Mutant Sl Alleles of Mice Affect Susceptibility to Friend Spleen Focus-Forming Virus

Abstract. *Mice of genotypes* Sl/Sl^d, $Sl/+$, *and* $Sl^d/+$ *(but not* Sl^T/Sl^T *or* $Sl^T/+$*) were resistant to spleen focus-forming virus. The "environment" in which hemopoietic target cells for this virus develop was not conducive for infection or focus formation, or both, but the target cells were not altered directly. The same genes appear to regulate hemopoiesis and leukemogenic transformation.*

The component of Friend leukemia virus complex which initiates spleen focus formation in mice (SFFV) appears to be closely associated with the component initiating splenic erythropoiesis (*1*). Alleles at the *W* (dominant spotting) locus control the availability or quality, or both, of target cells for SFFV, since mice bearing certain mutant alleles at this locus are refractory to this virus, unless they have been previously implanted with bone marrow cells from normal littermates (*2*). This report describes the refractoriness to SFFV conferred by mutant alleles at the *Sl* (*Steel*) locus (*3*, *4*) which affect hemopoiesis in mice by an entirely different mechanism (*5–7*).

Congenic C3H/He mice of genotypes $Sl/+$, $Sl^T/+$, Sl^T/Sl^T, and $+/+$ were obtained for breeding and for experiments from the animal colony of L. B. Russell; *Sl* is the originally observed mutant at the *Sl* locus (*4*), and Sl^T is a translocation, T(4;?)8Rl, having one break at, or very close to, the *Sl* locus (*8*). Mice of genotypes $Sl^d/+$ were obtained from E. S. Russell (*3*) on the C57BL/6 background, but the mutant allele was transferred onto the C57BL/10ScSn (B10) background by outcrossing and repeated backcrossing. Female C3H/He-$Sl/+$ and male B10-$Sl^d/+$ mice were mated to produce (C3H × B10)F_1 mice of genotypes $+/+$, $Sl^d/+$, $Sl/+$, and Sl/Sl^d. Mice of both sexes and of various ages were used. The susceptibility of $+/+$ mice to the lines of SFFV used decreased slightly after the mice were 3 months old.

Two lines of SFFV, known as BSB and BB6, were adapted to grow and to induce spleen foci in C57BL/Ha mice (*2*). A third line of SFFV (PSE) was passaged through Ha/ICR Swiss mice. Virus preparations used for infection were cell-free filtrates obtained from 10 percent suspensions of homogenized spleen from mice infected with SFFV. The virus was titrated by the spleen-focus assay method (*9*). Titers so obtained in vivo reflect the host susceptibility to the virus as well as the number of infectious viral units inoculated.

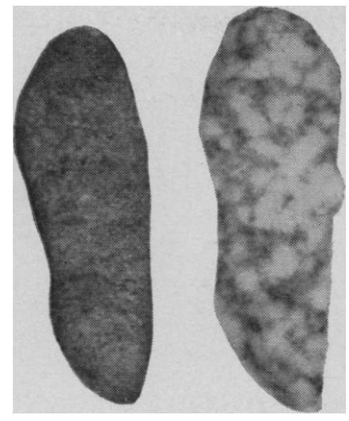

Fig. 1. Spleens from (C3H/He × C57BL/10)F_1 mice 9 days after infection with SFFV-BSB (50 focus-forming units). (Left) Sl/Sl^d genotype; (right) $+/+$ genotype.

The susceptibility of C3H, B10, and (C3H × B10)F_1 mice of the various *Sl* genotypes to the PSE, BSB, and BB6 lines of virus is summarized in Table 1. No foci were detected in the spleens of two Sl/Sl^d mice, while their $+/+$ cage mates developed about 50 foci per spleen (see representative spleens in Fig. 1). Also $Sl/+$ and $Sl^d/+$ mice were relatively resistant to the focus-forming activity of the viruses, each of three and five comparisons, respectively, showing a highly significant difference from the corresponding $+/+$. Like the *W* locus (*2*), the *Sl* locus somehow controls murine susceptibility to the early biologic effects of SFFV.

However, Sl^T/Sl^T and $Sl^T/+$ mice were as susceptible as their $+/+$ cage mates of similar age, none of three comparisons showing a significant difference. The red blood cell counts, hematocrits, and erythrocyte size-distribution plots on one Sl^T/Sl^T and eight $Sl^T/+$ mice were not significantly different from normal. It is of interest that Sl^T is a translocation. If the coat-color phenotype associated with Sl^T is the result of position effect, it could mean that the other pleiotropic changes normally associated with the *Sl* locus do not respond to this position effect.

