THE ORIGIN OF ALVEOLAR MACROPHAGES IN MOUSE RADIATION CHIMERAS*, ‡

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Most of the known bactericidal properties of the lungs reside in the phagocytic and lytic potential of the alveolar macrophages (1, 2). Besides maintaining the sterility of the alveolar surface, alveolar macrophages also participate in the clearance of dust and debris from the nonciliated portions of the lung (3, 4). As these important roles of the alveolar macrophage were recognized, many investigators attempted to determine the origin of the cells (reviewed in Bertalanffy [5, 6]).

Several different cells have been suggested as the progenitor of the alveolar macrophage; the sources of macrophages most frequently mentioned have been hematopoietic tissues (7, 8) and intrapulmonary tissues (9, 10). Recently, Pinkett et al. (11) utilized mouse irradiation chimeras in which donor hematopoietic cells were marked by the T_6 chromosome and concluded that two-thirds of alveolar macrophages were of hematopoietic origin. Virolainen (12) applied the T_6 method to organ culture and concluded that all dividing free cells were of hematopoietic origin. Unfortunately, the use of a chromosomal marker visible only during mitosis limited these studies to dividing cells and did not permit morphologic evaluation of these cells. Brunstetter et al. (13), using reciprocal chimeras in which donor hematopoietic cells either carried or lacked an identifiable esterase marker, concluded that alveolar macrophages were primarily of bone marrow origin. However, their analytical method was qualitative and necessitated the pooling of all free cells recovered in the lung washing.

In view of these conflicting reports, it was apparent that the experimental approach to this problem must include: (*a*) a specific marker to identify the origin of individual alveolar macrophages, (*b*) the utilization of an unselected or randomly selected popu-

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lation of cells, and (*c*) a method which includes morphologic evaluation of the marked cells. These criteria are best satisfied by adapting the immunologic approach used by Balner (14) and Goodman (15) to determine the origin of peritoneal macrophages. Mouse irradiation chimeras were used in which the marker to identify the cellular origin of individual cells is a single, known antigenic specificity difference between donor bone marrow cells and recipient somatic cells. By using cytotoxic, monospecific antibody to identify donor cells, it is possible to study unselected free cells obtained by lung washing and at the same time observe the cellular morphology in the cytotoxic test to be certain that only mononuclear macrophages are studied.

Using this approach, this investigation determines the origin of alveolar macrophages in mouse irradiation chimeras at selected time intervals after irradiation and bone marrow transplantation. The origin of alveolar macrophages is also determined in chimeras after the inhalation of an iron oxide aerosol.

Materials and Methods

Chimera Production.—C57B6/AF$_1$, C57B10D2 ♁, and Ajax ♂ mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Mice were kept five to a cage and allowed free access to food and water. C57B6/AF$_1$ mice were used as recipients since these animals lack antigenic specificity 31 on the *H-2* locus. The F$_1$ generation (bred in our animal colony) of C57B10D2 ♁ × Ajax ♂ were used as donors since these mice have antigenic specificity 31. Recipients were irradiated with 900 R generated by a General Electric Maximar 250 kvp machine (General Electric Company, Schenectady, N. Y.) delivering 100 R/min. 15–20 million bone marrow cells were obtained by aspiration from the long bones of a donor animal, suspended in 0.5 ml of sterile medium 199x (Grand Island Biological Co., Grand Island, N. Y.), and injected into the tail vein of an irradiated recipient mouse. The animal then developed into a chimera in which all cells of donor (and therefore hematopoietic) origin contain the antigen while all other tissues such as the pulmonary epithelium or endothelium lack the antigen.

Preparation of Cell Suspensions.—Mice were anesthetized with pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, Ill.) given by intraperitoneal injection (50 mg/kg). The abdominal aorta was transected to exsanguinate the animal and the chest cavity was opened to allow the lungs to collapse. The trachea was cannulated with polyethylene tubing and alveolar macrophages were obtained by ten *in situ* pulmonary lavages (each composed of 1 ml of physiological saline at 37°C). A temperature of 37°C maximized the viability of the alveolar macrophages recovered. The lung washings were centrifuged at 1750 rpm in an International Clinical Centrifuge (Model CL) (International Equipment Company, Needham Heights, Mass.) and the macrophages were then resuspended in physiological saline to a concentration of approximately 3×10^6 cells/ml. Of these cells about 95% appeared to be mononuclear alveolar macrophages. Bone marrow cells were obtained by aspiration from the femur and suspended in saline at a concentration of approximately 3×10^6 cells/ml.

