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Received June 8, 1959. P.S.E.B.M., 1959, v101.

Implantation of Normal Blood-Forming Tissue in Genetically Anemic Mice, Without X-irradiation of Host.* (25089)

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Numerous experiments(1-7) have demonstrated that blood-forming tissue may be successfully transplanted into heavily X-irradiated normal mice, but this phenomenon has never been demonstrated with adult untreated hosts. Transfers of blood-forming tissue between normal donors and genetically anemic hosts, reported here, indicate that, at least under certain circumstances, implantation of blood-forming tissue from another individual may be achieved without X-irradiation of recipient. The successfully implanted hosts include not only adult anemic mice similar to those in which implantation of isologous normal hematopoietic cells following mild (200r) whole-body radiation has already been reported(8,9), but also juvenile hosts of this genotype (WW^v) and of the lethally anemic genotype (WW). The anemia encountered in both of these genotypes has been reported (10) as a normochromic, macrocytic type. Although experiments using heavily irradiated normal hosts have demonstrated successful implantation of both homologous and isologous hematopoietic cells, homologous normal blood-forming cells have not been effectively implanted in adult anemic hosts after 200 r and much heavier radiation doses(11). These mice are extremely radiosensitive with median lethal doses in range of 200 r to 300 r(8). The low frequency of spontaneous survival to adulthood observed both in WW^v mice (25-31%) and in WW mice (0-10%)(12) make it especially doubtful whether juvenile animals of these genotypes could withstand heavy doses of radiation even as a prelude to tissue implantation. Thus, radiation to induce acceptance of blood-forming tissue appears unsuitable as therapy for these genetically anemic mice. It seemed advisable, therefore, to test for the possibility of implantation of isologous normal cells without radiation of the host. A critical test for success of implantation is possible in these genetically anemic hosts, since erythrocytes derived from normal blood-forming tissues, isologous with

* This work supported by contract with U. S. Atomic Energy Comm., and by grant to Roscoe B. Jackson Memorial Laboratory from Am. Cancer Soc.

† The junior author wishes to express gratitude to the Guggenheim Fn. and to Calif. Inst. of Technol. for assistance.

TABLE I. Success and Duration of Implants of Isologous Normal (*ww*) Embryo Liver Hematopoietic Cells in Non-Radiated Anemic Hosts.

Donor-host combination	No.	Surviving	Successful	Duration (days)
Adult hosts, WW^v (20/24)				
C57BL I.V. into WK × C57BL	3	3	2	610, 700
WB I.V. into WB × C57BL	2	2	2	310*
WC × C57BL I.P. into WC × C57BL	19	17	16	180*
Juvenile hosts, WW^v (9/14)				
C57BL I.P. into 11-20 day WB × C57BL	11	9	7	780, 800, 700, 310*(4)
WC × C57BL I.P. into 6 day WC × C57BL	3	2	2	300, 475
Juvenile hosts, *WW* (4/15)				
WB I.P. into 11-13 day WB	6	2	2	75, 310
WC " " 7-13 " WC	6	2	2	45, 220
WK " " 11-12 " WK	3	0	0	

* Still alive at compilation of data, or used for other experiments after indicated interval.

the host except for *W*-series genes, may be recognized by their characteristic cell size as indicated below.