If refractoriness to SFFV is an intrinsic property of hemopoietic progenitor cells, then this property should be transferable by grafting *Sl* or *W* anemic marrow cells into irradiated, but

Table 1. Susceptibility of congenic mice with mutant alleles at the *Steel* locus to SFFV. Strains of mice are those mentioned in the text; ages are indicated in parentheses. Results are given as means ± standard error, and the number of mice is given in parentheses.

Genotype	Virus dose (FFU)*	Number of foci per spleen	
		PSE line of virus	
		C3H/He (5 to 11 months)	
$+/+$	4	4.0 ± 2.1 (2)	
$Sl^T/+$	4	15.0 ± 6.2 (5)	
Sl^T/Sl^T	4	20 (1)	
		BSB line of virus	
		C57BL/10 (2 to 3 months)	(C3H × B10)F_1 (13 months)
$+/+$	4	3.8 ± 1.1 (10)	5.6 ± 1.5 (7)
$Sl/+$	4		1.0 ± 0.8 (3)
$Sl^d/+$	4	0.0 ± 0.0 (10)	0.5 ± 0.4 (2)
		BB6 line of virus	
		C3H/He (5 to 11 months)	C57BL/10 (2 to 4 months)
$+/+$	4	4.3 ± 1.5 (3)	5.2 ± 1.5 (6)
$Sl^d/+$	4		0.0 ± 0.0 (4)
$Sl^T/+$	4	4.0 ± 1.4 (2)	
$+/+$	24	23.8 ± 4.2 (9)	23.7 ± 5.8 (6)
$Sl/+$	24	3.0 ± 0.9 (4)	
$Sl^d/+$	24		0.2 ± 0.2 (5)
$Sl^T/+$	24	17.0 ± 6.1 (3)	
		BB6 line of virus	
		(C3H × B10)F_1 (2 months)	
$+/+$	50	48.8 ± 0.7 (5)†	
$Sl/+$	50	10.7 ± 3.5 (3)	
$Sl^d/+$	50	9.5 ± 0.4 (2)	
Sl/Sl^d	50	0.0 ± 0.0 (2)	

* Input virus doses as titrated in $+/+$ mice of the same age and strain. † Number of foci were too numerous to count accurately in these mice.

otherwise normal, recipient mice. Strain (C3H/He × C57BL/Ha)F_1 male mice (12 weeks old) were exposed to 900 r of x-radiation and infused intravenously with 1.9 to 3.8 × 10^6 bone marrow cells taken from (i) (C3H × B10)F_1 mice of genotypes +/+, *Sl*/+, *Sl*^{d}/+, or *Sl*/*Sl*^{d} or (ii) (B10 × WB/Re)F_1 mice of genotypes *W*/+ or *W*^{v}/+. While progenitor cells of both *W*/+ and *W*^{v}/+ mice function deficiently (*4*), only *W*^{v}/+ mice are refractory to SFFV (*2*). Four weeks after transplantation, the marrow grafts of donor mice bearing one or more *Sl* mutant alleles did not confer refractoriness to SFFV to recipients (Table 2, *A*). However, there is some indication that *W*^{v}/+ marrow grafts rendered recipient mice more refractory to SFFV than did their *W*/+ counterparts (Table 2, *B*), although the difference is not significant ($P = .06$). These data suggest that progenitor cells of *Sl* mice are intrinsically susceptible to SFFV, while the opposite is probably true for progenitor cells of *W*^{v}/+ (and, presumably, *W*^{v}/*W*) mice.

If *Sl* anemic mice are refractory to SFFV because of an abnormal hemopoietic "environment" (*7*), then susceptibility to SFFV should *not* be transferable to such mice by normal (+/+) marrow cells. Preliminary experiments revealed that irradiated *Sl*/+ (unlike *Sl*/*Sl*^{d}) mice accepted marrow grafts like +/+ mice. Therefore, (C3H × B10)F_1 mice of genotypes +/+, *Sl*/+, and *Sl*^{d}/+ (8 weeks old) were exposed to 650 r of x-radiation and infused with 29.7 × 10^6 normal (+/+) (C3H/He × C57BL/Ha)F_1 marrow cells. Four weeks after transplantation, mice of *Sl*/+ and *Sl*^{d}/+ genotypes remained refractory to SFFV (Table 2, *C*). This result is in keeping with the abnormal environment hypothesis.

