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Counting Alveolar Macrophages (AM) from Expectorate Samples of Exposed Workers as a Test for Lung Irritation from Occupational Exposure

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Alveolar macrophages (AM) have a natural function in the human body in keeping the lung tissue sterile and in dealing with any foreign material contaminating the airways. AM increase in number when the lungs are exposed to inhaled particles or gases. The increase is related to the degree of irritation caused by foreign material (Bergström et al. 1979, Rylander et al. 1979). Further on Pratt et al. (1969) and Harris et al. (1975) demonstrated that the number of AM counted after lung lavage in samples from persons chronically exposed to cigarette smoke, was significantly higher than in samples from not so exposed persons. These investigations have shown that the number of AM changes in relation to the quantity and the type of compounds inhaled, and that this change can be measured by differential counting of the total number of free lung cells, or of AM alone in samples recovered by lung lavage.

We have developed a method by which AM are counted in expectorate samples from exposed workers.

Pilot investigations have been made in a coke plant, and an iron works (Nilsen et al. 1984), on welders in a ship building yard, and on asphalt workers (Mylius and Gullvåg, 1984).

MATERIALS AND METHODS

A primary aluminium reduction plant was chosen, because the kind and degree of chemical pollution of the working atmosphere had already been relatively well investigated (Bjørseth et al. 1978, Rob personal communication, Fig. 1) and respiratory disorders had been watched over (Vale et al. 1980).

The primary aluminium reduction plant consists of three potrooms, a foundry, an anode paste plant, and a maintenance department. Expectorate samples were obtained from 143 workers selected at random from these different parts of the plant. Samples from a control group consisting of inhabitants of Trondheim, smokers and non-smokers, were also investigated. The control group (36 persons) consisted of people of approximately the same age and sex as the exposed workers. All persons who took part in the investigation also filled in a questionnaire to provide information

on their place of work, previous exposure to air pollution at work, state of health, and smoking habits. Persons found to be suffering from e.g. heart ailments, or who had a routine use of medicine or who smoked only at parties etc., were excluded from the control group. Each person was instructed to cough vigorously and the expectorate produced was collected. Four drops of the non-transparent parts of the sputum with high viscosity were selected from each expectorate sample and eight smears were made. These were fixed immediately, using Adams Spray Cyte fixative and stained according to the Papanicolaou-method. The number of AM in the smears were counted under a light microscope, using magnification of 500 X. A single field across the middle of the smear was counted, and the mean value for all eigth slides of each sample was calculated. The smears were designated as being representative of the lower part of the airways when either cylindric epithelial cells or AM, or both were found. The counting procedure with regard to number of persons, number of drops and number of sampling days has been evaluated in detail on the basis of variance analyses. The Wilcoxon rank sum test (single-tailed) was used to work out the statisticial significance of the differences between groups. Level of significance used $p \leq 0.05$.

In addition to the counting of AM all smears were screened for atypical cells and metaplasia (Garret 1973). This screening was also used as a control for the negative samples, where 0 AM had been registered by the AM test.

RESULTS AND DISCUSSION

The AM are easy to identify in the smears. The persons counting have sometimes checked up the counts of each other.

All groups were subdivided into smokers and non-smokers respectively and the potroom workers were subdivided further according to the operations performed. In the potrooms there is a significant difference in AM numbers between the exposed groups and the corresponding control groups. The highest numbers of AM were found in the samples from the workers in the potroom number 1 and 2, both smokers and non-smokers in the different job categories had significantly more AM in their samples than the controls. The potroom workers had the highest median number of AM compared to the other workplaces in the plant.

Values for all the non-smoking potroom workers taken together were significantly higher than those for the non-smokers in potroom 3. Potroom 3 had been modernized and the pollution here substantially reduced in comparison with 1 and 2 and we think this is well expressed by the AM counting method.
The big difference between exposed smokers and non-smokers in all groups and the fact that the median AM numbers for exposed smokers are higher than those for smoker controls + exposed non-smokers should be noticed. The conclusion must be drawn

that cigarette smoking and work place pollution act synergistically. Either the production of AM is affected and/or the clearance from the lung tissue is reduced leaving more macrophages in the lung tissue. The fact that cigarette smoking strongly reduces the ciliary movements in the bronchial epithelium is well established. (Figure 1.)

