HEMATOPOIETIC ORIGIN OF MACROPHAGES AS STUDIED
BY CHROMOSOME MARKERS IN MICE*

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Bone marrow has been implicated as the main source of precursor cells for macrophages in inflammatory reactions and in peritoneal exudate. Radioautographic studies have shown that peritoneal cells (1) and mononuclear cells in foci of acute nonbacterial inflammation (2–4) and in delayed hypersensitivity reactions (5, 6) are derived from rapidly and continuously dividing precursors, and that these cells can be obtained from transfused bone marrow (7). Using the cytotoxic activity of specific isoantibodies in mouse radiation chimeras free peritoneal cells were found to be of donor type (8, 9). This technique could not be applied for typing of tissue macrophages (8). The origin of alveolar macrophages has not been established unequivocally. The cells lining the alveoli (10, 11), pulmonary mesenchymal cells (11–13), extrapulmonary reticuloendothelial cells (14), and blood monocytes (11, 15) are involved as possible sources for alveolar macrophages. Pinkett et al. (16) studied the origin of alveolar macrophages using mouse radiation chimeras in which the hematopoietic donor cells were identified by marker chromosomes. This study suggested that two-thirds of the dividing alveolar cells arise from the hematopoietic system and one-third are of pulmonary origin. Using marker chromosome technique, Howard et al. found that during intense reticuloendothelial proliferation in the liver in graft versus host reaction (17) and after stimulation with Corynebacterium parvum (18), dividing cells in liver macrophage preparations are derived from thoracic duct cells and from bone marrow. However, it has been shown that to any detectable extent exudate macrophages are not derived from thoracic duct lymphocytes (3, 7).

The application of an organ culture system in which pure populations of macrophages can be obtained in vitro (19) and the finding that macrophages can be induced to proliferate in vitro by factor(s) in conditioned medium (20) make it possible to apply the chromosome marker technique for in vitro studies concerning the origin of macrophages in different tissues.

Studies in which a chimeric state is established in lethally irradiated mice by hematopoietic cells with marker chromosomes demonstrate that macrophages

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from different sources are all derived from hematopoietic tissues. Experiments analyzing the source of macrophages in radiation chimeras given injections of cells from various tissues of two genetically different donors suggest that only hematopoietic tissues contain precursor cells for macrophages.

Material and Methods

Mice.—The mice used for the experiments were CBA mice (Jackson Laboratories, Bar Harbor, Me.), the histocompatible homozygous CBA.TyT6 strain (obtained initially from Professor B. Ephrussi) which carries two easily recognizable minute chromosomes, and F1 hybrids between these strains. All recipient mice were 2- to 3-month-old males. Donor cells were obtained from adult males except for thymus and liver cells which were from 1-day-old mice.

Production of Chimeric State.—Recipient mice were either X-irradiated (220 kv, 0.25 mm Cu, 1.0 mm Al), or γ-irradiated using a dual-source 137Cs irradiator (Isotopes Inc., Westwood, N.J., GR-7) at a dose rate of 180 rads per minute.

Cell suspensions were made from femur marrows by aspirating repeatedly in a plastic syringe in phosphate-buffered saline (PBS). Other donor tissues were first minced with scissors and then aspirated. The number of nucleated cells was counted in a hemocytometer after dilution with 2% acetic acid. Various amounts and combinations of cell suspensions were injected intravenously into irradiated recipients within 8 hr after irradiation. Control mice irradiated with 900 R but not injected died within 14 days. No endogenous spleen colonies were found in these mice.

Macrophage Cultures.—Mice were killed at various intervals after irradiation and injection of cells. 3 days before killing, the mice were injected intraperitoneally with 2 ml of 1% hydrolyzed starch suspension. Peritoneal cells were collected and cultured on cover slips as described previously (20).

Alveolar macrophages were collected by Pinkett’s method (16), except that no irritation was used. Cells from lung washings were plated in 35-mm plastic Petri dishes which were provided with cover slips. These were cultured in the same way as were the peritoneal macrophages.

