INVESTIGATION OF AUTONOMY OF ACTION OF THE WHITE GENE IN ALLOPHENIC MICE

V. V. Osipov and M. P. Vakhrusheva

UDC 575.222.75

An allophenic mouse and three allophenic embryos were obtained by fusion of eight-cell Mi^{wh}/Mi^{wh} and +/+ embryos. The color of the hair and pigmented epithelium of the eye showed chimerism. The Mi^{wh}/Mi^{wh} melanoblasts die during development of the embryo. Variations in color of the chimera depend on interaction between normal melanocytes and the surrounding cells of the dermis, which consists of clones with the Mi^{wh}/Mi^{wh} or +/+ genotype and a mixture of cells of these genotypes. Differences were observed in the development of the right (chimeric) and left (normal) eyes and normal clones grew more rapidly in the allophenic mouse. Pigmentation of the eyes was reduced in the two allophenic embryos compared with normal. This indicates the autonomous action of the white gene in mice.

KEY WORDS: allophenic mice; pigmentation; genotype of cell clones.

Methods have been developed recently whereby various experiments can be performed at virtually any stage of mammalian embryogenesis. One such method is the obtaining and study of allophenic mice. The allophenic mouse is an animal which contains two or more genetically different cell populations arising from two or more different fused zygotes. Allophenic mice (genetic chimeras) were first obtained by Tarkowski [12] by fusing two early embryos with different genotypes. Later this method was modified by Mintz [4-6]. Allophenic mice have now been obtained in several laboratories. They are convenient objects for the study of problems of genetic control of differentiation of cells and organs, the relationship between the phenotype of the cell and its genotype, the clonal bases of formation of the phenotype of an organ, carcinogenesis, and other problems in biology and medicine.

The object of the present investigation was to obtain allophenic mice and to use this model to study autonomy of action of the white gene (Mi^{wh}).

EXPERIMENTAL METHOD

Allophenic mice were obtained by Tarkowski's method [12] modified by Mintz [4-6], by aggregating two embryos of different genotypes. Spontaneously ovulating mice homozygous for the mutant white gene (Mi^{wh}) and female mice of the normal inbred C57BL/Mib (+/+) strain were crossed with males of the same genotype. On the 3rd day after discovery of a vaginal plug the Mi^{wh}/Mi^{wh} and C57BL/Mib donor females were killed (the day of discovery of the vaginal plug was regarded as the first day of pregnancy). The embryos were flushed out of the oviducts with warm physiological saline on to a watch glass. The zona pellucida was removed from the eight-blastomere embryos with pronase. Embryos of the two genotypes were brought into contact in Biggers' medium [1] under mineral oil and kept in a container at 37°C, through which a gas mixture (5% CO_2 in air) was passed to maintain the pH of the medium. The aggregated embryos, having developed to the blastocyst stage, were implanted surgically into the uterine cornu of a pseudopregnant female mouse of inbred line A/He. Pseudopregnant inbred A/He female mice were obtained by crossing with C57BL/Mib males previously verified for sterility. Sterile males were obtained by dividing and ligating the spermatic cords.

Three allophenic 14-day embryos and one newborn male allophenic mouse were obtained; the latter was fostered by a mother which had her own newborn progeny. The weight of the embryos and mice of the genotypes studied was determined. Their eyes and skin were investigated in histological preparations obtained by the usual method.

Laboratory of Experimental Genetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 3, pp. 365-368, March, 1978. Original article submitted May 23, 1977.

