

# Chemiluminescence and Antibody-dependent, Cell-mediated Cytotoxicity between Human Alveolar Macrophages and Peripheral Blood Monocytes in Smokers, Nonsmokers, and Lung Cancer Patients\*

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Some evidence suggests a capability of peripheral blood monocytes to destroy tumor cells, while this ability by human alveolar macrophages, the main defense cells in the alveoli, is still debatable. We measured the chemiluminescence and antibody-dependent, cell-mediated cytotoxicity in the PBMs and HAMs of 12 lung cancer patients and 20 healthy subjects; the latter included ten smokers and ten nonsmokers. The PBMs were prepared by using a Ficoll-Hypaque density gradient, then separated by Petri-dish adherence. The HAMs were taken during the bronchoalveolar lavages. The chemiluminescence in the HAMs of smokers was significantly higher than nonsmokers, ( $p < 0.05$ ), which did not occur in the PBMs. Chemiluminescence in HAMs from the lung cancer patients was also significantly higher than the smoker control subjects ( $p < 0.01$ ). However, the lung cancer patients had significantly lower ADCC activity than the smokers in the control group ( $4.52 \pm 2.96$  vs  $8.27 \pm 2.83$  percent;  $p < 0.05$ ). The chemiluminescence in the PBMs showed no statistical

difference between the lung cancer patients and smoker control, but PBMs of the lung cancer patients had significantly lower ADCC activity than the smoker normal control group. The HAMs from lung cancer patients produced more superoxide anion, for an increased chemiluminescence reaction was noted, although ADCC activity was lower than in smokers, *ie*, HAMs were ineffective in killing tumors. Environmental factors such as cigarette smoking affect HAM's function by causing an increase of superoxide anion production. The chemiluminescence and ADCC activity in the PBMs does not always correspond to the HAMs findings. These results suggest that PBMs can not accurately reflect or predict the HAMs' function in lung diseases. (Chest 1989; 95:553-57)

PBM = peripheral blood monocytes; HAM = human alveolar macrophages; ADCC = antibody-dependent, cell-mediated cytotoxicity; FCS = fetal calf serum; PBS = phosphate-buffered saline; MPO = myeloperoxidase; PHA = phytohemagglutinin; ConA = concanavalin A

For many years macrophages and monocytes were considered to play an important role in preventing oncogenesis,<sup>1-3</sup> based on much evidence from a variety of *in vivo* and *in vitro* studies. In human studies researchers found that the presence of infiltrating macrophages or monocytes in the histologic section of certain types of tumors, such as breast cancer, had a lower incidence of axillary lymph node metastasis and subsequently a better prognosis. In addition, patients with positive axillary lymph nodes who exhibited histiocytosis had a lower incidence of distant metastasis.<sup>4,5</sup> Second, trypan blue (a macrophage lysosomal enzyme inhibitor) and silica (a specific macrophage toxin) seemed to decrease the resistance of mice and rats to transplantable tumors.<sup>6,7</sup> Third, BCG, which is a macrophage-activating agent, could increase the resistance to transplantable tumors in mice.<sup>8</sup>

The *in vitro* studies of the past also supported the role of macrophages in the resistance of oncogenesis. Hibbs et al<sup>9</sup> and Keller et al<sup>10</sup> noted that activated murine macrophages were cytotoxic and cytostatic against tumor cells. Numerous researchers found that sensitized T-lymphocytes in the presence of germane tumor antigens secreted a cytophilic protein that would activate macrophages and enhance tumoricidal activity.<sup>11,12</sup>

From BAL, recently developed for examination and research, pulmonary alveolar macrophage (PAM) is the main component in the cells obtained.

We attempted to elucidate the following questions: Does cigarette smoking affect superoxide anion production and the ADCC of HAMs and PBMs in healthy individuals? Do patients with bronchogenic carcinoma (CA) have impaired superoxide anion production and decreased HAM cytotoxicity? Does the alteration of *in vivo* microenvironment (*eg*, acute or chronic inflammation) affect the function and cytotoxicity of PBM or HAMs? Finally, can the examination of the PBMs' function alone accurately reflect and predict the HAMs' function?

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We performed BAL to obtain the HAMs from the lung fluid and drew peripheral blood samples to determine the PBM of the respective groups. The chemiluminescence and ADCC per group were analyzed and comparisons made between the respective PBMs and HAMs from each group.

