The activity does not correlate with the endotoxin content of the samples. Using cotton samples we found that the IL-1 stimulant could be removed from the cotton by washing with water but not acetone.

Introduction

Occupational exposure to vegetable dusts occurs in a variety of worksites. Such exposures have the potential to produce immunopathological reactions which could lead to chronic lung diseases such as asthma or hypersensitivity pneumonitis (17). While in some occupational lung diseases there is evidence to suggest that an immune response to inhaled antigens occurs and may contribute to the pathogenesis of the lung disease, there are other occupational lung diseases associated with organic dusts in which the role of immune reactions is less obvious. For example, in grain handlers and cotton mill workers precipitating antibodies to appropriate antigen extracts are not frequently seen in symptomatic individuals, i.e. persons who display respiratory symptoms on exposure to these dusts (4,8). While the presence of antibodies to an inhaled antigen does not necessarily mean that an immunopathological reaction is responsible for the respiratory symptoms, the lack of antibody could indicate that classical immunological reactions (allergy, or immune complex reactions) are not involved. This would suggest that these dusts may contain inflammatory, but non-immunogenic substances.

In an attempt to better define the inflammatory potential of certain vegetable dusts, we have tested the ability of aqueous extracts of these dusts to stimulate alveolar macrophages to produce interleukin 1 (IL-1). IL-1 is a soluble protein (mol. wt. 15,000) produced by monocytes and macrophages which has a number of biological activities. IL-1 is thought to function in the initiation of immune response (12), to stimulate the release of acute phase reactants (20), to promote fibroblast proliferation (19), and to be an endogenous pyrogen (5,14). Thus, IL-1 appears to be an important mediator of inflammatory reactions, and analysis of production of IL-1 may provide some insight into the inflammatory potential of vegetable dusts.

Materials and Methods

Preparation of Extracts

Samples of grain dust were obtained from active grain port terminals in the Duluth-Superior region of the United States as previously described (18). Dust samples of spring wheat, durham wheat, oats, and barley were used in this study. A 10% (W/V) mixture was prepared in sterile, non-pyrogen free water, and incubated for one hour at room temperature. The extracts were clarified by centrifugation (1000 g for 20 minutes), filtered through a 0.45 µm pore size filter (Millipore), and then lyophilized. The resulting residue was weighed and redissolved in sterile, non-pyrogenic saline (0.15 M NaCl) at a concentration of 1 to 5 mg of dissolved solids per ml. Samples of the stock suspensions were stored at -80°C until used.

Two samples of hand picked cotton, Stoneville closed boll bract intact (CBI) and Stoneville closed boll bract removed (CBR) (4), and a sample of bract (8) were used in this study. These samples were first ground to yield a fine powder (9), and the powders extracted as described above for the grain dusts. Extracts were stored at -80°C until used.

Samples of baled cotton were obtained from the People's Republic of China (15), and from the USDA Cotton Quality Research Station in Clemson, South Carolina (MQ-135). Extracts of baled cotton samples were prepared by placing 300 mg samples of cotton in 10 ml of sterile, non-pyrogenic free water, and gently agitating the mixture for one hour at room temperature. The samples were centrifuged for 10 minutes at 1000 g, the supernate collected and stored at -80°C until used.

