Characteristics of the CFU-s population in mice carrying the Sl^j allele

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Abstract. A tentative characterization of haemopoietic stem cells with respect to their organ distribution, seeding fraction and colony formation in the spleen, radiosensitivity and humoral regulation was attempted in mice heterozygous for the mutant allele Sl^j and in their normal littermates. Sl^j/+ mice were characterized by a deficient CFU-s content of the blood and spleen and had slightly lower femoral CFU-s numbers. This CFU-s distribution could not be explained by differences in seeding efficiency 'f' between CFU-s of Sl^j/+ and +/+ origin in lethally irradiated recipients used in the CFU-s assay. The seeding fraction of CFU-s of +/+ origin did not differ in +/+ and Sl^j/+ recipients. However, in irradiated Sl^j/+ recipient mice a 30% decrease was observed in the number of the colonies derived from splenic and femoral CFU-s of both +/+ and Sl^j/+ origin. The serum level of SHSF (splenic haemopoiesis stimulating factor) was decreased in Sl^j/+ mice, but significantly increased in Sl/Sl^d mice, as compared to their respective normal +/+ littermates. Endogenous colony formation in Sl^j/+ spleens was deficient in comparison to that observed in +/+ spleens, and distinct sex differences were observed. However, mutant and normal CFU-s from spleen and bone marrow had a similar survival following *in-vitro* γ irradiation.

Femurs and spleens of both Sl^j/+ and +/+ origin were implanted into both Sl^j/+ and +/+ hosts. Six weeks later the Sl^j/+ grafts contained less CFU-s than the +/+ grafts.

These data show that the splenic stroma of Sl^j/+ mice is not defective in its capacity to lodge injected CFU-s but is deficient in its ability to maintain CFU-s under 'steady-state' conditions and stimulate their colony formation in a 'perturbed state'. Some of the characteristics of Sl^j/+ mice segregate them from Sl/Sl^d mice, i.e. a deficient splenic CFU-s content, normal seeding fractions 'f' of CFU-s from spleen and bone marrow in the presence of an almost compensated anemia, and decreased serum levels of SHSF. The study of the Sl^j trait may be a useful extension of the current Sl/Sl^d model for exploration of hereditary defects in haematopoietic stroma.

From a series of alleles detected at the highly mutable 'steel' locus on chromosome 10 in mice (Russell & Bernstein, 1966) the 'Steel-Dickie' (Sl^d/Sl^d or Sl/Sl^d) allele combinations have

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received much attention in the past 26 years with respect to the associated haematological anomalies (Russell, 1979). The Sl^d/Sl^d, Sl/Sl^d and all heterozygote mice survive birth, but both Sl/Sl and Sl^j/Sl^j genotypes result in pre-natal death. Adult mice, homozygous for the 'steel' locus, are characterized by severe macrocytic normochromic anaemia, lack of hair pigmentation and sterility. The observations that Sl/Sl^d mice have an essentially non-erythropoietic spleen, a quantitative deficiency of early erythrocytic precursors in spleen, bone marrow (Wiktor-Jedrzejczak *et al.*, 1979) and blood (McCarthy & MacVittie, 1975) and a defective microenvironment for macroscopic erythrocytic spleen colonies (McCulloch *et al.*, 1965; Wolf, 1974) in conjunction with high serum erythropoietin levels (Bernstein, Russell & Keighley, 1968) have supported the view that the major disruption of erythropoiesis is early and more severe than in the granulocytic cell population (McCarthy, Ledney & Mitchell, 1973; Cole *et al.*, 1975).

The haematopoietic stroma in the deficient mice is considered to lack a positive proliferative stimulus for colony formation (Wolf, 1978a, b) and this defect is probably located in a relatively radioresistant stromal cell class (Fried *et al.*, 1973a). A small complement of normal cells appears to be adequate to ensure normal erythropoiesis permanently in mosaic mice carrying a preponderance of the 'steel' locus (Mintz & Cronmiller, 1978). Alleviation of the anaemia can only be produced by transplanting a normal spleen but not by normal stem-cell transfusions (Bernstein, 1970).

