J. Embryol. exp. Morph. Vol. 36, 2, pp. 425–430, 1976
Printed in Great Britain

Comparative analysis of cell distribution in the pigment epithelium and the visual cell layer of chimaeric mice

By S. SANYAL[1] AND G. H. ZEILMAKER[2]
From the Departments of Anatomy[1] and Physiology[2]
Medical Faculty, Erasmus University, Rotterdam

SUMMARY

In chimaeras of both *rdrdCC*↔+ +*cc* and *rdrdcc*↔+ +*CC* combinations two types of distribution were observed. In a majority of the chimaeras both retinal layers were chimaeric; whereas in a few cases the pigment epithelium was chimaeric but the visual cell layer was made of + + cells only. No spatial relation was observed in the distribution of the cells in the two layers. The two eyes of the individuals were nearly always identical with regard to occurrence of chimaerism in the two layers. The findings are discussed in the light of the possible site and mode of expression of the *rd* gene.

INTRODUCTION

In a preliminary study (Sanyal & Zeilmaker, 1974) we have observed a close correlation in the occurrence of chimaerism in the pigment epithelium and the visual cell layer of the retina and in the two eyes of individuals from a series of *rdrdCC*↔+ +*cc* chimaeric mice. These observations have been extended in a larger sample and similar data have been obtained from a series of *rdrdcc*↔+ +*CC* chimaeras. This paper presents comparative data on the frequency, extent and relationship of chimaerism in the two retinal layers of the chimaeras of these two series.

MATERIAL AND METHODS

Mice from C3HfHeA *rdrdCC*, Balb/cLiA + +*cc* and their congenic variants C3H + +*CC* and Balb/*crdrdcc* (Sanyal & Bal, 1973) were used. The chimaeras were produced by aggregation of 8-cell embryos. The zona pellucida was removed partially in 0·5 % pronase (Calbiochem) (Mintz, 1962) and finally with a narrow pipette. Aggregation was performed in droplets of medium under mineral oil with the help of a Leitz micromanipulator. The fused morulae were

[1] *Author's address*: Department of Anatomy HB 12, Erasmus University Rotterdam, P.O. Box 1738, Rotterdam, The Netherlands
[2] *Author's address*: Department of Endocrinology, Growth and Reproduction HB 15, Erasmus University Rotterdam, P.O. Box 1738, Rotterdam, The Netherlands

grown *in vitro* till the blastocyst stage. Groups of 6–10 blastocysts were transferred to the uterine horns of recipient females on day 2 of pseudopregnancy (plug = day 0).

The eyes were removed when the offsprings were 20–26 days old and fixed in Carnoy's fluid. Serial paraffin sections of 8 μm thickness were stained with haematoxylin and eosin. Cellular genotypes were ascertained in the pigment epithelium by the presence or absence of melanin and in the neural retina by difference in the thickness of the outer nuclear layer (Figs. 1, 2, 6, 7). To determine the chimaeric or unigenotypic composition of each eye all the sections were examined.

RESULTS

Frequency of chimaeric distribution

Balb/c + +cc↔*C3H* rdrdCC. Two types of chimaeras were observed in a total of 29 offsprings (Table 1) from chimaeric blastocysts. In the first type, both eyes of four (13·8 %) animals showed chimaerism in the pigment epithelium only; the neural retina in all cases was identical to the Balb/c + + phenotype.

Table 1. *Frequency of chimaeric distribution in pigment epithelium (PE)/neural retina (NR)*

Donor genotypes	No. of mice	PE Balb/c / NR Balb/c	↔ / Balb/c	↔ / ↔	↔ / C3H	C3H / C3H
Balb/c + +*cc* \ C3H *rdrdCC* /	29	6 (20·7)	4 (13·8)	16 (55·2)	—	3 (10·3)
Balb/c *rdrdcc* \ C3H + +*CC* /	38	10 (26·3)	—	21 (55·3)	2 (5·3)	5 (13·1)

↔ denotes presence of cells from both donor genotypes. Balb/c and C3H denote presence of cells from the corresponding genotype only. Figures in parentheses indicate percentage.

