CHIMERIC DRIFT IN THE ERYTHROCYTE POPULATION IN BALB/c—C57BL/10 AND BALB/c—B10.D2 MICE

L. M. Fedorov and B. V. Konyukhov

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The ratio between the numbers of cells of the parental genotypes in the peripheral blood of chimeric mice is often unstable and changes with age. It has been shown [6] that in C57BL/6—C3H aggregation mouse chimeras a spontaneous increase in the relative percentage of C57BL/6 erythrocytes takes place with age in the blood erythrocyte population. Later, fluctuations of genotypic composition (by "genotypic composition" we mean the relative percentages of cells of the parental genotypes in the tissues of the chimera) of erythrocyte and leukocyte populations were observed alternately in favor of one or other parental genotype [11]. To designate spontaneous changes in the genotypic composition of blood cell populations in the chimeric organism, some workers [11] have used the term "chimeric drift." Chimeric drift is unconnected with the animal's hair color or sex [10] and is not determined by differences between cells of the parental lines for alleles of the Fv-2 locus, controlling the early stages of erythropoiesis [4]. The causes of chimeric drift are not yet clear.

The aim of the present investigation was to determine whether the appearance of chimeric drift in the erythrocyte population is connected with differences between chimeric cells with respect to haplotypes of the H-2 complex and (or) disturbance of endogenous immunologic tolerance.

EXPERIMENTAL METHOD

To obtain aggregation chimeras, BALB/c ($H\text{-}2^{dd}$), C57BL/10 ($H\text{-}2^{bb}$), and B10.D2 ($H\text{-}2^{dd}$) mice, congenitally resistant, and differing from each other only for haplotypes of the H-2 complex, were used. Chimeric mice were obtained by aggregation of 8-10-cell embryos by the methods described previously [1, 7]. Every month 50 µl of blood was taken from the caudal vein of the chimeras. Erythrocytes were isolated by centrifugation of the blood cell suspension in a Ficoll density gradient (d = 1.09) at 900g for 10 min. To detect chimerism, glucose phosphate isomerase (GPI) isozymes, which have about equal activity but which differ in electrophoretic mobility in starch gel [8], were used as cell markers. The gels after electrophoresis were photographed on x-ray film and, after densitometry of the negatives, the relative percentages of the parental components was determined on the densitograms by measuring the area occupied by peaks of the corresponding isozymes [5]. In addition, the electrophoretic gels of the chimeric samples were compared with those of blood samples with known relative percentages of BALB/c and C57BL/10 erythrocytes (Fig. 1a). Skin from the tail of animals of both parental lines was transplanted into the chimeras at the age of 4-5 months; grafts of each genotype were transplanted to the dorsal surface of the trunk, which has both pigmented and nonpigmented hair cover. The state of the grafts was observed for 5-6 months.

EXPERIMENTAL RESULTS

Altogether 16 chimeras were obtained by the aggregation method: 8 BALB/c ($H\text{-}2^{dd}$)—C57BL/10 ($H\text{-}2^{bb}$) and 8 BALB/c ($H\text{-}2^{dd}$)—B10.D2 ($H\text{-}2^{dd}$), as well as 25 single-component animals: 21 C57BL and 4 BALB/c. In both combinations one parental component was represented by BALB/c, the other by one of the congenitally resistant lines, differing from each other only with respect to haplotypes of the H-2 complex. The two genetic combinations therefore differed from each other only relative to haplotypes of the H-2 complex of one of the com-

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, N. I. Vavilov Institute of General Genetics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 344-347, September, 1988. Original article submitted January 14, 1988.