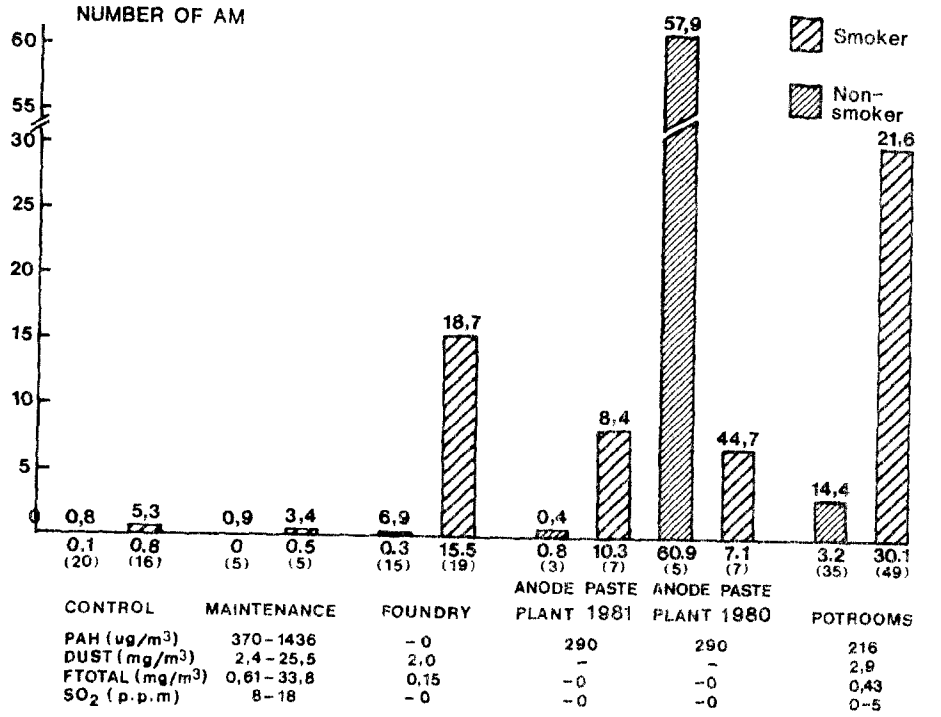

Figure 1. Median AM values for the workers grouped according to their main places of work. The actual values shown at foot of columns together with the number of persons in each group shown in brackets. Mean deviation above the column. The number of air analyses made to investigate the degree of pollution varied from 2 to 11. The maintenance department is represented by the analyses for the crane reparators' place of work only.
F = fluoride.

Some of our results need further explanatory comments, e.g. the discrepancy between the two investigations of the anode paste plant in 1980 and 1981 respectively. Non smokers among workers in the anode paste plant in 1980 had a remarkably high number of AM.

Besides the fact that the group is too small to be dealt with separately, an additional explanation was suggested by the plant physician: 3 of the 5 non-smokers who had a surprisingly high number of AM were in fact smokers who recently had stopped smoking. Furthermore one suffered from an allergic disease and another had just previously recovered from an attack of pneumonia. They had been transferred from the potroom to the anode paste plant because of lung disease and had also stopped smoking for that reason. Two of these 3 persons with a high number of AM in the samples took part in the 1981 study. On this occasion their values were more similar to the other exposed workers.

Another example of results which do not fit in with the common trend, are the smokers among potmen/foremen in potroom 3 who in fact have more than twice the number of AM than the potmen/foremen in potroom 1 and 2. (Figure 2.)