The grid organ culture method of Jensen et al. (19) was used to obtain cultures from tissue macrophages. Small pieces of bone marrow, spleen, cervical lymph nodes, thymus, lung, and liver were placed on the supporting grids, and cells which dropped from the explants were cultured on cover slips. Cover slip cultures were removed from the grid culture system at various time intervals after initiation of the cultures and grown in separate culture dishes. The medium was double strength Eagle’s basal medium with 10% fetal bovine serum, and the gas phase 5% CO2 in air.

To induce macrophages to divide, cover slip cultures were grown in medium which was supplemented with 25–50% of used medium from L cell cultures (20).

Chromosomal Analysis.—Before harvesting cover slip cultures for chromosome preparations, the living cultures were observed through an inverted microscope with phase optic. Cultures with fibroblastic growth were discarded. Colcemid, 0.5 µg/ml, was added to the cultures. 4-6 hr later cover slips were washed in PBS, treated with hypotonic solution (1 part Hanks’s solution, 4 parts water) for 15 min, fixed in methanol-acetic acid, air-dried or ignited over flame, and stained with Giemsa. Duplicate cultures were harvested without hypotonic treatment for morphologic observations.

For chromosome analysis of the whole bone marrow of the chimeric mice, bone marrow from one femur was suspended in 2 ml of medium and incubated for 5 hr in the presence of 0.5 µg/ml Colcemid. Chromosome preparations were made according to the Moorhead method (21).
RESULTS

Macrophage Cultures.—In the present study the name macrophage is used for a large phagocytic cell in vitro which profoundly differs from fibroblasts, epithelial cells, and leukocytes. Morphologically, these cells are rounded or have a few pseudopods, the cytoplasm is fairly large, and the nucleus is small with indistinct nucleoli (Fig. 1). These cells have a high acid phosphatase content. They cannot be detached from glass surface by trypsin treatment, and very few cells divide when cultured in regular tissue culture media. When exposed to "macrophage growth factor" (MGF), which is present in "conditioned" medium from homologous fibroblastic cultures, these cells stretch and send out more pseudopods within a few hours (Fig. 2). During the first days of incubation in conditioned medium a large part of the cells (up to 98% of peritoneal macrophages) enter the cell division cycle (20).

Peritoneal exudate and lung washings contained, initially, many leukocytes in addition to macrophages, but change of medium after 2 hr of incubation removed almost all cells other than macrophages. In peritoneal and alveolar cell cultures the number of fibroblastic cells was less than 1/5000 the number of macrophages.

Organ cultures of bone marrow produced large numbers of macrophage during the first days in vitro. Cover slips underlying the explants were changed after $\frac{1}{2}$, 1, and 2 days, and cultured separately. Spleen, lymph node, thymus and lung cultures started to produce more macrophages after only about 1 wk in vitro. The cover slips were usually changed at weekly intervals. However, spleen from a 2-wk-old chimera which was undergoing active hematopoiesis behaved as bone marrow during the first days in vitro. Liver cultures produced only few macrophages; chromosomal analysis of these cells was not successful.

Some cover slip cultures obtained from organ explants other than bone marrow were "contaminated" by fibroblasts at early stages of cultivation, but after subsequent cover slip changes pure macrophage cultures could usually be obtained. As judged by observations of the living cultures and of duplicate cultures which were stained with Giemsa stain, the cultures used for chromosome preparations were essentially free of cells other than macrophages.

Cover slip cultures were usually harvested for chromosome preparations after 3-5 days' incubation in conditioned medium at the time when the first mitotic wave occurred (Figs. 3 and 4). As found previously (20), the incubation time needed for the induction of cell proliferation was dependent on the cell number and/or density in the cover slip cultures.

Lethally Irradiated CBA Mice Given Injections of Hematopoietic Cells from $T_{s}T_{a}$ Donors.—Chromosomal analysis of mitoses in whole bone marrow incubations revealed that injections of $T_{s}T_{a}$ bone marrow or spleen cells into lethally (900 R) irradiated mice lead to the appearance of dividing cells in bone marrow which are exclusively of the donor type. 25 or more bone marrow metaphases were analyzed in each animal, and all had the marker chromosomes.
Hematopoietic Origin of Macrophages

Results from the chromosome analysis of macrophage cultures from various sources are given in Table I. Without any exceptions, all dividing macrophages had the marker chromosomes. They were found both in “young” chimera (2 wk after the injection of bone marrow cells) and in a chimera established 1 yr earlier.