The evidence obtained so far indicates that the anaemia has a dual nature and is due not only to a defect in the haematopoietic microenvironment but as well as to a 5–10-fold increased periodic loss of blood *via* the intestinal tract (Kales, Fried & Gurney, 1966; Wolf, 1978a). In our opinion, such a dramatic blood loss might obfuscate the study of the stromal defect in Sl/Sl^d mice, since experimentally induced anaemia in mice has been shown to be associated with considerable changes in the population size of CFU-s, BFU-e and CFU-e in spleen and femur (Hara & Ogawa, 1976, 1977; Ploemacher, Van Soest & Vos, 1977). In a previous study (Brockbank & Ploemacher, 1983), we have shown that $Sl^j/+$ mice have a normal low erythrocyte loss *via* their gut. The Sl^j locus has been demonstrated to have effects upon haematopoiesis in fetuses homozygous for the Sl^j allele (Cole *et al.*, 1975) and in allophenic mice formed by injection of OTT 6050 male teratocarcinoma (129 strain) transplant line germ cells into normal WH blastocysts (Mintz & Cronmiller, 1978). Both groups of authors agree on the notion that the primary lesion in the anaemia resides in the haematopoietic microenvironment. To investigate the applicability of the Sl^j model in stroma-related research we have characterized the CFU-s population of adult 129 mice, heterozygous for the Sl^j allele and their normal littermates, with respect to organ distribution, seeding behaviour, colony formation, radiosensitivity and humoral regulation. Attempts were also made to investigate stromal function and humoral CFU-s regulation in these mice. Our results indicate that the $Sl^j/+$ gene complement is associated with a stromal defect, which causes a significant deficiency of CFU-s in their spleen, blood and bone marrow.

MATERIALS AND METHODS

Animals

129/SvSl^{j}CP mice of both sexes were derived from animals originally obtained from the Jackson Laboratory, Bar Harbor, Maine, U.S.A. $Sl^j/+$ and normal $+/+$ mice were bred at the Laboratory Animal Centre of the Erasmus University by natural matings between male $Sl^j/+$ and female $+/+$ mice, yielding 50% $+/+$ and 50% $Sl^j/+$ segregants. Selection of the defective mice could be made from their lighter grey-brownish coat (agouti) with scattered grey hairs and diminished pigmentation of their feet and tail end. The mice were used at 12–20 weeks of

age. Male C57BL/Rij mice, 48–54 weeks of age, were purchased from the Dutch Reactor Centre in Petten, and 4–10 week-old male WCB6F1/J mice were obtained from Jackson Laboratory and maintained at the Erasmus University under clean conventional conditions. The animals were maintained under constant light. Food pellets and acidified (pH 2·5) water were available *ad libitum*.

Blood analyses
Blood was withdrawn from the retro-orbital sinus under ether anaesthesia and collected in heparinized tubes. Standard procedures were employed to measure red blood cells (RBC) and nucleated cell numbers in a Coulter particle counter and to determine the haematocrit (Hct) in an International microcapillary centrifuge. The mean cell volume (MCV) of RBC was calculated from the preceding data.

Assays for *in-vivo* colony formation and CFU-s seeding efficiency
A Gammacell 40 Caesium 137 irradiation unit (Atomic Energy of Canada Ltd, Ottawa) was used to irradiate animals and cell suspensions *in vitro*. Total body irradiation at a dose rate of 1·31 Gy min^{-1} was used for recipients in the *in-vivo* colony-forming assays. A modified logarithmic transformation was used for evaluation of the endogenous colony counts to correct for their skewed distribution (Smith, Budd & Cornfield, 1966).

The CFU assay of Till and McCulloch (1961) was performed using both 9 Gy irradiated Sl^J/+ and +/+ mice as recipients. The 24-hr seeding efficiencies 'f' of CFU-s were determined according to the method of Siminovitch, McCulloch and Till (1963). Briefly, femoral marrow or spleen cells from pools of three Sl^J/+ or +/+ mice were injected i.v. into three or four intermediate recipients with Sl^J/+ or +/+ gene complements. Twenty-four hr later the mice were killed, their spleens removed, and 1/5 to 1/10 of a spleen was than injected into 10 +/+ secondary recipients. The CFU-s content of the original cell suspensions was determined in 10 primary recipients by the *in-vivo* CFU-s assay. *In-vitro* irradiated spleen and marrow cells were transplanted into lethally irradiated recipient mice within 3 hr following irradiation. In both the transplant assay and the endogenous spleen colony assay spleens were fixed in Bouin Hollande's fluid on day 8 following irradiation. Diameters of spleen surface colonies were then estimated in tenths of a mm using an inverted microscope and a fine-scale transparent matrix.

