The Immune Response in Mice of Genotypes W/W^v and Sl/Sl^{d1} (34161)

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Mutations at the *w* and *sl* loci have pleiotropic effects with defects being observed in hemopoiesis, pigmentation, and gametogenesis (1). Mice of genotype Sl/Sl^d or W/W^v are severely anemic, have no pigment-producing cells in their skin, and are sterile. The cellular bases for the defects are different for each mutation. The W/W^v mice have defective hemopoietic stem cells (2) and defective pigment-producing cells (3). In Sl/Sl^d mice there is a defect in the environment which prevents normal hemopoietic (4) and pigment-producing (3) cells from proliferating. Both mutants are also refractory to infection with Friend leukemia virus; as with hemopoiesis and pigmentation, the lack of susceptibility to virus infection was shown to be a cellular defect in W/W^v (5) and a host defect in Sl/Sl^d (6).

It is of interest to determine whether or not the immune response in W/W^v and Sl/Sl^d mice is also defective. It has been proposed that differentiated cells in the immune system are derived from the same stem cells that supply differentiated cells in the hemopoietic system (7–9). Since the function of hemopoietic stem cells is defective in these mutants, one might anticipate the existence of a defective immune system as well. However, there have been conflicting reports about the immune response in mice homozygous for *W* or *Sl* mutations. For example, McCulloch *et al.* (10) found a normal immune response in both W/W^v and Sl/Sl^d mice, but Shearer and Cudkowicz (11) reported a defective response in W^v/W^v mice. In an attempt to clarify the conflicting data concerning the immune response in W/W^v and Sl/Sl^d mice, we tested the ability of both mutants to respond to sheep erythrocytes (SRBC) in two different assays. In the first test, mice were immunized with SRBC and the number of plaque-forming cells in their spleens measured 4 days later. In the second test, we measured the synergism between bone marrow and thymus cells using the method described by Claman *et al.* (12).

Materials and Methods. Because mice homozygous for either the *W* or *Sl* mutations are sterile, mice of the desired genotypes must be produced as F1 hybrids from parents heterozygous for these alleles. Mice of genotype W/W^v were produced by crossing WB–$W/+$ and C57BL/6–$+/W^v$. Similarly, mice of genotype Sl/Sl^d were produced from crosses of WC–$Sl/+$ and C57BL/6–$+/Sl^d$. The W^v and Sl^d mutations were used because of the poor viability of mice homozygous for *W* and *Sl* alleles. Some W/W^v mice were purchased from Jackson Laboratory, Bar Harbor, Maine. The remaining W/W^v mice and all of the Sl/Sl^d mice used in these experiments were bred in the animal colony at the Ontario Cancer Institute. This breeding program was initiated with mice obtained from Jackson Laboratory.

Mice were irradiated in a ^{137}Cs irradiator (13). All mice to be used as recipients in transplantation assays were given a whole-body dose of 950 rads at a dose rate of 108 rads/min. Following irradiation and transplantation, mice with the exception of those of genotype Sl/Sl^d were kept three to a case. The Sl/Sl^d mice were kept one per cage in an attempt to improve their poor survival following irradiation. All mice were allowed food and water *ad libitum*.

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TABLE I. PFC Response in Mice of Different Genotypes.

Genotype	PFC/spleen (SE)[a]
+/+	93,000 (76,000–110,000)
+/*W*	92,000 (63,000–130,000)
+/*Sl*	150,000 (130,000–170,000)
W/*W*^{v}	140,000 (130,000–160,000)
Sl/*Sl*^{d}	200,000 (170,000–250,000)

[a] The values shown are the geometric means of individual measurements made on 5 mice; the values in parentheses enclose two standard errors.

The antigen used for all of the experiments was sheep erythrocytes (SRBC) (Woodlyn Farms, Guelph, Ontario). Before use either as antigen or an indicator in the assay for antibody-producing cells, the SRBC were washed three times in phosphate-buffered saline (PBS).

Spleen cell suspensions from individual mice were prepared by placing the whole spleen in 0.5 ml of cold CMRL 1066 (14) containing 5 units/ml of heparin and forcing the spleen in and out of 1 ml plastic syringe. Samples of this suspension were assayed for antibody-producing cells by the plaque-forming cell (PFC) method described by Jerne *et al.* (15). Because each spleen was suspended in a constant volume of medium and a known volume of this suspension was assayed for PFC it was possible to calculate the number of PFC/spleen without making cell counts on each individual suspension.