0007-4888/78/8503-0395$07.50

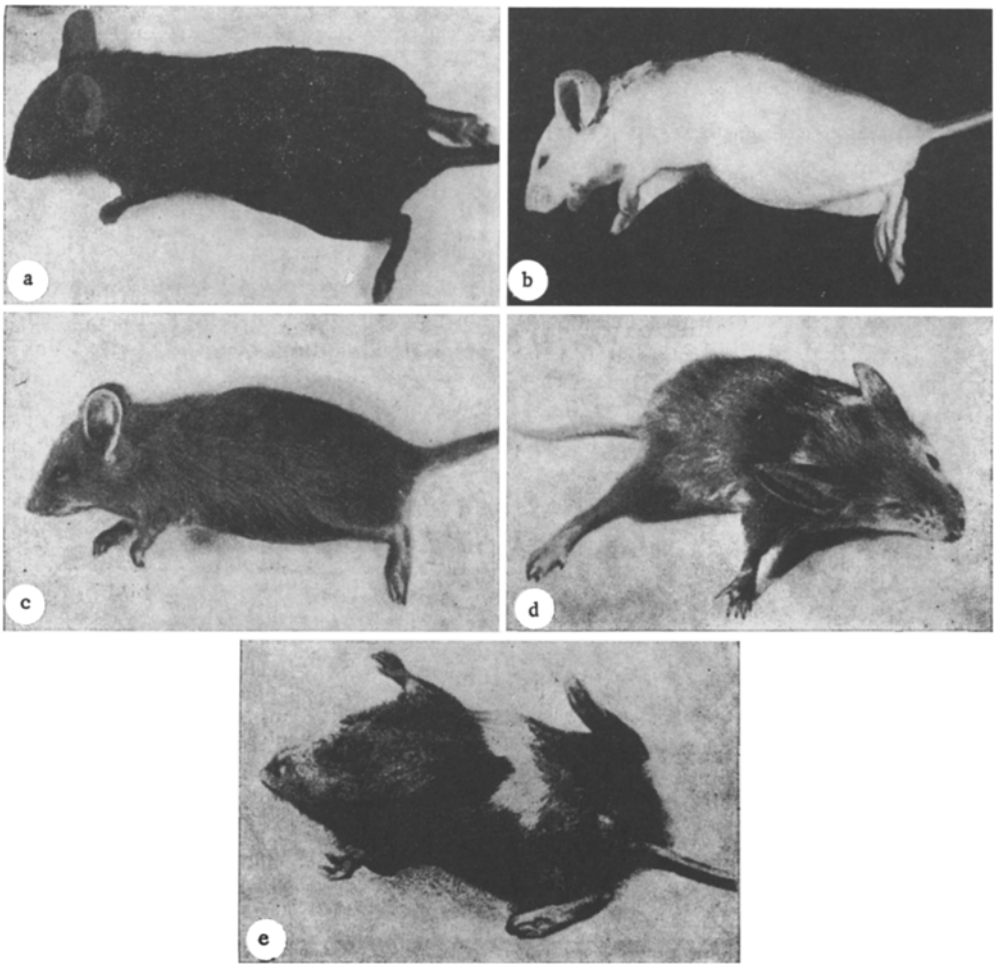

Fig. 1. Mice of different genotypes: a) C57BL/Mib (+/+); b) Mi^{wh}/Mi^{wh}; c) $Mi^{wh}/+$; d, e) allophenic $Mi^{wh}/Mi^{wh} \leftrightarrow +/+$ mouse.

EXPERIMENTAL RESULTS

Chimerism was manifested in the experimentally obtained allophenic $Mi^{wh}/Mi^{wh} \leftrightarrow +/+$ mouse in the color of its hair and eyes. The hair of the chimera consisted of alternate black and gray strips with small and symmetrical white spots behind the ears and large white and black spots on the abdomen (Fig. 1).

The melanocytes and certain components of the dermis are known to be derivatives of the neural crest [2]. Melanocytes are absent from the hair bulbs of Mi^{wh}/Mi^{wh} mice, for the melanoblasts of this genotype die during differentiation of the dermis [7-9]. All variations of hair color in the chimera are evidently connected with the distribution of the number of melanocytes of the +/+ genotype and their interaction with the surrounding tissues, i.e., with cells of the hair follicle and cells of the dermal layer of the skin. Alternation of black, gray, and white stripes and spots in the color of the hair in the chimera reflects the character of distribution of clones of ectomesenchyme in the developing dermis, for normal melanoblasts and melanocytes are found in the dermal layer consisting of clones of +/+ and Mi^{wh}/Mi^{wh} cells and a mixture of them. Black stripes and spots appear in the case of a normal dermis and normal melanoblasts. Gray stripes and spots are formed by interaction between normal melanoblasts and Mi^{wh}/Mi^{wh} dermis or a mixture of normal dermal cells and Mi^{wh}/Mi^{wh} dermal cells. White spots evidently arise when there are no melanocytes in the hair follicles. This can be observed during delayed migration of melanoblasts, when the hair follicles form before the melanoblasts can reach them. White spots on the abdomen and general weakening of pigmentation are observed in $Mi^{wh}/+$ mice, but they differ from chimeras in that black spots and stripes are never observed.