Cytotoxic Tests.—Mice were sacrificed for cytotoxic tests at 7, 14, 21, 28, and 35–50 days and 4, 5, 8, and 11 months after the irradiation and transplantation of bone marrow. To determine the presence or absence of the antigenic specificity, cytotoxic tests similar to that originally described by Gorer and O'Gorman (16) were used. Aliquots (0.025 ml) of cell suspension were incubated for 10 min at 37°C with 0.025-ml aliquots of monospecific anti-31 antibody obtained from Dr. Henry Winn, Massachusetts General Hospital or from Dr. George Snell, Jackson Laboratory, Bar Harbor, Maine. Three dilutions of antibody, 1:2, 1:4, and 1:8, were used for each determination. After this incubation, the cells were centrifuged at

1500 rpm in an International Centrifuge (Model UV) (International Equipment Co.) and the supernatant discarded. Then 0.025 ml of absorbed rabbit complement was added to the cells and the mixture incubated for 30 min at 37°C. The cells were studied for viability by the trypan blue dye exclusion method in which cells containing the antigen are damaged by the antigen-antibody complement reaction, and thus become permeable to trypan blue. Cells lacking the antigen exclude trypan blue. Each test of a cell type from each animal included a medium only, an antibody only, and a complement only control as well as the three test dilutions of antibody and complement. In each control test and at each antibody dilution, a minimum of 200 cells was counted. Cells obviously not alveolar macrophages such as ciliated cells were excluded from the determination. Data were considered acceptable only when the three controls yielded per cent kills (positively stained cells) which were less than 20%. The antibody was tested at all three dilutions in donor and recipient animals. Antibody was considered effective if more than 80% of the donor cells were killed and less than 20% of the recipient cells were killed.

We found that the unabsorbed rabbit serum was not suitable as a complement source since it was highly toxic to alveolar macrophages. We adapted the technique described by Boyse et al. (17) in order to make nontoxic, yet effective, complement. We found it necessary to decrease the ratio of rabbit serum to mouse tissue from 7:1 to 3:1. We were most successful when we used serum obtained from COBS rabbits obtained from the Charles River Breeding Laboratories, Wilmington, Mass.

Cell Counts.—The white blood cell count and the total number of alveolar macrophages harvested from the lungs were measured on every mouse in which the cytotoxic tests had been performed. Samples of venous blood were diluted with 2% acetic acid and counted in an AO Spencer Bright-Line hemacytometer (American Optical Corp., Scientific Instrumental Division, Buffalo, N. Y.). The pooled lung washings were thoroughly mixed and counted in the same hemacytometer. The concentration of alveolar macrophages was multiplied by the volume of lavage fluid recovered and the results expressed in terms of cells per gram of body weight.

Morphology.—Additional chimeras were prepared and sacrificed at the same intervals as those used for the cytotoxic tests. After the lung lavage to harvest the alveolar macrophages, the cell suspension was centrifuged and the resulting pellet of cells was fixed using Karnovsky's formaldehyde-glutaraldehyde fixative, dehydrated, embedded in Epon-Araldite, sectioned at 1 μ, and stained with toluidine blue. 100 cells were counted from randomly selected fields and scored by size and by the presence or absence of vacuoles and granules.

Aerosol Exposure.—Iron oxide particles were continuously generated by the combustion of iron pentacarbonyl in a 680°C furnace. The aerosol was diluted with air and entered a 50 liter Lucite chamber where mice, individually housed in a metal cage, breathed the aerosol spontaneously for 2 hr. The average mass concentration of the aerosol in the exposure chamber was 172 $\pm$ 3 (SE) μg/liter. Samples were taken by an electrostatic precipitator and analyzed by electron microscopy; the particles were agglomerates of various shapes; the longest dimension of 95% of the particles was less than 1.0 μ.

