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HEMOPOIETIC STEM CELL MIGRATION
IN MICE OF GENOTYPE $S1/S1^d$
FOLLOWING A SINGLE INJECTION OF
BORDETELLA PERTUSSIS VACCINE

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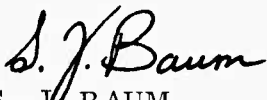
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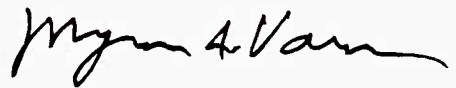
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S1/S1^d FOLLOWING A SINGLE INJECTION OF
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FOREWORD
(Nontechnical summary)

A gene in mice found at the Steel loci is highly mutable, having given rise to over 30 new genes. Mice carrying mutations ($S1/S1^d$) at the Steel loci are black eyed, white, infertile and severely anemic. These mice are extremely sensitive to ionizing radiation, the $LD_{50/30}$ dose is less than 200 rads. The double mutation at the Steel loci is believed to lower the concentration of a stromal element necessary for maximum hematopoietic stem cell growth. Thus, following whole-body irradiation the hematopoietic stem cell population of $S1/S1^d$ mice does not renew itself as quickly as in normal mice and the production of blood cells is reduced. Therefore, $S1/S1^d$ mice are more susceptible to bacterial infections for a longer period of time than are normal mice.

The present work is concerned with the mechanism of hematopoietic stem cell growth in mice in response to bacterial infection. It was found that when $S1/S1^d$ mice are challenged with a single intraperitoneal injection of 20×10^9 heat killed bacteria, the hematopoietic stem cells in the circulation and spleen do not respond in a normal fashion. The importance of this observation is that in normal mice, challenged with various bacterial preparations, the total hematopoietic stem cell compartment is increased several fold in situ by cellular replication and by the process of stem cell migration, i. e., stem cells leave the marrow, enter the circulation and seed the spleen. Apparently this type of response (cellular migration) is severely restricted in $S1/S1^d$ mice and might explain, in part, the sensitivity of these mice to ionizing radiation.

ABSTRACT

The ability of a single injection of Bordetella pertussis vaccine to cause an increase in the number of circulating transplantable colony forming units (CFU) in mice of genotype S1/S1^d and their normal congeneric littermates was studied. Seventy-six hours after injection of B. pertussis vaccine, the number of circulating CFU per milliliter of blood in normal mice increased from approximately 45 CFU/ml to over 300 CFU/ml, while in S1/S1^d mice the number of circulating CFU increased from approximately 22 CFU/ml to only 57 CFU/ml. Thus, the previously reported restriction of splenic CFU growth in S1/S1^d mice following multiple injections of endotoxin is discussed in the present work not only in terms of a defect in splenic stromal elements necessary for maximum CFU growth, but also in view of current concepts of CFU migration from marrow to spleen via the circulation.

I. INTRODUCTION

It has been reported that daily injections of endotoxin in normal mice of the strain (WC x C57BL/6J) F_1 cause a threefold increase within 72 hours in the colony forming units (CFU) of the spleen; while, in mice of the same strain but of genotype S1/S1^d treated similarly, no increase in the number of splenic CFU was observed.¹² This difference has been attributed to mutations or deletions of genes found at the Steel loci which, in some manner, appear to alter the nature or concentration of some splenic stromal element which, in turn, results in poor CFU growth.¹¹

The nature of this stromal defect has been the subject of several recent papers. Altus et al.¹ reported that the defect in the hematopoietic response of S1/S1^d mice is in the tissue matrix rather than the hematopoietic stem cell or humoral regulating mechanism. Fried et al.⁷ compared the growth of splenic implants from donors of genotype +/+ and S1/S1^d in normal mice and concluded that the defective CFU growth factor is cellular in nature, is less radiosensitive than CFU, supports the growth of CFU and possibly can differentiate into CFU.