## MATERIALS AND METHODS

### Patient Selection

From March 1985 to December 1986, 12 lung cancer patients were studied. Their mean age was 54 years, ranging from 40 to 65 years. Among these 12 cases were three cases of primary bronchogenic squamous cancer, three cases of primary bronchogenic adenocarcinoma, two cases of bronchogenic small cell cancer, one case of large cell cancer, and three cases of metastatic cancer. The diagnosis of lung cancer was determined through either a histologic or a cytologic specimen from the sputum, bronchial brushing or biopsy, or lung aspiration or thoracotomy. The AJC definitions of TNM stages was used to determine the exact staging of each patient.<sup>13</sup> All of the patients were stage 3, and none of the subjects had previously been treated by chemotherapy or radiation therapy. The mean smoking duration was 12.9 pack-years.

The control group consisted of 20 healthy subjects, of which ten were nonsmokers and ten smoked. All ten smokers had a history of more than five pack-years and together had a mean smoking history of 10.8 pack-years. All 20 of the subjects in the control group visited our hospital and received a bronchoscopic examination. All had chest tightness or a foreign body sensation in the throat but no other specific chest complaints. In each case the chest x-ray film and the pulmonary function test both showed normal results.

### BAL Technique

All of the subjects received IM administration of 0.4 mg of atropine, 40 mg of meperidine (Demerol), and topical lidocaine (Xylocaine). The bronchoscope was then passed transnasally and wedged on the right middle lobe or left lingular lobe of the subsegmental bronchial orifice in the control group. In the cancer group, the bronchoscope was wedged in the subsegmental bronchus of the lesion site. Then 50-ml aliquots of normal saline solution was infused, and, using gentle suction (less than 40 mm Hg), was retrieved. This procedure was repeated four times with a total of 200 ml of solution being administered. The infused solution and recovery rate of the lavage is shown in Table 1.

### Chemiluminescence Study

The preparation of the cells included the following procedures. The HAMs were separated by Petri-dish adherence. The PBMs were obtained from the PBMs after Petri-dish adherence. The

**Table 1—Cells Recovered from BAL Fluid of Nonsmokers, Smokers, and Lung Cancer Patients\***

	Nonsmokers (n = 10)	Smokers (n = 10)	Lung CA (n = 12)
Age, yr	24.2 ± 3.6	29.3 ± 3.8	54.7 ± 6.3
BAL return, %	57.0 ± 7.6	57.5 ± 6.9†	39.5 ± 7.2†
Cell count, × 10 <sup>6</sup> /dl	2.86 ± 0.85†	4.08 ± 1.58†	7.82 ± 1.83†
Cell differential, %			
Macrophages	88.2 ± 2.9	91.3 ± 3.9	87.2 ± 7.4
Lymphocytes	7.8 ± 2.8	4.8 ± 2.3	9.3 ± 5.9
Neutrophils	3.6 ± 1.1	3.5 ± 2.6	4.3 ± 1.2
Eosinophils	0.4 ± 0.3	0.3 ± 0.3	0.6 ± 0.84

\*All data expressed as mean ± SD.

†Fisher's LSD test; p < 0.05.

PBMs were then prepared using a Ficoll-Hypaque density gradient from the heparinized peripheral blood. In both instances, the HAMs and the PBMs were adjusted to 5 × 10<sup>6</sup>/ml and then suspended in medium-199.

The zymosan opsonization was performed using the method perfected by Easmon et al.<sup>14</sup> Briefly, the zymosan (Sigma) was suspended in a phosphate buffer saline solution (PBS, pH 7.2) at a concentration of 20 mg/ml, and then was boiled in a water bath for 10 min. After centrifuging at 1,000 rpm for 5 min, the supernatant was discarded, and the 20 mg of pelleted zymosan was resuspended in 0.5 ml of serum and incubated at 37°C for 30 min. After incubation, the opsonized zymosan was washed three more and then resuspended in PBS to a concentration of 20 mg/ml.

Chemiluminescence was measured with a luminometer (LKB, Wallace) which was described by Easmon et al.<sup>14</sup> The HAMs (0.1 ml) or PBMs (5 × 10<sup>6</sup> cells) were then added to curvettes containing 0.7 ml Lucigenin (10<sup>-4</sup> M, LKB Wallace) and 0.2 ml zymosan solution. The samples were then transferred to a luminometer (LKB 1251 Wallace) with an automatic temperature control. With the samples under 37°C, the chemiluminescence was measured at 2-min intervals for up to 50 min.