### Table I

**Incidence of TnT6 Marker in Bone Marrow and in Cultured Macrophages of Lethally Irradiated* CBA Mice Injected with Cells from TnT6 Donors**

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>TnT6 cells injected</th>
<th>Age of chimera (days)</th>
<th>Incidence of marker in bone marrow</th>
<th>Incidence of marker in cultured macrophages from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peritoneal fluid</td>
<td>Lung washing</td>
</tr>
<tr>
<td>10</td>
<td>15 × 10⁶ bone marrow cells</td>
<td>14 30/30</td>
<td>30/30</td>
<td>30/30</td>
</tr>
<tr>
<td>3</td>
<td>25 × 10⁶ “” “” “” “”</td>
<td>25 30/30</td>
<td>30/30</td>
<td>30/30</td>
</tr>
<tr>
<td>7</td>
<td>15 × 10⁶ “” “” “” “”</td>
<td>26 60/60</td>
<td>60/60</td>
<td>60/60</td>
</tr>
<tr>
<td>9</td>
<td>10 × 10⁶ “” “” “” “”</td>
<td>59 60/60</td>
<td>60/60</td>
<td>60/60</td>
</tr>
<tr>
<td>168</td>
<td>10 × 10⁶ “” “” “” “”</td>
<td>368 100/100</td>
<td>100/100</td>
<td>100/100</td>
</tr>
</tbody>
</table>

* 900 R, X-ray (mice 1-10) or ³²⁵Cs (mice 147-171).

### Table II

**Incidence of TnT6 Marker in Bone Marrow and in Cultured Macrophages of Sublethally Irradiated* CBA Mice Injected with Bone Marrow Cells from TnT6 Donors**

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>No. of TnT6 bone marrow cells injected</th>
<th>Age of chimera (days)</th>
<th>Incidence of marker in bone marrow</th>
<th>Incidence of marker in cultured macrophages from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peritoneal fluid</td>
<td>Lung washing</td>
</tr>
<tr>
<td>154</td>
<td>10⁶</td>
<td>35 42/45</td>
<td>42/45</td>
<td>6/7</td>
</tr>
<tr>
<td>159</td>
<td>10⁶</td>
<td>42 96/100</td>
<td>96/100</td>
<td>23/24</td>
</tr>
<tr>
<td>170</td>
<td>10⁶</td>
<td>63 97/100</td>
<td>97/100</td>
<td>67/67</td>
</tr>
<tr>
<td>189</td>
<td>3 × 10⁵</td>
<td>38 19/20</td>
<td>19/20</td>
<td>28/30</td>
</tr>
</tbody>
</table>

* 500 R, ³²⁵Cs source.

Sublethally Irradiated CBA Mice Given Injections of Bone Marrow Cells from TnT6 Donors.—Following sublethal irradiation (500 R) and injection of 10⁶ or 3 × 10⁵ TnT6 bone marrow cells, the host marrow was only partially repopulated by donor cells. The percentage of donor type metaphases was on the average 96% (Table II).

As shown in Table II, the incidence of marker presence in dividing macrophages followed very closely that found in bone marrow cells.
Lethally Irradiated Mice Given Injections of Bone Marrow Cells and Cells from Various Tissues with Different Genotypes.—In these experiments the recipient mice were injected with $10^6$ $T_eT_e$ bone marrow cells together with various amounts of $T_e$ cells obtained from tissues of $F_1$ hybrid mice (when recipients were CBA) or of CBA cells (when recipients were $F_1$ hybrids). No host type metaphases were found in any of the chromosome preparations.

