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In Vitro Release of Arachidonic Acid and *In Vivo* Responses to Respirable Fractions of Cotton Dust

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It was considered that the fall in lung function seen after exposure to cotton dust may be attributable in part to the activity of arachidonic acid metabolites, such as leucotrienes as well as to the more established release of histamine by cotton dust. However, we found that cotton and barley dusts elicited poor release of arachidonic acid from an established macrophage like cell line compared with that observed with other organic dusts.

In the experimental animal, pulmonary cellular responses to both cotton and barley dust were similar to those evoked by moldy hay and pigeon dropping dusts, although after multiple doses a more severe response was seen to cotton and barley. Since both moldy hay and pigeon droppings elicit a greater arachidonic acid release than cotton or barley, a role for arachidonic acid in inducing the cellular response is less likely than other factors.

There are limitations to our conclusions using this system, i.e., the arachidonic acid may be released in a nonmetabolized form, although it is noted that the two dusts with the greatest arachidonic acid release produce their clinical responses in humans largely by hypersensitivity mechanisms.

Introduction

While the clinical response to certain organic dusts is attributable to hypersensitivity reactions, the activity of dusts such as those generated during cotton processing is in part a function of the dusts' ability to cause the release of pharmacologically active components from cells (*1*). For other dusts, e.g., grain, other biologically active components may be nonspecifically generated, e.g., complement activation (*2*).

Part of the response is a fall in lung function, as in acute farmer's lung, although a reduction in some lung function parameters may be seen after exposure to cotton (*1*) and grain (*3*) without an obvious clinical response in the exposed subject. One of the most widely studied mediators in cotton dust exposure is histamine although in terms of a pulmonary response there are other possible mediators such as leucotrienes whose effect on lung function is several orders of magnitude greater than histamine (*4*). Also leucotrienes and other arachidonic acid metabolites, such as prostaglandins, when introduced intravenously can cause parenchymal lung injury (*5*).

Thus we sought to determine whether organic dusts such as cotton, moldy hay, and barley could cause leucotriene and prostaglandin precursor release and to relate these findings to the ability of these dusts to induce lung injury in the experimental animal.

Materials and Methods

Dusts Studied

Three dusts encountered in agriculture and associated with pulmonary disease were studied. These were cotton, barley, and moldy hay. Pigeon droppings, a dust also associated with pulmonary disease similar to that caused by moldy hay, was also included as an example of a material causing reactions mainly by hypersensitivity mechanisms.

Respirable Dust Fractions

Cotton dust was obtained as a gift from Dr. P. Sasser (Cotton Inc, Raleigh, NC, USA). Moldy hay and pigeon droppings were obtained locally. Barley dust was collected from dust generated during the transfer of barley

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grain from storage silos via conveyor belts and collected from clean surfaces close to the conveyor belts. Respirable fractions of dusts were prepared by agitating each sample and drawing the dust cloud through a Casella Hexhlet horizontal elutriator at 50 L/min. Respirable fractions were sterilized by overnight incubation in ethanol for the *in vitro* studies and by gamma irradiation for the *in vivo* studies.

Arachidonic Acid Release

The method for cell culture and arachidonic acid release was identical to that used in our previous studies (*6*). Briefly, P388D1 cells (a murine macrophagelike cell line) were grown until 80–90% confluent in 24 well Multiplates (Falcon Plastics). The cultures were then treated with (5,6,8,9,11,12,14,15-^{3}H) arachidonic acid (4 Ci/mmole, Amersham International PLC) at 0.25 μCi/well for 24 hr. The unincorporated label was removed by washing three times with medium. The material under investigation was made up in suspension in medium immediately before use and added to the monolayers. After 24 hr the distribution of radioactivity between the medium and cell layer was determined by scintillation counting. Cell death was investigated by phase contrast microscopy using an inverted microscope and by measuring the release of lactic dehydrogenase (LDH) into the medium using a commercial kit (LD-L no. 22G, UV Sigma). Statistical analysis was by Student's *t* test.

Pulmonary Response to Dusts

Each dust was resuspended at 2 mg/mL in sterile saline and 0.5 mL deposited in the lungs of ether anaesthetised Wistar rats via long blunt ended steel needle introduced into the trachea through the glottis.