Thus, there is ample support for the original concept that the lack of splenic CFU growth in S1/S1^d mice in response to multiple injections of endotoxin is due to poor in situ CFU proliferation. However, Monette et al.¹³ have reported that, following a single intraperitoneal injection of Bordetella pertussis vaccine, stem cell growth in the spleen might occur not only by in situ proliferation but also by migration of CFU from marrow to spleen via the circulation. Growth by the latter mechanism might account for up to 60 percent of the total increase in number of splenic CFU's.

Inasmuch as McCulloch et al.¹² could detect no increase in the splenic CFU compartment of S1/S1^d mice pretreated with endotoxin and Wolf¹⁸ has reported that the seeding or trapping of CFU from the circulation by the spleen is perfectly normal in S1/S1^d mice, it is possible that not only splenic CFU proliferation but also migration of CFU from marrow to spleen is restricted in these mice.

Thus, the purpose of this study was to determine what effect mutations at the Steel loci have on the size of the circulating stem cell population during periods of severe hematopoietic stress.

II. METHODS

Animals. The experiments were carried out using a stock of F₁ mice obtained by crossing WC males with C57BL/6J females. These mice were reared at the AFRRRI. Host mice, 5 to 10 months old, were randomized according to weight, age and sex. Experimental mice of the normal genotype (+/+) and the mutant (S1/S1^d) were obtained by crossing mice of genotype C57BL/6J-+/S1^d and WC-S1/+. These mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. All experimental mice were male and between 10 to 13 weeks of age. In some cases only littermates were used and this is noted in the results.

Bordetella pertussis vaccine treatment. B. pertussis vaccine was purchased from Eli Lilly and Company, Indianapolis, Indiana. Male mice of the genotype S1/S1^d or +/+ were challenged with a single intraperitoneal injection of approximately 20 x 10⁹ killed organisms.¹³ At various time intervals thereafter, groups of pertussis treated mice were euthanatized and splenic, femoral and peripheral blood suspensions prepared. Blood was collected from the jugular vein using EDTA as an anticoagulant. Spleens

were pressed through a stainless steel wire mesh into Fisher's medium for leukemic cells of mice; femurs were flushed out, also, with Fisher's medium for leukemic cells of mice. The cellularity of these suspensions was measured by visual counting in 1 percent acetic acid.

Assay for colony formation. Recipient mice were bilaterally irradiated at a distance of 1.82 meters from the AFRRI ^{60}Co source, at an absorbed dose rate of 153.8 rads/min and to a total dose of 950 rads. After exposure and intravenous injection of the various cell suspensions into groups of 7 to 10 mice, the mice were housed three per cage and were allowed acidified water and food ad libitum. Nine days later they were euthanatized. The spleens were removed, fixed in Bouin's solution, and the number of nodules per spleen determined.¹⁷

III. RESULTS

Changes in the number of CFU in the blood, spleen and marrow following B. pertussis vaccine treatment. An average increase of over 290 CFU/ml of blood was observed in normal mice 76 hours after a single intraperitoneal injection of B. pertussis vaccine. During this same period of time an average increase of only 34 CFU/ml of blood was found in S1/S1^d mice (Table I).

The changes in the splenic CFU compartment paralleled those of the blood. In the spleens of normal mice the number of CFU increased threefold to sevenfold, while in S1/S1^d mice the number of CFU increased less than threefold (Table II).

The number of marrow CFU was relatively unaffected by B. pertussis vaccine treatment (Table III).

