# MIXED HEMATOPOIETIC AND PULMONARY ORIGIN OF 'ALVEOLAR MACROPHAGES' AS DEMONSTRATED BY CHROMOSOME MARKERS

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The origin of the large mononuclear cells which occur in the alveolar spaces of normal lungs, and in lungs subjected to irritant stimuli, has not been established conclusively. Earlier workers have implicated cells from the alveolar lining epithelium,[1,6] the capillary endothelium,[2,3] the mesenchyme of the alveolar walls,[4–6] and blood leukocytes,[6–9] as possible sources. In the present communication, the term 'alveolar macrophage' will be used to designate these large free intra-alveolar mononuclear cells.

Prior investigations have generally depended upon convential morphologic criteria for their conclusions. In the present study, which was limited to determining the origin of dividing alveolar cells, the relative proportions of blood-derived cells in the alveolar macrophage population was determined by the method of chromosome analysis on lung washings of mouse chimeras.

## Material and Methods

Chimeras were obtained from Leonard J. Cole, United States Naval Radiological Defense Laboratory, San Francisco, Calif. To produce the chimeric state, CBA mice were x-irradiated (900 r) and given injections of bone marrow cells from the histocompatible homozygous $T_6$ strain which carries two easily recognizable minute chromosomes (Fig. 1). The hematopoietic tissues in these mice were repopulated by donor cells with marker chromosomes, and the presence of these markers in dividing cells was an indication of their hematopoietic origin.

One to 4 months after chimerism had been established, alveolar macrophages were harvested from 19 mice. To increase the yield of cells, two-thirds of the mice were given injections of 10 $\mu$l Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) into the tail vein 3 to 6 days before being killed.[6] Six hours prior to harvesting the cells, all mice received 0.2 mg colchicine intraperitoneally. Approximately half the mice were anesthetized with ether for the colchicine injection.

This investigation was supported by Grant No. 2-TI-CA-5022-08 from the National Institute of Health, United States Public Health Service and by Public Health Service Research Career Award 5-K3-GM-15004 and Research Grant CA-04659 from the National Cancer Institute.

Presented at the Sixty-third Annual Meeting of The American Association of Pathologists and Bacteriologists, March, 1966, Cleveland, Ohio.

Accepted for publication, December 31, 1965.

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In collecting the cells, mice were lightly etherized and exsanguination was accomplished by transection of the abdominal aorta. The lungs were allowed to collapse by puncturing the diaphragm. An opening was made in the trachea into which was inserted a blunt 20-gauge needle connected to the tip of a 3-way stopcock with 2 syringes attached. One syringe contained Earle's balanced salt solution (BSS), the other being empty. Several ml of BSS were slowly injected through the trachea into the lungs, expanding them to just fill the thorax.[10] This first volume of fluid was left in the lungs for 10 to 20 minutes and then gently aspirated into the receiving syringe. A second filling with BSS was removed within a few seconds. The cells recovered from the lung washings, along with samples of bone marrow, spleen and mesenteric lymph nodes were processed for chromosome study according to the method of Nowell, Ferry and Hungerford.[11]

In the preparations of the recovered alveolar macrophages, all metaphases were examined for the presence of the $T_6$ marker chromosomes. In the hematopoietic tissues of each subject, 25 mitoses were examined and classified as either host (CBA) or donor ($T_6$) cells.

The washed lungs of the chimeras were fixed, *in situ,* with Zenker's fixative which was slowly injected through the trachea to expand the lungs to fill the thoracic cavity. The trachea was then ligated and the thoracic contents carefully removed and immersed in Zenker's solution overnight. Representative blocks were embedded in paraffin, cut at 5 $\mu$ and stained with hematoxylin and eosin or with Masson's trichrome stain.

In addition to conventional examination, an adaptation of the point-count technique for estimation of percentage volume composition [12] was applied to the sections. In fields chosen at random, the following cell types were enumerated: 1) large mononuclear cells free in the alveolar spaces [alveolar macrophages]; 2) blood elements free in the alveolar spaces, which included erythrocytes, neutrophils and typical small lymphocytes; 3) alveolar wall cells, both the vacuolated and non-vacuolated types and the endothelium-like cells as classified by Bertalanffy [5]; 4) other fixed cells including vascular and bronchiolar cells, lymphoid tissue and histiocytes. One to two hundred cells were evaluated in each section, and the percentages of the various classes were taken to reflect the relative proportions of such cells in the lung parenchyma. The results of these analyses were compared with those obtained from similar preparations made from a group of control mice which were not subjected to the lung washing procedure.

