GENE CONTROL OF HEMATOPOIESIS

I. Erythrocyte Mosaicism and Permanent Immunological Tolerance in Allophenic Mice

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Mammalian genetic mosaicism, in the constitutional sense, was first detected in erythrocytes of cattle twins. We owe its discovery to Owen (1), who suggested that placental vascular anastomoses served as the vehicle of erythropoietic cell exchange in fetal stages. Further examples of mosaicism, involving various cell populations, have since been found in man and other mammals. The reason for genotypic admixtures in an individual is often obscure and the condition itself is exceptional. Its functional counterpart—cellular phenotypic heterogeneity despite presumed identity of genotype—is, however, a commonplace of differentiation, within as well as among cell types. Constitutional mosaicism, insofar as it inevitably creates functional mosaicism, can therefore provide ways of analyzing the normal mechanisms which underlie differential gene expression and subsequent cell interactions.

Spontaneous constitutional mosaics, despite their great interest, are not only rare, but are restricted in their array of useful genetic markers. The working range of both genetic and chronologic possibilities has been successfully extended for certain systems (e.g., immunologic, erythropoietic) by a number of techniques such as transplantation (2) or postradiation infusion (3) of cells of "foreign" genotypes. The difficulty which remains in these instances is that the genotypic confrontations must of necessity postdate specific developmental origin of the system in question. Thus the mechanisms responsible for its establishment and its primordial kinetics may be bypassed in the experiment.

In order to overcome these limitations, methods were devised (recapitulated in the next section) that yield fully viable mice in which distinctive cellular genotypes have coexisted from earliest embryonic life onward (4–10). In these

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studies, embryonic cells were found to remain developmentally labile during the preimplantation period, at least into the early blastocyst stage (4–6, 9). Blastomeres from two (or more) genetically dissimilar cleavage stage embryos could therefore be experimentally aggregated into one group in vitro, and cultured to form a single embryo. When the genetic composite reaches the morula or blastocyst stage, it is allowed to complete its prenatal life by transferring it to the uterus of a foster mother. Approximately one-third of all transferred embryos survive, and almost 1000 healthy, vigorous adults (comprising 38 different genotypic combinations) have been produced in this manner (references 10–13 and unpublished data from Mintz). Each animal originates from two embryos and thus from four parents instead of the usual two (7, 9–10). Since genetic mosaicism has preceded cell specialization, any tissue can ultimately include two genetic subpopulations of cells whose lineages have jointly participated in ontogeny. Entire clonal histories can in fact be detected by means of appropriate genetic markers, and interactions between clones can be discerned. For example, all pigment cells in the adult coat appear to be clonally derived from only 34 genetically determined primordial melanoblasts (10), and genotype-specific clonal selection is capable of markedly modifying their archetypal relations (11). Comparisons between the artificial mosaics and ordinary, single-genotype mice have, moreover, suggested the hypothesis that phenotypic clonal heterogeneity may be widespread or universal in all cell types, even within a single genome; and that selection among the variant subpopulations may be a major and orderly mechanism of development and aging in mammals (10–12).

The biology of these animals clearly differs in most essential respects from that of all other so-called experimental "chimeras." The term *allophenic mice* has been introduced to emphasize this distinction and to specify their chief biological characteristic: orderly coexistence of different cellular phenotypes, each of known genotype, in any or all tissues throughout development (10-11).

Of the various kinds of allophenic mice that have been "synthesized," many are presumed to contain red blood cells of two independent genotypes. The present report is based on genetically determined differences in strong histocompatibility (*H-2*) antigens, and is the first in a series of studies employing blood cell markers in allophenic mice to examine problems of gene control of normal hematopoiesis and of hereditary anemias.

The following questions will be considered here: (*a*) whether erythrocyte mosaicism can in fact be experimentally imposed in the mouse from the very onset of erythropoiesis; (*b*) whether *H-2* antigenic differences among erythrocytes, under these conditions, are permanently tolerated immunologically; (*c*) whether there is any indication of selection between the erythrocyte types; and (*d*) whether erythrocyte proportions are related to composition of other organs. Preliminary evidence on some of these points has been given elsewhere (14).

