Alveolar Macrophages as Biomarkers of Pulmonary Irritation in Kitchen Workers

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Objectives: Alveolar macrophages (AM) are used as a biomarker of pulmonary irritation due to occupational exposure in the AM test. The aim of this study was to investigate whether there is a co-variation between the number of AM and exposure to cooking fumes.

Materials and methods: The study group consisted of 62 volunteers. People who worked in a kitchen preparing hot meals were considered as occupationally exposed (35 persons). The exposed group was further divided into highly and slightly exposed persons according to the levels of fat aerosols and aldehydes in the working atmosphere. People who were not preparing hot meals were considered as unexposed (27 persons). The number of AM was counted in smears prepared from expectorate samples from each participant. Samples were taken on three different days.

Results: Highly occupationally exposed persons had a higher number of AM in their samples than both slightly occupationally exposed persons and unexposed persons. Highly exposed smokers had a statistically significantly higher number of AM compared with both slightly and unexposed smokers ($P \leq 0.05$).

Conclusion: The results suggest an increase in the number of AM due to exposure to cooking fumes and a synergistic effect between occupational exposure and smoking.

Keywords: alveolar macrophages; cooking fumes; kitchen workers; smoking; synergism

INTRODUCTION

Alveolar macrophages (AM) act as a primary cellular defence against inhaled foreign material (Ando et al., 1984) and it has been shown that an increase of AM in the lungs correlates with the total load of pollution, including cigarette smoke (Rylander et al., 1979; Gullvåg et al., 1985). The relationship between smoking and an increase in the number of AM is well established (Pratt et al., 1969; Golde, 1977; Sibille and Reynolds, 1990). Studies have also shown that smoking and occupational exposures to particulate matter and gases have a synergistic effect on the level of AM (Rylander et al., 1979; Mylius and Gullvåg, 1986). A method by which AM are counted in expectorate samples was developed during an investigation in workers from an aluminium reduction plant in Norway (Gullvåg et al., 1985).

Although there are limited data on the emission of cooking fumes into the work atmosphere, it is well known that these fumes may contain irritants or other harmful substances. Epidemiological data indicate that cooks have an increased incidence of cancers in the respiratory tract (Coggon et al., 1986; Lund and Borgen, 1987; Foppa and Minder, 1992). An association between exposure to cooking fumes and allergic rhinitis (Ng and Tan, 1994), chronic cough and phlegm among Chinese women has also been found (Ng et al., 1993). In addition, we have shown in a parallel study that Norwegian kitchen workers may have an increased risk of respiratory symptoms (K. Svendsen et al., submitted for publication).

During frying at high temperatures, fat enters the atmosphere by volatilization and condenses to respirable aerosols. If the aerosols are inhaled and deposited in the alveoli, they will induce AM to phagocytose and thus activate a cascade reaction that further increases the number of AM in the lungs (Bowden, 1971; Cherniak and Cherniak, 1983; Schlesinger et
al., 1997). In addition, cooking fumes contain harmful gases, e.g. aldehydes, which can affect the AM activity (Rylander et al., 1979). Thus it can be expected that exposure to cooking fumes could result in an increase in the number of AM.

The aim of the present study was to examine whether exposure to cooking fumes in kitchens results in an increase in the number of AM in kitchen workers.

**MATERIALS AND METHODS**

**The study group**

The study group consisted of 62 volunteers (males and females) from central Norway. Thirty-five participants working in kitchens where hot meals were prepared (grill restaurants, hotel kitchens, hamburger chains and fast food stores) were considered as being exposed. The exposed group was further divided into highly and slightly exposed persons. The exposure levels to fat aerosols and aldehydes in different types of kitchens had earlier been determined by personal sampling (Svendsen et al., 2002). All workers in fast food stores and grill restaurants were defined as highly exposed and the workers in hotel kitchens and hamburger chains were defined as slightly exposed. This classification was made in accordance with our measurements of the levels of fat aerosols in the different kinds of kitchens, using the geometric mean of fat aerosol in all kitchens to discriminate between kitchens with ‘high’ and ‘low’ exposure levels. This implies that kitchens with a geometric mean below 0.28 were classified as slightly exposed and kitchens with a geometric mean over 0.28 were classified as highly exposed. The mean exposure levels in the different types of kitchens are given in Table 1.

The unexposed group included six people working in kitchens where exposure to cooking fumes was thought to be at a minimum level (cafés bars and canteens). In addition, 21 students who matched the exposed group in age, sex and smoking habits supplemented the unexposed group. All the participants filled in a questionnaire that asked for information on their age, place of work, actual tasks, state of health and smoking habits.