Results. To test the normal immune response 4×10^8 SRBC were injected intravenously into mice of various genotypes. Four days later, at the time of the peak PFC response in normal mice, spleens were removed and assayed individually for their content of PFC. The data in Table I give the results of a typical experiment. The values shown are geometric means of the number of PFC/spleen from 5 mice of each genotype. Although there are significant differences in the response of mice of different genotypes, there is no indication that the presence of the mutations suppresses the PFC response. In fact the highest responses were found in the mutants.

Bone marrow–thymus synergism (12) provides another way to test the immune response. To confirm that the synergism can be used as an assay for immunological activity in suspensions of bone marrow and thymus cells, we carried out the follwing experiment: Varying numbers of bone marrow cells from either normal or W/W^v donors were mixed together with SRBC and thymus cells from normal donors. This mixture of cells was injected intravenously into irradiated, normal recipients; 9 days later, spleens from the survivors were assayed individually for PFC. The linear relationship between the number of PFC per spleen and the number of bone marrow cells transplanted indicates that bone marrow–thymus synergism is a satisfactory, quantitative assay for the immunological capacities of these tissues (Fig. 1). The data clearly indicate that bone marrow from W/W^v donors is almost twice as active in this assay as is bone marrow from normal donors.

Since bone marrow–thymus synergism provides a quantitative assay of the immune response, bone marrow and thymus cells from W/W^v donors were tested in all possible

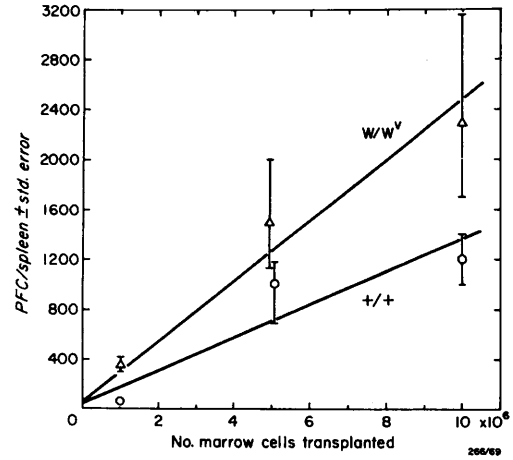

FIG. 1. Titration of bone marrow cells from normal and W/W^v donors. Different groups of irradiated recipients were given 3.5×10^7 thymus cells from normal mice and varying numbers of bone marrow cells from normal or W/W^v donors. Antigen was given as described for Table II. The data in the graph shows the geometric mean of the number of PFC/spleen in mice given varying numbers of normal (○) or W/W^v (△) bone marrow cells. The bars indicate one standard error on each side of the geometric mean.

TABLE II. Bone Marrow–Thymus Synergism Using W/W^v Donors and Sl/Sl^d Recipients.[a]

Expt. no.	Source of bone marrow	Source of thymus	Genotype of recipient	PFC/spleen (SE)[b]
1	+/+	—	+/+	17 (16–19)
	W/W^v	—	+/+	27 (22–34)
	—	+/+	+/+	19 (16–24)
	—	W/W^v	+/+	180 (90–340)
	+/+	+/+	+/+	650 (500–840)
	W/W^v	+/+	+/+	2200 (1600–3000)
	+/+	W/W^v	+/+	630 (500–790)
	W/W^v	W/W^v	+/+	1200 (810–1700)
2	+/+	—	+/+	11 (8–17)
	—	+/+	+/+	24 (14–40)
	+/+	+/+	+/+	1100 (880–1400)
	+/+	—	Sl/Sl^d	9 (6–15)
	—	+/+	Sl/Sl^d	2 (1–3)
	+/+	+/+	Sl/Sl^d	900 (680–1200)

[a] Bone marrow (5×10^6) and/or thymus cells (5×10^7) and SRBC (1×10^8) were injected intravenously into irradiated (950 rads) recipients; 5 days after transplantation the mice also received an intraperitoneal injection of SRBC (1×10^8); 4 days later the spleens from all survivors were assayed individually for their content of PFC.