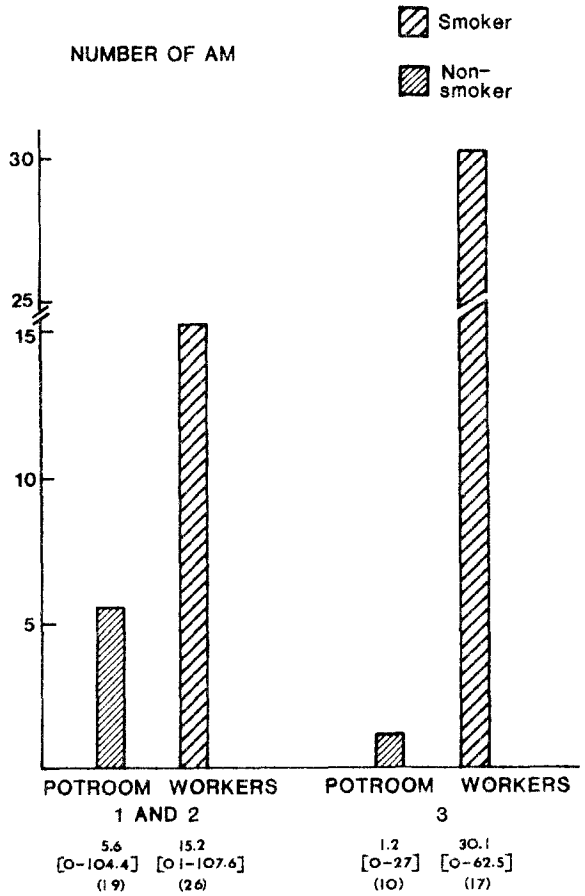

Figure 2. Median AM values for the smokers and non-smokers in potrooms 1 and 2 and in potroom 3 separately. Actual values shown at foot of columns together with overall ranges in square brackets and number of persons shown in round brackets.

The most acceptable explanation for this discrepancy is the fact that workers having indications of respiratory disorders had been moved from potroom 1 and 2 to the better conditions in potroom 3. These workers may be more sensitive to the air pollution than those who are still able to work in potroom 1 and 2 without discomfort. The smokers in potroom 3 had in fact also worked in the plant longer than the smokers in potroom 1 and 2 (respective mean values 4.7 years and 2.2 years).

In the maintenance group the figures both for smokers and non-smokers were on the level of the controls. This finding is rather remarkable as workers from the maintenance department in addition to ordinary repairs perform the most dirty jobs in the potrooms (crane-repairing under the potroom ceilings, Fig. 1), but doing so they always use special protection masks. This underlines the effect of protection gear, but the smallness of the groups (Fig. 1) may also have influenced results.

The degree of exposure to occupational pollution will depend on the use of respirators by the exposed persons, which will vary considerably and will greatly influence the results of the individual AM-count. The respirators used in the plant are of the 3M-type (with Al_2O_3 filter for fluorides), with a good cleansing effect (Rob personal communication) and capable of removing up to 93% of the dust in the working atmosphere. While many of the workers use them when performing some of the jobs they found it difficult to give precise information about the daily length of time they were used. We assume that the AM test integrates the varying use of respirator and yields a resultant of the sum of the positive and negative factors which influence AM production. Even if the plant has a continous monitoring by air filter sample analyses, many factors make it difficult to evaluate the pollution inhaled by one special worker. However, there seems to be a gradient in median numbers of AM for the different groups which to a certain extent parallels the values of pollutants. When trying to establish a pollution dose/AM-response relationship we found it more difficult to pinpoint the dose than the response. The AM number may therefore be of great value as a dose indicator. It can be assumed (Cockroft et al. 1982, Jensen 1980, Martin 1973) that a high number of AM in the lungs per se represents a hazard due to leakage of enzymes such as lysozyme and elastase. A high number of AM may in fact contribute to the development of lung diseases. Further work will show if the number of AM can be used also as a health indicator.

The sensitivity of the AM test is rather high. From a total number of 143 workers 24 smears were not designated as representative for the lower respiratory tract. Nine of these were on the other hand shown to be representative when using the ordinary screening procedure. The false negatives indicated by this counting method were 6%. A 0-sample is not necessarily a failure but could indicate that a person is healthy enough to have difficulties in producing AM or cylindrical cells in his sputum.

Our experience so far indicates that the method gives reproducable results and the following conclusions may be drawn:

1. The AM test reflects the pulmonary irritation from industrial air pollution and can be used as a dose indicator.
2. The AM test reflects changes in the load of industrial air pollution due to technological improvements, different kinds of respirators and varying use of respirator.
3. The AM test reflects the increase in irritation from the industrial air pollution combined with smoking.
4. The AM test may also contribute to the medical surveillance of workers in polluted industries.

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