IL-1 Production

Alveolar macrophages were obtained from 10-20 week old rats by pulmonary lavage using Hanks balanced salt solution (HBSS) lacking calcium, magnesium and phenol red. The cell suspension was washed once, counted and resuspended to a cell density of 1×10^6 cells per ml in Medium 199 (M-199, GIBCO, Grand Island, NY) containing 2% heat-inactivated fetal bovine serum (FBS) and antibiotics (100 unit/ml penicillin, 100 µg/ml streptomycin). Cell suspensions were cultured for 2 hours at 37° in 5% CO_2 at a cell density of 4×10^5 cells per cm^2 surface area of the culture vessel. After this incubation, the medium was removed from the cultures, the plates rinse with fresh media to remove nonadherent cells, and fresh medium containing test extract was added to the macrophage culture plates. For the grain dust, CBI, CBR, and bract extracts the concentrated stock extracts were diluted with M-199 to obtain the desired concentration. With the baled cotton extracts a 1/10 dilution of the extracts with M-199 was used. The macrophages were then incubated for 18 hours at 37°C in 5% CO_2. The spent media from these cultures were collected, centrifuged at 1000g for 10 minutes, filtered through a 0.45µm filter and stored in sterile vials at -70°C until assayed for IL-1 content. As a positive control macrophages were exposed to a known IL-1 stimulant, E-coli 0111:B4 lipopolysaccharide (LPS) (Difco Laboratories, Detroit, MI) at 10 µg/ml. A positive control and a negative control (medium only) were included in each assay.

Endotoxin Assay

The gram negative bacterial endotoxin content of the samples was determined by a spectrophotometric modification of the Limulus amebocyte lysate gel test (Pyrostat; Millipore Corp., Bedford, MA). Each sample was analyzed in duplicate and results determine by reference to a standard curve using a linear regression program. Results are expressed as nanograms of U.S. Reference Endotoxin.

Assay for Il-1 Activity

The Il-1 content of the macrophage culture medium was assayed using a mouse thymocyte proliferation assay (11). Briefly, the thymus glands of young CD-1 mice were removed and gently teased apart in RPMI-1640 media containing 10% FBS, 5mM glutamine, 20 µM 2-mercaptoethanol, and antibiotics. Tissue debris was removed by filtration through a 100 mesh wire screen, and the resulting cell suspension was counted and adjusted to 10×10^6 cells per ml. To the wells of a 96 well culture plate 100 µl of the thymocyte suspension were placed in each well along with either 100, 50, or 25 µl of the macrophage culture supernate being tested. Each well was brought up to 200 µl total volume with the RPMI-1640 media. The cells were then incubated for 48 hours at 37°C in 5% CO_2, pulsed with 1.0 µCi of ^{3}[H]-thymidine (New England Nuclear, Boston, MA) per well for 6 hours, and harvested with the aid of a multiple sample cell harvester (PHD Cell Harvester, Cambridge Technology Inc., Boston, MA). The radioactivity of each sample was determined using a Beckman LS-9000 liquid scintillation counter. Each concentration of macrophage media was tested in replicates of four, and at least two assays for IL-1 activity were performed on each macrophage-derived sample. In addition to the positive and negative

IL-1 controls, extract controls were included in which samples of the extracts were added directly to the thymocyte culture to match the concentration of extract that would be carried over with the macrophage culture supernate.

Results

Initial experiments were performed using samples of dusts which we had in sufficient quantities to prepare large quantities of the extract so that some biochemical characterization of the extracts could be accomplished. Table I lists the protein and endotoxin contents of these samples and shows that endotoxin and protein concentration are not related. In order to compare the relative potency of these extracts to stimulate IL-1 production, we tested the extracts using the same concentrationn of dissolved solids. As shown in Table II, the oats and wheat dust extracts were potent stimulators of IL-1 production while the cotton dust extracts yielded markedly lower values. Comparison of the data shown in Tables 1 and 2 reveal that the ability of the extracts to stimulate IL-1 production does not correlate with either the protein or endotoxin content of the samples. The data obtained with the cotton extracts suggest that the IL-1 stimulation might be associated with the bract, but additional experiments are necessary to clarify this point.

Because these data provided some evidence that cotton dust extracts could act as stimulants for IL-1 production, we tested extracts prepared from a variety of baled cotton samples. In table 3 the results obtained using extracts of baled cotton from several world-wide sources are presented. These extracts were selected for testing based upon their endotoxin content. The results obtained show that some of the extracts can stimulate IL-1 production but that this activity is not directly correlateed with endotoxin content. It should be noted that these extracts were simply diluted in culture medium and tested. No attempt to standardize them with respect any constitutent was made, and thus, these results should not be compared with those shown in Table II to determine relative potency. Because these cotton extracts were prepared at the same time and in identical manner, they do indicate that the different cottons do vary in their potency.