The weight of the allophenic mouse at birth and at subsequent times of development was a little less than normal. The newborn allophenic mouse weighed 1.05 g, at the age of 7 days it weighed 3.10 g, 14 days 6.30 g, and 20 days 7.10 g. Normal mice of the corresponding age weigh 1.52 ± 0.02, 3.71 ± 0.09, 8.96 ± 0.19, and 10.21 ± 0.25 g. The smaller weight of the allophenic mice at birth and in the later stages of development and the mosaic constitution of these mice do not reduce their viability [3, 7, 11, 12].

Chimerism in the mice also was manifested in the size of the eyes and the color of the pigmented epithelium. The right eye weighed 11 mg and the left eye 12.2 mg. The eyes of normal 20-day mice weigh 17.2 ± 0.9 mg, eyes of $Mi^{wh}/+$ mice weighed 14.9 ± 0.85 mg, and eyes of Mi^{wh}/Mi^{wh} mice of the same age weighed 9.0 ± 0.7 mg. The pigmented epithelium in the left eye was virtually indistinguishable from normal. The right eye was somewhat smaller than the left, and the lids were not completely opened. The color of the whole pigmented epithelium was considerably diminished in intensity and, in addition, there were several small areas in which the pigmented epithelium was not colored. Individual differences in the pigmentation of the eyes in allophenic mice, and preferential development of normal clones over mutant have been observed by some workers [9, 10].

The weight of the allophenic embryos (220 ± 21 mg) was a little less than the weight of normal embryos (250 ± 12 mg) at the same time of development. Chimerism of the $Mi^{wh}/Mi^{wh} \longleftrightarrow + /+$ embryos was shown by a decrease in the intensity of pigmentation of the eyes compared with normal. Of the three embryos, a clearly visible bilateral decrease in the intensity of pigmentation of the outer layer of the optic cup was found in only two. In one embryo the intensity of pigmentation of the eyes was the same as in normal embryos at the same period of development.

Manifestation of the effects of the white gene in the allophenic embryos and mouse indicates the autonomous action of this gene.

The experimental method of obtaining allophenic mice can provide an unambiguous answer to the question of autonomy of action of the gene in a small number of animals. This is one of the advantages of this method.

LITERATURE CITED

1. J. C. Biggers, W. K. Whitten, and D. C. Whittingham, in: Methods in Mammalian Embryology (ed. by J. Daniel), San Francisco (1971), p. 86.
2. S. Horstadius, The Neural Crest, New York (1950).
3. A. McLaren and P. Bowman, Nature, 224, 238 (1969).
4. B. Mintz, Science, 138, 594 (1962).
5. B. Mintz, Science, 148, 1232 (1965).
6. B. Mintz, in: Methods in Developmental Biology (ed. by F. Wilt and N. Wessells), New York (1967), p. 379.
7. B. Mintz, Proc. Nat. Acad. Sci. USA, 58, 344 (1967).
8. B. Mintz, Genetics, 61, Suppl., 41 (1969).
9. B. Mintz, Fed. Proc., 30, 935 (1971).
10. B. Mintz et al., Genetics, 64, Suppl., 43 (1970).
11. E. T. Mystkowska and A. K. Tarkowski, J. Embryol. Exp. Morphol., 20, 33 (1968).
12. A. K. Tarkowski, Nature, 190, 857 (1961).