**Personal characteristics**

Since an increase in the number of AM has been shown to correlate with increased cigarette smoking (Nilsen and Engen, 1985), the groups of highly, slightly and unexposed persons were further divided into smokers, ‘intermediate’ smokers and non-smokers. Persons smoking >10 cigarettes/day were defined as smokers and persons smoking 1–9 cigarettes/day were defined as ‘intermediate’ smokers. In addition, the people had to have smoked continuously for a minimum period of 1 yr in order to be defined as a smoker. Non-smokers were defined as persons who had never smoked or stopped smoking more than 5 yr before the study. Data for the study group are given in Table 2.

**AM test**

Expectorate samples were obtained from each participant at the end of the working day on three different days. Owing to practical problems, the samples were not collected on three consecutive days, although this is recommended. The participants were instructed to cough vigorously and the produced expectorate was collected. Eight smears were made from four non-transparent droplets of expectorate from each sample (24 smears/person in total). The smears were immediately fixed (Labofix Spray Fixative; Labonord) and stained according to the Papanicolau method (Koss, 1979). AM were counted under a light microscope in one field of vision line across the middle of each smear, at 500× magnification (Gullvåg et al., 1985). Cell types other than AM, e.g. neutrophils and eosinophils, were not taken into account in this study. The material on the smears was designated as representative of the lower part of the airways if either cylindrical epithelial cells or AM or both were observed. Observations of poly-nucleated AM were noted. The two persons counting the AM randomly cross-checked each other’s counts. Participants who delivered three non-representative expectorate samples were excluded from further determination of the results.

**Statistical analysis**

All analyses were performed with SPSS 10.0 for Windows (Microsoft Corp., 1999). Differences in the number of AM between the groups were assessed using the non-parametric Mann–Whitney U-test as the data were non-normally distributed. All statistical tests were two-tailed. P values <0.05 were considered statistically significant.

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**Table 1. Results from personal samples of fat aerosols and aldehydes (formaldehyde, acetaldehyde and acrolein) performed in different kinds of kitchens grouped into categories according to the measured contents of exposure and in the group of all kitchens**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Kitchen</th>
<th>Fat aerosols (mg/m³)</th>
<th>Sum of aldehydes (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High</strong></td>
<td>Fast food stores</td>
<td>1.40 (1.89)</td>
<td>114.35 (36.34)</td>
</tr>
<tr>
<td></td>
<td>Grill restaurants</td>
<td>0.30 (0.80)</td>
<td>51.03 (34.11)</td>
</tr>
<tr>
<td><strong>Slight</strong></td>
<td>Hotel kitchen</td>
<td>0.21 (0.28)</td>
<td>43.45 (31.10)</td>
</tr>
<tr>
<td></td>
<td>Hamburger chain</td>
<td>0.10 (0.12)</td>
<td>34.94 (14.20)</td>
</tr>
<tr>
<td>All kitchens</td>
<td></td>
<td>0.28 (0.98)</td>
<td>57.00 (41.61)</td>
</tr>
</tbody>
</table>

From Svendsen et al. (2002). The results are given as geometric means with the standard deviations in parentheses.
RESULTS

Sixty-two persons participated in the study. Of the total number of smears, 96.8% were designated as representative. Fifty-seven persons delivered representative samples on all three days. One person was excluded because of the lack of representative material in all samples. Three people contributed representative smears on only two days and one person on one day only. The number of AM (median) in the expectorated samples from the study group, divided into three exposure categories, are given in Table 3.

The number of AM is highest in the group of highly exposed persons. The differences in the number of AM between highly exposed and slightly exposed persons and between highly exposed and unexposed persons are statistically significant ($P \leq 0.05$) (Table 3).

The persons in the high exposure group had a higher number of AM (median) than the persons in the minor exposure group in all smoking categories (Table 4). Expectorate samples from highly exposed smokers had the highest number of AM. The difference in AM numbers between the smokers in the high exposure and minor exposure groups is statistically significant ($P \leq 0.05$).

More of the persons in the high exposure group had polynucleated AM in their expectorated samples than in the minor exposure group (Table 4). All highly exposed smokers had polynucleated AM in their samples. One non-smoker in the minor exposure group had polynucleated AM in one of the expectorated samples.

We observed a considerable variation in the number of AM in expectorated samples collected on different days from the same person.

DISCUSSION

The differences in the number of AM (median) between highly and slightly exposed persons and between highly and unexposed persons are statistically significant ($P \leq 0.05$) (Table 3). These results support a relationship between exposure to cooking fumes and an increase in the number of AM in the lungs. However, slightly exposed persons and unexposed persons had nearly the same number of AM (median) (Table 3). It is possible that the levels of pollution measured in kitchens where the slightly exposed persons work are too low to give an increase in the number of AM.