### ADCC Assay

The ADCC assay described by Trinchieri et al.<sup>15</sup> was used with minor modifications. The HAM or PBM was adjusted to 2 × 10<sup>6</sup>/ml in a RPMI 1640 and then supplemented with 10 percent fetal calf serum (FCS) as effector cells. The erythrocytes were obtained from young white Leghorn chickens, suspended in 5 × 10<sup>6</sup>/ml, and then incubated with 100 μCi of <sup>51</sup>Cr/ml for 1 h at 37°C. The cell suspension was then washed with phosphate-buffered saline solution (PBS) three times and resuspended to 2 × 10<sup>6</sup>/ml to make the target cells.

The cytotoxic test was performed on round-bottom microplates. In each well, 2 × 10<sup>4</sup> target cells (0.1 ml) were mixed with 2 × 10<sup>6</sup> effector cells (0.1 ml) to which was added 20 μl goat antichickerythrocyte antibodies. Each microplate included the following controls: (a) maximal release (M) by adding to the well, 0.12 ml 2 percent Triton (Sigma) USA; and (b) spontaneous release (S) in the presence of effector target cells but without antiserum. The plates containing both experimental and control assays were incubated for 4 h at 37°C in a humidified atmosphere containing 5 percent CO<sub>2</sub>. At the end of the incubation, the plates were centrifuged at 400 × g for 15 minutes at 4°C. From each well, 0.1 ml of supernatant was collected and counted for <sup>51</sup>Cr activity in a well-type gamma counter. In the following formula, (E) is the experimental release and is the actual number of <sup>51</sup>Cr cpm obtained in the experimental wells. Specific <sup>51</sup>Cr release (R) is expressed as the percentage of net experimental release over net total release as indicated in the following formula:

$$(\%)R = \frac{E - S}{M - S} \times 100$$

### Data Analysis

In Tables 1 and 2 and Figure 1, we used the Fisher's LSD test to compare the variances of these three groups. All values were expressed as the mean ± SD with significance accepted as p less than 0.05.

## RESULTS

The percentages of lavage fluid return, total cell counts, and differential cell counts taken from the lavage fluid are shown in Table 1.

The results that compare the chemiluminescence and ADCC of PBMs and HAMs between smokers and nonsmokers are shown in Table 2 and Figure 1. It is

**Table 2—Comparison Among Nonsmokers, Smokers and Lung Cancer Patients Regarding the Chemiluminescence and ADCC Using Human Alveolar Macrophages and Peripheral Blood Monocytes\***

	Nonsmokers (n = 10)	Smokers (n = 10)	Lung Cancer (n = 12)
<b>BAL</b>			
Chemiluminescence (ΔMV)	10.78 ± 2.81*	18.64 ± 4.65*	26.91 ± 5.02*
ADCC (%)	10.37 ± 3.03	8.27 ± 2.83*	4.52 ± 2.96*
<b>PB</b>			
Chemiluminescence (ΔMV)	592.61 ± 133.46	601.12 ± 135.63	592.79 ± 201.99
ADCC (%)	20.43 ± 3.44	19.64 ± 4.4	2.81 ± 5.68*

all data were expressed as mean ± SD.

\*Fisher's LSD test: p<0.05.

ΔMV = millivoltage.

obvious that in healthy individuals, whether they smoke or not, the chemiluminescence and ADCC of the PBMs groups were higher than the respective HAMs groups and was statistically significant. In comparing smokers and nonsmokers, the difference in the chemiluminescence and ADCC of PBMs was of no statistical significance. However, the chemiluminescence of HAMs in smokers was higher than nonsmokers and this difference was of statistical significance. On the other hand, the ADCC of smokers was slightly lower than that of nonsmokers. However, the difference was statistically insignificant.

