GENOTYPIC COMPOSITION OF VARIOUS TISSUES AND ORGANS OF BALB/c—C57BL/10 AND BALB/c—B10.D2 MOUSE AGGREGATION CHIMERAS WITH MANIFEST CHIMERIC DRIFT

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In adult mouse aggregation chimeras of certain genetic combinations and, in particular, C3H↔C57BL/6 and BALB/c↔C57BL/10 peripheral blood erythrocytes of one parental genotype with age replace erythrocytes of the other parental genotype [1, 2]. It has been shown that for this process to take place, disturbance of endogenous immunological tolerance and differences between chimeric cells with respect to haplotypes of the H-2 complex are unnecessary [1, 2]. It is also known that on combination of embryos of different lines of mice, cells of one line in the chimeric embryo obtain a selective advantage. As a result, among the parental animals most are of the one-component type (these animals consist entirely of cells of only one genotype), and the ratio of the parental components in the tissues of the chimeras is shifted strongly toward one of them. For example, on aggregation of embryos of C57BL/10 and BALB/c mice, 24 one-component animals (19 were C57BL/10 and five were BALB/c) and 18 chimeras, most of which contain more than 75% of the C57BL/10 component, were obtained [4]. When C3H↔F_1(SJL×129) aggregation chimeras were obtained, F_1(SJL×129) cells obtained the advantage [4]. Consequently, in chimeric animals of certain genetic combinations, selective advantage of one cell clone over the other was observed at different stages of ontogeny. The aim of the present investigation was to examine the relationship between selective advantage of the cells of one genotype during embryonic development of the chimeric organism and with a unidirectional change in the genotypic composition of the blood erythrocyte population in adult chimeras.

EXPERIMENTAL METHOD

Aggregation chimeras were obtained by the use of BALB/c albino mice and C57BL/10 and B10.D2 mice, which have black fur. BALB/c↔C57BL/10 and BALB/c↔B10.D2 chimeric mice were obtained by the method in [3]. Chimerism in the peripheral blood erythrocyte population, spleen, and brain was determined by electrophoresis of glucose phosphate isomerase (GPI) isozymes [5]. Erythroid cells were isolated from the blood and spleen by centrifugation of cell suspensions in a Ficoll density (d = 1.09) gradient at 900g for 10 min. Chimerism of bone marrow was determined in XX↔XY chimeras by differential C staining of the chromosomes, using the Y chromosome as the cell marker [6]. To determine the genotypic composition of the sex cell population, chimeras were crossed with BALB/c mice and the content of gametes of the parental genotypes in the gonads of the chimeric animals was judged from segregation in the progeny. From each fertile chimera 7-10 litters were obtained at intervals of 1 month. Chimerism of the fur was determined visually. Correlation for distribution of cells of parental genotypes in the chimeric tissues was determined by Spearman's rank correlation coefficient r_s.

EXPERIMENTAL RESULTS

In accordance with preliminary analysis of the animals' fur (at the age of 1 month) 8 BALB/c↔C57BL/10 chimeras, 8 BALB/c↔B10.D2 chimeras, and 25 one-component animals (21 — C57BL, 4 — BALB/c) were obtained. Since genetic combinations BALB/c↔C57BL/10 and BALB/c↔B10.D2 differed only a little and chimeric drift of the same character was found in chimeras of both combinations, all the chimeras were combined into one group for analysis.

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TABLE 1. Percentage of Cells of BALB/c Genotype in Different Organs and Tissues of BALB/c—C57BL/10 and BALB/c—B10.D2 Chimeras

No. of chimeras	Sex of chimeras	Fur	Erythrocyte population		Spleen	Bone marrow	Gametes	Brain
			first month of life	last month of life				
1	Male	1	0	0	0	—	—	0
9	»	3	0	0	0	—	0	0
2	»	13	8	40	43	—	3	10
10	»	6	15	43	39	—	0	10
3	»	22	15	35	32	—	0	40
11	»	10	30	52	53	—	0	30
12	Female	47	43	73	71	—	0	45
13	Male	55	42	90	88	87	100	37
4	»	45	50	78	90	94	0	40
5	»	40	48	100	100	—	0	46
6	»	60	58	100	100	—	81	60
14	»	52	61	79	85	—	93	40
7	Female	53	66	100	100	—	4	45
8	Male	68	66	88	89	—	—	70
15	Female	75	73	100	100	—	9	60
16	»	90	96	100	100	—	52	95

