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Alveolar Macrophages from Expectorate Samples: A Stress Signal from Occupational Pollution

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Alveolar macropages (AM) clean the inner surfaces of the lungs to maintain their sterility. There is a positive correlation between the amount of inert dust cleared away and the number of phagocytizing cells (Labelle and Brieger 1960). Dust exposure increases the recruitment of macrophages into the alveoli in a bifasic way (Grant et al. 1976), first via circulating monocyte migration and later via interstitium macrophage proliferation and migration (Adamson and Bowden 1980; 1981; Bowden and Adamson 1978; 1980). The respons of the macrophages after gas exposure is less studied, but generally the number of macrophages present in the deep respiratory tract varies with the chemical properties and amount of dust and gas exposure (Bowden 1973). The macrophages are cleared away from the alveoli by the mucociliary escalator up the tracheobroncheal tree (Green 1973) and are either swallowed or coughed up.

We suggest using the number of AM from expectorate samples for measuring the strain on the lungs from dust and gas pollution in the working environment, as an increased number of AM is the body's respons to the total load of pollution (including smoking).

We have investigated expectorate samples from workers at a Norwegian coke plant and an iron works. By making comparisons among differently exposed groups of workers and a control group, all groups being divided in smokers and non-smokers, a gradient of AM respons may be discerned. The group averages indicate the AM

reaction to the total load of pollution and reveal a possible interaction between smoking and workroom pollution. The individual values may suggest extra sensitivity in some exposed persons.

The aim of this study is to show that AM from expectorate samples can be used as indicator cells providing a simple method to study the human reaction to pollution.

MATERIALS AND METHODS

AM were obtained from expectorate samples from employees functioning under a variety of working conditions at a coke plant and an iron works. The workers answered a short questionnaire emphasizing smoking habits, the number of years they had been exposed to occupational pollution, use of respirators and medical history (heart failure and lung disease). Persons with heart failure were excluded from the investigation. The workers were instructed how to cough.

For light microscopical counting, AM samples were obtained simply by picking out aggregations of cells which could be discovered as lumps of different color and viscocity in the expectorated fluid. The aggregations were divided into drops with approximately uniform size. Smear slides were made out of one drop (two slides) from each sample. The smears were fixed with a spray fixative (Spray-Cyte, Clay Adams), and stained according to the Papanicolaou method. AM were counted in the central 2,4 cm^2 of each slide.

Statistical comparison of the results from different working groups were made with Student's t-test. This test requires normally distributed data. The results from the countings were transformed with logarithms to obtain a better approximation to the normal distribution.

The contols are counted with a slightly improved method; AMs are

registered in only one line of field of vision across the middle of the smears.

RESULTS AND DISCUSSION

Table 1 shows the mean number of AM in the expectorate samples from the workers at the coke plant. It is clearly shown that the smokers have an increased number of AM compared with non-smokers. The differences are significant ($p < 0,05$) for all working categories. The "side of oven" working area was not tested, because it was represented only by one worker.

Table 1. Mean number of AM in expectorate samples from workers at the coke plant.

	Number of AM ± SD and number of persons n			
Working category	Smokers	n	Non-smokers	n
Top of coke oven	1581 ± 954	(8)	89 ± 104	(6)
No respirator	2782 ± 1226	(2)	183 ± 163	(2)
Respirator	1181 ± 623	(6)	41 ± 62	(4)
Side of coke oven	816 ± 795	(14)	56 ± 82	(1)
Others	637 ± 703	(8)	85 ± 105	(6)
Mean	972 ± 916	(30)	85 ± 105	(13)
Control	168 ± 336	(16)	26 ± 54	(20)

43 out of the 59 samples (i. e. 73%) were characterized as representative - containing cells from the lower airway regions. Dividing the samples according to smoking habits showed that 79% of the smokers and 60% of the non-smokers produced representative samples. No significant differences between the different working categories of non-smokers were found. The mean number of AM from exposed non-smokers were, however, higher than that from the control non-smokers.

The results from the controls are not tested statistically against

the exposed groups, because AM from the controls are counted in a slightly different way (Nilsen and Engen, unpublished data). The means are, however, comparable when multiplying the control values with 30. Our studies in other industries verify that the differences between exposed workers and controls are real (Mylius and Gullvåg 1983; Mylius et al. 1981).

The exposed smokers had much higher numbers of AM than the sum of AM from exposed non-smokers and the non-exposed smokers. This means that the occupational air pollution and smoking together increase the number of AM in a synergistic way.

The "top of coke oven" working area showed the highest total response with a mean of 1581 AM in the smokers group. The "side of oven" and "others" working categories had a mean of 816 AM and 637 AM respectively. The differences between top of oven and the other two working categories are significant ($p = 0,01$ and $p = 0,025$).

