Genetic Differences in Hemoglobin as Markers for Bone Marrow Transplantation in Mice. (24465)

RAYMOND A. POPP, G. E. COSGROVE, JR. AND R. D. OWEN
(Introduced by A. C. Upton)
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.*

Immunological, histochemical, and cytological methods have been used to demonstrate the transplantation and repopulation of erythrocytic(1-4), platelet(5), thymocytic (6), and white blood cell(7,8) precursors from bone marrow cells of homologous or heterologous origin. The immunohematological systems now used to type mouse erythrocytes are based on differences at the *H-2* locus(9,10). This system permits distinction of cells of donor origin from those of the recipient when donor and recipient are of distinguishable *H-2* genotypes. Although chromosomal markers(7) may be used, in cases in which donor and recipient are indistinguishable at the *H-2* locus, the technic cannot be applied to the circulating red cells. An independent method for distinguishing erythrocytes is, therefore, potentially useful in study of bone marrow transplantation, particularly where the *H-2* markers cannot be used. With paper electrophoretic technics, Gluecksohn-Waelsch *et al.* (11) identified inheritable hemoglobin differences in mice. Starch gel electrophoresis, described by Smithies(12), can also be used for identifying hemoglobin differences in mice (Fig. 1). This preliminary report indicates that erythrocytes of mouse chimeras can be typed by using inheritable differences occurring in mouse hemoglobin.

Methods. Mice used as donors and recipients in this experiment were strains C57BL S, 101, and (C57BL/S × 101)F_1. Erythrocytes of C57BL/S mice are of *H-2*^{b} genotype and have the "single" type of hemoglobin, whereas erythrocytes of 101 mice seem to be of *H-2*^{k} genotype and have the "diffuse" type. Erythrocytes of (C57BL/S × 101) F_1 also have diffuse hemoglobin. Donor bone marrow or splenic suspensions were injected intravenously and intraperitoneally, respectively, into X-irradiated recipients. Blood samples for serological typing and starch gel electrophoresis were obtained by severing the tail vein.

Results. Table I gives comparative results obtained in the 20 mice cross-examined by serological technics(3) and by electrophoresis. The data show that, when bone marrow or splenic suspensions from a donor with one type of hemoglobin are transplanted into irradiated mice that differ at the *H-2* locus and have another type of hemoglobin, the hemoglobin in the recipients' circulating erythrocytes, characterized by starch gel electrophoresis, corresponds with the type of the red blood cells as characterized serologically. The electrophoretic method has not yet been refined sufficiently to detect small quantities (20% or less) of the single type hemoglobin when 80% or more of the red blood cells have the diffuse type. However, both hemoglobin type and red cell serotype seem to be under the autonomous control of the genotype of the nucleated cell from which the erythrocyte was derived. Makinodan and Anderson(13) also found that hemoglobin in mouse-grown

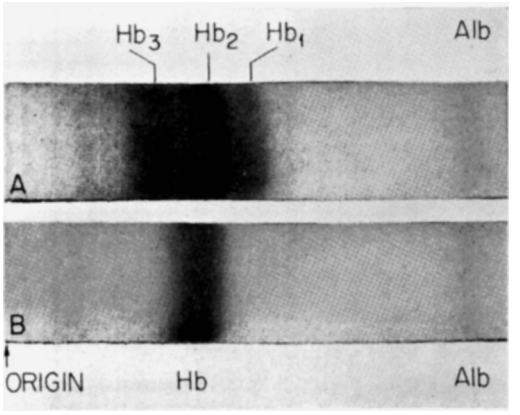

FIG. 1. Comparative patterns of mouse hemoglobins after electrophoresis in starch gel. Strip A shows the "diffuse" type of hemoglobin of strain 101 mice that resolves into 3 electrophoretically separable fractions. Strip B illustrates the "single" type of hemoglobin of C57BL/S mice (R. Popp and W. St. Amand, unpublished data).

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TABLE I. Comparison of Serotype and Hemoglobin Type in X-irradiated Mouse Chimeras.

