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Chemotactic Responsiveness of Human Alveolar Macrophages: Effects of Cigarette Smoking

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Pulmonary alveolar macrophages from six cigarette smokers demonstrated higher random migration and greater chemotactic responsiveness to casein than did macrophages from seven nonsmokers. These observations are consistent with the concept that pulmonary macrophages are metabolically activated by cigarette smoking.

Chemotactic responsiveness of phagocytic cells, particularly of polymorphonuclear leukocytes, is defective in a number of clinical conditions in which host defense to bacterial infections is impaired (9, 11). Mononuclear phagocytes can respond to chemotactic stimuli, but chemotaxis of more specialized cells, such as pulmonary alveolar macrophages (PAM's), has not been as extensively studied (6, 10).

Previous studies of human PAM's obtained by bronchial lavage have revealed that cigarette smoking is associated with alterations in morphology (5), increased lysosomal (7) and microsomal enzyme content (2), higher resting metabolism (5), increased migration, and diminished responsiveness to migration inhibitory factor (12). Published reports indicate that the capacity for phagocytosis and bacterial killing are unaffected (3, 5).

The following study assesses the effects of cigarette smoking on migration and chemotactic responsiveness of PAM's in vitro. PAM's were obtained by bronchial lavage from 13 healthy volunteers, including seven nonsmokers (age, 23 to 26; mean, 24.4 years) and six cigarette smokers (age, 19 to 26; mean, 22.0), as previously described (12). These smokers had a mean cumulative cigarette exposure of 7.66 pack-years. Informed consent was obtained in writing from all volunteers prior to their inclusion in the study.

Quantitatively and qualitatively, the cells obtained by lavage (Table 1) were similar to those of previous studies (5, 7, 12).

For each subject, the lavage suspensions containing pulmonary cells were pooled and centrifuged in the cold at 180 $\times$ *g* for 10 min, washed twice in 0.15 M NaCl, then suspended at a concentration of 5×10^5 cells/ml in tissue culture medium 199 (TCM) containing antibiotics (100 U of penicillin and 100 μg of streptomycin per ml) and 10% fresh autologous serum, adjusted to pH 7.4 with 30 mM *N*-2-hydroxyethylpiperazine - *N'* - 2 - ethanesulfonic acid buffer.

Migration and chemotactic responsiveness of PAM's were measured in triplicate by using previously described (4, 8) modified Boyden chambers, with further modification of the cell compartment to include a double membrane system. Briefly described, the upper cell compartment consisted of a transected disposable test tube (Falcon no. 2027), to which a 13-mm diameter membrane filter (Millipore Corp., NCWP 01300), having a mean pore size of 14 μm and a 150-μm thickness, was glued with formulation no. 1 MF cement (Millipore Corp.). A second filter of 3-μm mean pore size and 13-mm diameter was cemented in place immediately below the first filter with formulation no. 2 MF cement.

The PAM suspension (containing 2.5×10^5 cells in 0.5 ml) was pipetted into the upper cell compartment. To test random migration, the lower compartment (a 2-dram glass medicine vial) contained 2.0 ml of TCM. To test chemotactic responsiveness, 0.1% casein was included.

Preliminary studies revealed that PAM's rarely migrated through a 14-μm filter before 18 h. In these studies, chambers were incubated for 24 h at 37 C, after which the bottom filters were removed, fixed, stained, and cleared (1). For each filter, the number of macrophages resting on the lower membrane in five randomly chosen $\times$100 microscopic fields were counted. The PAM's were unable to traverse the lower filter of

3-μm porosity under these incubation conditions.

The random migration of PAM's from smokers was more active than migration of PAM's from nonsmokers (Fig. 1). Smoker PAM's reached a density of 21.1 ± 2.7 cells/field compared with 9.7 ± 2.2 nonsmoker macrophages/field (Student's t test, $P < 0.01$). When casein was present in the lower compartment, the smoker PAM's were found to be more responsive to this chemotaxis stimulus than were the nonsmoker PAM's (Fig. 2). The chemotactic index (the ratio between migration toward casein and random migration) for smoker PAM's was 3.43 ± 0.60 as compared with 2.13 ± 0.15 for nonsmoker macrophages ($P = 0.05$).

PAM's from smokers were previously noted to migrate from capillary tubes at a faster rate than PAM's from nonsmokers. However, it was not possible to assess chemotactic responsiveness with the capillary tube system (12).

The findings presented indicate that smoking does not impair the ability of PAM's to respond to a chemotactic stimulus. Indeed, cigarette smoking is associated with an increased PAM population capable of an enhanced response. Because the lavage material from these young, asymptomatic smokers is regularly sterile for bacteria and viruses and because polymorphonuclear leukocytes (which are characteristically found in material lavaged from chronic bronchitis patients) are not found, the changes in PAM's observed are probably associated with smoking rather than being secondary to another process, such as chronic, lower respiratory infection.

The preservation of chemotactic responsiveness of PAM's from cigarette smokers suggests

TABLE 1. *Results of bronchial lavage with 250 ml of sterile saline*

Subjects	Lavage recovered	Cells			
		Total obtained[a]	Macrophage	Small mononuclear	Viability[b]
Nonsmokers (n = 7)	149 ± 13.2	2.3 ± 0.6 × 10^7	87 ± 2.6%	12 ± 2.8%	93 ± 1.6%
Smokers (n = 6)	151 ± 6.6	11.0 ± 2.0 × 10^7	91 ± 1.7%	6.5 ± 1.5%	96 ± 0.3%

[a] Significant difference between smokers and nonsmokers (Student's t test, $P < 0.01$).
[b] Percentage of cells excluding trypan blue dye after 5 min of incubation.

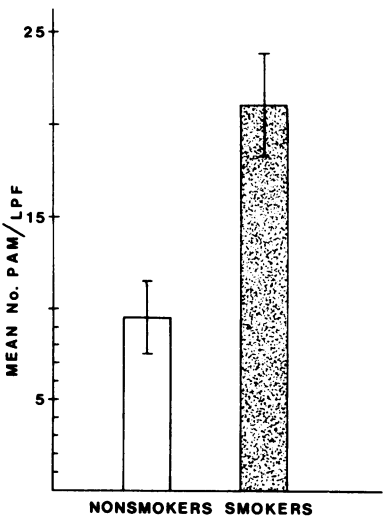

FIG. 1. *Random migration of human pulmonary alveolar macrophages, expressed as the mean number of PAM's per microscopic field migrating through a 14-μm membrane filter during 24 h of incubation at 37 C.*

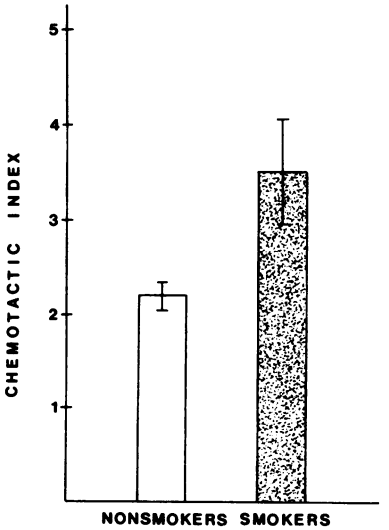

FIG. 2. *Chemotactic responsiveness of human pulmonary alveolar macrophages to 0.1% casein, expressed as the chemotactic index (the ratio between migration in a casein gradient and random migration). All incubations were for 24 h at 37 C.*

that any association between smoking and defective pulmonary defense mechanisms must be explained on another basis.

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