Note occurrence of two types of chimaeras in both series. Information on the coat colour chimaerism of the animals is described in the text.

All these animals showed chimaeric coat colour. In the second type, 16 (55·2 %) animals showed chimaerism in both pigment epithelium and neural retina. Except for one mouse, of which one eye was entirely of C3H *rdrdCC* composition, all animals showed chimaerism in both eyes. Two of the animals of this type were completely agouti while the rest were chimaeric in their coat colour. In six (20·7 %) animals, both the retinal layers were identical to the Balb/c phenotype; one of these had chimaeric coat and the others were completely albino. In three (10·3 %) animals, the retinal layers showed the C3H phenotype only; one of them had chimaeric coat and the others were completely agouti.

Balb/c rdrdcc↔*C3H* + +CC. Out of 38 animals obtained in this series, two (5·3 %) showed chimaerism in the pigment epithelium only and possessed a neural retina entirely of + + phenotype in both eyes. These animals had a

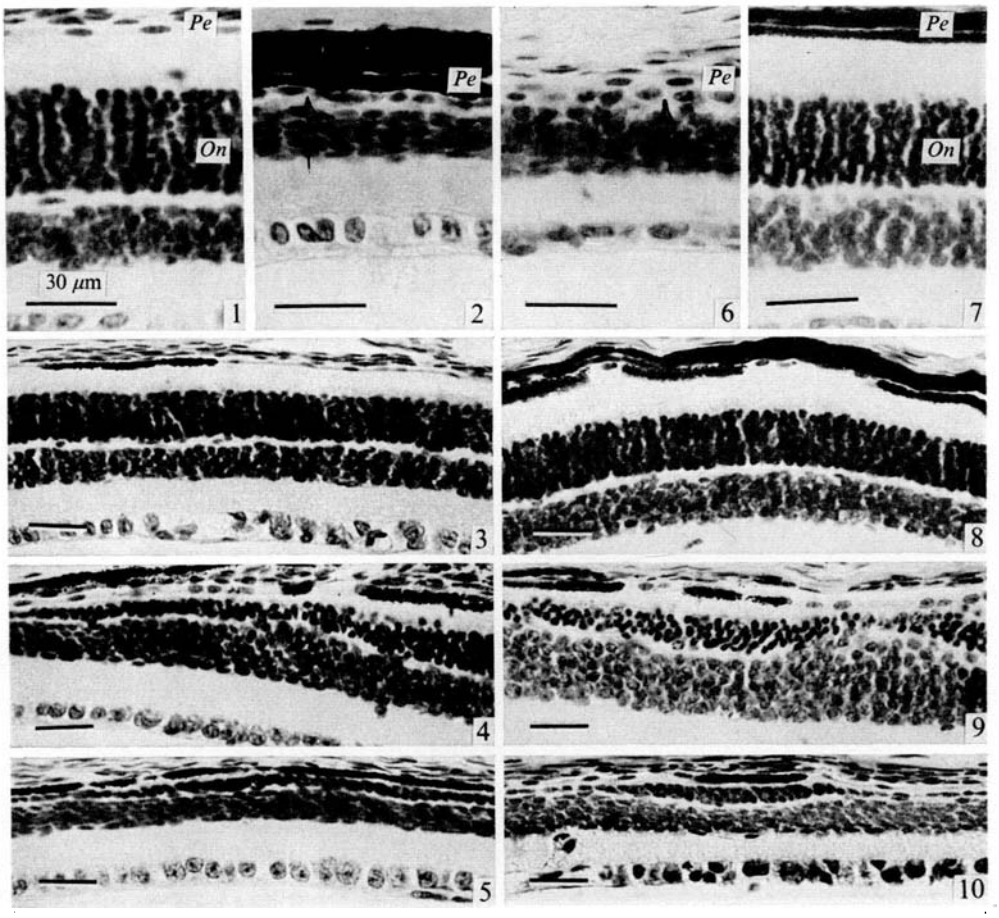

Fig. 1. Balb/c $++cc$ retina. The pigment epithelium (*Pe*) is devoid of melanin. In the neural retina, the perikarya of the visual cells are seen in the thick outer nuclear layer (*On*).