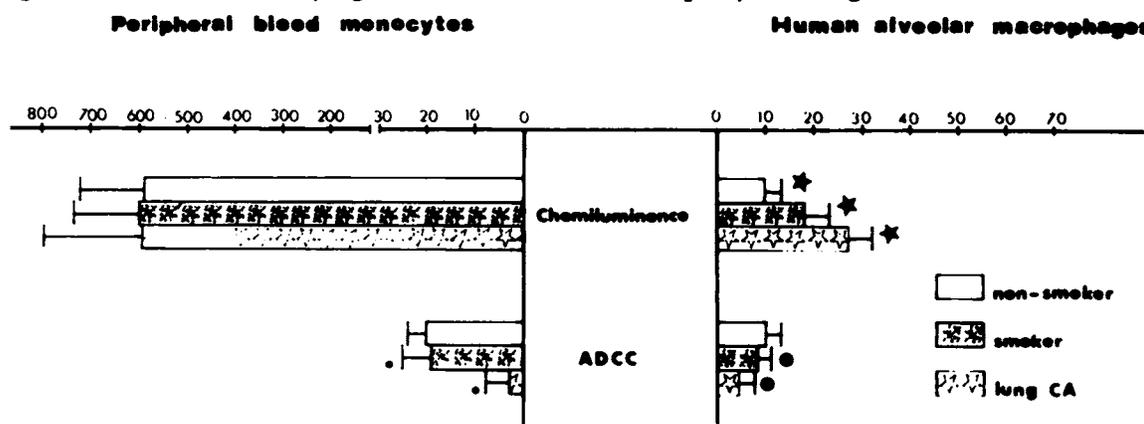
Table 2 and Figure 1 also disclose the results comparing the chemiluminescence and ADCC between healthy smokers and patients with lung cancer. Although the PBMs of lung cancer patients had higher chemiluminescence than HAMs, as in healthy smokers, the ADCC showed no difference between PBMs and HAMs. The difference in the chemiluminescence of PBMs in lung cancer patients compared with healthy smokers was of no statistical significance, but the ADCC showed a significant decrease. On the other hand, the HAMs of lung cancer patients had increased chemiluminescence and a decrease of ADCC, especially when compared with healthy smokers, thus making the difference statistically significant.

## DISCUSSION

Over the past few years, many studies have shown that cigarette smoking can affect the different functions of the HAMs. However, the ability of HAMs (which are the main defense cells in the alveoli) to kill tumor cells is still controversial.<sup>16</sup>

Given this incongruity, we began this research in an attempt to elucidate the function of PBMs and HAMs in tumor defenses. We measured the chemiluminescence and ADCC of 12 lung cancer patients and 20 healthy subjects. Of the 20 healthy subjects, there were ten nonsmokers and ten smokers.

On measuring the chemiluminescence of the 20 healthy subjects, it was immediately evident that the PBMs' chemiluminescence was significant higher than in any of the respective healthy HAMs groups. Weissler et al in 1986<sup>17</sup> and Kemmerich in 1987<sup>18</sup> pointed out that HAMs can produce more superoxide anion, which is the initial toxic oxygen species generated during a respiratory burst, than PBMs in normal healthy non-smoking subjects. The earlier study by Weissler et al<sup>17</sup> also demonstrated that HAMs' chemiluminescence, to measure the toxic oxygen compounds' interaction with myeloperoxidase (MPO) and halide ions, is far less than that found in the PBMs. They explained this discrepancy as being due to the maturation of the



**FIGURE 1.** Comparison of nonsmokers, smokers, and lung cancer patients in regard to respective chemiluminescence ΔMV and ADCC (%) using human alveolar macrophages and peripheral blood monocytes. All data expressed as mean ± Fisher's LSD test; \*, •, \* p<0.05.

HAMs taken from the PBMs. As they mature, HAMs lose their MPO activity. Therefore, the low HAMs' chemiluminescence, especially when compared with that found in the PBMs, is due to its lacking MPO. These findings are compatible with the results of this study in that the HAMs' chemiluminescence in both healthy (smokers and nonsmokers) groups and the lung cancer patients was significantly lower than all of the respective PBMs groups.

A second observation was that the ADCC in the HAMs of both the smokers and nonsmokers was also substantially lower than their respective healthy PBM groups. This finding differs from the observations of Whitcomb,<sup>19</sup> which showed that HAMs were more efficient at mediating ADCC than PBMs. This can be true only when effector-to-target-cell ratio was 1:10. However, when Whitcomb changed the effector-to-target-cell ratio to 10:1, the results of the ADCC of the PBMs were reversed, compatible with our result. Further, the lower HAM chemiluminescence may help to explain the lower ADCC in the HAMs than the PBMs. The findings of Weissler et al<sup>17</sup> also corroborate our results, for they found that HAMs had lower tumor-killing ability than PBMs in healthy nonsmokers.

