Spleen-Colony Formation
in Anemic Mice of Genotype WW

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Abstract. The hemopoietic cells from anemic mice of genotype WW* are less able by 200-fold to take part in colony formation in the spleen than cells from the normal littermates of genotype ww. The genetic defect shows itself in the colony-forming cells, since cells from normal littermate mice form colonies in the spleens of unirradiated mice of genotype WW*. Use of animals of genotype WW* as recipients improves the spleen-colony method by removing bias resulting from the death of irradiated recipients.

A macrocytic anemia occurring in mice of genotype WW* has been studied extensively (1, 2). A remarkable feature of the anemia in these animals is that it can be cured permanently by the intravenous infusion of hemopoietic tissue from isologous fetal liver from animals of genotype ww (3). The marrow spaces of the recipient anemic mice become populated by the descendants of the infused cells, and normal numbers of peripheral erythrocytes result from the proliferation and differentiation of these cells. Thus, isologous marrow grafts can grow and function in mice of genotype WW* in much the same way as in heavily irradiated hosts.

Mice of genotype WW* are very much more susceptible to the short-term lethal effects of total-body x-irradiation than are mice of the normal ww genotype. Bernstein (4) found the LD 50/30 (lethal to 50 percent in 30 days) for the anemic mice to be in the range 250 to 350 roentgens, in contrast to values near 700 to 750 roentgens for their normal littermates. However, WW* anemic mice successfully implanted with isologous normal ww blood-forming tissues show LD 50/30 values approaching those of normal ww individuals (5), indicating that mice of genotype WW* might be deficient in those cells which promote survival in irradiated ww animals.

We have suggested (6, 7) that the cells responsible for regenerating the hemopoietic tissues and hence for survival of irradiated mice may be recognized by their capacity to form macroscopic colonies in the spleens of mice irradiated with 900 roentgens of x-rays. These cells have been termed "colony-forming" cells, since, at present, they can be detected only by this property. Three types of evidence have indicated that the colony-forming cells play a part in the survival of animals exposed to total-body radiation. (i) The cellular descendants of colony-forming cells include the erythrocytic, granulocytic, and megakaryocytic precursors whose functions are necessary for life (8). (ii) The kinetics of repair of colony-forming capacity in the spleens of mice exposed to sublethal doses of total-body radiation appears to correlate well with the kinetics of repair of radiation injury as measured by changes in the LD 50/30 (9). (iii) The LD 100/30 (9) is approximately equal to the dose required to eliminate colony formation by "endogenous" colony-forming cells (10) present in the spleens of animals exposed to total-body radiation. The hypothesis that colony-forming cells are important in the regeneration of hemopoietic tissue, taken together with the work of Bernstein (3-5), led to the predictions that anemic mice of genotype WW* would be deficient in colony-forming cells, and that transplants of normal, isologous hemopoietic cells might be able to colonize the spleens of unirradiated anemic mice. Our results indicate that both these predictions were correct.

Severely anemic mice of genotype WW*, along with their hematologically normal littermates of genotype ww, were obtained from the Jackson Laboratory, Bar Harbor, Maine. These mice were hybrids obtained by crossing animals of the genotype WB-Ww with mice of genotype C57BL/6-Ww*. Thus, littermates may be considered to differ from each other genetically only at the W locus. In addition, C57BL/6 mice obtained from the annex colony of the Ontario Cancer Institute were used.

The spleen-colony method of assaying for the proliferative capacity of cells derived from hemopoietic tissue has been described (11, 12). In the first experiment, we tested for the capacity of cells derived from mice of genotype WW* to form colonies in irradiated C57BL/6 recipients. Accordingly, cell suspensions were prepared from the marrows (11) and spleens (13) of groups of nine to ten donors of genotype WW* and ww, and each suspension was injected intravenously into a group of heavily irradiated (900 to 1000 rad) C57BL/6 mice. After 9 to 11 days, the survivors were killed, their spleens were fixed in Bouin's solution, and visible colonies were counted. From Table 1, it is evident that the yield of nucleated cells obtained from marrow and spleen of normal and anemic mice was very similar, and that cells obtained from mice of genotype ww had normal colony-forming capacity, since the number of colonies observed per 10^5 cells injected was similar to that observed in other strains (11, 13). In contrast, cell suspensions from mice of genotype WW* were very deficient in colony-forming capacity since only a few very small colonies were observed even when large numbers of nucleated cells were injected. Of these, it is possible that a proportion were the

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cells recovered per spleen or two femurs</th>
<th>Nucleated cells injected</th>
<th>Spleen colonies ± std. error</th>
<th>Colonies per 10^5 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>4.4 x 10^6</td>
<td>1.0 x 10^6</td>
<td>12.2 ± 2.3</td>
<td>12.2</td>
</tr>
<tr>
<td>WW*</td>
<td>3.0 x 10^6</td>
<td>8.5 x 10^6</td>
<td>0.4 ± 0.1*</td>
<td>0.005</td>
</tr>
<tr>
<td>WW</td>
<td>1.7 x 10^6</td>
<td>5.0 x 10^6</td>
<td>4.1 ± 0.9</td>
<td>0.82</td>
</tr>
<tr>
<td>WW*</td>
<td>1.7 x 10^6</td>
<td>3.0 x 10^6</td>
<td>1.1 ± 0.3*</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Colonies were small in size and difficult to count.
result of survival of endogenous colony-forming cells derived from the hemopoietic tissues of the heavily irradiated recipient mice, though the average number of surviving endogenous colonies after a total-body radiation dose of 900 rad is usually considerably less than one colony per spleen (6, 10). In any event, it may be concluded that the colony-forming capacity of cells from anemic mice is less by at least 200-fold than that of cells from their normal littermates.