Materials and Methods

Allophenic Mice.—34 adult allophenic animals were analyzed. Each was derived from a pair of experimentally conjoined early cleavage-stage eggs, one member of which was either of the C3H (10 cases) or C3Hf (24 cases) inbred strain, and the other member was of the C57BL/6 strain. The strain designations of cells thus combined into single individuals (symbol ↔) will be represented as C3H(f) ↔ C57BL/6, and the separate use of C3H will include both the C3H and C3Hf alternatives, since they behaved indistinguishably in the present study. In brief, the eggs are explanted at about the eight-cell stage and are pronase-treated to lyse the surrounding zona (4, 5). All the blastomeres of each donor pair are then aggregated at 37°C, on a warm stage or in a temperature-controlled hood, into one group, which develops into a unified double-sized embryo (5, 6). The composite blastocyst or morula is returned surgically the next day to the uterus of a pseudopregnant recipient 1 day less advanced than the embryo donors. Total embryo size is soon reduced to normal, and development often continues to birth (7–10).

It is important to emphasize that the size regulation sometimes results in apparent exclusion of cells of one of the two genotypes, or in retention of extremely disparate proportions of cells of the respective genotypes. Histocompatibility constitution is irrelevant in these unilateral exclusions, as the same phenomenon occurs between members of coisogenic strains which differ only with respect to alleles at a single pigmentary locus (10). Mosaicism must therefore always be demonstrated *in each tissue independently*, and never assumed, and considerable variability among individuals is encountered (10, 11). The term *allophenic* is retained for all mice of multiembryo origin because of the difficulty of conclusively demonstrating total loss of one component strain.

The 34 adults tested for erythrocyte mosaicism were part of a much larger population of C3H(f) ↔ C57BL/6 mice, from which arbitrary numbers of representatives of each coat color class were chosen, including three all-agouti (A/A, as in C3H and C3Hf), five all-black or nonagouti (a/a, as in C57BL/6), and 26 two-color (both A/A and a/a). Total sex distribution (14 females, 20 males) simply reflects avoidance of drawing blood from females with young litters at the time of sampling.

Serological Tests.—Each animal received from one to four hemagglutination tests for each of the two possible erythrocyte antigen types (produced by the $H\text{-}2^k/H\text{-}2^k$ genotype in the C3H or C3Hf strains and by the $H\text{-}2^b/H\text{-}2^b$ genotype in C57BL/6). At the time of the first test, the mice ranged in age from 2–7½ months. When agglutination tests were repeated, the age span within an individual covered variable periods, up to 5½ months (Tables II, III). Absorption tests were done chiefly on animals whose red cells gave indications, in hemagglutination, of reacting with both *H-2K* and *H-2B* antisera, as a further check on the presence of two kinds of cells. Absorption as well as agglutination tests were conducted with a single sample of blood taken from each of 16 of the animals; in cases of multiple bleedings, this was always the last sample collected. The animals were killed for other studies at ages ranging from 6 months up to 2 yr 7½ months.

The isoantisera used were prepared by C57BL anti-C3H, and B10.D2 or C3H anti-C57BL immunizations, and were known from tests on appropriate backcross progeny to contain antibodies specific for *H-2K* and *H-2B* antigens, respectively. The dextran-human serum technique of red cell agglutination (15) was used throughout. Artificially prepared red cell suspensions containing known proportions of C3H and C57BL/6 cells supplied the necessary information on quantitative resolution of the antisera for detecting minority populations of cells. Under the conditions employed, agglutination by the minor component in artificial mixtures could be detected down to a 25–30% level by microscopic examination. Minimal detec-

tion by this method (as in EH26, Table III) thus signifies approximately 25% of the low-level strain. Allophenic blood yielding hemagglutination evidence of only one erythrocyte strain might, therefore, include the other strain at a level below 25% of the total in that animal.