RESULTS

Replacement of Macrophages by Donor Hematopoietic Cells.—Mouse irradiation chimeras were sacrificed for analysis at 7, 14, 21, 28, and 35–50 days and 4, 5, 8, and 11 months after lethal irradiation and bone marrow replacement. The relationship between per cent of lung cells and per cent of bone marrow cells of donor origin *versus* time is shown in Fig. 1. Each point represents the results of studying eight animals; only data meeting the control criteria described in the Materials and Methods section were used. The ranges shown are

the standard error of the mean. In donor control animals, mean kill of marrow cells was 86% and of lung macrophages 84%. In recipient controls, the mean kill of marrow cells was 10% and of lung macrophages 14%. At 7 days after irradiation, 60% of the marrow has been replaced by donor cells while 36% of the lung macrophages are of donor origin. At 14 days, marrow replacement has increased to 72% and lung macrophages of donor origin have increased to 57%. At 21, 28, and 35–50 days, the per cent kill of marrow cells is not significantly different from the per cent kill of lung macrophages. Also, per cent kill at these periods is not significantly different from the per cent kill in the donor controls, indicating essentially complete replacement of recipient hematopoietic tissue by donor bone marrow. Three chimeras studied at 4 and 5 months after irradiation had a per cent kill in lung cells and a per cent kill in marrow cells which

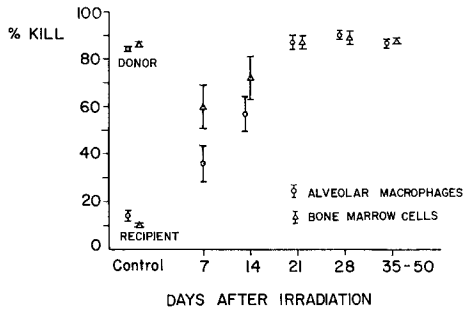


FIG. 1. Per cent kill in alveolar macrophages and bone marrow cells as a function of time after irradiation and transplantation. The means ± 1 standard error are displayed.

were not significantly different and these were also not significantly different from donor controls. Four chimeras were studied at 8 and 10 months. Two of these did not maintain complete chimerism. However, the per cent kill of lung cells in all these animals was still not significantly different from the per cent kill of marrow cells, regardless of the per cent chimerism.

Since these animals received a hematopoietically lethal dose of irradiation, it was important to monitor hematopoietic parameters as well as to determine pulmonary macrophage number and morphology. Fig. 2 illustrates the response of the peripheral white blood cell count after 900 R in mice which received and did not receive bone marrow transplantation. In chimeras, the white count initially drops to less than 300 cells/mm^3 at 3 days postirradiation and transplantation; it begins to recover by day 7, by day 21 it approaches the normal range, and by day 28 and thereafter it is not significantly different from that of control mice. Animals receiving 900 R and not injected with bone marrow all die within 14 days with white counts persisting below 300. In chimeras, the number of alveolar macrophages decreases slightly at 7 and 14 days (see Fig. 3), but returns to control levels by 21 days. In mice receiving irradiation only, the

number of alveolar macrophages does not differ from control until day 7 when it becomes significantly lower and remains low until death.

Morphology.—The appearance of the cells recovered in the lung washings was examined in 1-μ plastic sections stained with toluidine blue. At all times, although the cells varied both in staining characteristics and size, more than 90% of the cells were mononuclear with abundant cytoplasm and a variety of lysosomal granules and vacuoles. Thus they appeared to be alveolar macrophages.

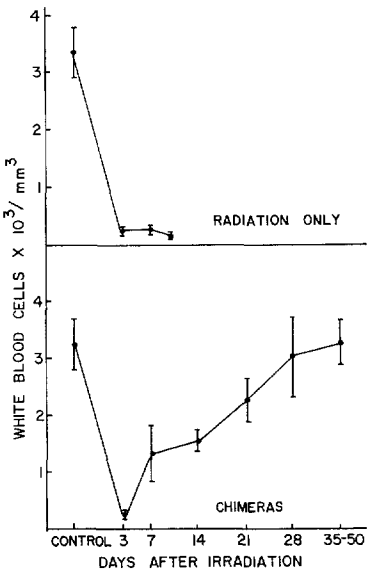


FIG. 2. Peripheral white blood cell counts as a function of time after irradiation. The means ± 1 standard error are displayed.

