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MASTER

Chimerism Induced by Intergenotype
Transplantation of Mouse Bone Marrow†

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Early work at The Jackson Laboratory indicated that W-series anemic mice could be permanently cured of their anemia by the injection of normal bloodforming tissues. Since that time we have undertaken numerous studies of the conditions necessary for successful transplantation. These investigations invariably lead us to conclude that the W-genetic defect in hematopoiesis in the adult is an intrinsic defect of their hematopoietic stem cells and that it does not affect extrinsic physiological or metabolic processes upon which successful hematopoiesis depends¹⁻⁴. These findings stimulated us to use W anemic recipients (W/W^V) to determine the location of the steel series defect in blood formation. Marrow from histocompatible steel anemics (Sl/Sl^d) was injected intravenously into W/W^V mice with the result that W/W^V mice developed a nonanemic blood picture. These and other studies revealed that steel marrow has normal numbers of stem cells and that these cells can permanently cure W anemics of their macrocytic anemia. All of the available evidence indicated that in steel anemics the defect is an extrinsic one.

The intergenotype transplantation method thus provided us with a means of determining whether a given genetic defect in hematopoiesis is expressed intrinsically or extrinsically with regard to erythrocyte precursors. We extended this method to analyze the primary site of gene action in other murine anemias (Jaundice, Hemolytic anemia, Normoblastosis, Flexed-tail anemia, Hertwig's anemia, and Microcytosis). The reader is referred to four extensive recent reviews¹⁻⁴ for a description of the anemias employed in this investigation. Suffice it to say that all are different, being

caused by different single autosomal recessive or semi-dominant genes located at different loci. Three are macrocytic hypoplastic anemias, and four are hemolytic disorders.

To carry out this investigation, we synthesized genetic stocks in such a way as to permit us to transplant marrow from the anemic in question to W-series anemics without involving any of the known histocompatibility barriers. Usually the donor and recipients were congenic (i.e., WBB6F₁-1s/1s marrow transplanted to WBB6F₁-W/W^V). Rarely the donor was one of the parental strains used in producing the F₁ hybrid recipient (i.e., FL/Re-f/f to B6FLF₁-W/W^V). Where possible, additional genetic markers were added, which would not interfere with transplant success but which could aid in determining whether the transplanted cells were successful in implanting and proliferating. For example, marrow from C57BL/6-an/an, Hbb^s/Hbb^s (cells carrying a homozygous marker for the single type of hemoglobin) was injected into WBB6F-W/W^V, Hbb^d/Hbb^s (W-anemic mice with phenotypically diffuse hemoglobin). Mice were injected intravenously with marrow cell suspensions in physiological saline. The numbers of nucleated cells introduced varied from 1×10^6 to 1×10^8 with most recipients receiving 5×10^6 cells.

To qualify as a definitive change either in the direction of therapeutic success or toward conversion to the donor phenotype two or more of the recipient's hematological values must exceed pretreatment values by more than two standard deviations. Preliminary observations appear in Table 1.

Within the confines of these experiments the values presented indicate:

1. That injections of normal (+/+) marrow cured W/W^V anemics completely and permanently with concurrent changes in the hemoglobin pattern to that of the donor type.

2. $\underline{W/W^V}$ marrow is without apparent affect on $\underline{W/W^V}$ recipients.

3. Marrow from Hartwig's anemia ($\underline{an/an}$) has a grossly observable effect on $\underline{W/W^V}$ hosts in only 50% of the cases; but since donor and recipient have similar macrocytic anemias one would be hard pressed to distinguish between them on the basis of peripheral blood values. Deliberate use of a hemoglobin marker revealed, however, that in 9 of 11 recipients tested 390 days after transplantation only donor type hemoglobin was present; suggesting that in Hartwig's anemia there is an intrinsic defect in erythroid stem cells.

4. In contrast, the transplantation of erythroid precursors from steel anemic ($\underline{Sl/Sl^d}$) mice to $\underline{W/W^V}$ hosts permanently cure them of their anemia. Red cell numbers and sizes also become normal; indicating that steel stem cells have normal potential for development but are prevented from doing so in their indigenous environment. Thus, in the case of steel anemia the genetic defect is viewed as being extrinsic to its erythroid stem cells.