Many studies have shown that cigarette smoking might affect the function of HAMs. The study by Harris et al<sup>20</sup> showed that the HAM of cigarette smokers had a greater proteolytic enzyme activity (*eg*, elastase-like esterase and protease activity) than nonsmokers. Compared with nonsmokers, smokers had a higher oxygen requirement, glucose utilization, and a difference in ultrastructures. This in effect means an increase in cellular activity in the aerobic pathway.<sup>21</sup> This finding was supported in our research in that smokers had a higher chemiluminescence in HAMs. However, higher chemiluminescence does not always mean that the cells were functioning more effectively. This can also be seen in other studies,<sup>22</sup> where the authors found that the HAMs of smokers impaired antigens and stimulated lymphoproliferation. Daniele et al<sup>23</sup> demonstrated that there was no difference between smokers and nonsmokers in proliferative response of peripheral blood lymphocytes to phytohemagglutinin (PHA), and concanavalin A (ConA). However, they did notice a decrease in the response of lung lymphocytes to PHA and ConA among smokers. The pulmonary lymphocyte function could affect macrophage function; therefore, impaired pulmonary lymphocyte function would probably induce impaired HAMs' function. Yeager et al<sup>24</sup> also demonstrated that HAMs' pinocytotic activity was found to be decreased in smokers.

In our study, the HAMs' ADCC was found to be decreased in smokers when compared with nonsmokers. However, this difference was small and of no

biostatistical significance, and might be because the amount of smoking was not large enough to statistically affect our smokers.

HAMs, by their very nature, are a main inflammatory cell of the lung. However, their exact role in the immune surveillance system and in the prevention of oncogenesis remains to be determined. In lung cancer patients, Weissler et al<sup>17</sup> demonstrated that monocytes obtained from their lungs were significantly more cytotoxic than those taken from normal, healthy individuals. In this study the ADCC found in the HAM of the lung cancer patients was impaired, especially when compared with the healthy smokers and nonsmokers. The reason for this discrepancy between this study and the results of the Weissler project may be attributed to the fact that all of the subjects in this study were in an advanced stage (III) of cancer. Weissler et al<sup>17</sup> did not indicate the stage of the patients at the time of the project. Many other studies have indicated a similar finding. Lemarie et al<sup>25</sup> demonstrated impaired HAM chemotaxis in cancer patients. Plowman et al<sup>26</sup> reported that bronchogenic cancer patients had pinocytotic activity that could not be restored by employing either levamisole or cyclosporin A. Bordignon et al<sup>27,28</sup> showed that the defective tumoricidal capacity in the HAMs of lung cancer patients could not be affected by interferon but was restored through lymphokines. All of these reports supported the findings of this study that the HAMs of the lung cancer patients had substantially higher chemiluminescence but lower ADCC. In this study the ADCC of PBMs measured from lung cancer patients was significantly lower than that of the healthy patients. This result was different from the study by Rhodes et al<sup>29</sup> who found an interesting reaction in that lung cancer patients had increased PBM Fc-receptor function but a depressed Fc-receptor function in the HAMs.<sup>27</sup> In explanation, the increased Fc-receptors in PBM in their study contrasts with the low ADCC result in the lung cancer patients of our study.<sup>29</sup> This may be attributed to the fact that our subjects were all in stage III. This is especially important because Rhodes et al had chosen lung cancer patients who were free of metastasis. Lin et al<sup>30</sup> had found that patients at different lung cancer stages demonstrated different natural killer (NK) activity. Therefore, it is possible that the advanced stage of the patients in our study affected the results.

One may draw several conclusions from the data. First, HAMs and PBMs do not have congruent functions. At times, their functions may seem similar or seem to run parallel. Therefore, it is important to realize that PBMs cannot be used to accurately reflect or predict HAMs value. Second, in healthy individuals, the HAMs' chemiluminescence is less than that found in the PBMs. This is possibly due to the loss of MPO during the maturation of the HAMs. Third,

without doubt environmental factors such as smoking affect the condition and function of the HAMs. Fourth, the HAMs taken from lung cancer patients produced more superoxide compounds, for an increased chemiluminescence reaction was noted. However, the ADCC activity was lower than in the smoker control. This means that the HAMs were ineffective in killing tumors, at least in stage III lung cancer patients.

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