Over 80 photographs of the cells were taken at various times after the irradiation and bone marrow transplantation. Systematic examination of these photographs revealed that there were some transient changes in alveolar macrophage morphology, but that the cells from chimeras 28 days post-irradiation or older resembled those from normal mice. At 7 days after irradiation, macrophages recovered from lung washings were also similar to controls. At 14 and 21 days after irradiation, although the cells were still almost all mononuclear, they varied strikingly in size and vacuolation. At these times there were prominent transient increases in vacuolation and size. In normal mice, approximately 20% of the alveolar macrophages had more than two clear vacuoles. In 14- and 21-day chimeras, 36.3 and 48.2% of the cells were vacuolated. Similarly in normal mice, about 2% of the macrophages were larger than 20 μ. However, in 14- and 21-day chimeras, 6.0 and 16.4% of the macrophages

were larger than 20 μ. Thus the most prominent transient changes observed were increase in vacuolation and increase in size.

Figs. 4 *a* and *b* are typical photographs of macrophages recovered from 28-day chimeras; now the transient changes described are much less apparent. By 35 days (see Figs. 4 *c* and *d*), the morphology was almost the same as that seen in control animals. Fig. 4 *a* contains one very large, heavily vacuolated cell which was characteristic of the 14- and 21-day chimeras.

Also prominent in most cells are abundant dark-staining granules, most of

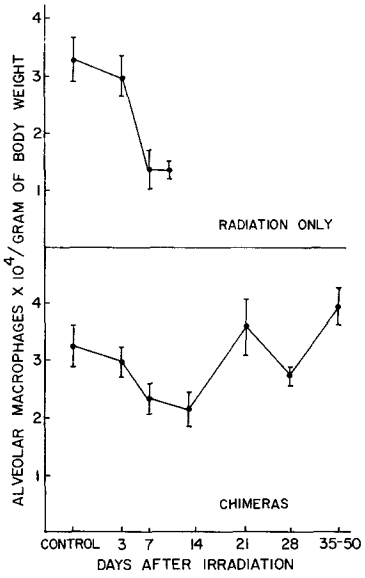
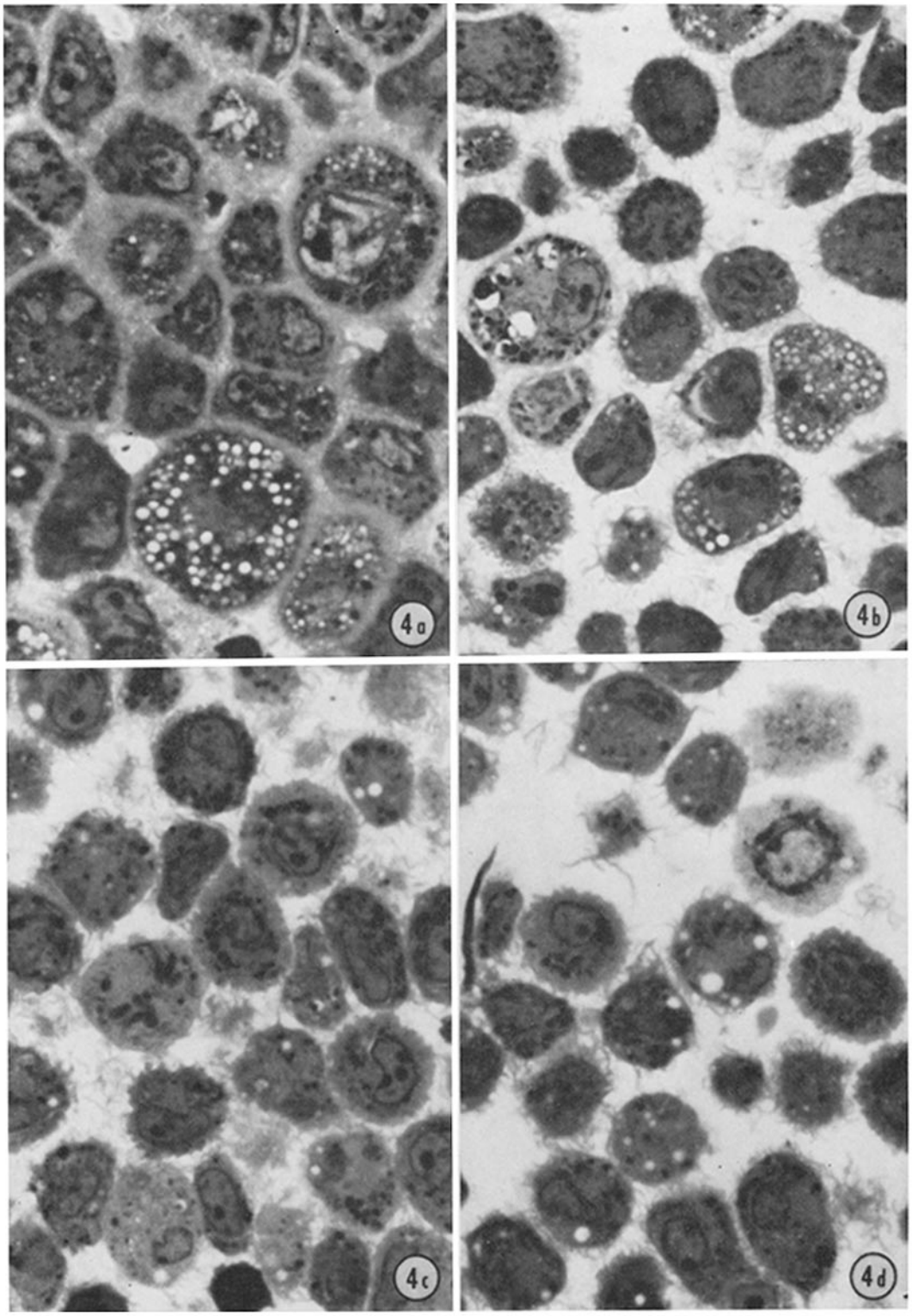


