Examination of Mechanisms responsible for Organic Dust-Related Diseases: Mediator Release induced by Microorganisms. A Review

S. Norn¹, P. Clementsen¹, K.S. Kristensen¹, P. Stahl Skov², H. Bisgaard², S. Gravesen³

Abstract

Microbial content in dusts such as bacteria, endotoxins and fungal spores are thought to be important causative agents for the symptoms in organic dust-related diseases. Microorganism-induced mediator release was therefore examined in human cells. Bacteria were found to trigger the release of histamine and leukotriene B₄ from bronchoalveolar cells, and in suspensions of dispersed lung and tonsillar cells they induce the release of histamine and prostaglandin D₂. Basophil histamine release was triggered by both bacteria and their endotoxins. Furthermore, histamine release caused by allergic as well as non-allergic reactions was enhanced by bacteria, endotoxins and fungal spores of moulds. These effects of dust components may be crucial for the symptoms in organic dust-related diseases, since the mediators are of key importance to the broncho-obstructive and inflammatory events in these disorders.

Introduction

The content in dusts of microorganisms such as bacteria, their endotoxins and spores from moulds and other microfungi are believed to be important causative agents for the symptoms in organic dust-related diseases (Lecours et al., 1986; Malmberg, 1990). Exposure to organic dusts has long been known to cause pulmonary diseases among farmers; these disorders are associated with industrial fermentation and work in waste deposit stations. The symptoms vary from rhinitis, irritation of the upper airways or increased mucous secretion with cough to chronic bronchitis, allergic alveolitis and asthma (Rylander, 1986). Exposure to very high concentrations of microorganisms may cause a toxic febrile reaction even in apparently nonsensitized subjects (Karlsson and Malmberg, 1989). Allergic as well as non-allergic reactions are crucial for these disorders by releasing various mediators such as histamine, prostaglandins and leukotrienes which are of key importance to broncho-obstructive and inflammatory events in the diseases. This review of our investigations will therefore focus on microorganisms and mediator release, i.e. the capability of the microorganisms to trigger mediator release or to enhance mediator release.

Methods

Human cellular studies on mediator release and its reinforcement were performed as follows:

1. Peripheral blood leukocytes containing approx. 2% basophilocytes. The cells were obtained by Ficoll-Hypaque gradient centrifugation, washed
twice, and suspended in a buffered (pH 7.4) solution of Tris-AMC (albumin, magnesium, calcium) (Espersen et al., 1984). The capability of microorganisms to trigger histamine release from the basophilocytes was examined by incubating the leukocytes for 40 min at 37°C with whole formalin-killed bacteria or their cell wall components (endotoxins, peptidoglycan), influenza A virus or fungal spores of moulds. The histamine release was determined spectrofluorometrically (Espersen et al., 1984) and expressed as a percentage of the total basophil histamine content of the sample. Potentiation, i.e. synergistic enhancement of mediator release by the microorganism, was examined by incubating the leukocytes for 40 min at 37°C with stimulator (triggering histamine release) in the presence and absence of the microorganism (potentiator). The stimulators included specific antigens (house dust mite and grass pollen allergen from Pharmacia, Sweden) or anti-IgE antibody (rabbit-anti-human IgE, e-chain, from Behringwerke AG, Germany), both representing IgE-mediated reactions, while non-immunological reactions were represented by stimulators such as calcium ionophore A23187 (Calbiochem AG, Switzerland) or Staphylococcus aureus, prepared according to Espersen et al. (1984).

2. Bronchoalveolar cells were obtained by bronchoalveolar lavage using a fibreoptic bronchoscope. The fluid was filtered through sterile gauze and the cells were recovered by centrifugation, washed twice and suspended in a buffered solution of Tris AMC (Clementsen et al., 1991). The cells were stimulated with Staph. aureus, for 20 min at 37°C. Histamine release was estimated by the fluorometric method of Shore et al. (1959) and generation of leukotriene B4 was determined in a competitive radioligand assay using a specific leukotriene B4 antibody (NEN Nuclear) according to Clementsen et al. (1989a).

3. Cells from lung parenchyma or tonsils were obtained by enzymic dispersion of the tissue, i.e. the tissue was chopped with scissors, washed and treated for 30 min at 37°C with pronase and chymopapain to destroy the network (Church et al., 1987). The freed cells were washed, suspended in the buffered solution and incubated for 30 min at 37°C with various bacteria. Histamine was measured by a single isotope histamine methyltransferase assay and prostaglandin D2 by radioimmunoassay (Church et al., 1987).