Materials and methods. Three series of experiments, differing in *W*-series genotype or age of hosts, were performed. In the first series, the anemic hosts were 24 adult (4-6 month) WW^v mice from 3 different F_1 hybrid crosses. In the second, the hosts were 14 juvenile (6-20 day) WW^v mice from 2 F_1 hybrid crosses, with their normal littermates, and in the third, the hosts were 15 juvenile (7-13 day) *WW* mice from 3 different inbred strains, with their normal littermates. In all 3 series, the implanted hematopoietic cells were obtained from livers of 15½ day old (normal *ww*) embryos, either completely isologous (except for *W*-genes) with the host, or from one of the parent-strains. The implantation technic involved injection of saline suspension of 8 x 10^6 nucleated cells, given intravenously in early experiments, and intraperitoneally in later experiments. The mice were of the following types: C57BL/6Jax, C57BL/6W^vw, (produced by repeated backcrossing of W^vw to C57BL/6Jax), and 3 inbred strains, WB, WC, and WK, maintained heterozygous for *W*(11). WW^v mice obtained from mating of W^vw parent isozygous with C57BL/6 and a *Ww* parent from WB, WC, or WK, were used as hosts in first and second series. They invariably have a severe macrocytic anemia at all ages (erythrocyte counts of young adults, 6-7 x 10^6 rbc/mm^3 in contrast to 11-12 x 10^6 rbc/mm^3 for littermate controls; mean erythrocyte volumes, 50-60 μ^3 in contrast to 39-45 μ^3 for normal littermates). In inbred strains in third experimental series, live newborn *WW* animals (erythrocyte counts approximately 1.5 x 10^6 rbc/mm^3 in contrast to 5 x 10^6 rbc/mm^3 in normal littermates) appear in almost the expected percentage(*12*). Nearly all die within 2 weeks of birth, but a very few, especially in the WC strain, survive to adulthood. These remain extremely anemic having erythrocyte count at 2-4 months of 4-5 x 10^6 rbc/mm^3 and M.C.V. of 70-85 μ^3. Erythrocyte counts and hematocrit determinations were made on all adult recipients before treatment, and at regular intervals after injection. In second and third series, determinations of erythrocyte levels were omitted before treatment to avoid trauma to the delicate juvenile hosts, but were started 45 days post-treatment, when all survivors were nearly full-grown mice, and were continued at regular intervals.

Results. Of 24 adult WW^v hosts implanted with fetal hematopoietic liver cells, 22 survived more than 30 days after injection (Table I), and 20 of these, from all 3 experiments, gave hematological evidence of permanent cell implantation. Two of the successfully implanted animals (WK x C57BL) survived more than 600 days after treatment, maintaining normal blood pictures to the end of their lives. Additional information on lifespan was not available since 2 of the groups (WB x C57BL, WC x C57BL) were terminated after 310 and 180 days, to use the material for another purpose. However, blood-pictures were followed in detail for 180 days in the largest series, *ww*(WC x C57BL) into WW^v(WC x C57BL). Two of the 19 adult

TABLE II. Erythrocyte Levels and Mean Cell Volumes of 16 Adult WW^v Hosts Successfully Implanted with Isologous *ww* Cells.

Days post treatment	R.B.C./mm³ 10^6 Mean	Range	Mean cell vol Mean	Range
0	6.44	5.48- 7.72	61.0	52-66
30	7.02	5.96- 7.96	58.8	52-69
60	9.41	8.16-10.70	47.9	43-54
90	9.33	6.92-10.58	45.8	42-53
120	9.31	8.30-10.54	46.2	37-53
180	9.69	8.58-11.08	44.3	34-48

hosts, with low pretreatment erythrocyte levels (5.24 and 4.79 x 10^6 rbc/mm³) died less than 60 days after treatment, and a third, also with low pretreatment level (5.24 x 10^6 rbc/mm³) survived through 180 days without evidence of implantation, and with a continuing drop in total erythrocyte count. The other 16 hosts, with higher pretreatment levels (Table II), all showed successful implantation of *ww* hematopoietic cells. There was little change in blood-picture 30 days after treatment but, between 30 and 60 days, erythrocyte numbers increased markedly and mean cell volumes decreased correspondingly (Table II), approaching values characterizing normal *ww* adults. Mean values at 90, 120, and 180 days after treatment were very similar to those at 60 days, with considerable variation between successive counts on the same animal.

TABLE III. Blood-Picture of WW^v and Littermate *ww* Mice after Injection of Isologous Embryo Blood-Forming Tissue into Juvenile Hosts.