FIG. 3. Alveolar macrophage counts as a function of time after irradiation. The means ± 1 standard error are displayed.

which are probably lysosomes. In Figs. 4 *c* and *d* (35 days) one can see typical alveolar mcrophages which are now more uniform in size. They are also somewhat smaller than the 21- or 28-day cells and thus probably represent a younger and uniformly aged population of macrophages. In the center of Fig. 4 *c* can be seen a cell which appears to be an alveolar macrophage in mitosis.

None of the animals in this study had evidence of active rejection of the bone marrow or evidence of graft-*versus*-host reaction. Similarly, the lungs of all the animals studied were examined grossly, and no evidence of disease was found in any animal included in this study. Representative animals were chosen at random for histologic study using paraffin sections. No microscopic evidence of pulmonary parenchymal disease was found in the animals studied.

Therefore, by 28 days after irradiation, chimerism was well established in the animals, the peripheral white blood count had returned to normal, and


4a
4b
4c
4d

lung macrophages had reached control levels both numerically and morphologically. At this time the per cent of lung macrophages of donor origin was the same as the per cent of marrow cells of donor origin. Based on these facts, it can be concluded that the alveolar macrophages in mouse irradiation chimeras were entirely of hematopoietic origin.

Aerosol Exposures.—Eight chimeras were exposed to an iron oxide aerosol of 172 μg/liter for 3 hr. 13 matched chimeras were selected as controls (not exposed). The animals were sacrificed 24 or 48 hr after the exposure. As is shown in Fig. 5, there was a significant increase in the number of alveolar

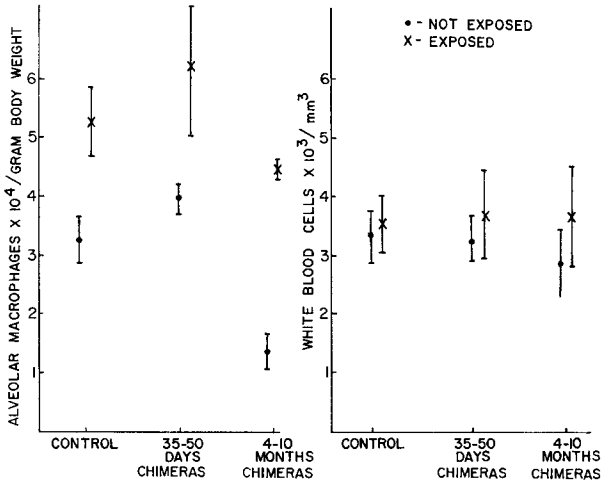


FIG. 5. Changes in alveolar macrophage counts and white blood cell counts after the inhalation of iron oxide particles. The animals were sacrificed 24 and 48 hr after the exposure. The means ± SE are displayed.

macrophages harvested by pulmonary lavage in the mice that breathed the aerosol. The percentage increase was greatest in the 4–10-month chimeras. Although the mean white blood cell concentration increased slightly in each of the groups, there were no significant differences from control values.