Rats were sacrificed in pairs (one male, one female) at 8 and 24 hr and 2, 4, 8, 16, and 32 days and at 3 months using barbiturate overdose. Excised lungs were inflated with formalin, and fixed tissue was sectioned and stained with hematoxylin and eosin.

Another group of rats was subjected to three doses of dust at one monthly intervals and sacrificed after the last dose at the same time intervals as the single dose animals.

Results

Arachidonic Acid Release

After 24 hr incubation, phagocytosis of all the dusts had occurred but there was no evidence of cytotoxicity when the cultures were examined by phase contrast microscopy or by LDH release.

The release of arachidonic acid by the dusts is shown in Figure 1. Both cotton and barley dust caused less than 5% of labelled arachidonic acid to be released and reached maximum release at 160 μg/mL with no increase at 320 μg/mL. Pigeon droppings produced a greater release than the barley and cotton dust suspensions, but moldy hay dust produced an almost linear release, more than three times that of barley and cotton dust at 320 μg/mL.

Pathological Changes

In general all four dusts produced a similar pattern of response. Initially, dust was taken up by alveolar macrophages, and by 8 hr polymorphonuclear leucocytes (PMNs) started to influx into the areas of dust deposition. The PMN influx continued up to 2 days but decreased at 4 days and was virtually absent at 8 days. Over the same time course there was a continuing influx of monocytes/macrophages starting at day 1 and continuing at day 4 and day 8 with the appearance of foreign body granulomas and giant cells at about day 8. The number and size of these granulomas decreased by 16 days, and by 32 days and 3 months very little residual activity was observed.

The animals receiving three doses produced an identical PMN monocyte/macrophage, giant cell response as the single dose animals, but two other features were noted: a superimposition of the acute reaction to dust onto lesions remaining from the previous two exposures and a more severe overall response.

The reactions observed were graded as slight, moderate, or severe using a combination of both the intensity of pulmonary involvement and the area of involvement in the various lobes of the lung.

The results are summarized in Table 1 and demonstrate the shift towards a more severe reaction in the multiple dosed animals.

Discussion

The results confirm our earlier work (*6*) and also show that barley dust causes a poor release of arachidonic acid, similar to cotton dust.

However, for arachidonic acid release to be meaningful in terms of leucotriene-induced bronchoconstriction, conversion of arachidonic acid would have to follow the lipoxygenase metabolic pathway to yield leucotrienes (*5*). No evidence of increased leucotrienes was observed in unconcentrated lung lavage fluid following inhalation challenge by cotton plant bract extracts in human volunteers (*7*), whereas cotton dust extract challenge in rabbits produced detectable $PGF_{\alpha 2}$ and thromboxane B2 (*8*), thus possibly the lipoxygenase pathway is not preferentially activated by cotton products.

The role of leucotrienes in cotton dust exposure causing bronchoconstriction seems less likely than that of histamine as the inhibition of the cotton dust extract-induced contraction of guinea pig ileum by the H1 histamine blocker, mepyramine, (*7*), and other work (*9*) indicates involvement of histamine.

A role of arachidonic acid metabolites in inducing pathological change also seems less likely than other, as yet undetermined factors. If one considers three parameters of the pulmonary response, i.e., PMN involvement, giant cell and granuloma formation, then the more