Implantation method
Spleens and femurs were removed after killing implant donors by cervical dislocation. They were freed of fat and tendons, respectively, and eight small incisions were made in the splenic hilus to ensure optimal regeneration. The epiphyseal ends of the femurs were cut off and spleens and femurs were placed in ice-cold saline containing 10^6 U penicillin and 1·0 g of streptomycin litre^{-1} while the hosts were being prepared for the implantation. Host mice were anaesthetized with Nembutal. The abdominal skin was vertically incised over the midline and undermined for a distance of about 1½ cm in each direction using blunt-tipped forceps. The organs to be implanted were s.c. positioned as far from the incision as possible and the wound closed. Each host received one spleen or two femurs. Six weeks later, the implants were removed and their cellularity and CFU-s content determined.

Detection system for splenic haemopoiesis-stimulating factor (SHSF)
The serum activity of SHSF (Staber & Metcalf, 1980a, b) was quantified *in vivo* by measurement of the splenic CFU-s accumulation induced by injection of the respective sera. Blood was obtained sterilely from unperturbed +/+ and Sl^J/+ mice and clotting was allowed

for 1 hr at room temperature. After storage at 4°C overnight, serum was collected by two consecutive centrifugations at 500 g and stored at −20°C until use. The level of pyrogens in the sera was estimated using the *Limulus* amoebocyte lysate assay as described by Thomas *et al.* (1981). Four days after +/+ and Sl^j/+ mice had been i.p. injected with 0·3 ml of the respective sera, their spleens were removed and the CFU-s content determined using the CFU assay of Till and McCulloch (1961).

RESULTS

Colony-forming ability of +/+ and Sl^j/+ haematopoietic cells in normal +/+ and anaemic Sl^j/+ recipients

From Table 1 it is apparent that Sl^j/+ mice have a mild macrocytic anaemia. Red blood cell (RBC) numbers and MCV values showed no overlap in the two congenic groups, while Hct values differed slightly but significantly. No sex differences were observed in the peripheral blood picture. Leucocyte numbers in Sl^j/+ mice were similar to those found in their normal littermates. Significantly less circulating CFU-s were found in the Sl^j/+ blood.

No significant differences in the femoral and splenic cellularity were noted between +/+ and Sl^j/+ mice (Table 2). A slightly lower colony-forming ability (75%) of Sl^j/+ femoral cells

Table 1. Some haematological parameters in strain 129 mouse blood

	+/+		Sl^j/+	
	Males	Females	Males	Females
Hct (%)	49·99 (0·36)*	49·33 (0·64)	47·23 (0·65)	47·25 (0·67)
Erythrocytes ml^{-1} ($\times 10^9$)	8·64 (0·39)	9·43 (0·28)	7·72 (0·24)	8·10 (0·12)
MCV (μm^3)	55·68 (1·21)	51·83 (1·02)	60·31 (2·03)	58·13 (1·79)
Leucocytes ml^{-1} ($\times 10^6$)	11·48 (3·23)	11·68 (2·26)	11·53 (2·38)	10·88 (1·76)
CFU-s ml^{-1}	49 (3)†		19 (6)	

* Arithmetic mean of twelve experiments (1 SE).

† Arithmetic mean of four experiments (1 SE). In two experiments male mice were used. All mice were 20 weeks of age. Colony formation was assayed in normal +/+ mice.

Table 2. Femoral and splenic cellularity and CFU-s incidence in strain 129 mice

	Femur		Spleen	
	+/+	Sl^j/+	+/+	Sl^j/+
Cellularity $\times 10^7$				
males	2·77 (0·41)*	2·14 (0·28)	16·5 (0·5)	16·3 (0·9)
females	2·04 (0·18)	1·94 (0·22)	15·8 (1·3)	15·4 (1·0)
males + females	2·41 (0·25)	2·04 (0·17)	16·0 (0·8)	15·7 (0·7)
CFU-s 10^{-7} nucl. cells				
males	2279 (384)	2464 (758)	281 (62)	149 (60)
females	2124 (230)	1583 (304)	325 (33)	135 (22)
males + females	2221 (224)	1916 (440)	300 (30)	140 (22)

* Arithmetic mean (1 SE) of three (male) or eight (female) separate experiments. Colony formation was assayed in normal +/+ mice.