The absorption test was found to be more sensitive: as little as 10% of one type could be consistently detected within artificial mixtures of C3H and C57BL/6 cells. The two allophenics (EH29 and EH32, Tables I and III) that received absorption tests and had minimally detect-

TABLE I

Reactions of A.SW Anti-A Antibody with C3H or A Strain Erythrocytes after Absorptions with C3H + C57BL/6 Red Cell Mixtures or with Cells from C3H(f) ↔ C57BL/6 Allophenic Mice

Absorbing cells	Test cells	Dilutions of antisera*						Mosaicism
		1/5	1/10	1/20	1/40	1/80	1/160	
—	C3H	±	+	+	++	+	0	
—	A	+++	+++	+++	+++	+	±	
C3H + C57, 25%:75%	C3H	0	0	0	0	0	0	
	A	+++	+++	+++	++±	++	+	
C3H + C57, 10%:90%	C3H	N.T.	+	±	0	0	0	
	A	+++	+++	+++	++	++	+	
C57	C3H	±	+	++	+	±	0	
	A	N.T.	+++	+++	++±	++	+±	
C3H ↔ C57 EH22‡	C3H	±	0	0	0	0	0	+
	A	+++	+++	+++	++±	+	+	
C3H ↔ C57 EH31	C3H	0	0	0	0	0	0	+
	A	+	+++	++±	+++	+±	+±	
C3H ↔ C57 EH12	C3H	+	+±	+±	±	0	0	−
	A	+++	+++	++±	++	+±	+±	
C3H ↔ C57 EH29	C3H	0	0	+	+	0	0	+§
	A	N.T.	+++	++±	++±	++	+	

* 0 indicates no agglutination; ± to +++, gradations of agglutination reactions; N.T., not tested because of insufficient antibody.

‡ Animal code numbers correspond to those in Tables II and III.

§ C3H component minimally detectable.

able (10%) C3H cells would be expected to have disclosed only C57 in agglutination tests, and this was in fact the case. For absorption studies, an *H-2* serum obtained by immunizing A.SW mice with tissues of A strain (*H-2*^{a}) mice was used. The strains are congenic (16), i.e., they are presumed to differ only for alleles at the *H-2* histocompatibility locus. The antibody strongly agglutinated A cells to a titer of 1/640. The serum also agglutinated C3H strain red cells because of antigenic specificities shared by the *H-2A* and *H-2K* antigens, but had no antibodies cross-reactive with C57BL strain red cells. Absorption of the serum with C3H cells cleared reactivity for the absorbing cell but not for cells of the A strain. The specificity of absorption of C3H antibody by cells of putative mosaics could, therefore, be determined by tests of each absorbed reagent on A cells. In addition, absorption tests always included C57BL

TABLE II

C3H(f) ↔ C57BL/6 Allophenic Mice with One Genetic Population of Erythrocytes, from Agglutination and Absorption Tests*

Animal code No.	Coat color‡	Age	No. of serological tests for *H-2* type		Erythrocytes*	Skin graft tolerance*		γ-globulins§		Liver‖		Heart‖		Other‖, ¶	
			Agglutination	Absorption		C3H	C57	C3H	C57	C3H	C57	C3H	C57	C3H	C57
		months								%	%	%	%		
EH1 ♀	*A*	2–2½	2		C3H	+	0	+++	0	100	0			+	0
EH2 ♂	*A*	2½	1		C3H	+	0	+++	0	100	0			+	0
EH3 ♀	*A*	2	1		C3H	+	+	++	0	100	0			+	+
EH4 ♀	*A* + *a*	6	1	1	C3H					95	5	100	0	+	+
EH5 ♂	*a*	2–2½	2		C57	0	+	0	+++					0	+
EH6 ♂	*a*	4½	1		C57	0	+	0	+++	0	100			0	+
EH7 ♂	*a*	2	1		C57	0	+	0	++++	0	100			0	+
EH8 ♀	*a*	2	1		C57			0	++++					0	+
EH9 ♂	*a*	2–2½	2		C57	0	+	0	+++	0	100	0	100	+	+
EH10♂	*A* + *a*	5½	1	1	C57									0	+
EH11♂	*A* + *a*	4	1		C57	+	+	0	+++	0	100			0	+
EH12♀	*A* + *a*	4½–7	2	1	C57	+	+	+++	+++	100	0	50	50	+	+
EH13♂	*A* + *a*	4	1		C57	+	+	++	+++	100	0	50	50	+	+
EH14♂	*A* + *a*	4½–7	2	1	C57	+	+	++	+++	33	67	0	100	+	+
EH15♀	*A* + *a*	2½	1		C57			++	+++	99	1	0	100	+	+
EH16♂	*A* + *a*	2–2½	2		C57	+	+	0	++++	59	41	5	95	+	+
EH17♂	*A* + *a*	2–4	2		C57			0	++++	19	81	20	80	+	+
EH18♂	*A* + *a*	4½	1		C57	+	+	+	+++	41	59	5	95	+	+

Diagnostic markers:

* *H-2*^{k}(C3H), *H-2*^{b} (C57) alleles for histocompatibility antigens. Data in Tables II and III on "parental" strain skin graft acceptance (+) or rejection (0) are from B. Mintz and W. K. Silvers (1967. *Science.* 158:1484).