[b] Except for the groups with Sl/Sl^d recipients the number of PFC is the geometric mean of the number of PFC/spleen from at least 10 survivors. Because of the poor survival of irradiated Sl/Sl^d mice the groups with these mice as recipients contained fewer than 10 mice at the time of the assay. Only 2 mice survived in the group that received thymus cells; in each of the other two groups there were 5 survivors. The values in parentheses enclose two standard errors.

combinations with bone marrow and thymus cells taken from normal mice. The data from these experiments (Table II, Expt. 1) establish that bone marrow and thymus cells from W/W^v mice are not defective in this assay. In addition to the large response of W/W^v bone marrow in combination with normal or W/W^v thymus, we find a high response in mice given W/W^v thymus cells without bone marrow. Normal thymus cells generally give only background numbers of PFC (about 15 PFC/spleen), but recipients of W/W^v thymus cells usually have 200–400 PFC/spleen. The reason for this high response is not known. Perhaps the parathymic lymph nodes (16) are larger than normal in W/W^v mice, and the response of thymus cell suspensions is simply the result of large numbers of contaminating lymph node cells which are known to give a high PFC response in transplantation assays (17).

In another experiment bone marrow and thymus cells were tested for their ability to initiate an immune response in Sl/Sl^d recipients. McCulloch *et al.* (4) showed that hemopoietic stem cells proliferate poorly, if at all, in the spleens of irradiated Sl/Sl^d recipients. Since the radiation response of bone marrow–thymus synergism indicates that cell proliferation is involved (18), it is of interest to determine whether or not the synergism occurs in Sl/Sl^d recipients. As shown by the data in Table II, (Expt. 2), Sl/Sl^d recipients provide a satisfactory environment for the synergism between bone marrow cells and thymus cells to occur.

Discussion. The failure to find effects of the *W* and *Sl* mutations on the immune response is surprising in view of the data indicating that the same stem cells supply differentiated cells to both the hemopoietic and immune systems. For example, Wu *et al.* (9) found progeny of hemopoietic stem cells in both the lymph nodes and thymus of mice, and Micklem *et al.* (8) reported that bone marrow-derived cells are able to reconstitute the bone marrow, spleen, thymus, and lymph nodes of irradiated recipients with functional

cells. If hemopoietic stem cells give rise to differentiated progeny in the immune system, we would have predicted that the *W* and *Sl* mutations, both of which affect the function of hemopoietic stem cells, to have detrimental effects on the immune responses: Both tests reported in this paper showed that W/W^v and Sl/Sl^d mice have a normal immune response.

Although the data in Tables I and II could be interpreted to mean that the stem cells of the immune system are independent of those involved in the differentiation of the hemopoietic system, there are alternative explanations of the data which are consistent with the hypothesis of a common stem cell for both systems. The alternative interpretations of the data fall into two broad categories:

(a) The stem cells of the two systems are identical, but the regulation of the hemopoietic pathway is completely independent of regulation of differentiation in the immune system. Thus, one could propose that the *W* and *Sl* mutations affect differentiation only along the hemopoietic pathways and leave the immune system intact. Mutations affecting only one pathway of differentiation of hemopoietic stem cells have been observed: The flexed-tail mutation affects differentiation of erythropoietic cells and has no effect on the production of granulocytes (19), although both cells are derived from the same stem cell (20).

(b) Another possible explanation of the failure of these genes to affect the immune system involves the different degrees of cellular proliferation required in the various assays. Assays such as spleen colony formation by stem cells (2) or spleen focus formation by virus (5) require a great deal of cell proliferation. However, the differentiation of antibody-producing cells from their immediate progenitors requires only 4–7 divisions (17). Thus, if these mutations permit some proliferation to occur, one would expect assays which require only a few cell divisions to be only slightly affected. In this connection it is interesting to note that while bone marrow from W/W^v mice contains no detectable spleen colony-forming cells (2), colonies are observed when W/W^v marrow is cultured *in vitro* where only 50 cells are required to detect the formation of a colony (21).

There is no obvious reason for the discrepancy between our data and those reported by Shearer and Cudkowicz (11). The major difference between our experiments and theirs was in the mice used. They examined W^v/W^v mice and found a defective immune response to SRBC while we found that W/W^v mice have a normal response to SRBC. Perhaps the slightly different alleles tested by different investigators account for the different results.

Summary. Mutations at the *w* and *sl* loci in mice severely affect the function of the hemopoietic system. Because the stem cells of the immune system are thought to be identical with hemopoietic stem cells, mice of genotypes W/W^v and Sl/Sl^d were tested for their capacity to respond to sheep erythrocytes. The results indicate that these mutations do not have detrimental effects on the differentiation of the immune system. In fact, mice of genotype W/W^v mice respond better to sheep erythrocytes than do coisogenic, normal mice.

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