Table 3. Seeding efficiency (24 hr) of femoral and splenic
cells in 129 mice

Cell suspension from	Genotype		24 hr seeding*
	Donor	1st recipient	
Spleen	+/+	+/+	2·66 ± 0·27†
	Sl^j/+	+/+	3·70 ± 0·66
	+/+	Sl^j/+	2·94 ± 0·27
Spleen	+/+	+/+	2·47 ± 0·22
	Sl^j/+	+/+	2·41 ± 0·66
	+/+	Sl^j/+	3·92 ± 0·66

* The percentage of seeded CFU-s was expressed relative
to the number of colonies evoked by injection of cells into
+/+ recipients. Second recipients were normal +/+ mice.

† Arithmetic mean of three separate experiments ± 1 SE.

was found in females. In contrast, the colony-forming potential of the $Sl^j/+$ spleen cells was observed to be only 42–53% of +/+ spleen cells in both males and females.

From Table 3 it is apparent that the 24-h seeding efficiency of both $Sl^j/+$ bone marrow and spleen cells was not significantly different from that of +/+ cells. Also, +/+ bone marrow and spleen cells had similar 24-h seeding efficiencies in irradiated +/+ and $Sl^j/+$ recipient mice. These observations imply that the data presented in Tables 2, 4 and 5 have a realistic basis and do not require correction for differences in seeding efficiency.

In Table 4 the femoral and splenic CFU-s content is listed according to the sex of the mice. Irrespective of the genotype of the irradiated recipients, $Sl^j/+$ femurs were found to have slightly less CFU-s per femur and a significantly reduced splenic CFU-s content. The data also clearly demonstrate that the $Sl^j/+$ splenic microenvironment is defective in its ability to optimally stimulate colony formation of bone marrow or spleen cells whether derived from +/+ or $Sl^j/+$ animals. The deficient colony number per spleen cannot be explained by a lower 24-hr seeding efficiency of +/+ CFU-s in the anaemic recipients (Table 3).

Because of relatively large variations between the separate experiments influencing standard errors, the data from Table 4 have been expressed as percentages of the +/+ femoral

Table 4. Femoral and splenic CFU-s content in strain 129 mice

Genotype recipient*	Sex	Femur		Spleen	
		+/+	Sl^j/+	+/+	Sl^j/+
+/+	♀†	4627 (524)	3723 (529)	5457 (639)	1915 (355)
Sl^j/+		3236 (327)	2943 (369)	3406 (509)	1486 (300)
+/+	♂‡	6664 (382)	5178 (1196)	4191 (1067)	1797 (467)
Sl^j/+		4489 (498)	4305 (665)	2779 (280)	1332 (677)

* Lethally irradiated recipient in spleen colony assay.

† Presented are the arithmetic means (±1 SE) of eight separate experiments in which female mice of 15–54 weeks of age were used.

‡ Presented are the arithmetic means (±1 SE) of three separate experiments in which male mice of 11–39 weeks of age were used.

Table 5. Colony formation in +/+ and $Sl^j/+$ recipient mice

Genotype recipient	Femur		Spleen	
	+/+	$sl^j/+$	+/+	$sl^j/+$
+/+	100	82·42 (10·89)	100	36·93 (3·46)
$Sl^j/+$	70·07 (3·83)	65·74 (6·86)	65·98 (6·65)	26·86 (3·08)
ratio +/+ vs. $Sl^j/+$ recipient	70·07	79·76	65·98	72·73

Number of CFU-s organ^{-1} expressed as % +/+ CFU-s forming colonies in irradiated +/+ recipients.

Arithmetic mean of 10–11 experiments with 1 SE in parentheses.

and splenic CFU-s content as determined in +/+ recipients (Table 5). The observation that the colony number reduction in $Sl^j/+$ hosts was constant (approximately 30%) irrespective of the origin of the infused cells, suggests that +/+ and $Sl^j/+$ CFU-s have comparable sensitivities to the proliferative stimulus for colony formation exerted by the hosts' spleen.