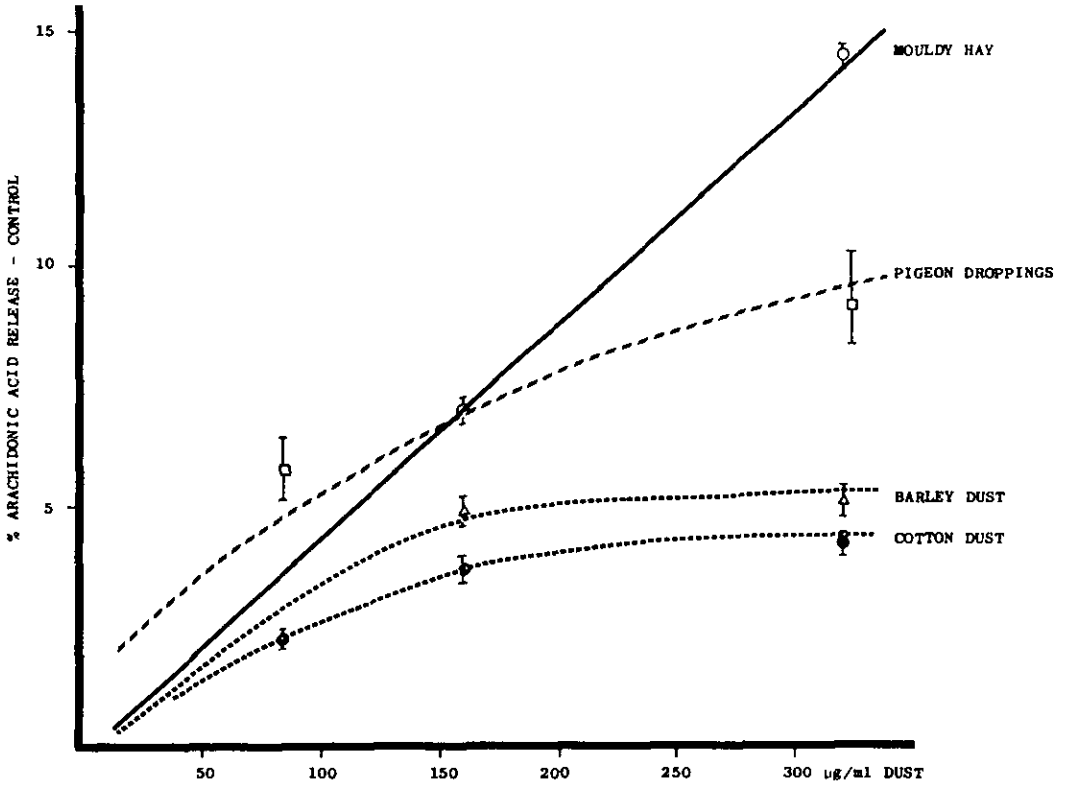

FIGURE 1. Release of arachidonic acid expressed as percentage total incorporated acid less control release (approx. 2–3%) in response to 24 hr incubation with respirable fractions of organic dusts. Note the relatively poor release induced by barley and cotton dust compared with pigeon droppings and moldy hay.

Table 1. Cellular responses of rats to dusts.[a]

No. doses dust	Reaction	PMN	GC	Grans
1	Slight	PD	PD	PD Ba
	Moderate	MHD Co	—	Co MHD
	Severe	Ba	—	—
3	Slight	—	PD	—
	Moderate	MHD PD	MHD Ba Co	MHD Co PD
	Severe	Co Ba	—	Ba

[a] Cellular responses of rats exposed to 1 mg respirable fraction of pigeon droppings (PD), moldy hay dust (MHD), cotton dust (Co) and barley dust (Ba). Overall results over 16 days are expressed arbitrarily as slight, moderate, or severe. PMN = polymorphonuclear infiltration; GC = giant cell formation; Grans = granulomata formation. Note the generally more severe response after three doses of each respective dust introduced endotracheally at intervals of 28 days.

severe responses are seen against barley and cotton dusts compared with pigeon droppings and moldy hay dust. In view of the known action of leucotrienes on PMNs and the *in vivo* cytotoxicity of prostaglandins on lung tissue, it is possible that our *in vitro* results have no *in vivo* counterpart or again that the released arachidonic acid is not metabolized to prostaglandins or leucotrienes.

The pathological results do emphasize the sequential nature of cellular events for all four dusts used and show clearly that cellular responses at any one time are largely dependent upon last exposure to dust. Lung biopsies as in farmer's lung (*10*) and pigeon breeder's lung (*11*) may not reflect very early cellular changes, since most are undertaken with hospitalized patients and taken some time after last exposure, i.e., the acute PMN response which is largely absent 4 days after dust exposure may not be observed.

Lung biopsies are also complicated by the effect of multiple doses. Our three dose animals were challenged at monthly intervals, and certain late cellular features were observed superimposed on the early response to the last dust dose, i.e., presence of granuloma (late response) on the early 8 hr PMN response, again emphasizing the need to relate lung biopsies to exposure patterns.

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