Legend. Nos. 1-8 and 9-16 correspond to BALB/c—C57BL/10 and BALB/c—B10.D2 chimeras respectively. All animals except chimeras Nos. 4 and 13 were killed at the age of 10 months. Chimeras Nos. 4 and 13 were killed at the age of 16 months. Relative percentage of BALB/c gametes determined relative to progeny obtained from chimera throughout its life.

To discover whether chimeric drift takes place in favor of BALB/c cells (demonstrated for the erythrocyte population) parallel in other rapidly renewed cell populations, the dynamics of gametogenesis in the chimeras was studied in the progeny obtained from them. A group of 16 chimeras consisted of 12 males and four females. Crossing the chimeric males with BALB/c females showed that all except chimeras Nos. 1 and 8 were fertile (Table 1). In three males (Nos. 2, 6, and 14) segregation in the progeny for fur color was observed (white and agouti), evidence of chimerism of the spermatozoa in their population. Consequently, these chimeras had an XY↔XY set of sex chromosomes. In these males, no increase in the relative percentage of BALB/c mice in the litters took place during 7-9 months of observation. The remaining seven males gave a progeny of only one genotype, and they were therefore classed as XX↔XY chimeras. Crossing chimeric females with BALB/c males showed that all four females were fertile, and in three of them, segregation in the progeny for fur color was observed (Fig. 1). In chimeric females Nos. 7, 15, and 16, with gametes of both parental genotypes, no increase in the number of BALB/c mice in the litters took place with age. Female No. 12 gave only pigmented mice in all the litters. These data indicate absence of selective advantage of BALB/c gametes relative to C57BL/10 (or B10.D2) gametes in the chimeras during gametogenesis.

The relative percentage of BALB/c erythrocytes in the blood of the chimeras in the first month of life correlated with the content of the BALB/c component in the fur (r_s = 0.917), but it increased sharply with time (Table 1). The relative percentage of BALB/c erythroid cells in the spleen and peripheral blood of the chimeras was similar on the day of sacrifice (r_s = 0.977) and was considerably greater than the content of erythrocytes of this genotype in the blood in the first month of life. The genotypic composition of the bone marrow was analyzed in chimeric males Nos. 4 and 13 which, according to preliminary data, had the XX↔XY set of sex chromosomes; in chimera No. 4, moreover, the BALB/c cells had an XX set of chromosomes, whereas chimera No. 13 had an XY set (Fig. 2). In both animals the relative percentage of BALB/c cells in the bone marrow was similar to that of BALB/c erythroid cells in the spleen and in the blood on the day of sacrifice and was considerably greater than their content in the blood in the first month of life (Table 1). The genotypic composition of the tissue of the cerebral hemispheres was analyzed in most chimeras aged 8-10 months after sacrifice of the