‡ *A*, agouti (C3H); *a*, nonagouti or black (C57).

§ Serum allotypes of the 7Sγ2a class of γ-globulins, with allelic regions differing in the C3H and C57 strains; quantitation based on micro-Ouchterlony precipitin tests from unpublished data by E. Weiler and B. Mintz.

‖ *Mdh-1*^{a} (C3H), *Mdh-1*^{b} (C57) alleles for enzyme variants of NADP-malate dehydrogenase. Heart analyses in Tables II and III are from unpublished data by B. Mintz and W. W. Baker; liver and "other" tissue analyses in Tables II and III are from unpublished data by B. Mintz.

¶ See text for additional markers and for "other" tissues tested. Presence (+) or absence (0) of each cell strain is indicated.

cells as a negative control, C3H cells as a positive control, and artificial C3H-C57BL admixtures in which the C3H cells represented 10% or 25% of the total suspension. For absorbing the antibody, antiserum was diluted 1/5 for absorption and mixed with two volumes of packed red cells that had been washed two times in saline, and again in 2% dextran. The mixture was incubated at 37°C for 45 min, during which time the samples were shaken at 15 min intervals. The results obtained in such absorption tests are indicated in Table I. A clear-cut diminution of reactivity of three tubes or more was considered positive evidence of the presence of *H-2K* antigens on the absorbing cells. Loss of two tubes of activity plus reduction in strength of agglutination in positive tubes (e.g., EH29) was considered to be evidence of minimally detectable mosaicism.

RESULTS AND DISCUSSION

Erythrocyte Mosaicism Throughout Development.—Of the 34 C3H(f) ↔ C57BL/6 allophenic mice tested, 18 (Table II) appeared to have only C3H or C57BL/6 erythrocytes while 16 (Table III) gave evidence of possessing erythrocytes of both strains. Among the 18 apparent nonmosaics, four (3 ♀♀, 1 ♂) were antigenically *H-2K* and 14 (3 ♀♀, 11 ♂♂) were *H-2B*; the *H-2*-antigen mosaics included 8 ♀♀ and 8 ♂♂. The sex classifications are based on external sex phenotype. One phenotypic female (EH22) proved, by sex chromosomal analyses, to be an XX ↔ XY mosaic, and two ostensible males (EH9, EH34) also contained XX along with XY cells (reference 12, and unpublished data from B. Mintz, D. Hungerford, and J. Morrow).

Detection of only one erythrocyte type in agglutination or absorption tests does not rule out the possibility that the other type may be present below the level of test sensitivity (25% and 10%, respectively), as stated in the section on Materials and Methods. In Table II, then, there are 4 animals shown by absorption to have no erythrocyte mosaicism down to the 10% level; the remaining 14 are known from agglutination to lack mosaicism down to the 25% level. It is very probable that at least some of these animals do indeed have red cells of only one strain. In red cell tests of other allophenic mice with markers detectable at the 1% level, instances of only one type were actually identified, some in individuals known to contain two genotypes of other kinds of cells, such as melanoblasts (B. Mintz and R. Niece, unpublished data).

The most likely interpretation of the test results in Table III is that all 16 animals listed contained two separate genetic populations of erythropoietic cells. While differential hemolysis of one erythrocyte type may be the ideal demonstration of separate cellular identity of the two antigen phenotypes, hemolytic procedures continue to pose technical problems in the mouse, revolving around variable resistance of cells of single individuals to hemolytic isoantibody (17). The absorption test, however, constitutes strong evidence for presence of two kinds of cells, and was applied in 12 of the 16 cases.

The question has arisen whether cell fusion between hematopoietic cells might occur, leading to a "hybrid" cell with a selective advantage over the "parental" ones. From analyses of a bull from a fraternal twin pair, Stone et al.