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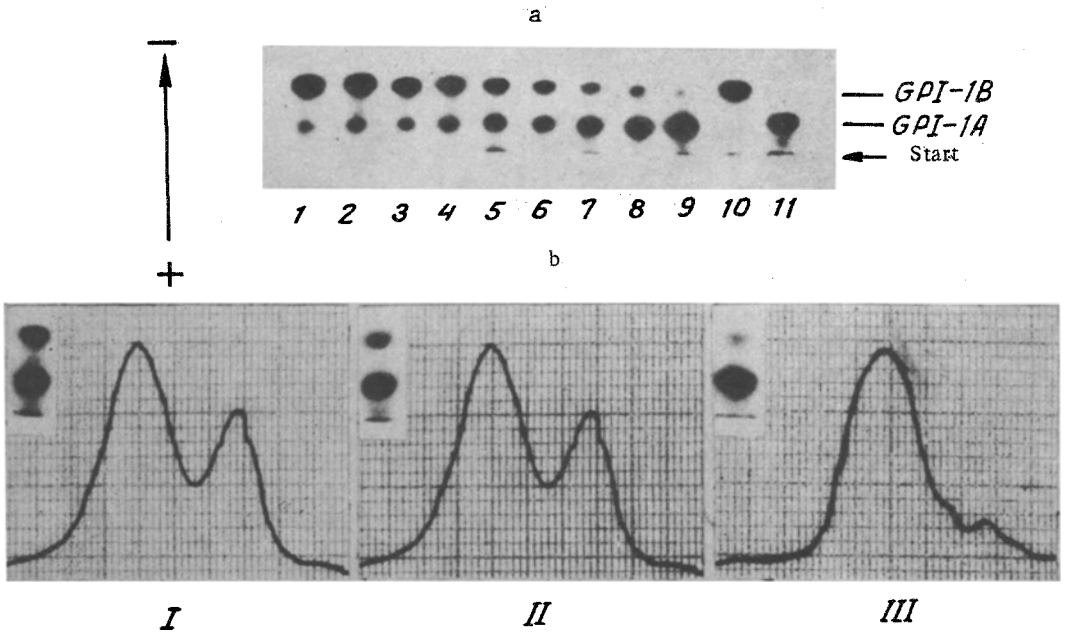
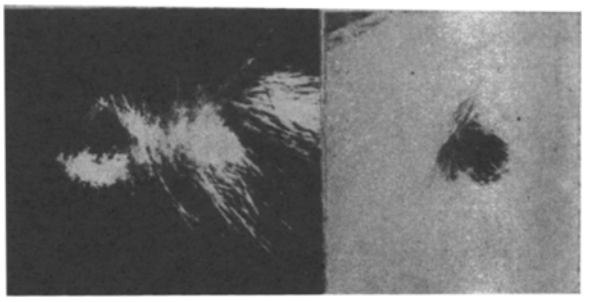


Fig. 1. Starch gel electrophoresis of GPI isozymes from mouse erythrocytes. a) Electrophoresis of hemolysates obtained from a mixture of erythrocytes containing (in %) 90:10 (1), 80:20 (2), 70:30 (3), 60:40 (4), 50:50 (5), 40:60 (6), 30:70 (7), 20:80 (8), 10:90 (9) C57BL/10 and BALB/c erythrocytes respectively; 10, 11) GPI isozymes of C57BL/10 and BALB/c mice; b) gels and corresponding densitograms of hemolysates of erythrocytes obtained from chimera No. 6 at the age of 2 months (I), 5 months (II), and 8 months (III).