Days post treatment	Mean, R.B.C./mm³ 10^6 WW^v (9)	*ww* (7)	Mean cell vol WW^v (9)	*ww* (7)
45	8.41	11.85	42.3	38.9
75	10.68	11.59	39.9	41.0
105	9.78	11.45	46.1	39.9
160	9.86	10.34	44.7	45.4
260	10.42	10.51	44.4	44.6

Juvenile WW^v hosts. Although no erythrocyte determinations were made before treatment of young WW^v animals, erythrocyte counts of approximately 3 x 10^6 rbc/mm³, and mean cell volumes of approximately 80-100 μ^3, were assumed at time of treatment on the basis of previous observation (12), in contrast to values of approximately 6 x 10^6 rbc/mm³ and 60-70 μ^3 for their normal littermates (12). Three of the 14 hosts died within 30 days after treatment, but 9 of remaining survivors became and remained essentially normal in blood-pictures (Table III). Erythrocyte levels for individual implanted anemics fluctuated for a considerable time, but after 75 and more days were always well above those of untreated anemic WW^v adults; mean cell volumes were, with only 2 exceptions (105 days), within the range of normal *ww* adults. The near-normal blood-cell levels of implanted anemics were maintained throughout their lives; 3 of 5 survived more than 700 days.

Juvenile WW hosts. Isologous intraperitoneal injections into 7-13 day old *WW* anemics were also successful in some cases, although the proportion of survivors was lower (4/15, Table I). It was assumed that erythrocyte counts at time of treatment were close to 2 x 10^6 rbc/mm³, with mean cell volumes of 80-100 μ^3 (10). Each of the 4 *WW* individuals which survived following injections of isologous blood-forming tissue developed a near-normal blood-picture, recognized in 3 cases 45 days after injection (Table IV), in the fourth not until 105 days after injection. Two of the implanted *WW* anemics had especially short lifespans (less than 105 days after implantation); the other 2 lived less than one year,

TABLE IV. Effect of Isologous *ww* Hematopoietic Cell Implant on Blood-Picture of *WW* Mice Inj. at 7-13 Days of Age. (Erythrocyte count untreated surviving adult *WW*, 4-5 × 10^6 R.B.C./mm³; M.C.V., 70-85 μ^3.)

	WW from WB strain				*WW* from WC strain			
	#1		#2		#3		#4	
Days post implant	R.B.C./mm³ 10^6	M.C.V., μ^3	R.B.C./mm³ 10^6	M.C.V., μ^3	R.B.C./mm³ 10^6	M.C.V., μ^3	R.B.C./mm³ 10^6	M.C.V., μ^3
45	4.83	62	11.78	41	9.77	40	8.41	46
75			10.69	42				
105	10.06	40	Died with in-		11.99	36	Died	
210	8.24	38	fection		9.44	42		
310	7.40	45			Died			

maintaining normal *ww* cell volumes, and erythrocyte numbers slightly below normal *ww* levels.

Discussion. All evidence presently available on genetically anemic (WW^v, WW) mice supports the hypothesis that isologous normal donor cells implant and continue to function autonomously according to their *ww* genotype. The present data clearly demonstrate that radiation to prepare the marrow "bed" is not essential for implantation of isologous normal embryonic blood-forming tissue, success having been obtained with WW^v adult and with WW^v and WW juvenile hosts without previous radiation treatment. The highly cellular marrow of WW^v adults(13) did not prevent implantation of isologous cells, the proportion of success (20/24) being similar to that found (16/18) following 200 r whole-body radiation (9). In adult animals injected without prior radiation, a considerable period of time, 30 to 60 days in contrast to 18 days with 200 r radiated hosts, elapsed before the effect of implanted blood-forming cells was apparent on the blood-picture of host. Failure of implantation in adult WW^v individuals with especially low pretreatment levels may mean that health of host plays some part in fostering growth of the implant. After a rise in erythrocyte count had been observed, large fluctuations in successive counts, extending over prolonged periods of time, were frequently observed, but always culminated in a permanent high level—only slightly lower than that for control *ww* adults or adult WW^v mice injected after exposure to 200 r.