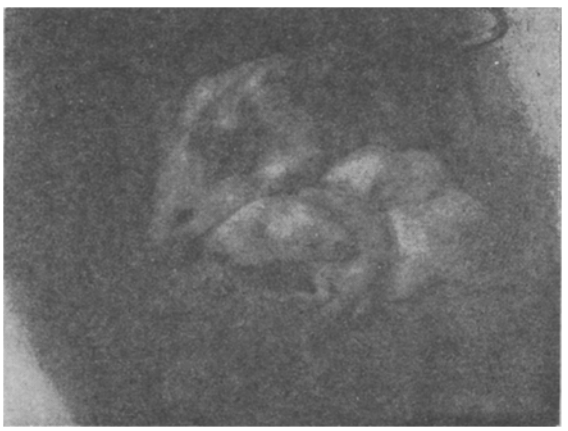

Fig. 1

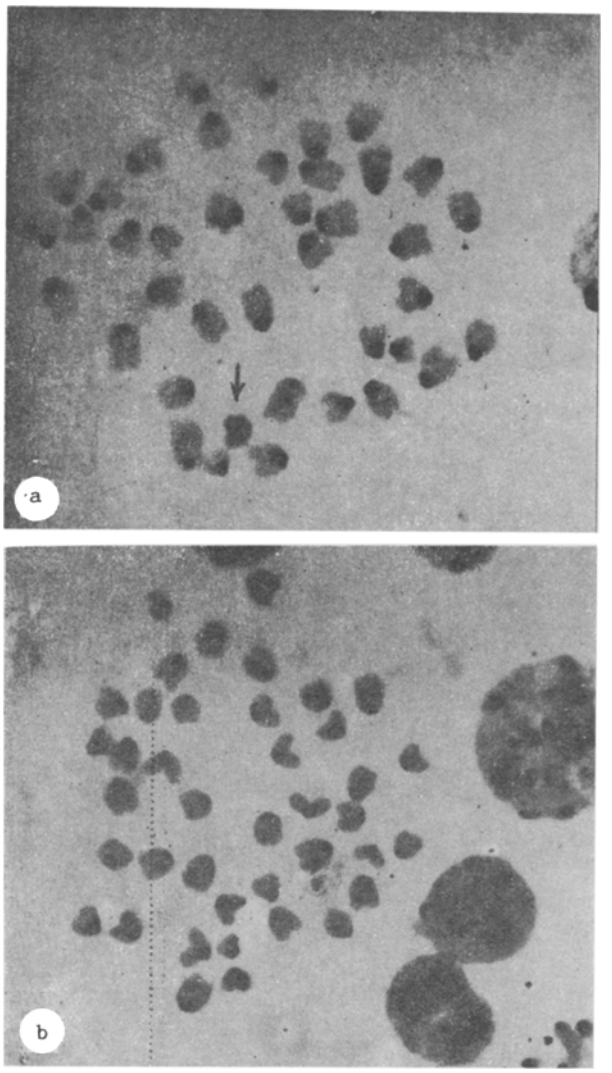

Fig. 2

Fig. 1. Female chimera No. 16 (BALB/c↔B10.D2) with progeny after mating with BALB/c male. Segregation in progeny indicates presence of BALB/c and B10.D2 oocytes in the female.

Fig. 2. Metaphase plates of bone marrow from chimera No. 4 (XX↔XY). a) XY plate. Arrow indicates Y chromosome, darkly stained, with no pericentric heterochromatin; b) XX plate. Differential C staining. 1000 ×.

animals and the relative percentage of the BALB/c component in the brain correlated with the content of this component in the fur (r_s = 0.981) and in the erythrocyte population in the first month of life (r_s = 0.896).

The content of parental components in the fur and in the erythrocyte population, as well as in the brain (after sacrifice of the animals at the age of 8-10 months) in BALB/c↔C57BL/10 and BALB/c↔B10.D2 chimeras in the first month of life was close in value. The presence of chimeras consisting predominantly of C57BL/10 (or B10.D2) cells and also of a large number of one-component C57BL/10 (or B10.D2) animals among those obtained by the aggregation method is evidence that BALB/c cells have no selective advantage over C57BL cells during embryogenesis of the chimera. The study of erythropoiesis and gametogenesis showed that during postnatal development of the chimeras renewal of the cell composition in different, rapidly renewed cell populations took place independently of each other, and replacement of one parental component by the other, as was shown for one rapidly renewed tissue, is not an essential property for other rapidly renewed chimeric tissues. Analysis of the genotypic composition of cell populations of the hematopoietic organs showed that cells of the BALB/c line in the bone marrow even at this stage obtain a selective advantage compared with C57BL cells. Chimeric drift in the peripheral blood erythrocyte population is thus not the result of an overall advantage of the parental component BALB/c over the C57BL component during prenatal

or postnatal development of chimeric mice, but is due to the selective advantage of BALB/c hematopoietic cells over C57BL cells in the chimeric hematopoietic organs.

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