The results from top of coke oven were also tested with respect to the cleaning effect of using a respirator (airstream helmet). The testing groups were relatively small. Both smokers and non-smokers not using a respirator had significantly more AM than those who used respirator ($p < 0,05$).

Table 2 shows the mean number of AM in sputa from workers at the iron works. The mean value of the smokers' AM is higher than the non-smokers', as was the situation at the coke plant, although the difference is not significant.

49 out of the 70 samples (i. e. 70%) were categorized as representative. Within the smoker and non-smoker groups the relative amounts of representative samples were 75% and 64% respectively.

The non-smokers at the plant had higher AM counts than the

controls (not tested), which indicate that inhaled workroom air pollution at the iron works leads to an increased number of AM within the respiratory tract.

Table 2. Mean number of AM in expectorate samples from workers at the iron works.

	Number of AM ± SD and number of persons n			
Working category	Smokers	n	Non-smokers	n
Iron works	1663 ± 2088	(33)	602 ± 683	(16)
Control	168 ± 336	(16)	26 ± 54	(20)

There is a trend in the results indicating that the use of a respirator (dust mask) reduces the number of AM in the exposed groups (Table 3). The decrease in AM numbers is however, not significant.

Table 3. Mean number of AM in expectorate samples from smokers and non-smokers at the iron works. The workers are divided in those using and those not using a respirator.

	Number of AM ± SD and number of persons n			
	Respirator	n	No respirator	n
Smokers	1350 ± 2100	(11)	1610 ± 1995	(22)
Non-smokers	357 ± 535	(9)	918 ± 760	(7)

As part of the lung protective system, AM react to the stress imposed on the lungs from pollution. Variation in the number of AM in expectorate samples may therefor reflect variation in pollution strain if a reproducable method is found for sampling and the differences in counts can be shown to be significant.

The results from the countings show that the workers at both the

coke plant and the iron works produced more AM than the controls. The AM counts of the non-smokers indicate that inhalation of the occupational pollution augments the AM respons. At the iron works the increase in AM numbers between exposed non-smokers and controls was 23 times, while it was 3 times at the coke plant. The difference seems to be the result of the higher strain of dust on the workers at the iron works. Both chemical composition and amount of pollution are important. The influence of the amount is clearly shown by the workers on the top of the coke oven. Those who do not use airstream helmet have more AM than those using a respirator.

At the coke plant the smokers had more AM than the non-smokers and a synergistic effect from agents in the workroom pollution and components of the cigarette smoke was observed which resulted in a conciderable increase in the number of AM. Agents present in larger amounts or only at the top of the oven cause a stronger reaction with smoking than chemicals also present at the other working areas, because the difference in AM numbers between smokers and non-smokers at the top of the coke oven was the highest in the coke plant, 17 times, while the plant average was 11 times. This implies that tar components (PAH/PPOM) or possibly gases (SO_x, NO_x, CO) strengthen the cumulative effect more than the dust alone.

The results from the iron works support the findings from the coke plant, even if the observed differences are not statistically significant. The lack of significance may be due to the fact that the total group at the iron works is composed of persons from different job categories inside the plant. These categories represent differences in amount of pollution, while the type of pollution is roughly identical. Because the various working places are not similar in pollution strain, the AM responses also differ from place to place. As a result, differences in certain parameters such as smoking and use of respirator, are less pronounced in the total group.

There are two studies which support our suggestion of using AM as a biological test. Bergström et al. (1979) have performed an inhalation toxicological study exposing small rodents to industrial dust in exposure chambers. They counted AM and leukocytes after pulmonary lavage and concluded that the number of white blood cells found in the lungs at an early stage of exposure can indicate the potential of a dust to produce inflammation. Nobutomo (1978) counted AM, neutrophile granulocytes and lymphocytes in sputum from two large populations in Japan exposed to various amounts of environmental air pollution (urban contra rural). If the number of cells in a chosen area of the slide counted was more than ten, he suggested that the pollution could have induced pathological changes. Our studies are different in that we investigate occupationally exposed workers and count AM only. On the basis of these and other evidence from the litterature (cf. e. g. Heppleston 1978; Jenssen 1980; Kimbel 1980) we also believe that an augmented number of AM may signify an augmented susceptibility to fibrosis, bronchitis and emphysema. The risk is difficult to quantify. It seems at the present stage safer to draw conclusions from groups of workers, even if individual high values may indicate the need for change of working place inside the plant. The number of AM, however, shows a total respons to the pollution produced by the living body itself.

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