## Results

Chromosome studies of the hematopoietic tissues in CBA mouse chimeras indicated that the tissues were almost completely repopulated with the donor ($T_6$) cells, evidence that the mice were true chimeras with respect to the blood forming elements. In 15 of the 19 chimeras, the $T_6$ markers were present in all spleen and bone marrow mitoses and in 96 to 100 per cent of the mesenteric node metaphases. In the 4 mice that deviated from this pattern, 1 or 2 dividing CBA cells were observed among the spleen and bone marrow mitoses; among the mesenteric node metaphases, 13 to 16 per cent of the cells were CBA.

As shown in Table I, of the dividing cells collected from the lungs in all mice studied, $\frac{2}{3}$ to $\frac{4}{5}$ arose in the hematopoietic system, having the $T_6$ marker chromosomes. This ratio of donor ($T_6$) to host (CBA) cells was not significantly altered by the injection of adjuvant 3 to 6 days

TABLE I

CHROMOSOME ANALYSIS OF THE ALVEOLAR CELLS RECOVERED FROM MOUSE CHIMERAS

| Treatment | Number of animals | Number of mitoses | Mean no. mitoses per animal | Number $T_6$ mitoses | Number CBA mitoses | % $T_6$ mitoses |
|---|---|---|---|---|---|---|
| No ether, no adjuvant | 5 | 12 | 2.4 | 9 | 3 | 75 |
| Adjuvant, no ether | 7 | 38 | 5.5 | 29 | 9 | 78 |
| Ether, no adjuvant | 2 | 31 | 15.0 | 22 | 9 | 71 |
| Ether + adjuvant | 5 | 71 | 14.0 | 45 | 26 | 64 |
| Totals | 19 | 152 | | 105 | 47 | |

before harvesting the cells, although this treatment did approximately double the number of dividing cells recovered. The brief period of ether anesthesia, experienced by some of the mice in connection with the colchicine injection, similarly appeared to cause an increase in the yield of alveolar macrophages. Two to 6 times as many dividing cells were recovered from the etherized chimeras than from those given colchicine without anesthesia. In addition, a slightly higher proportion of CBA mitoses was noted in the anesthetized animals as compared to those not exposed to ether. Irrespective of adjuvant or ether, however, it appeared that in all chimeras a high proportion of the dividing cells recovered from the lungs had origin in the hematopoietic system. Overall, the $T_6$ marker chromosomes were present in 72 per cent of the 152 dividing alveolar macrophages examined.

In 3 metaphases from 2 mice that had received injections of adjuvant 5 and 6 days before killing but had not been exposed to ether anesthesia, multiple chromosome abnormalities were observed. These anomalies were of the so-called unstable type, associated with radiation damage, consisting of dicentric chromosomes, acentric fragments and ring forms.

Examination of the histologic sections from the mice receiving adjuvant revealed focal areas of hypercellularity associated with large numbers of alveolar macrophages. These areas were characteristically located about the smaller branches of the pulmonary artery. They showed proliferation of the cellular elements of the alveolar wall, including both vacuolated and nonvacuolated wall cells as well as surface epithelial cells. Associated with this proliferation was a moderate infiltration of neutrophils, particularly surrounding the arterial branches, but only rarely were they seen free in the alveolar spaces. The lungs of mice exposed to ether 6 hours prior to killing, but not given injections of adjuvant, showed milder and more diffuse hypercellularity, composed of all cellular elements, and also showed a slight degree of interstitial edema in most areas. There were only a few neutrophils present in these sections. Mice that had not been exposed to either adjuvant or ether appeared normal in all respects.

In comparing those mice whose lungs had been washed with those which had not, there was no evidence that there had been any lifting up or stripping off of the surface epithelial layers. Neither was there a significant amount of edema evident.

The point-count analysis of the histologic sections indicated that in comparing the lungs that had been washed to recover alveolar macrophages with those that had not been washed, the only difference in relative cellular composition was in the numbers of free intra-alveolar mononuclear cells. In particular, the number of wall cells, which included the

surface epithelium, were essentially the same in the two groups. In those lungs that were washed, however, there were somewhat less than half the numbers of alveolar macrophages in the alveolar spaces. This difference was significant [$p < 0.001$].