Cytotoxic tests were performed on lung macrophages and bone marrow cells from exposed and unexposed chimeras in order to identify the origin of the macrophages. On the basis of the data in Table I, we conclude that the pool of alveolar macrophages was increased in exposed animals by cells of hematopoietic origin. This is apparent since no significant difference is seen in per cent

FIG. 4. (*a* and *b*): Alveolar macrophages recovered from 10 pooled lung washes of a 28 day chimera. Some are large and vacuolated. × 1200. (*c* and *d*): Alveolar macrophages recovered from a 35 day chimera. Cells are indistinguishable from control macrophages. Note mitotic figure in 4 *c*. × 1200.

of marrow cells of donor origin as compared with the per cent of pulmonary macrophages of donor origin in the exposed animals. The lower percentage of cells of donor origin seen in older chimeras, both exposed and not exposed, is due to the loss of chimerism in some of these animals. This is reflected in the increased standard error. Donor control animals are not significantly different from 35-day chimeras, exposed or unexposed.

TABLE I

Origin of Lung Macrophages after Aerosol Exposure

Animal group	Number	Per cent kill	
		Lung	Marrow
35-Day chimeras (not exposed)	6	86.4 ± 1.9	87.6 ± 1.3
35-Day chimeras (exposed)	4	81.4 ± 0.7	82.4 ± 0.9
5–11-Month chimeras (not exposed)	7	74.1 ± 10.4	70.4 ± 10.4
5–11-Month chimeras (exposed)	4	64.4 ± 17.2	63.9 ± 17.0
Donor controls (not exposed)	10	84.1 ± 1.5	86.1 ± 1.0

TABLE II

Per Cent Kill in Exposed Animals

Animal No.	After bone marrow injection	Lung	Marrow	Lung-Marrow
186	35 days	83.1	80.9	2.2
191	35 days	81.8	81.0	0.8
284	35 days	81.1	84.2	−3.1
205	35 days	79.4	83.5	−4.1
			Mean ± SE	−1.05 ± 1.51
189	10 months	13.8	13.8	0.0
198	11 months	71.7	71.2	0.5
196	5 months	85.4	84.0	1.4
197	6 months	86.7	86.7	0.0
			Mean ± SE	0.48 ± 0.33

The idea that alveolar macrophages have the same per cent kill as bone marrow and thus are of donor and hence hematopoietic origin is further supported by the individual data. In Table II, the results of individual exposed animals is tabulated. In spite of varying degrees of chimerism in the older animals, the lung and marrow cells always have the same per cent kill.

DISCUSSION

Since the beginning of this century, the attention of many investigators has focused on the origin of alveolar macrophages (reviewed in Bertalanffy, 5 and 6). Such diverse origins as the pulmonary capillary endothelium (18), the bronchial epithelium (19), and the pulmonary surface epithelium (type I pneumonocyte) (20) have all been hypothesized. Electron microscopy and other modern tech-

niques have not lent support to these possibilities; instead most authors have identified either the hematopoietic system or the great alveolar cell (type II pneumonocyte) as the primary sources of alveolar macrophages.

On the basis of morphological studies and on the uptake of dyes, more than a dozen investigators before 1940 suggested that the great alveolar cell or septal cell was the primary source of macrophages (see reviews 4–6). Recently Karrer (21), Nagaishi et al. (9), and Divertie and Brown (22) came to the same conclusion. In 1969, labeling experiments were interpreted by Evans and Bils (10) as indicating that the great alveolar cell functions as a stem cell, dividing to produce one cell that remains *in situ* and another that leaves and becomes an alveolar macrophage.

The data presented in this report do not support the conclusion that pulmonary tissues, such as the great alveolar cell, are the source of the alveolar macrophage. Morphologic studies using light or electron microscopy can describe anatomic similarities and differences between cell types, but cannot determine with certainty the origin of similar cells. Even the distinctive physiological and biochemical properties of the alveolar macrophages which are sometimes shared by other pulmonary cells may represent adaptation to a common environment rather than specification on the basis of common origin.