Additional studies were performed using a sample of cotton grown in the United States (MQ-135) which had been washed with acetone or water. As shown in Table 4 extracts prepared from either the unwashed cotton or acetone washed cotton were able to stimulate IL-1 production. Extracts from the cotton sample that had been washed with water were markedly less active. This would indicate that the IL-1 stimulant in cotton is a water soluble substance. The endotoxin content of these samples did not correlated with their ability to stimulate IL-1 production, again indicating that a substance other than endotoxin is responsible for this activity.

Discussion

The results of this study indicate that vegetable dusts can stimulate IL-1 production but vary greatly in their ability to do so. Until the active substance in these extracts is better defined chemically, it is difficult to compare the relative potency of the extracts. In the experiment in which cotton and grain extracts were tested together at a constant concentration relative to dry weights, it appears that the grain extracts were much more potent. This result should be viewed with caution. It may have been that by chance we selected a cotton sample of unusually low activity. Until a greater number of samples are tested, and a better definition of the active principle is obtained the relative potency of different vegetable dusts to stimulate IL-1 production cannot be clearly defined.

The extracts of baled cotton samples were prepared identically and tested at the same dilution, so that some comparisons between these samples can be made. It is evident from the results obtained with the cotton extracts that these samples vary greatly in their ability to stimulate macrophages to produce IL-1. Although endotoxin and LPS are known agonists for IL-1 production, as evidenced by the positive controls used in this study, there appears to be other factors present in these extracts which can stimulate the IL-1 response. The nature of the factors is unknown, but the data shown in Table IV and the fact that we use aqueous extracts demonstrate that it is water soluble. This observation provides a first step in developing protocols to use to biochemically characterize this activity.

The relationship, if any, between the results presented and the occupational lung diseases associated with these dusts is at best speculative. IL-1 has been shown to have a number of biologic activities, such as the induction of fever (5,14) and the release of acute phase reactants (19), which are responses generally associated with acute inflammation. IL-1 has been most throughly studied with respect to the initiation of an immune response (15). In immune reactions IL-1 acts on T-lymphocytes and triggers a series of cellular responses which lead to antibody production, the development of cytotoxic T-cells, and the release of other lymphokines (e.g. IL-2 and interferon) (16). The results obtained in this study indicate that vegetable dusts can stimulate IL-1 production; however, antibody responses to these dusts are not commonly seen (3,4). This apparent paradox may be explainable in terms of dose, route of exposure, or other environmental factors. In addition, immunoregulatory mechanisms may function in vivo to block or blunt the immune response to such environmental antigens.

Extracts of vegetable dusts in general, and of cotton dust in particular, have been shown to contain a variety of biologically active substances which produce certain responses in man and other animals. With extracts from cotton or cotton associated material the activities found include effects on serum proteins, (8,13), cells (1,6) and tissues (11). In addition, numerous biologically active compounds have been identified in cotton (2). The ability of cotton extract to stimulate IL-1 production by alveolar macrophages can be added to the list of biological activities associated with these extracts; however, the significance of this finding with respect to either the etiology or pathogenesis of byssinosis is unknown. This may just be an epiphenomena which can be observed in vitro but which may not occur or be of significance in vivo. Conversely, because of the multiple effects exerted by IL-1 it may play a role in the reaction to vegetable dusts in a currently unrecognized manner.

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References

1. Ainsworth, S.K. and Neuman, R.E. 1981. Chemotaxins in cotton mill dust: Possible etiologic agent(s) in byssinosis. Amer. Rev. Resp. Dis. 124:280-284.