ponent lines, so that the role of H-2^b and H-2^d haplotypes in the production of chimeric drift could be investigated. Preliminary determination of chimerism was carried out visually on the basis of hair color, but starting from the age of 1 month, on the basis of GPI isozymes of the peripheral blood erythrocytes. Chimerism of the erythrocyte population was observed in nearly all mice in which mosaicism of the hair cover was observed. The BALB/c erythrocytes were not found only in chimeras No. 1 BALB/c-C57BL/10 and No. 9 BALB/c—B10.D2, in which the BALB/c component was discovered only in the hair, and moreover, in a very small quantity. Animals with different ratios of erythrocytes of the parental genotypes were observed among chimeras of both genetic combinations. With age, a gradual increase was observed in the number of BALB/c erythrocytes in them (Fig. 1b). For instance, in chimeras No. 2 (BALB/c—C57BL/10) and No. 10 (BALB/c—B10.D2), which had about 10% of BALB/c erythrocytes during the first month of life, the number of the latter increased to 25-30% after 5-7 months. In mice with about equal ratios of erythrocytes of the two genotypes in the first month of life, an increase in the content of BALB/c erythrocytes also was observed with age. For example, chimeras No. 4 (BALB/c—C57BL/10) and No. 13 (BALB/c—B10.D2), which had 50 and 42% of BALB/c erythrocytes at the age of 1 month, contained 70 and 68% respectively of the erythrocytes of this genotype after 10 months. In chimeras Nos. 5, 6, and 7 (BALB/c—C57BL/10) and Nos. 15 and 16 (BALB/c—B10.D2), which had more than 50% of BALB/c erythrocytes in the first month of life, C57BL/10 (or B10.D2) erythrocytes could not be detected at all a few months later. In some chimeras, for example Nos. 6 and 14, fluctuations of genotypic composition of the erythrocyte population were sometimes observed in favor of the C57BL/10 (or B10.D2) genotype, but they were of short duration and did not as a whole change the direction of chimeric drift. Thus in chimeras of both combinations the increase in content of BALB/c erythrocytes with age took place independently of haplotypes of the H-2 complex and of the ratio between the parental components in the first month of life. The number of erythrocytes in the blood of the chimeras did not differ from normal during a long period of observation, and amounted to $(8.6\text{-}11)\cdot10^6/mm^3$.

Skin grafts of the parental lines took well on nearly all chimeras with different relative percentages of parental components. The take rate of skin of the parental genotypes did not depend on the phenotype of the area of the chimera's skin on which the graft was transplanted (Fig. 2). Rejection of BALB/c skin 12 days after transplantation was observed only in chimera No. 1 (BALB/c—C57BL/10), in which the BALB/c component was represented only in the hair, and even then, only in a very small quantity.



1 2 3

Fig. 2. Skin grafts from animals of parental lines surviving in BALB/c—C57BL/10. 1, 3) C57BL/10 grafts; 2) BALB/c graft.

Analysis of the genotypic composition of the erythrocyte population in chimeras of the two combinations thus showed a gradual increase in the relative percentage of BALB/c erythrocytes in them. A sharp shift of genotypic composition of the erythrocyte population in BALB/c—C57BL/6 chimeras in favor of BALB/c erythrocytes, induced by Rauscher leukemia virus, was demonstrated previously [2]. However, in intact BALB/c—C57BL/10 and BALB/c—B10.D2 chimeras changes in the genotypic composition of the erythrocyte population took place without any sharp shifts, and about equally, and the increase in the relative percentage of BALB/c erythrocytes rarely exceeded 5-7% in 1 month. The absence of any sudden fluctuations in the relative percentages of the erythrocyte population can evidently be explained by the unique buffer effect due to a relatively long lifespan of mouse erythrocytes, namely 45 days [9]. Consequently, the presence of one-way chimeric drift, at approximately the same rate of flow in chimeras of the two combinations, is evidence that the difference between cells of the chimera with respect to haplotypes $H\text{-}2^b$ and $H\text{-}2^d$ is not an essential condition for the onset of chimeric drift. Instability of the genotypic composition of the peripheral blood erythrocyte population in BALB/c—C57BL/10 and BALB/c—B10.D2 mice is likewise not the result of a disturbance of immunologic tolerance, for skin grafts from C57BL/10 and B10.D2 mice took just as well on the chimeras as did the skin of BALB/c mice, despite the selective advantage of BALB/c erythrocytes in the peripheral blood. The collapse of immunologic tolerance noted in NZB—CFW chimeras [3] can evidently be explained by a significant disturbance of their immune system, since NZB mice are characterized by hereditary autoimmune disease.

Reduction of the relative percentage of C57BL erythrocytes in the chimeras' blood was not accompanied by anemia, and was probably not the result of premature death of erythrocytes of this genotype. Chimeric drift is evidently due to interaction between cells of different genotypes at the early stages of hematopoiesis. One of the causes of chimeric drift may perhaps be the presence of different alleles of a certain locus, directly or indirectly controlling the rate of proliferation of a particular class of erythrocyte precursors, in cells of the BALB/c and C57BL genotypes. For example, the presence of an Stk (stem cell kinetics) locus, possibly controlling the rate of proliferation of hematopoietic stem cells, has been postulated [12].

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