### Table 1. Bacteria causing histamine release in vitro from human basophil leukocytes

<table>
<thead>
<tr>
<th>Gram-positive</th>
<th>Gram-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerococcus sp.</td>
<td>Branhamella catarrhalis</td>
</tr>
<tr>
<td>Corynebacterium sp.</td>
<td>Enterobacter cloacae</td>
</tr>
<tr>
<td>Group A and B strept.</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>Haemophilus influenzae</td>
</tr>
<tr>
<td>Staph. epidermidis</td>
<td>Haemophilus parainfluenzae</td>
</tr>
<tr>
<td>Strept. mitior</td>
<td>Klebsiella oxytoca</td>
</tr>
<tr>
<td>Strept. pneumoniae</td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>Strept. salivarius</td>
<td>Neisseria pharyngis</td>
</tr>
<tr>
<td></td>
<td>Proteus mirabilis</td>
</tr>
<tr>
<td></td>
<td>Proteus vulgaris</td>
</tr>
</tbody>
</table>

### Results

#### Mediator Release

Various gram-positive and gram-negative bacteria were found to trigger basophil histamine release (Table 1). The mediator release was obtained in leukocyte suspensions from normal individuals, children with intrinsic asthma, and allergic patients suffering from bronchial asthma and hay fever (Clementsen et al., 1990d; Espersen et al., 1984; Koch et al., 1982; Norn et al., 1985 and 1986a). A dose-dependent release was obtained with bacteria used in final concentrations from 0.6 to 10 mg/ml and the maximum release was from 13% to 37% of the cellular content of histamine in the basophilocyte (Figure 1). It is not known whether the groups of asthmatic and normal individuals show a difference in releasability or cell-sensitivity to bacteria. However, preliminary results in patients with

![Fig. 1 Basophil histamine release caused by bacteria in leukocyte suspensions from normal individuals. Klebsiella pneumoniae (1), Escherichia coli (2), Staphylococcus aureus (3), Streptococcus pneumoniae (4), Haemophilus influenzae (5) and Branhamella catarrhalis (6) (see text).](image-url)
chronic bronchitis reveal that leukocytes from the patients show higher basophil releasability than leukocytes from normal individuals to Haemophilus influenzae and Streptococcus pneumoniae (unpublished results). Peptidoglycan, the cell wall component in gram-positive and gram-negative bacteria, was found to be ten times more potent than the Staphylococcus aureus bacterium from which it was isolated (Norn et al., 1985). It is therefore possible that peptidoglycan might be a common factor responsible for mediator release induced by different bacteria. No histamine release was obtained by endotoxins isolated from E. coli and Salmonella bacteria (Norn et al., 1986a). However, in the presence of serum they can activate the complement cascade and thereby cause histamine release (Norn et al., 1986b).

Mediator release from cells in the respiratory tract was studied in superficially lying cells in the airway epithelium obtained by bronchoalveolar lavage (Clementsen et al., 1989a; Clementsen et al., 1991). When the cells were exposed to Staph. aureus in final concentrations of 0.6-10 mg/ml, the bacterium was found to trigger histamine release from the mucosal mast cells in half of the twenty non-atopic subjects. The release was dose-dependent, and in the highest concentration of the bacterium it amounted to 20% of the cellular content of histamine (Table 2). Furthermore, a dose-related increase in leukotriene B4 release was also found. In the highest concentration of Staph. aureus the leukotriene B4 production was increased significantly from a basal level of 67 to 221 pg LTBl per 10⁶ cells (Table 2). In dispersed lung parenchymal- and tonsillar mast cells from non-atopic subjects, histamine release was obtained by a wide spectrum of gram-positive and gram-negative bacteria including Staphylococcus epidermidis, Enterobacter cloacae, Proteus vulgaris, Klebsiella oxytoca and Escherichia coli; E. coli was found to generate also prostaglandin D₂ (Church et al., 1987).