Table I. Blood Cellularity and CFU Number Following Injection of B. Pertussis Vaccine

Experiment	Genotype	Number of donors		Control	Time interval			CFU Δ 76 h
					28 h	76 h	158 h	
1*	+/+	3	CFU/ml Recipients** WBC/ml	23.1 \pm 7.0 ^{‡§} 9/10 3.8 x 10 ⁶	22.6 \pm 7.3 6/10 2.9 x 10 ⁶	378.2 \pm 35.0 11/11 14.5 x 10 ⁶	110.2 \pm 18.4 9/10 7.0 x 10 ⁶	355
3	S1/S1 ^d	2	CFU/ml Recipients WBC/ml	8.0 \pm 2.0 5/10 4.0 x 10 ⁶		60.0 \pm 19.4 9/10 4.2 x 10 ⁶		52
4 [†]	+/+	2	CFU/ml Recipients WBC/ml	38.3 \pm 14.0 6/8 4.2 x 10 ⁶		344.0 \pm 31.9 5/8 8.5 x 10 ⁶		306
	S1/S1 ^d	2	CFU/ml Recipients WBC/ml	6.6 \pm 3.3 6/8 5.0 x 10 ⁶		40.5 \pm 16.3 4/8 6.2 x 10 ⁶		34
5 [†]	+/+	2	CFU/ml Recipients WBC/ml	75.0 \pm 12.3 6/7 9.8 x 10 ⁶		302.1 \pm 53.2 7/7 10 x 10 ⁶		227
	S1/S1 ^d	2	CFU/ml Recipients WBC/ml	53.1 \pm 10.9 6/7 12.3 x 10 ⁶		70.2 \pm 24.1 6/7 9.2 x 10 ⁶		17

* Mice reared at AFRRRI

† Littermates were used in this experiment

‡ Mean \pm S. E.

§ Not corrected for seeding efficiency

** Fraction survival of recipient mice

Table II. Spleen Cellularity and CFU Number Following Injection of B. Pertussis Vaccine

Experiment	Genotype	Number of donors		Control	Time interval 76 h	CFU Δ 76 h
3	S1/S1 ^d	2	CFU/spleen Recipients Cells/spleen	1,284 \pm 160 7/10 2.8 x 10 ⁸	725 \pm 174 8/10 2.9 x 10 ⁸	-559
4 [†]	+/+	2	CFU/spleen Recipients Cells/spleen	2,160 \pm 425 7/8 1.1 x 10 ⁸	17,580 \pm 676 6/8 2.5 x 10 ⁸	15,420
	S1/S1 ^d	2	CFU/spleen Recipients Cells/spleen	1,314 \pm 150 7/8 2.0 x 10 ⁸	3,520 \pm 1,061 6/8 1.6 x 10 ⁸	2,206

* Mice reared at AFRRRI

† Littermates were used in this experiment

‡ Mean \pm S. E.

§ Not corrected for seeding efficiency

** Fraction survival of recipient mice

Table III. Femur Cellularity and CFU Number Following Injection of B. Pertussis Vaccine

Experiment	Genotype	Number of donors		Control	Time interval 76 h	Δ CFU _{76 h}
2*	+/+	3	CFU/femur Recipients [§] Cells/femur	5,111 ± 840 ^{†‡} 7/10 2.5 x 10 ⁷	4,900 ± 828 10/10 1.7 x 10 ⁷	-211
3	S1/S1 ^d	2	CFU/femur Recipients Cells/femur	2,554 ± 252 7/10 1.6 x 10 ⁷	1,829 ± 633 10/10 0.9 x 10 ⁷	-625

* Mice reared at AFRRI

† Mean ± S. E.

‡ Not corrected for seeding efficiency

§ Fraction survival of recipient mice

IV. DISCUSSION

The important conclusion to be drawn from this work is that in mice of genotype S1/S1^d not only does a single injection of B. pertussis vaccine fail to stimulate the growth of the splenic CFU compartment, but it also effects less than a threefold increase within 76 hours in the number of circulating CFU. In normal congenic littermates this same vaccine treatment results in greater than a sevenfold increase in the number of circulating CFU. Therefore, the abnormal growth of the splenic CFU population in S1/S1^d mice in response to a single intraperitoneal injection of B. pertussis vaccine might be considered in terms of both in situ restriction of CFU proliferation due to a lack of some splenic stromal element and a repression of endogenous seeding of the spleen by marrow CFU via the circulation.