(18) have postulated that such cell hybridization may take place. In our allophenic mice, the possibility cannot be ruled out that "hidden hybrids" might be included among the red cells of the animals in Table III. From the cattle data, however, any hematopoietic cell mating would presumably be a rare event. If it occurs at all in blood-forming tissue of the mouse—and there is no critical evi-

TABLE III

C3H(f) ↔ C57BL/6 Allophenic Mice with Two Genetic Populations‡ of Erythrocytes, from Agglutination and Absorption Tests*

Animal code No.	Age	No. of serological tests for *H-2* type		Erythrocytes‡	Skin graft tolerance‡		γ-globulins‡		Liver‡		Heart‡		Other‡	
		Agglutination	Absorption		C3H	C57	C3H	C57	C3H	C57	C3H	C57	C3H	C57
	months								%	%	%	%		
EH19 ♀	6	1	1	C3H < C57									+	0
EH20 ♂	2–6½	3	1	C3H < C57			+++	++++					+	+
EH21 ♀	7½	1	1	C3H = C57			+++	++					+	+
EH22 ♀	2–7½	4	1	C3H < C57			+++	+++	100	0			+	+
EH23 ♀	6	1		C3H = C57					100	0			+	+
EH24 ♀	2–6½	3	1	C3H < C57			++	+++	100	0	85	15	+	+
EH25 ♀	2–6½	3	1	C3H < C57			++	+++	100	0	67	33	+	+
EH26 ♂	7½	1		C3H < C57§			+++	+++	100	0	80	20	+	+
EH27 ♀	6½	1	1	C3H = C57			++	++	100	0	95	5	+	+
EH28 ♂	6½	1	1	C3H = C57					100	0	40	60	+	+
EH29 ♂	4–7	2	1	C3H < C57§	+	+	0	++++	18	82			+	+
EH30 ♂	2–4½	3		C3H < C57	+	+	++	+++	51	49	0	100	+	+
EH31 ♂	4½–7½	2	1	C3H = C57			++	+++	43	57	33	67	+	+
EH32 ♂	2–7	2	1	C3H < C57§	+	+	+++	+++++	66	34	25	75	+	+
EH33 ♀	6½	1	1	C3H < C57			+++	+++	100	0	95	5	+	+
EH34 ♂	6	1		C3H = C57					98	2	75	25	+	+

* All animals are mosaic (*A* + *a*) for coat color.

‡ See Table II for key to diagnostic markers and for sources of data.

§ C3H component minimally detectable.

dence that it does (13)—it could hardly be expected to account for all 16 cases giving both *H-2K* and *H-2B* reactions. In addition, direct examination of some 10,000 metaphases from bone marrow of allophenic mice (reference 13, and unpublished data from B. Mintz, D. Hungerford, and J. Morrow) has yielded the following salient observations: (*a*) The normal diploid karyotype is the rule. (*b*) When homozygous translocation (*T6/T6*) and nontranslocation (+/+) cells coexist, only exceedingly rare presumptive "recombinants" (resembling

T6/+) are found; their incidence in relation to controls is not statistically significant, however, so that they supposedly do not represent fusions, but simply chromosomal accidents in a cell. (*c*) When XX and XY cells coexist (sometimes simultaneously bearing the *T6/T6* and +/+ differential as well), no "new" karyotypes are found.

Apart from the chromosome studies, a search for hybrid enzyme has been made in thousands of tissue samples (including blood) from allophenic mice derived from two homozygous allelic genotypes, each of which forms an electrophoretically distinctive variant of an enzyme (e.g., isocitrate dehydrogenase or malate dehydrogenase). With the conspicuous exception of skeletal muscle (19, 20), which is multinucleate, hybrid enzyme has not yet been found (13).

In short, the data support the conclusion, summarized earlier (14), that in some of our allophenic mice separate cells of entirely unrelated homozygous strains have contemporaneously shared in the establishment of the erythropoietic system and have been jointly responsible for its subsequent history. Since neither genotype is "graft" or "host," these mice are unique among experimental blood mosaics. Erythrocyte mosaicism can demonstrably be sustained for an entire lifetime: Four of the animals which still had both red cell types in their last test were killed because of terminal diseases only 1–45 days after that test.