2. Bell, A.A. and Stipanovic, R.D. 1983. Biologically active compounds in cotton: An overview. In: Proceeding of the Seventh Cotton Dust Research Conference. Wakelyn, P.J. and Jacobs, R.R. Editors. National Cotton Council, Memphis TN. pp. 77-80.

3. Boehlecke, B., Cocke, J., Bragg, K. Hancock, J., Petsonk, E., Piccirillo, R. and Merchant, J. 1981. Pulmonary Function Response to Dust from Standard and Closed Boll Harvested Cotton. Chest 79:77S-81S (Suppl.)

4. Broder, I., Davies, G., Hutcheon, M., Lezoff, A., Mintz, S., Thomas, P., and Corey, P. 1983. Variables of pulmonary allergy and inflammation in grain elevator workers. J. Occup . Med. 25:43-47.

5. Duff, G.W. andd Durum, S.K. 1983. The pyrogenic and mitogenic actions of interleukin 1 are related. Nature (London) 304:449-451.

6. Elissalde, M.H., Stipanovic, R.D., Bell, A.A., and Elissalde, G.S. 1983. Degranulation of mast cells by terpenoid aldehydes in cotton mill dust. In: Proceedings of the Seventh Cotton Dust Research Conference. Wakelyn, P.J. and Jacobs, R.R. Eds. National Cotton Council, Memphis, TN pp. 84-85.

7. Fedan, J.S., Franczak, M.S., Cahill,, J.F., Kosten, C.J. and Morey, P. 1983. Effects of cotton bract extract (CBE) on airway smooth muscle and nerves. In: Proceeding of the 7th Cotton Dust Research Conference. Wakelyn, P.J. and Jacobs, R.R. Editors. National Cotton Council, Memphis, TN pp. 93-96.

8. Kutz, S.A., Mentnech, M.S., Olenchock, S.A., and Major, P.C. 1980. Precipitation of serum proteins by extracts of cotton dust and stems. Environ. Res. 22:476-484.

9. Kutz, S.A., Mentnech, M.S., Mull, J.C., Olenchock, S.A. and Major, P.C. 1980. Acute experimental pulmonary responses to cardroom cotton dust. Arch. Environ. Health 35:205-210.

10. Lachman, L.B. 1983. Human interleukin 1: purification and properties. Fed. Proc. 42:2639-2645.

11. Lachman, L.B., Page, S.O. and Metzgar, R.S. 1980. Purification of human interleukin 1. J. Supramol. Struct. 13:457-466.

12. Maizel, A.L., Mehta, S.R., Ford, R.J., and Lachman, L.B. 1981. Effect of Interleukin 1 on human thymocytes and purified T-cells. J. Exp. Med. 153:470-475.

13. Mundie, T.G., Boackle, R.J., and Ainsworth, S.K. 1983. In Vitro alternative and classiccal activation of complement by extracts of cotton mill dust: A possible mechanism in the pathogenesis of byssinosis. Environ. Res. 32:47-56.

14. Murphy, P.A., Simon, P.L., and Willoughby, W.F. 1980. Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte activating factor made by rabbit alveolar macrophages. J. Immunol. 124:2498-2501.

15. Olenchock, S.A., Christiani, D.C., Mull, J.C., Ye, T-T., and Lu, P-L. 1983. Endotoxins in baled cottons and airborne dusts in textile mills in the People's Republic of China. Appl. Environ. Microbiol. 46:817-820.

16. Oppenheim, J.J. and Gery I. 1982. Interleukin 1 is more than an interleukin. Immunol. Today 3:113-118.

17. Parks, W.R. 1982. Occupational Lung Disorders, 2nd Edition. London; Butterworth andd Co., Ltd. pp. 359-453.

18. Peach, M.J., Olenchock, S.A., Sorenson, W.G., and Major, P.C. 1980. Relevance of grain dust collection techniques to respiratory disease studies. In: Occupational Pulmonary Disease: Focus on Grain Dust and Health. Dosman, J.A. and Cotton D.J., Editors. Academic Press, New York. pp. 507-512.

