Heterogeneity of the Radiosensitivity and Origins of Tissue Macrophage Colony-Forming Cells

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M-CFC/radiosensitivity/origin/89Sr

Previous studies suggest that the radiosensitivity and origin of tissue macrophage precursors differ from those of hemopoietic macrophage colony-forming units (CFU-Ms) committed to macrophage-lineage cells. We assessed the origins of tissue macrophage colony-forming cells (M-CFCs) in mice by comparing their kinetics and radiosensitivities in the normal steady state and under the conditions of bone marrow depletion by 89Sr-administration and/or splenectomy. The results indicate that the radiosensitive peritoneal M-CFCs elicited by thioglycollate are derived from bone marrow macrophage precursors; whereas alveolar M-CFCs, which are radioresistant, are self-sustained locally and independent of hemopoietic macrophage precursors. In contrast, highly radiosensitive liver M-CFCs are probably derived from CFU-Ms that appear to be propagated in the spleen in association with hemopoietic responses.

INTRODUCTION

Murine peripheral tissues outside hemopoietic tissues have macrophage(MΦ) precursors that proliferate and form MΦ colonies in vitro in agarose medium containing MΦ colony-stimulating factor(M-CSF) [1]. Such tissue MΦ colony-forming cells (M-CFCs), however, differ in radiosensitivity from hemopoietic colony-forming units (CFU-Ms) committed to MΦ-lineage cells. For example, alveolar M-CFCs isolated from the lung are more radioresistant than bone marrow CFU-Ms or peritoneal M-CFCs [2, 3]; whereas non-parenchymal liver M-CFCs are rather highly radiosensitive [4]. In addition, the total number of alveolar M-CFCs is sustained under normal conditions or under inflammatory conditions after selective depletion of radiosensitive bone marrow MΦ precursors and monocytes by a bone-seeking radiostrontium, 89Sr [5]; whereas peritoneal M-CFCs elicited by thioglycollate, which are relatively radiosensitive, are depressed by whole body γ-irradiation [6] as well as by 89Sr (unpublished data). These observations suggest that the different radiosensitivities of tissue M-CFCs reflect the heterogeneous MΦ populations that are dependent or independent of hemopoietic CFU-Ms, although the details of their origins, or ontogenic mechanisms largely remain to be elucidated.
We here compare the induction of heterogenous M-CFCs in pulmonary alveolar, peritoneal and liver non-parenchymal cells of mice under normal conditions and conditions of depletion of bone marrow CFU-Ms by $^{89}$Sr or by combination of splenectomy and $^{89}$Sr in order to define their origins. The results indicate heterogenous origins for the tissue MΦ precursors that are derived from bone marrow or are sustained locally independent of hemopoiesis.

**MATERIALS AND METHODS**

**Animals:** Female C3H/HeN mice, 8- to 12-weeks old, obtained from our animal breeding facility were kept under barrier-filtered air condition before and after experimental treatment as described below.

**Bone marrow depletion and splenectomy:** To induce monocytopenia and bone marrow depletion [7], groups of mice were injected intraperitoneally (ip) with 1.85 MBq of $^{89}$SrCl$_2$ (3.3–5.0 MBq/mgSr; Amersham International, UK). The control groups were administered ip with 1.3–2.0 mg of non-radioactive $^{88}$SrCl$_2$ in PBS. Groups of animals were aseptically splenectomized (Spx) under anesthesia with pentobarbital 30 days before $^{88}$Sr- or $^{89}$Sr-administration. The control and experimental groups of mice and treatment regimens are designated in the text respectively as: control ($^{88}$Sr), Spx/$^{88}$Sr (Spx), $^{89}$Sr alone ($^{89}$Sr), and Spx/$^{89}$Sr.

**Isolation and preparation of tissue MΦ:** On day 28 after Sr-injection, mice were exsanguinated under ether anesthesia to harvest tissue MΦ together with peripheral blood from the posterior vein, and the femoral bone marrow cells and spleen cells as described [5]. We performed bronchoalveolar lavage with Ca$^{++}$- and Mg$^{++}$-free PBS to isolate pulmonary alveolar MΦ, and peritoneal lavage to collect resident peritoneal MΦ from untreated mice and the elicited peritoneal MΦ from mice administered ip with 5% sterilized thioglycollate (TG; Difco, Detroit, MI) 5 days before harvest as described [2]. Liver MΦ was isolated by perfusing the liver in situ through the portal vein with Ca$^{++}$- and Mg$^{++}$-free Hanks' balanced salt solution (HBSS) and then with HBSS containing 0.2% pronase (Sigma, St. Louis, Mo), after which the nonparenchymal liver cells were minced and separated by centrifugation in a 30% metrizamide gradient solution (Sigma) as described elsewhere [4]. The numbers of cells obtained were counted by a Coulter counter (model ZM; Coulter Electronics, Hialeah, Fla), and the viability was estimated by dye exclusion assay with trypan blue. Differential counts of MΦ were made on cytocentrifuged preparations stained with Giemsa and non-specific esterase, and by indirect immunofluorescence with polyclonal anti-asialo GM1 (AsGM1) antibody (Wako Chemicals, Tokyo, Japan) and monoclonal F4/80 anti-MΦ antibody (ATCC, Rockville, MD) as described previously [2].