Permanent "Intrinsic" Immunological Tolerance of H-2 Differences.—Simultaneous presence of two red cell populations in which different *H-2* antigens are detectable automatically implies that the animals are immunologically tolerant of both types (14). These tolerant individuals can possess either similar or widely different proportions of the two kinds of red cells (Table III). Allophenic skin has also been shown, by grafting to "parental" strains, to contain separate cells (in varying proportions) differing in histocompatibility antigens; acceptance by the allophenics themselves of parental strain skin grafts thus also demonstrates tolerance (21). In all tolerant animals with *H-2* mosaicism directly discerned in circulating erythrocytes or in skin, tolerance has remained permanent (14, 21). No evidence whatever of runt disease or of so-called "allogeneic inhibition" (22, 23) has ever appeared in any of our allophenic mice, though at least 700 have by now lived out a full lifespan. It should be noted also that the tolerant animals possess a normal capacity for rejection of tissue containing foreign antigens, such as grafted skin of a third histocompatibility genotype (21). In addition, they have ultimately succumbed to the sorts of diseases—including neoplasias—which characterize their strains of origin (B. Mintz and A. Donnelly, unpublished data). We can again conclude (21) that while "allogeneic inhibition" may be a phenomenon of experimental interest in specialized in vitro and irradiation circumstances, it appears to play no role in the development and economy of intact, unirradiated, healthy animals.

The form of tolerance encountered in allophenic mice differs from the well-known experimentally "acquired" immunological tolerance (2) because, in

allophenic embryos as in normal development, the cells that produce unlike proteins are in each other's company from before the time their respective phenotypes are first expressed. The tolerance of allophenic mice has therefore been designated natural or "intrinsic" (21). The absence of autoimmunity in them fits the expectation predicted (24) from development of normal, single-genotype animals.

As pointed out in the skin graft study (21), nontolerant allophenics could conceivably harbor some cells of the graft-rejected type. We have since learned that an occasional nontolerant animal may in fact contain a small cache of tissue of the genotype of the rejected skin (detected by other markers). An example is EH9 (Table II), an all black mouse that rejected a C3H skin graft, had *H-2B* red cells (within test sensitivity limits), and contained only C57 cells in liver, heart, and other major organs (by the *Mdh-1* malate dehydrogenase [25, 20] isozyme marker). Nevertheless, bone marrow metaphases included 31 XX cells and 92 XY cells (unpublished data by B. Mintz, D. Hungerford, and J. Morrow). Cells of both inbred strains were therefore detected only in the bone marrow. Even if these metaphases were all in erythropoietic cells, that proportion of the minor type (approximately 25% in the dividing marrow cells) could escape detection in the blood with the hemagglutination method. Since a C3H skin graft was not accepted, either skin contains some tissue-specific and strain-specific antigen absent from marrow; or the C3H marrow cells are relatively secluded from the total system; or the *H-2* antigenicity of a small C3H marrow population in an otherwise "all-C57BL/6" animal is inadequate for purposes of immune recognition. A related observation in cattle is that skin grafts exchanged between dizygotic cattle twins with erythrocyte mosaicism can be slowly rejected (26).

Selection between Erythropoietic Cells of Different Phenotypes.—The data suggest that, in allophenic mice, C57BL/6 erythropoietic tissue may possess some selective advantage over C3H. There are 4 mice which show only C3H red cells, 14 with evidence only for C57, and 16 with both. It is possible that two in the C57 group (EH5 and EH9) may also have borderline amounts of C3H: On their first agglutination test, they were graded as C57 with questionable C3H; on a subsequent agglutination test, neither appeared to have C3H and no absorption tests were run to verify the earlier impression. We can perhaps exclude from the tabulation animals without known mosaicism of any kind (six mice identified in Table II), on the grounds that one cell strain may have been lost entirely during regulation of embryo size at implantation, in ways unrelated to any later erythropoietic cell selection. This would still leave two cases with C3H red cells, 10 with C57, and 16 with C3H plus C57. In the two-color group alone, there are 1 C3H, 9 C57, and 16 mosaic.