The mean diameter of the four largest colonies per spleen evoked by injection of +/+ bone-marrow cells and spleen cells in normal and defective recipients was not significantly different. Femoral cells formed colonies with a mean diameter of 1·12 mm (1 SE, 0·03) in +/+ recipients and 1·03 (0·03) mm in $Sl^j/+$ recipients. For splenic cells these data were respectively 1·08 (0·03) mm in +/+ recipient mice and 1·07 (0·04) mm in $Sl^j/+$ mice.

Haematopoiesis in implanted spleens and femurs

At 6 weeks' post-implantation, following the regeneration of the splenic and femoral stroma, the cellularity and CFU-s number (Table 6) of +/+ implants exceeded that of $Sl^j/+$ implants

Table 6. CFU-s content and cellularity of implanted +/+ and $Sl^j/+$ spleens and femurs as function of the host status

Organ content	Host (genotype/status)	Implanted organs			
		Spleen		Femur	
		+/+	$Sl^j/+$	+/+	$Sl^j/+$
CFU-s	+/+	58 (25)‡	22 (11)	1061 (122)	601 (151)
	$Sl^j/+$	120 (9)	37 (27)	715 (140)	608 (124)
	+/+ (irr)†	225 (16)	51 (4)	1054 (60)	832 (248)
Cellularity $\times 10^6$	+/+	14·28 (3·81)	8·90 (1·06)	8·38 (1·15)	8·29 (1·19)
	$Sl^j/+$	19·61 (2·66)	10·46 (2·59)	11·08 (1·18)	8·05 (0·86)
	+/+ (irr)	15·00 (0·08)	8·80 (1·98)	10·84 (1·23)	8·64 (2·77)

* Lethally irradiated +/+ mice were used in the CFU-s assay.

† Hosts received 5 Gy total body irradiation 2 days before organs were implanted.

‡ Arithmetic mean of four separate experiments (1 SE).

Table 7. Effects of serum from mice carrying the 'steel' allele and
their normal littermates on spleen CFU-s levels

Normal serum injected	Splenic CFU-s content ($\times 10^3$)		
	$+/+$ mice	Sl^j/$+$ mice	C57BL/Rij mice
None	4·2 (1·1)	1·8 (0·5)	6·6 (2·4)
$+/+$*	12·7 (1·4)	5·3 (0·8)	—
Sl^j/$+$	8·1 (0·4)	2·5 (0·7)	—
$+/+$†	—	—	9·8 (2·7)
Sl/Sl^d	—	—	18·2 (4·9)

* 0·3 ml of serum was injected i.p. Mice were killed 4 days
later. The serum batch was prepared by pooling the sera from 80
mice per experimental point.

† At 0, 6 and 24 hr 0·1 ml of serum was injected i.p. Mice were
killed at day 4.

‡ Arithmetic mean (± 1 SE) of three individually assayed mice.

in both $+/+$ and Sl^j/$+$ hosts. The CFU-s content of splenic implants in Sl^j/$+$ hosts and preirradiated $+/+$ hosts exceeded that in $+/+$ hosts. The latter observation indicates that Sl^j/$+$ mice, similar to irradiated mice, have higher levels of undefined systemic factors, which enhance the implant regeneration.

The SHSF level in normal serum of Sl^j/$+$ and Sl/Sl^d mice and their respective normal $+/+$ littermates

In order to investigate whether the serum of Sl^j/$+$ mice also contained higher levels of a factor which would activate CFU-s to proliferate in the spleen (SHSF—splenic haemopoiesis stimulating factor), 0·3 ml of normal $+/+$ or Sl^j/$+$ serum was injected i.p. into both normal and mutant mice. Four days later an enhanced splenic CFU-s content in $+/+$ and Sl^j/$+$ mice was observed (Table 7). The spleen of both Sl^j/$+$ mice and their normal littermates contained significantly more CFU-s following injection of $+/+$ serum than following Sl^j/$+$ serum. This observation suggests that Sl^j/$+$ mice do not show increased humoral stimulation of CFU-s proliferation in an attempt to compensate for the deficient splenic CFU-s content.

From Table 7 it is also apparent that normal mouse serum from Sl/Sl^d mice was significantly more potent than was serum from their normal $+/+$ littermates in evoking a splenic CFU-s accumulation in the spleen of C57BL/Rij mice. This relatively high serum SHSF level in Sl/Sl^d mice suggests that these stromal-defective mice exert an increased systemic stimulus for CFU-s proliferation in response to the severe macrocytic anaemia suffered by these mice.