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Host genotype</th>
<th>Cells injected in addition to $10^6$ $T_eT_e$ bone marrow cells</th>
<th>Age of chimeric state</th>
<th>Incidence of $T_eT_e$ cells in bone marrow</th>
<th>Incidence of $T_eT_e$ marker in cultured macrophages from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Peritoneal fluid</td>
</tr>
<tr>
<td>148</td>
<td>CBA</td>
<td>$20 \times 10^6$ $T_e$ spleen cells</td>
<td>59</td>
<td>28/31</td>
<td>49/51</td>
</tr>
<tr>
<td>177</td>
<td>$T_e$</td>
<td>$2 \times 10^6$ CBA newborn liver cells</td>
<td>34</td>
<td>18/23</td>
<td>71/100</td>
</tr>
<tr>
<td>190</td>
<td>$T_e$</td>
<td>“ “</td>
<td>52</td>
<td>14/50</td>
<td>18/47</td>
</tr>
<tr>
<td>150</td>
<td>CBA</td>
<td>$15 \times 10^6$ $T_e$ lymph node cells</td>
<td>55</td>
<td>9/9</td>
<td>5/5</td>
</tr>
<tr>
<td>160</td>
<td>CBA</td>
<td>“ “</td>
<td>100</td>
<td>49/49</td>
<td>28/28</td>
</tr>
<tr>
<td>176</td>
<td>$T_e$</td>
<td>$5 \times 10^6$ CBA newborn thymus cells</td>
<td>34</td>
<td>50/50</td>
<td>50/50</td>
</tr>
<tr>
<td>134</td>
<td>CBA</td>
<td>$13 \times 10^6$ $T_e$ peritoneal cells</td>
<td>30</td>
<td>50/50</td>
<td>50/50</td>
</tr>
<tr>
<td>149</td>
<td>CBA</td>
<td>“ “</td>
<td>55</td>
<td>15/15</td>
<td>14/14</td>
</tr>
</tbody>
</table>

* 900 Rd $^{32}P$Cs source.
‡ No host type metaphases were found.

When cells from other hematopoietic tissues, i.e. newborn liver and spleen, were injected together with bone marrow cells, the recipient marrow was repopulated by cells from both tissues used. Bone marrow cells were found to be about 400 times more effective than spleen cells in repopulating the recipient marrow. In a chimera which was established 1 month before chromosomal analysis, adult bone marrow cells were five times more effective than newborn liver cells in repopulating the marrow. In a 2-month-old chimera the ratio of genotypes from bone marrow and liver origin was approximately the same as...
the ratio of the number of injected cells. Again, the ratio of genotypes in dividing macrophages was approximately the same as in bone marrow cells (Table III).

Injections of newborn thymus cells, lymph node cells, or of peritoneal exudate cells, together with bone marrow cells, was not found to contribute dividing cells either to the whole bone marrow or to the macrophage population (Table III).

**DISCUSSION**

Chromosome marker techniques, especially the T₄ markers of CBA mice, have been widely applied in studies on the origin of cells under different experimental conditions (22, 23). The application of this technique is limited, however, in situations in which it is necessary to identify a specific cell type in a mixed population because recognition of the cell type of a spread metaphase is usually not possible. This makes it difficult to apply this technique for in vivo studies on macrophages, as macrophages are always mixed with other cells in the organism. In vitro studies using pure macrophage populations could be more revealing, but this is rendered difficult by the poor growth capability of cultured macrophages.

In the present study, the poor growth of macrophages in vitro was overcome by the use of "macrophage growth factor" which is present in conditioned medium from fibroblastic cell cultures (20). Essentially pure cultures of macrophages could be established from peritoneal exudate and lung washings, and from different organs by the grid organ culture method (19).

The results indicated a close relationship or possible identity of precursor cells for macrophages and for hematopoietic cells at the differentiation level at which the repopulation of the irradiated host takes place. This conclusion is based on the following findings: (a) All macrophages independent of their source were of donor type when hematopoietic cells were injected into lethally irradiated recipients. (b) When mixed cell injections of hematopoietic tissues from genetically different donors were given, the ratio of genotypes was the same in bone marrow cells and in macrophage cultures. (c) Thymus, lymph node, and peritoneal exudate cells which did not repopulate the marrow (at the level of the sensitivity of the methods used) were not found to contain precursor cells for macrophages either. (d) Precursor cells for macrophages and for bone marrow cells appeared to be equally sensitive to sublethal irradiation.