These proportions might not be borne out in a large random sample of the total C3H(f) ↔ C57BL/6 population; but a trend favoring C57 is also suggested

by the quantitative and chronological evidence. Among the 16 red cell mosaics (Table III), grading of test results by comparison with artificial mixtures yielded 10 with more *H-2B* than *H-2K* cells (including two with very low and three with barely detectable *H-2K*), six with roughly similar proportions of both, and none with more *H-2K* than *H-2B* cells. When tests were repeated on some individuals, EH24 had marked amounts of both cell types in a hemagglutination test at 2 months of age, but revealed *H-2B* with questionable *H-2K* at 4 months, and only *H-2B* by agglutination as well as absorption criteria at 6½ months of age. Therefore, in EH24, the C3H cells were probably present above the 25% level at 2 months, and either absent or less than 10% at 6½ months. (The liver in the same individual was all C3H). No animal with a trend in the opposite phenotypic direction was discovered in the present study. In most instances, the time period covered by progressive tests of one mouse was relatively short, and a substantially longer period had elapsed between birth and the first test in such individuals. If a selective shift was indeed occurring during adult life, it is reasonable to expect that it may have been in progress even before the first test and perhaps during prenatal development as well.

There are a number of loci which have been identified as active in erythrocyte precursor cells and which have different alleles in the C57BL/6 and C3H inbred strains. These include some genes concerned with red-cell-specific products such as hemoglobins, and others that are functional in many cell types (e.g., histocompatibility antigens, respiratory enzymes, etc.). The *H-2* distinctions serve only as a convenient marker; selection may conceivably operate between cells whose phenotypes differ at any or all of these loci, or at others still undefined.

Decisive evidence of shifts in genotypically mixed red cell populations has been obtained in long-range tests of cattle twins, though the direction of change in those instances could be interpreted as random (18). Some shift with age has also been found in human (27) and sheep (28) cases of red cell mosaicism. The changes were clearly shown not to result from an abrogation of immunological tolerance (18, 28).

From these and other studies conducted on allophenic mice (e.g., on melanoblasts [10], germ cells [12], and other cell types), there is increasing documentation for a generalization which has been proposed earlier (11): that selection between phenotypically different cells of any cell population in vivo may be a commonplace and may exercise a major role in producing a supracellular or "total" tissue phenotype. Moreover, such selection can exist as long as the phenotypes are expressed, and can therefore be sustained during part of embryonic life, through birth, and into old age, contributing enormously to a progressively changing total phenotype (11).

In blood of ordinary single-genotype animals, there is at least one possible genetic mechanism which might account for different erythroblast phenotypes, namely, in X-linked heterozygous genotypes of females (29, 30). There, random

activation of one allele in a cell can result in two phenotypic classes of cells upon which selective factors can operate (31, 32). As one of us has proposed (11), there may be other (unknown) genetic mechanisms in mammals which may also be responsible for production of phenotypically different clonal variants in other genotypes (autosomal or sex-linked, homozygous or heterozygous), thereby setting the stage for selection. On this hypothesis, the circulating blood may be a far more heterogeneous and dynamic tissue than we have previously supposed.

Relative Autonomy of Genotypic Composition of the Erythrocyte Population.— How does genotypic composition compare in blood and other tissues of allophenic mice? In Tables II and III, some of the data obtained in other tests are reported. Many additional C3H(f) ↔ C57BL/6 allophenics have been analyzed for genetic identity of γ-globulins, liver, heart, etc., and the total results in each of these systems will be considered in greater detail elsewhere.

The serum allotypes of γ-globulins of the 7Sγ2a class (33), which differ in the C3H and C57 strains (34, 35), were determined by micro-Ouchterlony precipitin tests (36). For many other systems, analyses were based on electrophoretic differences in strain-specific molecular variants of widespread respiratory enzymes, particularly malate dehydrogenase (25), by methods described elsewhere (20). That test is sensitive to the 5% level; in liver, any numbers implying finer discriminations are based on calculation of a weighted average for the entire organ, with actual results from individual lobes corrected for the weights of the lobes. The column labeled "other" summarizes whether or not mosaicism has thus far been found in any tissues other than those explicitly listed. This column covers some or all of the following, for each animal: skeletal muscle, lungs, mammary gland, kidneys, thymus (all of the preceding with the *Mdh-1* malate dehydrogenase marker); germ cells (by progeny tests for *A* and *a* coat color markers); and XX and XY sex chromosome karyotypes, in bone marrow and spleen (only for EH9, EH22, EH34).