Similar fluctuations and delay in establishment of a near-normal blood-picture were observed with implantation of juvenile WW^v hosts. Using juvenile hosts, 2 obvious factors, related to health of host, may contribute to mortality after injection. Some animals may die before the implanted cells can establish themselves, since only 25-31% of WW^v (and less than 10% of WW individuals) spontaneously survive the critical pre-weaning period of active growth(11). Moreover, the trauma of intraperitoneal injection may affect longevity of these poorly viable hosts. The difference in proportion of success with WW^v juvenile hosts (9/14) and with WW hosts (4/15) fits both these suggestions. The short lives of successfully implanted WW hosts suggest either that undesirable side-effects are produced by the operation as presently practiced, or that the lifespan of WW individuals may depend upon genic effects other than those determined by the implanted blood-forming tissue.

Many of these observations fit well with the hypothesis of competition between a large body of indigenous defective, slow-acting blood-forming tissue of the genetically anemic host(14) and an initially small implant of rapidly functioning blood-forming tissue from the genetically normal donor. This phenomenon of increasing effect from the implant may be different from that observed if both host and donor tissue had normal blood-forming capacity. If so, the difference would almost certainly favor permanent implantation after normal-into-anemic injections more than after normal-into-normal injections.

The demonstration that isologous implantation can take place without X-ray preparation of host solves some, but by no means all, of the problems of therapy of blood-forming defect of genetically anemic *W*-series mice.

It is a step forward to know that the marrow "bed" of the radiosensitive hosts need not be subjected to radiation, especially since no level of radiation has been observed which allows survival with homologous implantation (11). The small proportion of surviving WW^v and WW juvenile hosts suggests, however, that attempts should be made to minimize trauma to the host, and to develop methods assuring rapid implantation and proliferation of the injected cells.

Since it seems possible that extreme radiosensitivity might be encountered in cases of genetically-conditioned human hypoplastic anemias, the demonstration of implantation without radiation in genetically anemic mice is of interest. However, for this finding to have any carry-over into therapy of human genetic defects of hematopoiesis, it is necessary to find methods which will promote implantation of homologous blood-forming tissue, since with therapy of human genetic defects, it is nearly impossible to obtain isologous cells for implantation. Experiments

testing possibilities for implanting homologous normal cells in anemic hosts are in progress.

In spite of these limitations the successful cases of permanent isologous implantation of normal blood-forming tissues in genetically anemic mice, including the lethal *WW* type, without prior radiation treatment, suggest that if methods for circumventing the homograft reaction can be found, it may be possible to use cell implantation generally for the therapy of genetic defects in erythropoiesis.

Summary. Successful implantation, without x-irradiation or other pretreatment of host, of isologous blood forming tissue from normal (*ww*) mouse fetal liver in viable severely anemic recipients (20 of 24 adult WW^v mice, 9 of 14 juvenile WW^v mice) and in lethally anemic recipients (4 of 15 juvenile *WW* mice) has been demonstrated. Criteria of success were permanent increase in rbc/mm^3 to near-normal levels, and decrease of mean cell volume from macrocytic to normocytic levels. These changes appeared in WW^v adult anemic hosts longer after cell injection than in similar transplant experiments with WW^v hosts subjected to 200-r whole-body irradiation. These findings fit well with the hypothesis of competition between slow-acting indigenous blood forming tissue of genetically anemic hosts and initially small implants of rapidly functioning blood forming tissue from genetically normal donors. If methods for circumventing homografts can be found, cell implantation may be generally useful for therapy of genetic defects in erythropoiesis.

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Received June 3, 1959. P.S.E.B.M., 1959, v101.

Effect of Degree of Encapsulation upon Virulence of *Cryptococcus Neoformans.** (25090)

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Capsule formation has been associated with virulence of many microbial species such as *Diplococcus pneumoniae, Bacillus anthracis, Hemophilus influenzae* and *Klebsiella pneumoniae.* However, the chemical nature of capsular substances formed by different microbial species is dissimilar, varying from complex polysaccharides composed of units of glucose, fructose, galactose, mannose, sugar acids and amino sugars, to polypeptides such as polymers of glutamic acid. The capsule of *Cryptococcus neoformans* contains 2 complex polysaccharides; an amylose of the starch group, and a serologically active pentosan which upon hydrolysis, yields units of xylose, mannose, galactose and glucuronic acid(1). To date, no single chemical constituent of cap-

* Supported in part by grant from Natl. Science Fn.

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