### Table 2 Mediator release from human bronchoalveolar cells triggered by Staphylococcus aureus

<table>
<thead>
<tr>
<th>Staph. aureus mg/ml</th>
<th>0.6</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine¹⁾</td>
<td>9</td>
<td>5±1</td>
<td>16±2*</td>
<td>20±2*</td>
</tr>
<tr>
<td>Leukotriene B₄²⁾</td>
<td>4</td>
<td>72±15</td>
<td>94±27</td>
<td>158±39</td>
</tr>
</tbody>
</table>

¹⁾ Histamine release in per cent of the cellular content
²⁾ LTBI production, pg/10⁶ cells (basal = 67±15)

Mean ± SEM; *P<0.05 by paired Student’s t-test

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>% Enhancement by moulds (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10⁻³</td>
</tr>
<tr>
<td>Grass</td>
<td>C. globosum</td>
</tr>
<tr>
<td>Anti-IgE</td>
<td>A. terreus</td>
</tr>
<tr>
<td></td>
<td>C. globosum</td>
</tr>
<tr>
<td></td>
<td>M. racemosa</td>
</tr>
</tbody>
</table>

Histamine release induced by grass pollen allergen or anti-IgE antibody. Mean of 5-7 subjects. The enhancement was significant for all three moulds in both atopic and non-atopic persons (P<0.01 by paired Student’s t-test)

### Potentiation

When leukocytes from individuals not allergic to fungi were exposed to whole spores of the moulds Aspergillus terreus, Chaetomium globosum or Mucor racemosus, no basophil histamine release was obtained. However, Table 3 shows that the spores potentiate mediator release. A low histamine release (12%) was obtained when cells from grass-pollen-allergic patients were exposed to small amounts of specific antigen. In the presence of C. globosum (10⁻³ to 10⁻¹ mg/ml) the release was gradually increased to 25%, corresponding to an enhancement of 110% (P<0.01 by paired t-test). A similar potentiating effect was obtained by all three moulds when the IgE-mediated histamine release was triggered by anti-IgE antibody in cell suspensions from normal individuals (Table 3). Moreover, histamine release triggered by non-immunological mechanisms using stimulators such as calcium ionophore A23187 or Staph. aureus was enhanced by the spores (results not shown). Thus, Staph. aureus-induced histamine release was enhanced from 7% to 18% in the presence of C. globosum (10⁻² mg/ml) (P<0.01 by paired t-test, N=5) indicating that in normal individuals a mixture of bacteria and moulds can lead to aggravated mediator release although an allergic reaction is not involved. In all experiments a potentiating effect was obtained by small amounts of whole spores ranging from 10⁻³ to 10⁻¹ mg/ml.

A potentiating effect was also obtained by bacteria and their cell wall components (Clementsen et al., 1990a, b; Norn et al. 1986a, 1987). Table 4 shows the effect of Staph. aureus, E. coli and K. oxytoca as well as of endotoxins (E. coli and Salmonella typhimurium LPS) and of peptidoglycan isolated from Staph. aureus. Both allergic and non-immunological histamine release was enhanced by synergism. When cells from mite-allergic patients were
Table 4 Potentiation of basophil histamine release by microorganisms and their components

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Stimulator</th>
<th>Potentiator</th>
<th>% Enhancement*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mite sensitive</td>
<td>Mite</td>
<td><em>Staph. aureus</em></td>
<td>900</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em></td>
<td>400</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Peptidoglycan</em></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Influenza A virus</em></td>
<td>80</td>
</tr>
<tr>
<td>Grass sensitive</td>
<td>Grass</td>
<td><em>Klebsiella oxytoca</em></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. typhimurium LPS</em></td>
<td>110</td>
</tr>
<tr>
<td>Normal individuals</td>
<td>Anti-IgE</td>
<td><em>Staph. aureus</em></td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Calcium ionophore A23187</td>
<td><em>Staph. aureus</em></td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. typhimurium LPS</em></td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Influenza A virus</em></td>
<td>90</td>
</tr>
</tbody>
</table>

Mediator release was triggered by the stimulator and enhanced by the potentiator.

* Mean of 4-7 experiments, P<0.01 by paired Student's t-test

challenged with small amounts of specific antigen, inclusion of *Staph. aureus* increased the release and a maximum enhancement of 900% was obtained by 0.1 mg bacterium/ml (Table 4). In analogy, *E. coli* and *K. oxytoca* in concentrations of 1 and 5 mg/ml respectively enhanced the allergic histamine release by 400% and 100% respectively. The table also shows the enhancement of non-immunological mediator release triggered by calcium ionophore A23187. Endotoxins were found to potentiate in a wide range of concentrations from 1 pg to 100 ng/ml, possibly depending on the category of the patients, the LPS preparation and the stimulator triggering the mediator release. Experiments with influenza A virus showed no mediator release. However, the virus was able to enhance the release triggered by allergic and non-immunological mechanisms (Table 4) (Clementsen et al. 1989b; Clementsen et al., 1990c).