Clearly, the definitive method of study must involve a reliable and identifiable marker which will not be altered by the environment of the cell. Pinkett et al. (11) used mouse chimeras in which the hematopoietic cells contained a T_6 marker. However, to recognize this marker the cells must be undergoing cell division and thus only a highly selected population was studied (only the chromosomal spreads of 152 dividing cells in 19 mice or a mean of 8 cells per mouse were examined); it is also impossible to examine the morphology of the dividing cells in detail and to identify positively the scored cell as a macrophage. Furthermore, since only 6 hr elapsed between the colchicine injections (used to arrest the dividing cells in metaphase) and the lung washings, it is possible that macrophage stem cells present within the interstitium of pulmonary tissue were arrested in metaphase but were not released on to the alveolar surface during this period. Therefore, their conclusion that "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" is suspect. Virolainen (12) also used the T_6 chromosome system and studied the karyotype of dividing cells explanted to organ culture from the lungs of chimeras adding an additional selective factor, the ability of the cells to multiply in organ culture. He concluded that the origin of alveolar macrophages is entirely hematopoietic.

The availability of inbred mouse strains with known *H-2* histocompatibility differences at single specificities has provided a unique situation in which cells with an antigenic marker rather than a chromosomal marker can be transplanted. By proper selection of donor and recipient strains, graft-*versus*-host reactions and active rejection of the graft can be avoided. Using cytotoxic

tests with specific isoantisera, the origin of a much larger portion of the cell population can be determined with no selective factors involved. Also, morphologic examination of the marked cells can be done as the cells are evaluated for presence or absence of the marker antigen. Ciliated epithelial cells, polymorphonuclear leukocytes, and other contaminating cells can be excluded from the determination. This immunologic approach was applied to mouse irradiation chimeras by Balner (14) and Goodman (15) to determine the origin of peritoneal macrophages; we are not aware of its use in determining alveolar macrophage origin. Balner (14) found that all free peritoneal macrophages were of hematopoietic origin 6 wk after the irradiation transplantation. Goodman (15) also showed that the macrophages in both stimulated and unstimulated peritoneal cavities were derived from bone marrow.

We determined the per cent cells of donor origin (per cent kill) in the bone marrow and alveolar macrophages of ten donor controls, ten recipient control mice, and eight chimeras at each of the following time intervals: 7, 14, 21, 28, and 35–50 days postirradiation and transplantation of bone marrow. Chimeras were also studied at 4, 5, 8, and 11 months. In each animal at these times, the white blood cell count was determined as well as the total number of macrophages washed from the lung. Morphology of the alveolar macrophages was observed in the cytotoxic test and in plastic sections. Once chimerism was well established (after 21 days) we always found that the per cent cells of donor was identical in bone marrow and in alveolar macrophages. At this time macrophage numbers and morphology were normal. Thus we concluded that the alveolar macrophages, like peritoneal macrophages, were of entirely hematopoietic origin in mouse irradiation chimeras. Other studies have also concluded that the blood and bone marrow may be the primary source of alveolar macrophages.

Mecknikov (23) claimed that dust cells were derived from leukocytes of the macrophage type which had migrated very early in life from the bloodstream into the alveolar walls. Foot (24) claimed that all lung free cells were migratory blood cells which penetrated into the alveolus in response to a stimulus supplied by the presence of foreign matter. Ungar and Wilson (7) reported that the viable monocytes labeled by the ingestion of carbon, when injected into the circulation of guinea pigs, were concentrated in the lungs. Nagaishi et al. (9), using an electron microscope, concluded that some of the free cells are derived from monocytes.

More recently, Bowden et al. (25) and van Furth (8) using tritiated thymidine labeling, concluded that all alveolar macrophages are of hematopoietic origin. In addition, Brunstetter et al. (13) utilized irradiation chimeras and an esterase marker to study the source of alveolar macrophages. Their qualitative electrophoretic analysis of esterase in these animals also suggests a primary hematopoietic origin.

