Skin Graft Rejection in Mice Repopulated with Marrow of the Skin Donor Type: A *Skn* Gene in a Congenic Line

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**Abstract.** Genetically anemic *W/W* ~ mice and lethally irradiated wild-type mice were cured and populated by grafted marrow cells from donor mice of three congenic lines that differed at non-*H*-2 histocompatibility loci. Tail skin from mice of the same congenic lines was grafted 3–4 weeks later. In two cases, the recipients behaved as expected, no longer rejecting skin syngeneic with the marrow graft that had repopulated them. However, B6-*H*-24c skin was rejected by WBB6F1-~W/W~ mice that were cured with B6-*H*-24c marrow showing a mean survival time of 9.9 weeks. It was rejected somewhat faster, with a mean survival time of 5.9 weeks, by W/W ~ mice cured with marrow from other types of donors. Results were more variable in lethally irradiated WBB6F1-~+/+ recipients of B6-*H*-24c marrow, but they also rejected B6-*H*-24c skin. Both types of recipients remained chimeras after the skin was rejected, showing more than 90% of the B6-*H*-24c hemoglobin type. This is the first report of a *Skn* gene in a congenic line.

**Introduction**

Characterizing immune responses against specific antigens on different tissues is important in understanding the functions of the antigens as well as in defining the tissues. To compare antigens present in skin and in the immunohemopoietic system, we have studied skin graft survival on marrow-cell chimeric hosts. The chimeras were produced from unirradiated genetically anemic WBB6F1-~W/W~ mice or lethally irradiated WBB6F1-~+/+ mice that were populated by marrow grafts from donors congenic with the B6 strain. In cured *W/W* ~ recipients, erythrocytes and granulocytes become entirely donor type (Russell and Bernstein 1968, Murphy et al. 1973), while proliferating cells in the organs of the immune system become mostly, but not entirely, donor type (Harrison et al. 1979). In lethally irradiated recipients,
almost all of the proliferating cells in the hemopoietic and immune systems become
donor type (Micklem and Loutit 1966).

Lethally irradiated recipients saved and repopulated by allogeneic marrow cells
should accept skin grafts of the same type as the marrow donor, unless the skin
grafts differ in antigens not in any of the cells produced by the marrow graft. Evidence
for such antigens has been reported by a number of groups (Warner et al. 1965, Boyse et al. 1970, Silvers et al. 1976; reviewed by Steinmueller and Wachtel 1980), and genes specifying these skin-specific antigens were originally designated
Sk, later Skn. These have usually been defined by transplanting marrow from an F1
hybrid into one of the parental strains, and then testing skin graft survival of the
hybrid or other parent. For example, lethally irradiated B6 mice cured with B6AF1
marrow subsequently reject B6AF1 or A skin (Boyse et al. 1970). After several skin
grafts, the chimeras produce antibody that is selectively cytotoxic for epidermal but
not hemopoietic donor cells (Scheid et al. 1972). There are genes at two or more
segregating Skn loci that differ between the B6 and A strains (Wachtel et al. 1977),
and antigens of this type differ between the CBA, C3H and A strains (Fleming and
Silvers 1981). The latter authors also pointed out that the degree of immune
responses to antigens specified by Skn genes is genetically linked to the H-2 locus.

Detailed analysis of antigens specified by Skn genes would be greatly facilitated
by the availability of Skn congenic lines. These were not found in an extensive
screening of non-H-2 congenic lines for Skn loci (Steinmueller et al. 1978). We here
report evidence that the B6.C-H-24c or Hw54 congenic line differs from B6 for a Skn
gene.

Materials and Methods

Animals. Recipient mice were produced by mating WB/Re-W/+ and C57BL/6J-Wo/+ parents
to produce WBB6F1-Wo/Wo, Wo/+ and +/+ offspring. Genetically anemic Wo/Wo recipients were
used without further treatment, while normal WBB6F1-/-/+ recipients were lethally irradiated before
marrow cell injection. The donor mice were congenic with C57BL/6By (B6) mice and were produced by
Bailey (1975) using tail skin grafting to select for genes determining histocompatibility antigens from the
BALB/c strain. The congenic lines used were B6-H-24c (B6.C-H-24c), B6-H-17c (B6.C-H-17c), and
B6-H-25c (B6.C-H-25c). The H-24 locus is on chromosome 7 near the centromere, and the H-25 locus is
on chromosome 1 (Roderick and Davisson 1982). Recent information about these and many other
congenic lines has been summarized by Klein (1973). Donors from these strains are listed as differing from
B6 or WBB6F1 recipients at a single antigenic locus, although they may differ at several closely linked
antigenic loci. In these cases, the combined antigenic differences were tested.

Procedures. Chimeras were produced by IV injection of 1.0 \times 10^7 donor marrow cells into each 4- to
6-week-old recipient 3-4 weeks before tail skin grafting. Techniques used to prepare marrow cells have
been previously reported (Harrison and Doubleday 1976, Harrison and Astle 1982), as have tail skin
grafting techniques (Bailey 1963). All donors were 6-10 weeks old when marrow or skin was taken.
Lethally irradiated mice were given 700 rads of X-irradiation on the evening before marrow grafting as
has been previously described (Harrison 1981). Percentages of donor (B6) type and recipient (WBB6F1)
type hemoglobins were measured using previously published techniques (Whitney 1978, Harrison 1980),
and Wo/Wo mice were scored as cured when their erythrocyte counts and hematocrits increased by
approximately 40% and 20%, and mean erythrocyte volumes decreased by approximately 30%. The
competitive repopulation technique using hemoglobin markers has been described elsewhere (Harrison
1980, 1981, Harrison and Astle 1982), as has its use to quantitatively measure the effects of immune
responses on the repopulating ability of erythropoietic stem cells (D. E. Harrison, manuscript submitted).