Endogenous colony formation

Upon sublethal irradiation, surviving CFU-s formed colonies in the irradiated animal's spleen and bone marrow. Sl^j/$+$ mice of both sexes were characterized by a lower endogenous spleen colony formation than occurred in $+/+$ mice (Fig. 1). The average difference in the 3·3–7·0 Gy range was 5·7 for females and 3·1 for males. The endogenous CFU-s of $+/+$ origin were characterized by a D_{37} of 0·93 Gy for females and 0·84 Gy for males, whereas D_{37} values for Sl^j/$+$ females were 0·75 Gy and for males 0·77 Gy.
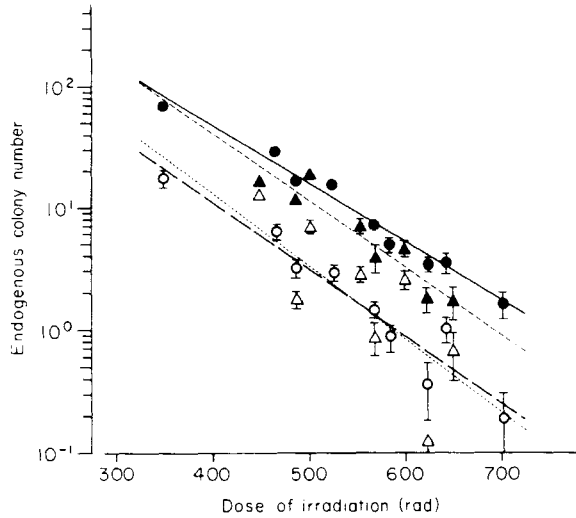
Fig. 1. Radiosensitivity of endogenous spleen colony-forming cells. A logarithmic transformation according to Smith *et al.* (1966) was employed to correct for the skewed endogenous colony distribution. Open symbols refer to $Sl^j/+$ mice ($D_{37} = 0.75–0.77$ Gy), solid symbols to $+/+$ mice ($D_{37} = 0.84–0.93$ Gy). The circular symbols represent females, the triangles males. Presented are the results of separate experiments as the mean colony count on ten spleens (1 SE) per experimental point. SE bars, which are not indicated in the figure, fall within the limits of the symbols used.
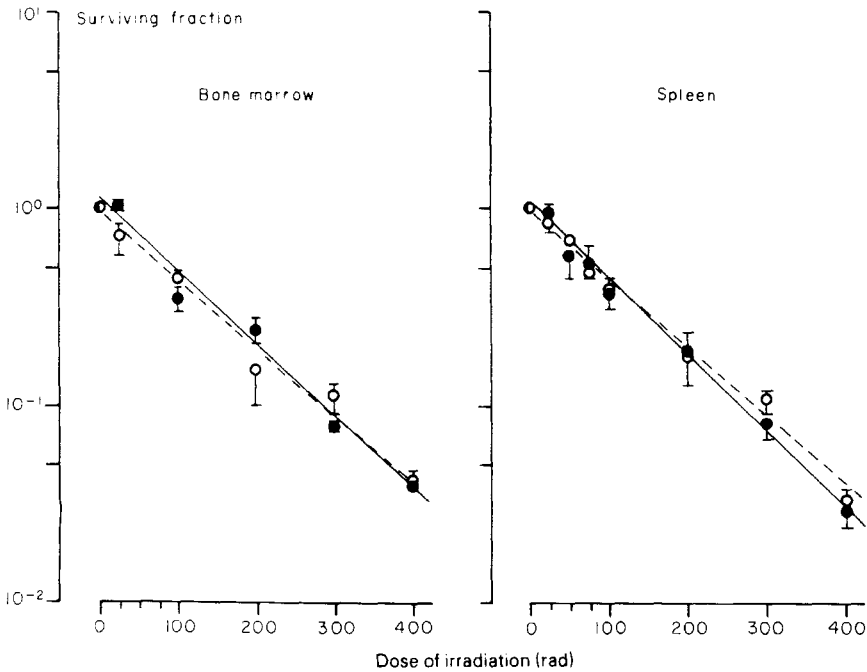
Fig. 2. Survival curve for *in-vitro* irradiated CFU-s from spleen and bone marrow. Open symbols refer to $Sl^j/+$ mice, solid symbols to $+/+$ mice. Presented are the arithmetic mean (± 1 SE) of nine individual experiments. Marrow CFU-s of $+/+$ mice had a D_{37} of 1.22 Gy and extrapolation number of 1.13. D_{37} for $Sl^j/+$ marrow was 1.37 Gy, extrapolation number 0.98. Spleen CFU-s of $+/+$ mice, D_{37} of 1.13 Gy, extrapolation number 1.10; $Sl^j/+$ spleen CFU-s had a D_{37} of 1.27 Gy and extrapolation number of 0.98.