Several studies have given evidence that the blood monocyte acts as the immediate precursor of macrophages (7, 26, 27). Bowden et al. (25) have postulated the existence of a stem cell pool in the marrow which is transported to the lung by the blood, divides and matures in the lung interstitium, and is then released from the interstitium onto the alveolar surface as a macrophage. They had demonstrated in their studies that the immediate precursor of the alveolar macrophage is a cell in the interstitium. Although

our studies reported here show that the ultimate source of alveolar macrophages is the bone marrow, it does not preclude the possibility that there may be a division: maturation compartment of hematopoietically derived cells in the alveolar wall. It is significant that Fig. 4 *c* contains a mitotic figure. Clearly some alveolar macrophages are capable of cell division. In addition, Chang (28) cultivated mouse peritoneal macrophages for over 200 days and showed they could divide. Fig. 1 demonstrated that marrow replacement with donor cells precedes replacement of pulmonary macrophages with cells of donor origin. This lag could be the period postulated by Bowden et al. when maturation and division in the interstitium is taking place. Similarly, Bowden et al. demonstrated an increase in macrophage number 21 days after irradiation. This was associated with increased interstitial mitosis at 14 days and interpreted to represent the presence of a synchronized population of cells on the alveolar surface which had previously divided in the interstitium. In Fig. 3 of this report, an increase in macrophage number at 21 days is also seen. Also, at this time many macrophages were large and vacuolated suggesting these were older cells. It is possible that the macrophage population in these chimeras were synchronized so that older cells were present at 21 days, and complete replacement time was about 28 days.

One limitation of techniques involving the use of radiation to produce chimeras should be mentioned. It is possible that the ability of pulmonary tissues to serve as a source of alveolar macrophages has been compromised by the 900 R radiation dose. Sections of the pulmonary parenchyma in 28- and 35-day and 3-month chimeras reveal, however, relatively normal cells in the alveolar wall which appear to be type II pneumocytes. In addition, the survival of many other nonhematopoietically derived cell renewal systems suggests that the results obtained in these chimeras may be representative of normal animals. Ideally, chimerism would be induced in the newborn with little or no irradiation, but consistent and complete chimerism is difficult to achieve without radiation.

The exposure of animals to increased numbers of particles by inhalation or intratracheal injection has been shown to alter the number of macrophages obtained by pulmonary lavage (29, 30). No previous studies have attempted to study the origin of macrophages in animals stimulated by particle inhalation. In spite of an increased pool size of macrophages harvested by lavage, we still found that macrophage origin was entirely hematopoietic in chimeras.

At a conference on Mononuclear Phagocytes (Leiden, Netherlands, September, 1969), a proposal for a new classification of mononuclear phagocytic cells was developed (31). The consensus was that there was adequate information to consider all highly phagocytic mononuclear cells and their precursors in a single system. Mononuclear phagocytes originate from a common progenitor cell in the bone marrow (promonocytes), they are transported by the blood (monocytes), and finally enter a variety of tissues and become macrophages. The experiments reported here are consistent with this proposed scheme.

SUMMARY

This investigation attempted to determine whether the primary source of alveolar macrophages is pulmonary or hematopoietic. We have utilized an

antigenic marker to identify cells of hematopoietic origin. Mouse chimeras were produced by irradiating C57B6/AF_1 mice (900 R) and then injecting them intravenously with B10D2/AF_1 bone marrow. The donor animal has an antigenic specificity on the *H-2* locus, not shared by the recipient. Alveolar macrophages were obtained by repeated lung washings with physiologic saline at 37°C. Cytotoxic tests were done on bone marrow and alveolar macrophages using anti-31 mouse antibody, absorbed rabbit serum as complement, and trypan blue exclusion as a test for viability. Animals were studied at 7, 14, 21, 28, and 35–50 days and 4, 5, 8, and 11 months after irradiation and bone marrow replacement. By 21 days after irradiation, 90% of the animals had greater than 80% replacement of marrow with donor tissue; and white blood cell and alveolar macrophage counts approached normal. At this time and at later intervals the per cent of donor cells in the lung free cell population was not significantly different from the per cent of donor cells in the bone marrow. Similarly, after aerosol particulate exposure, the percentage of marrow cells and alveolar macrophages of donor origin were not significantly different. This immunologic approach suggests that alveolar macrophages in radiation chimeras are entirely of hematopoietic origin.

Excellent technical assistance was provided by Miss Lucinda Mowitt. Dr. George Snell at the Jackson Laboratory provided us with anti-31 antibody. Dr. Henry Winn at the Massachusetts General Hospital also supplied us with anti-31 antibody and with invaluable advice regarding immunological techniques. Dr. Sergei Sorokin helped us photograph the alveolar macrophages. Dr. John Little permitted us to use his irradiation facilities.

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