5. Turning to an analysis of the hemolytic diseases, Hemolytic anemia ($\underline{ha/ha}$) and Jaundiced ($\underline{ja/ja}$), we discover that 90% of $\underline{W/W^V}$ mice injected with $\underline{ha/ha}$ marrow and 100% of $\underline{W/W^V}$ injected with $\underline{ja/ja}$ marrow take on the complete phenotypic characteristics of the donor with the development of hypochromia, splenomegaly, hyperbilirubinemia, and aberrant erythrocyte morphology (schistocytosis, normoblastosis, stipple cells, anisocytosis, and poikilocytosis).

6. In the case of Normoblastosis ($\underline{nb/nb}$), only 40% of the recipients gave evidence of temporary conversion to the donor phenotype by the appearance of circulating normoblasts and all reverted to the host phenotype within 90 days of transplantation. In retrospect, it is clear that the

investigator was a bit too impatient to get started. Donors were incompletely inbred and it is almost certain that marrow grafts failed as a consequence of histoincompatibility. The appearance of normoblastosis, though temporary, suggests that the defect is most probably intracorpuseular.

7. Finally, in the case of Microcytosis (mk/mk), a non-icteric hemolytic disorder, we once again encounter a defect which is intrinsic to erythroid stem cells and their progeny, for in every case macrocytic W/W^V recipients were converted to the microcytic hypochromic phenotype of the donor. Cell size distribution analysis 210 days after grafting revealed the presence of but a single population of extremely small cells ($MCV = 28.5 \pm 3.7 \mu^3$).

These results suggest, therefore, that in the genotypes an/an, ls/ls, ha/ha, nb/nb, and mk/mk, the genetic defect is expressed primarily within and confined to their erythropoietic cells, for on transplantation into a normal environment their erythroid progenitors continue to produce their characteristically abnormal cells.

In conclusion, we have demonstrated: 1. the utility of this method for distinguishing between intrinsic and extrinsic defects of erythroid precursor cells in mice; 2. that the method may be used to produce viable phenotypes which otherwise are neonatally lethal, and 3. that the method may be used to produce chimeras without employing X-irradiation or other marrow depleting treatments adversely influencing the process of hematopoiesis or other normal physiological processes.

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le 1. Changes in peripheral blood values induced by the intergenotype transplantation of marrow.

Donor-Recipient combination	Sample time	RBC ² ± σ	HT ³ ± σ	MCV ⁴ ± σ	Other Parameters
+/+ donors	average adult ⁵	9.77 ± 0.37	47.1 ± 1.3	48.3 ± 1.0	<u>Hbb^s/Hbb^s</u>
12 <u>W/W^v</u> recipients (100% converted ¹)	pretreatment	6.32 ± 0.61	40.6 ± 2.5	64.6 ± 4.8	<u>Hbb^s/Hbb^d</u>
	30 d later	9.11 ± 0.49	48.4 ± 1.3	52.9 ± 2.6	
	90 d later	10.77 ± 0.60	47.2 ± 2.6	43.9 ± 1.9	
	360 d later	9.44 ± 0.40	46.4 ± 1.6	49.1 ± 0.7	only donor-type hemoglobin
<u>W/W^v</u> donors	average adult	6.16 ± 0.43	40.3 ± 1.9	65.6 ± 4.3	
7 <u>W/W^v</u> recipients (0% converted)	pretreatment	6.41 ± 0.50	40.9 ± 1.8	61.9 ± 3.2	
	30 d later	6.45 ± 0.50	40.9 ± 2.0	63.8 ± 6.2	
	90 d later	6.14 ± 0.58	39.1 ± 2.4	63.8 ± 3.4	
	240 d later	5.87 ± 0.79	39.3 ± 1.5	64.4 ± 2.9	
<u>Sl/Sl^d</u> donors	average adult	4.23 ± 0.38	31.7 ± 2.4	73.6 ± 3.0	
39 <u>W/W^v</u> recipients (97% converted)	pretreatment	5.94 ± 0.58	39.2 ± 1.4	64.5 ± 5.7	
	30 d later	9.17 ± 1.02	48.0 ± 2.2	52.9 ± 5.4	
	90 d later	8.50 ± 1.35	46.2 ± 2.7	55.5 ± 6.7	
<u>an/an</u> donors	average adult	5.48 ± 0.85	34.4 ± 3.2	63.8 ± 5.5	<u>Hbb^s/Hbb^s</u>
23 <u>W/W^v</u> recipients (50% converted)	pretreatment	6.10 ± 0.29	39.5 ± 1.9	66.9 ± 5.3	<u>Hbb^s/Hbb^d</u>
	30 d later*	6.10 ± 0.53	40.3 ± 1.4	66.3 ± 2.4	
	90 d later*	5.85 ± 0.40	38.7 ± 1.5	66.4 ± 6.4	
	240 d later*	5.08 ± 0.78	33.4 ± 3.2	66.9 ± 8.3	at 390 d only donor-type hemoglobin ⁶
<u>ha/ha</u> donors	average adult	3.61 ± 0.71	23.6 ± 4.3	68.4 ± 14.2	serum bilirubin 4.4 mg%. MCHC 22.2
10 <u>W/W^v</u> recipients (90% converted)	pretreatment	6.20 ± 0.34	40.6 ± 1.3	65.8 ± 5.3	" " 1.2 mg%. MCHC 33.6
	30 d later*	5.38 ± 0.47	35.4 ± 3.1	66.2 ± 7.2	" " 2.8 mg%.
	90 d later*	5.60 ± 0.74	37.2 ± 5.5	66.2 ± 2.9	splenomegaly MCHC 22.8
	360 d later*	4.72 ± 0.69	35.8 ± 2.8	73.7 ± 8.0	