**Colony-forming cell assays:** Hemopoietic CFU-Ms were detected in vitro by 7-day cultures of 2 x 10$^4$ bone marrow cells or 4 x 10$^4$ spleen cells in soft agarose medium containing RPMI-1640 medium (Flow Laboratories, McLean, VA), 0.36% agarose (Sea Plaque; FMC Biopro-
ducts, Rockland, ME), 10% L-cell conditioned medium (L-CM) as a source of M-CSF [1], 10% fetal bovine serum (FBS; GIBCO, Grand Island, NY), and supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin in triplicate 35-mm tissue culture dishes (Falcon 3001; Becton-Dickinson Labware, Oxnard, CA). Tissue M-CFCs were detected by 14- to 21-day cultures of 1 × 10^4 alveolar cells (MΦ >95%), 1 × 10^4 TG-elicited peritoneal cells (MΦ >80%), 1 × 10^5 resident peritoneal cells (MΦ >60%), or 1 × 10^5 non-parenchymal liver cells (MΦ >60%) in soft agarose medium containing 10% L-CM in triplicate 35-mm dishes as described elsewhere [2, 5].

**Irradiation:** To assess the radiosensitivities of tissue M-CFCs in vitro, the cells isolated from mice were resuspended in ice-cold, FBS-free RPMI-1640 medium, and then were irradiated with a ^137^Cs-source at 1 Gy/min immediately before cultures for the CFC assays as described above.

**Statistics:** Comparisons between the control and treatment groups of animals were made by Student’s T-test in order to evaluate any significant differences.

**RESULTS**

**Hematology after ^86^Sr and splenectomy**

By day 28 after ^88^Sr-administration, total monocytes in the peripheral blood and bone marrow CFU-Ms had decreased to less than 10% of the control (^88^Sr) value, whereas splenic CFU-Ms increased up to 10-fold of the control value (Table 1). When mice first had been splenectomized (Spx/^89^Sr), ^89^Sr induced further depletion of monocytes and bone marrow

**Table 1.** Hematological Changes in Mice after Splenectomy (Spx) and ^89^Sr-Injection

<table>
<thead>
<tr>
<th>Cell and Tissue</th>
<th>Control</th>
<th>Spx</th>
<th>^89^Sr</th>
<th>Spx/^89^Sr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral blood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>white blood cells (×10^9/ml)</td>
<td>8.8±1.3^b</td>
<td>9.6±1.6</td>
<td>3.4±0.7^*</td>
<td>1.8±0.3**</td>
</tr>
<tr>
<td>monocytes (×10^9/ml)</td>
<td>4.3±1.5</td>
<td>5.4±1.4</td>
<td>0.5±0.5^*</td>
<td>0.1±0.1**</td>
</tr>
<tr>
<td><strong>Bone marrow (per femur)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total nucleated cells (×10^9)</td>
<td>4.1±0.1</td>
<td>4.1±0.2</td>
<td>1.2±0.4^*</td>
<td>0.8±0.2**</td>
</tr>
<tr>
<td>total CFU-M (×10^3)</td>
<td>11.3±1.9</td>
<td>13.1±1.2</td>
<td>0.8±0.3^*</td>
<td>0.1±0.1**</td>
</tr>
<tr>
<td><strong>Spleen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total nucleated cells (×10^9)</td>
<td>64 ±2.1</td>
<td>—</td>
<td>86±3.1^*</td>
<td>—</td>
</tr>
<tr>
<td>total CFU-M (×10^3)</td>
<td>6.0±1.6</td>
<td>—</td>
<td>87±21**</td>
<td>—</td>
</tr>
</tbody>
</table>

^a. Splenectomy (Spx) was done 30 days before ^88^Sr- or ^89^Sr-injection. Hematological examinations were made on day 28 after Sr-administration.