The data indicate that certain general trends exist which superficially obscure any autonomy in composition of an organ (11). There is an obvious and misleading tendency for an individual to have either extensive cellular mosaicism in many of its tissues, or else extensive predominance of one strain in many tissues. This tendency can be readily understood as depending upon whether very similar or very unequal numbers of cells of the component genotypes were retained in the allophenic embryo after regulation from double to normal size. As a further, somewhat spurious, complication, a tissue may occasionally and coincidentally be derived from an "unmixed" patch of embryo cells of one genotype.

Despite the latter kind of accidental variability, combined with the former type of variability attributable to total level of retained mosaicism, tissue-specific trends are a reality. Such trends have been uncovered by large-scale analysis and comparisons within and among animals of a given strain combination (reference 11 and unpublished data by B. Mintz). Single animals may

fall at different places in this total complex spectrum of overlapping random and specific variabilities. As the data show (Tables II and III), a great deal of individuality can therefore be achieved from one allophenic animal to another, although each has developed from the same paired combination of embryo strains.

In a general way, such a situation is favorable for tracing cell lineages and analyzing gene control. For example, composition of the erythrocyte population can differ markedly from that, say, of hair follicle cells in the same experimental group. This result is scarcely surprising, and presumably signifies that the two types of cells were genetically determined independently, by activation of cell-type-specific loci in different precursor cells.

In the particular case of blood development and blood physiology, the striking individuality among allophenic mice becomes an especially useful tool because it leads, in effect, to a manipulation of parameters which could not otherwise be experimentally achieved. For example, in view of the role of the liver as the hematopoietic source in fetal stages, and the later assumption of erythropoiesis by bone marrow, it is noteworthy that the adult liver often consists entirely or largely of a cellular genotype which is absent from the circulating erythrocyte population or present only in low frequency (e.g., EH12, EH13, EH15, EH22, EH24, EH25, EH26, EH33). Comparisons of erythrocyte phenotypes and γ-globulin allotypes are also of interest: There is considerably more concordance between them than between erythrocytes and adult liver or heart, though in this allophenic combination there seems to be more discrimination against C3H among red cells than among γ-globulin–producing cells (e.g., EH12, EH13, EH14, EH15).

Many other experimental possibilities for further work are presented by these permutations and combinations. There may, for instance, be animals with C3H and/or C57 erythrocytes which arise in foci with genetically different neighboring cells; or which exist in plasma to which the liver contributes proteins of the opposite strain; or which circulate through a heart or vessels of an entirely unrelated genotype.

The fact that primordial erythropoietic mosaicism can be established in intrinsically tolerant animals therefore affords many novel opportunities for investigation of gene control of hematopoiesis in normal mammalian development and in a variety of hereditary blood diseases.

SUMMARY

Erythropoietic cells of two unrelated strains, C3H (or C3Hf) and C57BL/6, can coexist throughout hematopoiesis in allophenic mice experimentally produced from aggregated, undifferentiated blastomeres of separate genotypes. The presence of two red cell genotypes in these circumstances signifies that the erythroid population must normally be multiclonal, i.e., derived mitotically from

at least two genetically determined cells. The two strains were detected by hemagglutination and absorption tests of erythrocytes for the specific histocompatibility antigens dictated by the $H\text{-}2^k$ and $H\text{-}2^b$ alleles.

Of 34 C3H(f) ↔ C57BL/6 allophenics tested, 16 had both red cell types; the remaining 18 showed only C3H or C57 red cells and included 12 mice with both cell strains present in some other tissues. All animals with evidence of two *H-2* phenotypes among circulating erythrocytes were permanently immunologically tolerant of both antigenic types and remained free of runt disease. They lived a full lifespan, up to 2 yr 7½ months of age.

The data suggest a possible specific selective advantage of C57BL/6 over C3H erythropoietic tissue. There is considerable individual variability, not only in proportions of antigenically distinct erythrocytes, but also in strain composition of other tissues in the same animals. A broad spectrum of distinctive situations is found, in which parameters are varied within or outside of the circulatory system. Allophenic mice can therefore serve as investigative tools for entirely new kinds of experimental studies of gene control mechanisms and blood physiology in normal hematopoiesis and in a number of hereditary blood diseases.

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