Donor-Recipient combination	Sample time	RBC ² ± σ	HT ³ ± σ	MCV ⁴ ± σ	Other Parameters
<u>ja/ja</u> donors	average adult	4.51 ± 0.70	23.8 ± 3.6	54.2 ± 3.1	spleen wt. 1670 ± 430 mg serum bilirubin 4.8 mg%
14 <u>W/W^v</u> recipients (100% converted)	pretreatment	6.27 ± 0.32	41.2 ± 1.4	65.4 ± 2.4	spleen wt. 85 ± 15 mg serum bilirubin 1.2 mg%
	30 d later	5.75 ± 0.49	32.2 ± 2.6	56.1 ± 3.7	
	90 d later	4.57 ± 0.60	23.8 ± 2.4	53.7 ± 7.3	spleen wt. 1540 mg serum bilirubin 3.2 mg%
	120 d later	4.53 ± 0.39	24.6 ± 2.5	54.4 ± 2.5	
<u>nb/nb</u> donors	average adult	5.21 ± 0.58	23.8 ± 2.6	44.7 ± 4.6	normoblastosis, hemoglobin 6.5±0.5 g %
10 <u>W/W^v</u> recipients (40% converted ⁷)	pretreatment	5.99 ± 0.47	40.6 ± 1.9	68.7 ± 6.6	no " " " 13.2±0.9 g %
	30 d later*	5.79 ± 0.36	33.4 ± 1.9	57.8 ± 0.9	normoblastosis, " 11.6 g %
	90 d later*	6.45 ± 0.26	40.8 ± 1.2	63.3 ± 2.1	
	360 d later*	6.04 ± 0.24	39.1 ± 1.8	64.7 ± 3.1	
<u>mk/mk</u> donors	average adult	15.76 ± 1.40	42.5 ± 3.6	27.5 ± 2.9	<u>Hbb^s/Hbb^s</u>
15 <u>W/W^v</u> recipients (100% converted)	pretreatment	6.05 ± 0.38	40.1 ± 1.7	66.0 ± 2.5	<u>Hbb^s/Hbb^d</u>
	30 d later	12.26 ± 1.69	40.4 ± 1.9	31.6 ± 1.6	
	90 d later	14.09 ± 1.15	39.9 ± 2.0	28.4 ± 2.0	only 1 population on Coulter B
	210 d later	13.07 ± 1.41	36.8 ± 2.5	28.5 ± 3.7	only donor type hemoglobin

1

Per cent of mice converted: (having hematological values more than 2 σ away from normal range for a critical parameter)

2

Red blood cells × 10⁶/mm³

3

Hematocrit in per cent

4

Mean cell volume in μ³

5

Values for untreated animals

6

In 9 of 11 mice checked

7

All reverted to pretreatment value

* Values for converted mice only