Recipient mice			Donor cells ($\times 10^6$ nucleated cells)					Post treatment (days)	Serotype		Hemoglobin type	
			Bone marrow		Spleen							
No.	Strain	Dose (r)	C57BL/S	(C57BL/S × 101)F_1	C57BL/S	(C57BL/S × 101)F_1		Recipient (%)	Donor (%)	Recipient	Donor
2	101	850	15				43	100		×	
2	(C57BL/S × 101)F_1	900	15				93	"		×	
2	"	"	15				"		100		×
1	"	"	15				142	3	97		×
1	C57BL/S	"		15			42		100		×
1	(C57BL/S × 101)F_1	400			30		93	100		×	
1	"	"			"		"	93	7		×
1	"	"			"		"		100		×
1	"	500			"		21	100		×	
1	"	"			"		"	64	36		×
1	"	"			"		"	62	38		×
1	"	"			"		"	80	20		×
1	"	600			"		93	100		×	
3	C57BL/S	400					35	"		×	
1	"	"					"	97	3		×
							120				

rat erythrocytes has physicochemical properties of rat hemoglobin. Origin (host or recipient) of the circulating erythrocytes can therefore be inferred from the hemoglobin type as well as from the serotype. Thus, after transplantation of bone marrow cells between strains of mice with identical *H-2* genotypes, one can infer whether the circulating erythrocytes are of donor or recipient origin if there are differences in their hemoglobin types; *e.g.*, C57BL/6 and 129/Rr are both *H-2*^{b}, the former has a single and the latter a diffuse hemoglobin.

Snell *et al.*(14) and Counce *et al.*(15) showed that the *H-2* locus strongly affects transplantability of tumors and skin in mice. Trentin(16) and Uphoff(17,18) suggested that the *H-2* antigens may also be important in reactions occurring after bone marrow transplantation into lethally irradiated mice. Bone marrow transplantations between sublines of mice indistinguishable at the *H-2* locus but with different hemoglobin types and varying degrees of heterogeneity at other loci may further help to elucidate the relative importance of the *H-2* locus and other histocompatibility loci in bone marrow transplantation.

Summary. Both hemoglobin type and red cell serotype seem to be autonomously controlled by the genotype of the nucleated cell from which the erythrocyte is derived. Thus, genetic differences in hemoglobin can be used as markers for bone marrow transplantation in irradiated mice. Hemoglobin typing may be particularly useful where the *H-2* markers cannot be used.

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Incorporation of Cystine and Lysine by Normal and "Cystinuric" Leukocytes. (24466)

F. F. BECKER AND H. GREEN (Introduced by B. W. Zweifach)
Dept. of Pathology, N. Y. University Bellevue College of Medicine

Cystinuria has been shown by Dent(1,2) to be a disease of renal tubules in which amino acids cystine, lysine, arginine and ornithine fail to be absorbed from the glomerular filtrate. Since these substances can be considered as structurally related di-amino acids(3) and their absorption from the glomerular filtrate is largely controlled by a single gene(4), it has been suggested that a single mechanism for absorption of these amino acids exists in the renal tubules. However, other kinds of body cells are capable of concentrating amino acids, such as muscle(5), white blood cells (6), tumor cells(7), and established cell lines grown *in vitro*(8). If the concentration mechanism of amino acids by such cells were related to that of the kidney tubules, these cells might also be affected in the cystinuric individual, for biochemical defects in genetic diseases are frequently widespread. The fact that such a general metabolic abnormality has not been found in cystinuric subjects does not argue against this possibility, for whereas the defect in kidney tubules results in obvious failure to transport amino acids against a concentraton gradient into the blood, the other body cells might continue to obtain sufficient amino acids from blood by diffusion alone, the blood level of these amino acids in cystinurics remaining normal or only slightly reduced(2,9). If however, such cells were placed in a medium containing a considerably lower amino acid concentration than normal blood, any latent defect of this kind in the cystinuric cells might become evident. In the present experiment, therefore, white blood cells of normal and cystinuric subjects were compared in their abilities to incorporate lysine and cystine from the medium, at concentrations below those existing normally in human blood, and at which rate of uptake depends upon concentration in the medium. At the lowest concentration tested, uptake of amino acids by normal and "cystinuric" leukocytes was the same.

Methods. The cystinuric subjects were a brother and sister in their forties who had an extensive family history of the disease; each had been operated on more than once for renal stones, but otherwise was in good health. Their urine gave a strong cyanide-nitroprusside test for cystine. White blood cells were separated from freshly drawn human blood by fibrinogen sedimentation as described by Skoog and Beck(10). They were resuspended in isotonic sodium chloride solution and washed twice. The final suspension was made in the balanced salt solution of Eagle, BSS,(11) and the cells incubated with gentle shaking in water bath at 37°C, under atmosphere of 5% CO_2, 95% O_2. At time zero, labeled amino