In some strains of mice, splenic CFU have a lower self-renewal capacity than do marrow CFU. In these mice it is possible that CFU constantly leave the marrow, enter the circulation, and seed the spleen.^{4,6,9,15} This endogenous seeding of the spleen by marrow CFU appears to be of special significance during some forms of

severe hematopoietic stress. The rate at which splenic CFU enter cell cycle following a single intraperitoneal injection of B. pertussis vaccine^{2, 13} or multiple injections of phenylhydrazine¹⁴ was insufficient to account for all the splenic CFU growth that was observed in CF mice. Because pretreatment with B. pertussis vaccine or phenylhydrazine causes a tenfold increase in the number of circulating CFU in CF mice, it was postulated that the bulk of splenic CFU growth was by splenic trapping of the increased number of circulating CFU.

However, Hodgson⁸ found that circulating CFU in BALB mice pretreated with phenylhydrazine have a lower self-renewal capacity than splenic CFU and therefore questioned how the increased numbers of circulating CFU could be the precursors of the increased number of splenic CFU. Because the bulk of splenic CFU in BALB mice enters cell cycle during phenylhydrazine-induced anemia, whereas in CF mice it does not, Hodgson⁸ suggested that the nature of splenic CFU growth, whether by endogenous seeding or in situ proliferation, is strain dependent.

In the present work a correlation between the lack of splenic CFU growth and a depressed number of circulating CFU was observed in S1/S1^d mice treated with B. pertussis vaccine. Whether one is the cause of the other or both are the result of a defect found elsewhere in the hematopoietic system is not known. Possibly, the lower number of circulating CFU in S1/S1^d mice could be a result of a restriction in the rate at which marrow CFU enter cell cycle in these mice.

The work of Lajtha et al.¹⁰ and Boggs et al.⁵ indicates that the endogenous repopulating CFU compartment might have as its origin the subpopulation of CFU in the marrow that is in cell cycle. Because this subpopulation comprises only a small fraction

of the total number of CFU found in the marrow,³ a restriction in the rate at which marrow CFU enter cell cycle would not necessarily be expected to reduce greatly the total number of marrow CFU. Thus, it is significant that Sutherland et al.¹⁶ and Fried et al.⁷ observed a reduced rate of CFU growth in femurs of S1/S1^d mice. Apparently this disorder affects only a small percentage of the marrow CFU, for in the present work and work of McCulloch et al.¹² and Wolf¹⁸ the number of CFU found in the marrow of S1/S1^d mice was only slightly less than the number found in the marrow of normal congenic littermates. If it is assumed that in (WC x C57BL/6J)F₁ mice a correlation exists between the endogenous repopulating compartment and the number of circulating CFU, it would be expected that a restriction in the rate at which marrow CFU enter cell cycle would depress the number of circulating CFU several fold.

In summary, it was found that following a single intraperitoneal injection of B. pertussis vaccine, mice of genotype S1/S1^d have a reduced circulating CFU population compared to their normal congenic littermates. Presumably, this reduced circulating CFU population is a result of a defect in a marrow stromal element which restricts the rate at which marrow CFU enter cell cycle. This might explain, in part, the lack of a normal increase in the splenic CFU population following treatment with various bacterial preparations.

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13. ABSTRACT

The ability of a single injection of Bordetella pertussis vaccine to cause an increase in the number of circulating transplantable colony forming units (CFU) in mice of genotype S1/S1^d and their normal congeneric littermates was studied. Seventy-six hours after injection of B. pertussis vaccine, the number of circulating CFU per milliliter of blood in normal mice increased from approximately 45 CFU/ml to over 300 CFU/ml, while in S1/S1^d mice the number of circulating CFU increased from approximately 22 CFU/ml to only 57 CFU/ml. Thus, the previously reported restriction of splenic CFU growth in S1/S1^d mice following multiple injections of endotoxin is discussed in the present work not only in terms of a defect in splenic stromal elements necessary for maximum CFU growth, but also in view of current concepts of CFU migration from marrow to spleen via the circulation.