19. Schmidt, J.A., Mizel, S.B., Cohen, D., and Green I. 1982. Interleukin 1, A potent regulator of fibroblast proliferation. J. Immunol. 128:2177-2182.

20. Selinger, M.J., McAdam, K.P., Kaplan, M.M., Sipe, J.D., Vogel, S.N., and Rosenstreich, D.L. 1980. Monokine induced synthesis of serum amyloid. A protein by hepatocytes. Nature 285:498-500.

Table I

General Characteristics of Some Extracts
Used to Stimulate IL-1 Production by Macrophages

Sample	Dissolved 1/ Solids (μg/ml)	Protein μg/ml	Endotoxin ng/ml
Oats	50	1.49	34
Spring Wheat	50	0.98	577
Durham Wheat	50	1.46	170
Barley	50	1.89	505
Cotton (BI)	50	4.00	2.6
Cotton (BR)	50	2.61	0.8
Bract	50	nd	1.4

1/ Residue from lyophilized samples were weighed, redissolved in saline, and diluted with M-199. Values shown are the final concentrations of dissolved solids, protein, and endotoxin of the samples in the macrophage culture media.

Table II

IL-1 Assay of Macrophage Supernate from Cells Exposed to Grain Dust or Cotton Dust Extracts

Dust Sample	Conc[1/] mg/ml	CPM ^{3}[H]-TdR[2/] Incorp.	Stimulation Index[3/]
Oats	50	71,029 ± 6839	50.6
Spring Wheat	50	64,556 ± 6424	46.0
Durham Wheat	50	70,185 ± 2414	50.0
Barley	50	12,208 ± 2298	8.6
Cotton (BI)	50	2,651 ± 1243	1.9
Cotton (BR)	50	3,178 ± 302	2.3
Bract	50	4,276 ± 1139	3.0
LPS (Pos. Cont.)	10	54,316 ± 2674	38.7
Media (Neg. Cont.)		1,404 ± 120	-

1/ Concentration of extract added to macrophages expressed as µg/ml of dissolved solids in media
2/ Counts per minute of tritated thymidine incorporated by thymocytes exposed to the macrophage culture supernate.
3/ Stimulation index is the fold increase over negative control.

Table III

IL-1 Assay on Macrophage Supernate from Cells Exposed to Extracts of Bulk Cotton from World-wide Sources

Cotton Sample from	Endotoxin[1/] ng/ml	CPM ^{3}[H]TdH[2/] Incorp.	SI[3/]
China, Shanghai 1	135.7	33,528	12.8
Pakistan	74.2	34,184	13.0
China, Shanghai 2	45.4	19,062	7.3
China, Xinjiang region	11.5	3,190	1.2
Morocco	0.2	3,286	1.3
LPS (Pos. Cont.)	-	60,918	23.3
Media (Neg. Cont.)	-	2,619	-

1/ Extracts were diluted 1/10 in culture media and endotoxin values are the concentrations in media added to macrophages
2/ Counts per minute of tritrated thymidine incorporated by thymocytes exposed to the macrophage culture supernates.
3/ Stimulation index

Table IV

IL-1 Assay of Macrophage Culture Supernate from Cell Exposed to Washed and Unwashed Bulk Cotton Sample from the United States

Sample (MQ-135)	Endotoxin[1/] ng/ml	CPM ^{3}[H]-TdH[2/] Incorp.	SI[3/]
Unwashed	566.4	87,020	76.9
Acetone washed	52.7	75,761	66.9
Water washed	15.7	5,959	5.6
LPS (Pos. Cont.)	-	80,143	70.8
Media (Neg. Cont.)	-	1,132	-

1/ Final concentration of endotoxin in culture media after 1/10 dilution of extracts
2/ Counts per minute of tritated thymidine incorporated by thymocytes exposed to macrophage culture supernates
3/ Stimulation index

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