## Discussion

The technique of lung washing can be assumed to provide a population of cells that are largely alveolar macrophages. Differential counts of cells collected by lung washing has indicated that better than 85 per cent of the cells collected can be classified as large mononuclears, the remainder being erythrocytes, neutrophils and bronchial epithelial cells (all of which can be assumed to be incapable of mitosis) and less than 10 per cent of cells resembling the small lymphocyte.[13] Further, the point-count data indicates that the only difference between the washed and unwashed lungs lies in the numbers of alveolar macrophages. Neither visual inspection nor point-count analysis gave any evidence of loss of surface epithelial cells as a result of the washing. Thus it would seem that the vast majority of the dividing cells examined were the large free mononuclear cells referred to as alveolar macrophages.

Chromosome analysis of dividing cells washed from the lungs of CBA mouse chimeras indicated that these cells originated from more than one source. Approximately one-third were of pulmonary origin, derived presumably from the alveolar epithelial lining or from mesenchymal wall cells. The other two-thirds had origin in the hematopoietic system, probably representing mononuclear leukocytes from the blood. The passage of cells into the alveoli from the lung capillaries was demonstrated in guinea pigs by Ungar and Wilson.[9] These investigators were able to illustrate in the alveolar walls and spaces, viable, stained peritoneal exudate cells which had been injected intravenously.

Monocytes have been the blood cells classically considered the source of the alveolar macrophages. Investigations of the potential of the small lymphocyte, however, have revealed that this cell, either under antigenic or nonspecific stimulation, can enlarge and become mitotically active.[14,15] Also, Gough, Elves and Israëls [16] have shown recently that macrophages can form *in vitro* from peripheral blood lymphocytes, both in the presence and absence of foreign stimuli. Thus, lymphocytes as well as monocytes may be blood elements from which some of the intra-alveolar cells originate.

These experiments have further indicated that both adjuvant introduced by the vascular route and ether *via* the airway were effective in increasing the numbers of dividing cells that could be recovered by lung washing. The proportions of blood- and of lung-derived cells were, how-

ever, not altered by adjuvant injection and only slightly changed by exposure to ether. The slight increase in host (CBA) cells noted in the etherized mice could have resulted from the desquamation of cells from the slightly edematous alveolar walls. It is unlikely that the point-count method would be able to pick up a change of this small magnitude.

Moore and Schoenberg [6] investigated the origin of non-dividing intra-alveolar cells collected from the lungs of adjuvant-injected rabbits. Using as their criteria the cytoplasmic ultrastructure of the cells, they reported that the maximum numbers of cells derived from the blood forming system occurred about 24 hours after adjuvant injection. Following this period the numbers of these cells declined while an increase occurred in the cells of mesenchymal and epithelial origin. The results of the present investigation are not in complete agreement with the findings of these authors. In our study, however, only dividing cells were assessed, and the response of these cells to the adjuvant was considered only to the sixth day after injection. The study by Moore and Schoenberg was continued for 4 to 6 weeks after administration of the adjuvant and perhaps, as the period after injection was lengthened, desquamation of non-dividing cells from the alveolar walls would account for the reported increase in the number of lung derived cells.

The few cells with multiple unstable chromosome abnormalities observed 5 days after adjuvant were undoubtedly CBA cells undergoing their first post-irradiation mitosis.[17] These could have been normally dormant alveolar wall cells coming into mitosis in response to the damage inflicted by adjuvant, and this activation of resting cells may indicate the availability of a repair mechanism within the alveolar wall. Such a response would be analogous to the cellular behavior occurring in the liver after chemical injury or partial hepatectomy. It is also possible that these radiation-damaged CBA cells were not of pulmonary origin but rather represented resting host lymphocytes which survived the irradiation (a few CBA cells were noted in the lymph nodes of the chimeras) and which were stimulated to proliferate in response to the adjuvant.

## Summary

The origin of alveolar macrophages was investigated in mouse chimeras in which the hematopoietic cells could be identified by marker chromosomes. By chromosome analysis it was found that in both normal lungs and in lungs exposed to an irritant, approximately two-thirds of the dividing cells which could be recovered by lung washing arose from the hematopoietic system and one-third were of pulmonary origin.

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We wish to thank Mrs. Carolyn Moore for providing technical assistance.

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[ *Illustrations follow* ]

## LEGEND FOR FIGURE

FIG. 1. A metaphase plate from a cell derived from a hematopoietic source recovered from the lungs of a CBA mouse chimera. Small $T_6$ marker chromosomes are designated by arrows. × 3,000.