Radiosensitivity of CFU-s

The γ-radiation survivial of *in-vitro* irradiated spleen and bone marrow CFU-s of $+/+$ and $Sl^j/+$ female mice is shown in Fig. 2. The survival curve for $+/+$ marrow CFU-s had a D_{37} of $1 \cdot 22$ Gy and an extrapolation number of $1 \cdot 13$ and did not differ significantly from that of $Sl^j/+$ marrow CFU-s (D_{37}, $1 \cdot 37$ Gy; extrapolation number $0 \cdot 98$). Spleen CFU-s from mutant and normal littermates also showed similar radiation-survival characteristics. The D_{37} of the survival curve for $+/+$ spleen CFU-s was $1 \cdot 13$ Gy with an extrapolation number of $1 \cdot 10$, while $Sl^j/+$ spleen CFU-s ahd a D_{37} of $1 \cdot 27$ Gy and an extrapolation number of $0 \cdot 98$.

DISCUSSION

The present data form a first attempt toward a characterization of the CFU-s population in adult 129 mice carrying the $Sl^j/+$ and $+/+$ gene complements. They extend the observation on a microenvironmental lesion caused by the Sl^j allele. The $Sl^j/+$ mice have a mild but definite macrocytic anaemia. Their circulating and splenic CFU-s were found to be reduced (being 39 and 42% of $+/+$ mice respectively) and the femoral CFU-s content (82%) was subnormal (Tables 1 and 5). It might be argued that the reduction in splenic and peripheral-blood CFU-s seen in $Sl^j/+$ mice is not due to reduced numbers of true pluripotent CFU-s but to reduction of erythroid-committed stem-cell progenitors relative to myeloid committed progenitors, which may contribute to colony formation (Kretchmar & Conover, 1970). However, the BFU-e and CFU-g/m population size have been demonstrated to be similarly reduced both in spleen and femur of $Sl^j/+$ mice, rendering this possibility unlikely (Brockbank & Ploemacher, 1983).

It has been suggested that there is a strong stimulus for differentiation in Sl/Sl^d mice, which sustains a normal splenic CFU-s content despite the apparent defect of the splenic stroma (Wolf, 1974; Bernstein *et al.*, 1968; McCarthy, Ledney & Mitchell, 1977; Harrison, Malathi & Silber, 1975; McCarthy, 1977). We propose that this strong stimulus may be absent in $Sl^j/+$ mice, due to the mild nature of the anaemia, resulting in a decreased CFU-s population, which is most evident in the spleen. Although we realize that only with extreme caution may one compare $Sl^j/+$ and Sl/Sl^d mice, due to their different genetic background, direct support for this hypothesis has been obtained in the present experiments, which showed that $Sl^j/+$ mice had decreased serum levels of SHSF, whereas the SHSF level was increased in Sl/Sl^d mice as compared to their respective normal $+/+$ littermates. The SHSF has been suggested to be a single factor (Staber & Metcalf, 1980b), which activates CFU-s to proliferate in the spleen. The accompanying rise in splenic levels of various haemopoietic progenitor cells has been held not to be mediated by the corresponding types of CSF (Staber & Johnson, 1980). It seems unlikely that in $Sl^j/+$ and Sl/Sl^d mice the stromal defect is mediated by a defective SHSF or stem-cell-activating factor (SAF, Cerny, 1975) regulation, because both mutant mouse strains have a normal enhanced serum level of these regulators following lipo-polysaccharide injection (Ploemacher *et al.*, submitted for publication).