Grill restaurants were classified as highly exposed, although the geometric mean of fat aerosols measured in these kitchens was not much higher than the geometric mean of all kitchens (Table 1). However, the variation in measured exposure levels on consecutive days in the same grill restaurants show that these

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Table 2. Description of the study group

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Sex</th>
<th>Age (SD)</th>
<th>Smoking habit</th>
<th>No. of yr in current kitchen [median (min–max)]</th>
<th>No. of yr as kitchen worker [median (min–max)]</th>
<th>Hours per week in kitchen [median (min–max)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Male</td>
<td>29 (6.7)</td>
<td>Smoker</td>
<td>2 (0–10)</td>
<td>4 (1–21)</td>
<td>36 (15–70)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>24 (7.8)</td>
<td>‘Intermediate’</td>
<td>2 (0–21)</td>
<td>2 (1–17)</td>
<td></td>
</tr>
<tr>
<td>Slight</td>
<td>Male</td>
<td>24 (3.9)</td>
<td>Non-smoker</td>
<td>2 (15–70)</td>
<td>36 (13–40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>24 (7.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>Male</td>
<td>29 (7.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>24 (7.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
workers are frequently exposed to higher levels of cooking fumes.

The groups of highly, slightly and unexposed persons are thought to be similar in all ways except for the differences in the levels of exposure to cooking fumes. The groups consisted of young people, mainly men, who had worked for a relatively short period of time. The results suggest that the differences in the numbers of AM between the group of highly exposed persons and the groups of slightly and unexposed persons are mainly caused by exposure to cooking fumes (Table 3).

The results from the AM test in this study show a relatively low number of AM compared with the numbers found in the expectorate from industrial workers (Nilsen et al., 1984; Gullvåg et al., 1985; Mylius and Gullvåg, 1986). However, the participants in the present study are younger and probably healthier than the workers in the earlier studies. In addition, the pollution from coke plants, iron works and aluminium reduction plants is expected to be more harmful to the lungs than cooking fumes and is thus expected to give a more pronounced biological response in the lung.

The AM defence system responds quickly to all inhaled matter. The defence is activated within 24 h after the deposition of foreign airborne matter (Adamson and Bowden, 1980). Conversely, the number of AM is reduced in the absence of pollution due to ciliary and lymphoid transport of loaded AM and the lack of immigrating AM (Schlesinger et al., 1997). This leads to fluctuations in the number of AM over a short period of time.

The persons in the high exposure group attained a higher number of AM (median) than the persons in the minor exposure group in all smoking categories (Table 4). Highly exposed smokers attained the highest level of AM, which is statistically significantly higher ($P \leq 0.05$) than the number of AM in the group of minor exposure smokers. The number of AM (median) found in the samples from the highly exposed smokers is greater than the sum of the number of AM (median) in the minor exposure smokers and the number of AM (median) in the highly exposed non-smokers. These results suggest a synergistic effect between cigarette smoke and cooking fumes.

In some cases, ‘intermediate’ smokers are neither smokers nor non-smokers and thus may be excluded from this kind of study. However, in this study we chose to keep the ‘intermediate’ smokers because of the relatively low number of participants. In studies from aluminium plants a group size of 10 persons was considered to be sufficient to give an estimate of the total pollution load (Nilsen and Engen, 1985). The group size in this study ranged from 7 to 13 persons (Table 4). Consequently, some of the groups are probably a bit too small to give a good estimate of the pollution load of cooking fumes.

Table 4 shows that more highly exposed persons had more polynucleated AM in their expectorate samples than the persons in the minor exposure group. These results indicate that highly exposed persons have more active AM than the persons in the minor exposure group, i.e. polynucleated AM have higher phagocytotic activity in response to an increased pollution load (Cohen and Cline, 1971). In addition, more smokers had polynucleated AM in their samples than ‘intermediate’ smokers and non-smokers.

We observed a variation in the number of AM obtained from the expectorate samples from the same person according to their work during the immediately preceding days. If a person had had one or more days free, he had a lower number of AM than after a week at work. This can, to some extent, explain the large range of the individual means. Individual metabolic variation may contribute to increased variability. Some sensitive individuals may react more strongly to certain exposures than others. However, it must be emphasized that the AM test functions only as a biomarker of pulmonary irritation from occupational exposure at a group level owing to the differences in the individual level of AM. Nevertheless, in some cases when extremely high values are observed, the individual result in the AM test can be an expression of the level of sensitivity to inhaled pollutants. In all groups, some people had a considerably higher number of AM in their expectorate samples than the rest of the group (maximum values, Table 3), which suggests that some people in this study had a higher sensitivity to inhaled matter than others. What caused this increase in the AM level is unknown, but in
another study (Gullvåg et al., 1985) a remarkably high number of AM was found in a person who suffered from an allergic disease and in another person who had just recovered from an attack of pneumonia.

The results from this and previous studies indicate the need for biological monitoring methods to be used in parallel with conventional chemical measurement when the content of lung irritants in the working atmosphere is evaluated. A biological test such as the AM test indicates both the degree of exposure and the workers’ reaction to it.

CONCLUSIONS

We found an increase in the number of AM in kitchen workers from fast food stores and grill restaurants and these results may reflect the higher level of fat aerosols in these kitchens.

In addition, these results suggest a synergistic effect between occupational exposure to cooking fumes and smoking on the level of AM.

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