Preliminary experiments in which hematopoietic spleen colonies (24) of different cellular composition have been cultured in vitro indicate that pure erythroid colonies are poor producers of macrophages whereas myeloid and some mixed colonies form large numbers of macrophages (25). The maturation of macrophages thus seems to be related to the myeloid series of differentiation.

In regard to peritoneal macrophages, the present results are in agreement
with studies in which immunological (8, 9) and radioautographic (1) methods have been applied for the identification of the origin of these cells. The present results indicate that as soon as 14 days after irradiation and administration of bone marrow cells all dividing macrophages were of donor type. However, Balner (8) found that the peritoneal cells were completely replaced by donor-type cells only about 6 wk after the establishment of chimerism. These findings thus suggest that host macrophages which possibly survive up to 6 wk are radiosensitive and not capable of cell division.

Concerning alveolar macrophages, there is a discrepancy between the present results and those of Pinkett et al. (16) who used the same chromosome marker technique, but collected alveolar cells after colchicine treatment in vivo. A possible explanation could be "contamination" by other types of cells after in vivo arrest of mitoses. Because the proliferation rate of free alveolar macrophages is low (13), even a small contribution of mitotic cells, e.g. from pulmonary epithelium, could explain their finding that one-third of the mitotic cells in lung washings were of pulmonary origin. However, if alveolar macrophages would, in fact, have a dual origin, the pulmonary population could lack the ability for in vitro division and would have remained undetected in the present experiments.

The rapid production of dense macrophage cultures from bone marrow and from spleen undergoing active hematopoiesis suggests hematopoietic tissues contain substantial numbers of immediate macrophage precursors capable of "transforming" into macrophages without cell division. Lymphoid organs and lung apparently contain fewer precursor cells initially, but in vitro conditions seem to favor the proliferation of these cells and their differentiation into macrophages.

It is important to emphasize that macrophages may be a heterogeneous population, and that the present results apply only for those macrophages which do respond in vitro to "macrophage growth factor." The fact that liver explants do not produce many macrophages in vitro could suggest a difference between fixed reticuloendothelial cells and free cells, e.g. peritoneal macrophages, almost all of which can be induced to cell division in vitro.

SUMMARY

The origin of macrophages was studied in mouse radiation chimeras by chromosome marker technique. Macrophage cultures were established from peritoneal exudate, from lung washings, and from organ cultures of bone marrow, spleen, lymph node, thymus, and lung. Cultured macrophages were induced to divide by adding conditioned medium from L cell cultures.

In chimeras which were lethally irradiated and given injections of bone marrow or spleen cells, dividing macrophages were of donor type, independent of the source of the macrophages. When chimeras were established by injections
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of a mixture of bone marrow cells and cells from other hematopoietic tissues of
two genetically different donors, the ratio of cells with different genotypes was
approximately the same in bone marrow cells and in macrophage cultures.
Thymus, lymph node, and peritoneal exudate cells were not found to contain
precursor cells for macrophages. Precursor cells for macrophages and for bone
marrow cells appeared to be equally sensitive to sublethal irradiation.

The results indicate that macrophages from different sources can all be de-

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EXPLANATION OF PLATES

PLATE 99

**Fig. 1.** Peritoneal macrophages cultivated in Eagle’s medium for 5 days. 2% glutaraldehyde fixation. Phase contrast, × 350.

**Fig. 2.** Duplicate culture of same peritoneal macrophages cultivated in conditioned medium for 5 days. 2% glutaraldehyde fixation. Phase contrast, × 350.
(Virolainen: Hematopoietic origin of macrophages)
PLATE 100

FIG. 3. Chromosome preparation of macrophages of bone marrow explant origin. Cultivated in conditioned medium for 4 days, and treated with Colcemid for 5 hr. Giemsa, × 225.

FIG. 4. High power view of the same culture as Fig. 3 showing the presence of the T6 marker chromosomes (arrows) in three metaphases. × 1400.
(Virolainen: Hematopoietic origin of macrophages)