$Sl^j/+$ mice were demonstrated to have a deficiency of endogenous spleen colony formation following sublethal doses of irradiation (Fig. 1). At least two factors may have contributed to this observation, i.e. (a) differences in the original splenic population size, and (b) differences in radiosensitivity of CFU-s. The latter possibility has to be disregarded since no differences in survival rate of $+/+$ and $Sl^j/+$ spleen and marrow CFU-s were observed following *in-vitro* γ irradiation (Fig. 2). With respect to the first possibility, Table 5 implies that a 60% reduction of endogenous colony formation may be expected in $Sl^j/+$ spleens irrespective of possible differences in radiosensitivity. The observation that the reduction of the endogenous spleen colony formation was larger than 60% in the female $Sl^j/+$ mice may be due to a difference in

the defective splenic support for the survival or proliferation of sublethally irradiated CFU-s. Similarly, such a difference in splenic support may be responsible for the observed sex differences. This observation strongly suggests that the local microenvironment is able to influence the survival rate of *in-vivo* irradiated CFU-s.

Two observations in the present study contributed to the notion, that $Sl^j/+$ mice have a defective stroma of the haemopoietic organs. At first, normal and mutant CFU-s from both spleen and marrow formed less colonies in $Sl^j/+$ spleens than in $+/+$ spleens, The inhibition of colony formation was not caused by lower seeding fractions of these CFU-s into the $Sl^j/+$ spleens (Table 3), but apparently the seeded CFU-s were less stimulated than in normal $+/+$ spleens to form detectable colonies. Secondly, spleens and femurs of $+/+$ origin regenerated better than $Sl^j/+$ spleens and femurs as measured by the implanted organ's cellularity and CFU-s content. The haematopoietic cells which grow in such implants originate predominantly from host CFU-s (Amsel & Dell, 1971; Fried *et al.*, 1973b; Adler *et al.*, 1980), whereas the supporting microenvironmental tissues are of donor origin (Friedenstein *et al.*, 1968; Tavassoli *et al.*, 1973; Wolf, 1982). It has been shown that increasing doses of X-rays given to the implant donors increasingly impair the ability of the implants to support haemopoiesis (Fried *et al.*, 1976; Ploemacher & Van Soest, 1979) and that freezing the tissues prior to implantation abolishes this ability (Fried *et al.*, 1973a). These observations support the concept that the stromal factors determining implant regeneration are cellular. The present findings therefore suggest that in $Sl^j/+$ mice the stromal cell population, which is necessary to support CFU-s proliferation and maintenance, is either smaller than in normal mice and sparsely distributed, or its individual component cells are less effective, or both. This concept is consistent with the observations on the defective stroma in Sl/Sl^d mice (Fried *et al.*, 1973a).

Splenic implants carried by $Sl^j/+$ hosts and preirradiated $+/+$ hosts contained more CFU-s than those in normal $+/+$ hosts (Table 5). This indicates that $Sl^j/+$ mice, similar to irradiated mice, have higher levels of as-yet-undefined systemic factors responsible for enhancement of splenic implant regeneration. Such systemic factors probably do not include SHSF (Table 7). The non-responsiveness of femoral implants to these factors might be explained by the observation that the total splenic mass in any one animal is controlled by certain factors (Crosby, 1963; Metcalf, 1963; Tavassoli *et al.*, 1973), whereas that of the bone-marrow mass is not (Chertkov *et al.*, 1980). This suggests that the spleen is more sensitive for the action of growth enhancing factors.

We conclude that the $Sl^j/+$ spleen shows defective CFU-s maintenance under 'steady-state' conditions and that the local proliferative stimulus for colony formation (as measured by the number of developing colonies) is decreased or repressed by some unknown mechanism. At the same time the splenic stroma of $Sl^j/+$ mice is not defective in its capacity to lodge injected CFU-s. This evidence supports previous studies suggesting that lodging and proliferative stimulus for colony formation operate as distinctive functional entities within the haematopoietic microenvironments (Wolf, 1974, 1982; Ploemacher, Brons & Van Soest, 1982). Among differences in a series of haematological parameters in $Sl^j/+$ and $+/+$ mice, the subnormal endogenous colony formation in the spleen is among the most characteristic features of the defect. The phenotypic expression of the Sl^j allele at the level of the haemopoietic microenvironment and the CFU's population size is different to that of the Sl^d allele. The $Sl^j/+$ gene combination therefore appears to be an interesting tool in haematological research.

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