^b. Data represent mean ± SD of 6 animals per group. Asterisks indicate significant differences in each category, as compared to the control (^88^Sr) (*p<0.05, **p<0.01).
CFU-Ms to less than 1% of the control value. Splenectomy alone (Spx), however, did not produce significant changes in monocytes and bone marrow CFU-Ms as compared to the control values.

**Total cell yield recovered from mice after $^{89}$Sr and splenectomy**

As shown in Table 2, the total numbers of MΦ recovered from the alveolar, peritoneal (resident), and liver tissues did not differ from those of the control ($^{88}$Sr) after Spx and/or $^{89}$Sr-administration, except for the thioglycollate (TG)-elicited peritoneal MΦ. The number of TG-elicited peritoneal MΦ increased to 20-fold of resident peritoneal MΦ in the control ($^{88}$Sr), and to about 14-fold after $^{89}$Sr, but negligibly after Spx/$^{89}$Sr. Splenectomy alone (Spx), however, did not affect the total numbers of MΦ recovered from the different tissues or inflammation sites.

**Table 2. Total Numbers of Macrophages (MΦ) Recovered from Mice after Splenectomy (Spx) and $^{89}$Sr-Injection**

<table>
<thead>
<tr>
<th>Tissue MΦ</th>
<th>Control</th>
<th>Spx</th>
<th>$^{89}$Sr</th>
<th>Spx/$^{89}$Sr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolar M Φ ($\times 10^5$)</td>
<td>3.8±0.8$^b$</td>
<td>3.5±0.8</td>
<td>3.9±0.9</td>
<td>3.7±0.8</td>
</tr>
<tr>
<td>Resident peritoneal M Φ ($\times 10^4$)</td>
<td>6.1±1.1</td>
<td>5.5±1.0</td>
<td>6.0±1.0</td>
<td>5.5±1.0</td>
</tr>
<tr>
<td>Elicited peritoneal M Φ ($\times 10^5$)</td>
<td>13.1±1.3</td>
<td>13.1±1.7</td>
<td>8.9±2.2$^*$</td>
<td>0.3±0.2$^{**}$</td>
</tr>
<tr>
<td>Liver M Φ ($\times 10^6$)</td>
<td>7.8±1.2</td>
<td>7.4±0.5</td>
<td>7.6±0.8</td>
<td>7.0±0.8</td>
</tr>
</tbody>
</table>

$^a$. Groups of mice were splenecotomized (Spx) 30 days before $^{88}$Sr- or $^{89}$Sr-injection, and tissue macrophages (M Φ) were recovered on day 28 after Sr-administration. To obtain elicited peritoneal M Φ, groups of mice were given thioglycollate intraperitoneally on day 23 after Sr-administration.

$^b$. Data represent mean ± SD of 6 animals per group. Asterisks indicate significant differences in each category, as compared to the control ($^{88}$Sr) (*p<0.05, **p<0.01).

**Changes in tissue M-CFC after $^{89}$Sr and splenectomy**

The plating efficiencies, lag period before replication, and radiosensitivity of tissue M-CFCs from normal mice differed with the cell source. Alveolar M-CFCs and TG-elicited peritoneal M-CFCs were detected from day 10 to 14 after plating on agarose medium, and their respective plating efficiencies in 21-day cultures were 2–3% and 3–4%. In contrast, the plating efficiencies of the resident peritoneal cells and non-parenchymal liver cells were very low, being less than 0.01% in the 21-day cultures. Moreover, only a small number of liver M-CFCs was detected early on day 3 to 7 after plating. These formed larger colonies, while the majority of the liver M-CFCs formed smaller colonies that grew slowly between day 7 to 10 of cultures. As compared to the bone marrow CFU-Ms detected from day 3 to 7 of cultures, the dose-related regression curve of the surviving fractions of these tissue M-CFCs after in vitro irradiation was different as shown in Fig. 1. The estimated $D_0$ value was the highest (2.0) for alveolar M-CFCs; 1.2 for TG-elicited peritoneal M-CFCs, and 1.0 for bone marrow CFU-Ms, whereas it was the lowest (0.8) for liver M-CFCs, indicative of the highest radiosensitivity being that of liver M-CFCs as reported elsewhere [4].
The induction of tissue M-CFCs was also compared between groups of mice after $^{89}$Sr and splenectomy (Fig. 2). The total numbers of alveolar M-CFCs and resident peritoneal M-CFCs were not affected by splenectomy and/or $^{89}$Sr-injection. In contrast, TG-elicited peritoneal M-CFCs were greatly decreased to about 25% of the control values after $^{89}$Sr, and also depleted after Spx/$^{89}$Sr as expected. The total liver M-CFCs were significantly reduced after Spx/$^{89}$Sr, but not after $^{89}$Sr or Spx alone. The induction of large-size liver Mφ colonies, however, was more apparent after $^{89}$Sr-treatment and was slightly suppressed by splenectomy alone (data not shown).