The results shown in Table 1, coupled with the demonstration by Bernstein and Russell (3) that normal cells from mice of genotype \( ww \) can be transplanted into unirradiated hosts of genotype \( WW^v \), suggested that colony-forming cells from normal \( ww \) mice might give rise to colonies in the spleens of unirradiated, anemic mice. Therefore, known numbers of femoral marrow cells obtained from ten mice of \( ww \) genotype were transplanted into groups of unirradiated mice of genotype \( WW^v \); after 10 days the recipient animals were killed and their spleens were fixed in Bouin’s solution. Figure 1 shows a spleen of an unirradiated \( WW^v \) mouse 10 days after intravenous injection of \( 2 \times 10^5 \) marrow cells from normal \( ww \) mice. It is apparent that colonies analogous to those observed in irradiated hosts were formed in the spleen of the unirradiated \( WW^v \) recipient. Thus \( WW^v \) mice might prove valuable as recipients in the techniques of assaying spleen-colony formation, since all of these unirradiated animals might be expected to live for the 10 days necessary for macroscopic colonies to be formed, whereas many irradiated recipient animals would die during this period (Table 2). However, their usefulness as recipients would depend on the demonstration of a linear relationship between the number of nucleated cells injected and the mean number of colonies per spleen observed. The results of two experiments in which this linear relationship is demonstrated are presented in Table 2.

Figure 2 shows that a linear relationship exists between the number of nucleated cells injected into \( WW^v \) mice and the number of colonies observed, similar to that found when irradiated mice were used as recipients (11, 12). Further, it may be seen that the mean number of colonies observed per \( 10^5 \) cells injected when unirradiated \( WW^v \) mice were used as recipients was not significantly different from the mean number when irradiated C57BL/6 animals were used (Table 2).

The results indicated that mice of the genotype \( WW^v \) are suitable as recipients for the measurement of colony formation by hemopoietic cells from littermate animals with genotype \( ww \). Further, these nonirradiated recipients have the advantage over irradiated normal recipients in that the results obtained are not biased because of loss of animals from death by irradiation during the period of the assay, and fewer animals per group will yield results of equivalent precision to those obtained with irradiated hosts. It is anticipated that this improvement in the spleen-colony assay technique will broaden greatly the applications which may be made of this method in the investigation of hemopoietic cell function (14). Our results support the view that colony-forming cells play an important role in the regeneration of hemopoietic tissue after total-body radiation. Anemic \( WW^v \) mice fail to recover from modest doses of total-body radiation. Anemic \( WW^v \) mice fail to form spleen colonies when injected into irradiated, genetically normal, animals. The genetically anemic mice are capable of supporting colony formation by cells derived from mice of normal genotype. Thus, the hemopoietic tissues of genetically anemic mice either are deficient in cells with colony-forming ability, or they contain cells which require a stimulus for colony formation which is not provided under the conditions of our experiments.

Russell and co-workers (15) have demonstrated that erythropoiesis is defective in irradiated \( WW^v \) mice. Since normal spleen colonies contain large numbers of erythrocyte precursors (8), a deficiency in erythrocytic differentiation could prevent normal colony development. Thus, it is possible that the reduced colony-forming ability of cells from \( WW^v \) hemopoietic tissue is a reflection of inadequate erythropoiesis. However, deficient \( WW^v \) hemopoietic tissue is still able to provide the continuing supply of cells necessary for maintenance of a constant, though reduced, red cell population and a nearly normal marrow cellularity (2). The anemic mice are also able to respond well to the erythropoietic stimulus of hypoxia (16) and, to a much lesser extent, to the administration of exogenous erythropoietin (17). Our results support the suggestion (18) that the "stem cells" responding to erythropoietin (19) are not identical with the "stem cells" that give rise to spleen colonies.

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References and Notes

14. We have found that the facility with which the colonies may be counted in *WW* mice is improved by irradiation of the hosts with 200 rad prior to the injection of cells. This treatment does not introduce any appreciable mortality.
20. We thank Dr. E. S. Russell for making available the mice of differing *W* genotype, and Miss R. Wyncoll, R. Course, J. Hicks, A. Galberg, R. Kuba, F. Mik, and P. Csordas for technical assistance. Supported by the Defence Research Board (grant 9350-14), the National Cancer Institute of Canada, the Medical Research Council, Canada (grant MA-1420), and the National Research Council of Canada (grant T-1714).