Since *Sl*/+, *Sl*^{d}/+, and *W*^{v}/+ mice are slightly anemic (*3*) and *Sl*^{T}/+ mice are normocytic, it is conceivable that anemia alone could have caused refractoriness to SFFV. Normal (C3H × C57BL/Ha)F_1 male mice (8 weeks old) were bled from their tail veins each day for 21 days (about 50 μl per day). Susceptibility to SFFV (BSB line) was determined after the last bleeding. Hematocrit values of the mice that were bled were 37 to 40 percent (normal is 42 to 48 percent). The spleens of these mice contained 0, 0, 1, 1, 1, 2, 3, 9, 9 (mean 2.9) foci, and the spleens of their nonanemic age controls contained 0, 0, 1, 2, 2, 3, 5, 5, 5, 6 (mean 2.9) foci. Thus, blood-loss anemia did not render mice refractory to SFFV.

Table 2. Susceptibility of mice to SFFV-BSB 4 weeks after x-irradiation and infusion of bone marrow cells. SFFV-BSB is the line of SFFV adapted to C57BL/Ha mice; all recipients received 12 focus-forming units as titrated in C57BL/Ha mice. Within group A, the mean counts from mutant donors were not significantly different from wild-type donors. In group B, the *P* value was .06; and in group C, the mean counts of mutant recipients were significantly lower ($P < .001$) than wild-type recipients. Results are means ± standard error; number of mice is given in parentheses.

Donor marrow		Recipient	
Genotype	No. cells infused (× 10^6)	Genotype	No. of foci per spleen
A. (C3H × B10)F$_1$ marrow cells injected into (C3H × C57BL)F$_1$ recipients			
+/+	1.98	+/+	4.7 ± 2.0(10)
Sl/*Sl*^{a}	1.96	+/+	6.1 ± 1.0(13)
+/+	3.21	+/+	6.3 ± 2.0(8)
Sl/+	3.78	+/+	3.1 ± 0.6(8)
Sl^a/+	3.38	+/+	2.8 ± 1.0(5)
B. (B10 × WB)F$_1$ marrow cells injected into (C3H × C57BL)F$_1$ recipients			
W/+	3.00	+/+	6.6 ± 3.4(7)
W^v/+	1.86	+/+	0.2 ± 0.1(12)
C. (C3H × C57BL)F$_1$ marrow cells injected into (C3H × B10)F$_1$ recipients			
+/+	29.7	+/+	6.7 ± 0.3(3)
+/+	29.7	*Sl*^{a}/+	0.0 ± 0.0(3)
+/+	29.7	*Sl*/+	0.3 ± 0.3(3)

In summary, the *Sl* locus affects the environment of SFFV target cells, so that they are either not available for infection or not able to form spleen foci once infected. Susceptibility to SFFV appears to be a sensitive indicator of hemopoietic function by progenitor cells. It is of interest to recognize that steps in normal differentiation and in neoplastic transformation can be influenced by the same genetic loci, by the nature of the hemopoietic environment, and by the nature of progenitor cells themselves.

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References and Notes

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8. L. B. Russell, *Mouse News Letter* **29**, 72 (1963). The abbreviated symbol, *Sl*^{T}, is used for a translocation that so far has shown no recombination with the *Sl* locus. Animals of genotype *Sl*^{T}/+ resemble *Sl*/+ in coat color, are semisterile, and show a ring of four chromosomes at meiosis; *Sl*^{T}/*Sl*^{T} are black-eyed white and viable, and males are fully fertile. Compounds of *Sl*^{T} with *Sl*, or with a large number of other mutations at the *Sl* locus, are black-eyed white, viable, and semisterile.
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Reticuloendothelial Blockade: Effect of Puromycin on Opsonin-Dependent Recovery

Abstract. *Reticuloendothelial blockade induced by the administration of a gelatinized "reticuloendothelial test lipid emulsion" is due to a loss of opsonic activity in the plasma. Recovery from blockade, which is associated with restoration of plasma opsonins, was inhibited by the administration of puromycin. The effect of puromycin appears to be mediated by inhibition of opsonin formation rather than a puromycin-induced macrophage defect in phagocytosis.*

Particle-induced blockade of the reticuloendothelial system (RES) has been the subject of considerable investigation. Recent evidence has suggested that reticuloendothelial blockade is a manifestation of a loss of plasma opsonic activity, that is, loss of factors which promote phagocytosis (*1*) and not, as others have suggested (*2*), a direct saturation of the reticuloendothelial cells. If the blockade is a depletion of opsonic protein, then replen-