DISCUSSION

Because radioactive $^{89}$Sr is preferentially distributed in skeletal bones and selectively irradiates bone marrow cells for relatively longer periods [8, 9], hemopoietic stem cell proliferation is inhibited and, as a result, the recruitment of radiosensitive bone marrow-derived Mφ precursors into extra-hemopoietic tissues is persistently suppressed in mice that received $^{89}$Sr. Our results also indicate that circulating monocytes and bone marrow CFU-Ms are more greatly depleted by the combination of splenectomy and $^{89}$Sr-injection (Spx/$^{89}$Sr) than by $^{89}$Sr-injection alone, but splenectomy alone (Spx) did not alter the levels of monocytes and bone marrow hemopoiesis. Such difference in the effectiveness of $^{89}$Sr may be related to its specific activity.
distributed [10] or to the later restoration of bone marrow hemopoiesis by splenic stem cells [8].

The cell yields of pulmonary alveolar Mφ, resident peritoneal Mφ and liver Mφ (Kupffer cells) from mice did not differ from the values of the controls or Spx, even when there was monocytopenic and bone marrow-ablated conditions produced by 89Sr- and Spx/89Sr-treatments. These findings support the hypothesis that tissue Mφ are maintained independent of hemopoietic Mφ precursors and monocytes [7, 11, 12]. The peritoneal Mφ elicited by thioglycollate were, in contrast, highly sensitive to bone marrow depletion but were not affected by splenectomy alone. Taken together with previous results reported in mice not only by 89Sr-injection [13] but also by whole-body γ-irradiation [6], the elicited peritoneal Mφ are mostly derived from radiosensitive bone marrow Mφ precursors and monocytes.

Concomitant with decrease in elicited peritoneal Mφ, peritoneal M-CFCs, which are relatively radiosensitive, were markedly reduced by 89Sr-treatment, and were further depleted by
Spx/$^{89}$Sr. The population of resident peritoneal M-CFCs remained very low even under the normal condition, being almost invariable after $^{89}$Sr- or Spx/$^{89}$Sr-treatment. DNA synthesis by the elicited peritoneal MΦ was much higher than that of the resident peritoneal MΦ, but was depressed to the values for the resident peritoneal MΦ after bone marrow depletion, as described elsewhere [9, 13]. Taken together, these observations suggest that the elicited peritoneal MΦ are highly proliferative in comparison to the resident peritoneal MΦ and that the formation of peritoneal M-CFCs is closely associated with the elicited peritoneal MΦ derived from bone marrows but not with the resident peritoneal MΦ. Alveolar M-CFCs, which show the highest radioresistance, however, were sustained locally independent of bone marrow depletion by $^{89}$Sr and/or splenectomy, suggesting the self-renewal of alveolar MΦ [14, 15].

Non-parenchymal liver cells in the normal condition also contained M-CFCs which were scarcely detectable in agarose cultures at low plating efficiency. The previous study [4] has shown that liver M-CFCs do not grow well in agarose cultures, the stronger anchorage-dependent growth of liver M-CFCs than other tissue M-CFCs. Moreover, the liver M-CFCs in agarose cultures in the experiments reported here showed the highest radiosensitivity, and some proliferated more rapidly and formed large-size MΦ colonies. The large-size liver M-CFCs were more often induced after $^{89}$Sr, but were suppressed by Spx/$^{89}$Sr. The total number of liver M-CFCs was, however, almost constant after $^{89}$Sr or Spx alone. Taken together with the above findings, the data suggest that most liver M-CFCs detected in agarose cultures, if not all, are probably hemopoietic CFU-Ms which appear to propagate in the spleen in association with hemopoietic responses. Although the liver MΦ (Kupffer cells) had less ability to form anchorage-independent MΦ colonies, they maintained their population size after bone marrow depletion or splenectomy. Therefore, the possibility cannot be excluded that the liver MΦ themselves can proliferate under certain conditions such as glucan-induced granuloma formation independent of the hemopoietic CFU-M responses as shown by Yamada et al. [12].

In conclusion, the heterogenous origins of tissue M-CFCs that have different radiosensitivities and lag periods before replication are indicated. Radiosensitive, elicited peritoneal M-CFCs and large-size liver M-CFCs either are derived from bone marrow or increase in association with hemopoietic responses of the spleen; whereas radioresistant alveolar M-CFCs are self-sustained and independent of hemopoiesis.

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REFERENCES