All these microorganisms contain long carbohydrate chains on the surface consisting of galactose, N-acetylglucosamine and other sugars. Endotoxins and peptidoglycan also contain these sugars. We assume that the long sugar chains are responsible for the potentiating effect. Thus poly N-acetylglucosamine (chitin) 10^4 mg/ml mimicked the potentiating effect of the microorganisms by enhancing the histamine release caused by calcium ionophore by 110% (N=10; P<0.01 by paired t-test). The hypothesis is also supported by the finding of a blocking effect of the monosaccharides galactose and N-acetylglucosamine which by competition abolished the potentiating effect of bacteria and endotoxin (Clementsen et al., 1990b).

Discussion

Organic dusts contain bacteria, endotoxins, fungal spores and allergens such as mites and animal dander (Lecours et al., 1986; Malmberg, 1990; Malmberg et al., 1990; Rylander, 1987). It is well known that allergens via sensitization and mediator release can be responsible for the symptoms in organic dust-related diseases. However, the microorganisms are also suspected of being causative agents and the possibility of microorganism-induced mediator release as a pathogenic mechanism was investigated in this paper.

Bacteria were found to trigger release of histamine and leukotriene B4 from cells in the airway epithelium obtained by bronchoalveolar lavage. The mediator release may be of importance for the development or exacerbation of pulmonary diseases since histamine is assumed to increase the epithelial permeability by opening tight junctions and thereby promoting the entrance of allergens, microorganisms and other insulting agents (Boucher et al., 1977; Hogg, 1981) and leukotriene B4 may facilitate airway inflammation by its chemotactic properties. The risk of impairment of the epithelial barrier is higher in patients with bronchial asthma where an increased number and sensitivity of epithelial mast cells are observed (Clementsen et al., 1991). Entrance of the noxious agents in the lung parenchyma may now trigger release of mediators and neurotransmitters leading to bronchoconstriction, inflammation and mucous secretion. In fact, we found that various bacteria trigger histamine release from mast cells derived from lung parenchyma
and tonsils, and furthermore, cause generation of prostaglandin D2. Basophil leukocytes are also target cells in airway diseases since the basophil appears to participate in the late-phase airway obstructive reaction (Liu et al., 1991). Both gram-positive and gram-negative bacteria were found to trigger histamine release from these cells. Such a mediator release may also occur by endotoxins since they are able to trigger histamine release via complement activation.

Another important aspect is the combined effect of allergens, microorganisms and other noxious agents leading to aggravation of mediator release by a potentiating effect. Bacteria, endotoxins, virus and fungal spores of moulds were found to potentiate histamine release. The aggravation of mediator release by these potentiators was obtained when mediator release was triggered by allergic as well as non-immunological reactions. The latter include an example of bacteria-induced histamine release which was enhanced by moulds. This is essential since organic dust-related diseases are provoked or aggravated by both allergic and non-allergic events. Since dusts contain stimulators triggering mediator release (bacteria and allergens) as well as potentiators (bacteria, endotoxins, fungal spores) speeding up the release of mediators, this may explain the inflammatory and broncho-obstructive symptoms obtained by exposure to organic dust. A humid and poor indoor climate may therefore cause a symptom-free allergic asthma to manifest disease, since a low allergic mediator release is speeded up by endotoxins and microorganisms in the inhaled dust. This may also occur in non-allergic hypersensitive individuals where microorganisms and "irritating" agents are suspected of releasing significant amounts of histamine whereas this is not the case in normal individuals. The reason may be multifactorial i.e. depending on a facilitated entrance of the deleterious agents caused by a defect in the airways epithelium, impairment of the mucosal immune barrier (Kilian et al., 1988; Sørensen and Kilian 1984) or impaired mucociliary clearance. Further, both the number (Lozewicz et al., 1988) and the sensitivity (Leung et al., 1986) of mast cells in the airways are increased and changes in the network of cytokines and immunological events may also contribute.

The deleterious mixture in dust of both bacteria, endotoxins, microfungi and allergens constitutes a high risk for the development of organic dust-related diseases, especially the lung diseases resulting from work in swine and chicken confinement buildings and waste deposit stations. It may therefore be important to test organic dust in indoor climate for microorganisms and to minimize the amount of noxious agents by cleaning and ventilation. Whether this could be controlled in vitro by testing histamine release or its potentiation has yet to be examined.

Acknowledgements

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