Fig. 2. C3H *rdrdCC* retina. The pigment epithelium (*Pe*) is heavily pigmented. In the neural retina, the *rd* gene causes selective death of the visual cells after initial development resulting in reduction of the outer nuclear layer to a single row (arrow).

Fig. 3. Retina showing chimaerism in pigment epithelium only; the layer of the visual cells is similar to Balb/c $++$.

Fig. 4. Retina showing chimaerism in both layers.

Fig. 5. Another chimaera showing increased proportion of pigmented C3H cells in the pigment epithelium and reduced proportion of visual cells in the neural retina.

Fig. 6. Balb/c *rdrdcc* retina. The pigment epithelium (*Pe*) is devoid of melanin. In the neural retina, the layer of the visual cells is reduced to a single row (arrow).

Fig. 7. C3H $++CC$ retina. The pigment epithelium is heavily pigmented. The neural retina shows the thick layer of visual cells.

Fig. 8. Retina showing chimaerism in the pigment epithelium only. Note presence of albino cells between stretches of pigmented cells. The layer of the visual cells is similar to C3H $++$ as in Fig. 7.

Fig. 9. Retina showing chimaerism in both layers.

Fig. 10. Chimaeric retina with more albino cells in the pigment epithelium and fewer visual cells in the neural retina.

chimaeric coat. In 21 (55·3 %) mice, chimaeric distribution was recorded in both pigment epithelium and neural retina. Excepting one individual, all animals in this group showed similar chimaerism in both eyes and were also chimaeric in coat colour. The exceptional mouse was completely albino and the retinal layers of one eye were similar to the Balb/c *rdrdcc* phenotype.

In ten (26·3 %) animals, both retinal layers were identical with Balb/c *rdrdcc* phenotype. Of these, one had some pigmented hairs but the rest were completely albino. In five (13·1 %) other animals, the retinal layers were as in the C3H + +CC phenotype with completely agouti coat colour.

Histological observations

Although no exact quantitation or spatial reconstruction of cell distribution was undertaken, by careful examination of serial sections, it was possible to make some conclusions based on visual estimation. In chimaeras, of which the visual cell layer was of + + phenotype (Figs. 3, 8), the pigment epithelium was predominantly populated by + + cells and the proportion of *rdrd* cells was correspondingly small. In animals showing chimaeric distribution in both layers, relative proportions of cells from the two genotypes varied considerably among the individuals (Figs. 4, 5, 9, 10). In the layer of visual cells, areas with normal thickness as in + + mice, areas with a single row of nuclei as in *rdrd* and areas of varying intermediate thicknesses were easily recognized. In the two animals showing unilateral chimaerism, both retinal layers in the chimaeric eye were predominantly populated by cells of the genotype encountered in the non-chimaeric eye. No spatial correspondence was observed in the distribution of cells in the two retinal layers. In other words, cells of one genotype, in the pigment epithelium, were often overlying cells of the other genotype in the neural retina.

Chimaerism in the melanocyte population of the choroidal layer of the eye was observed in many cases but apparently there was no correlation with the ehimaeric distribution in the retinal layers.

DISCUSSION

The foregoing results show a high degree of correlation in the occurrence of chimaerism in the pigment epithelium and the layer of visual cells and between the two eyes of the chimaeric individuals. In all animals with chimaeric distribution in the layer of visual cells, the pigment epithelium was also chimaeric. But lack of any spatial relation in the distribution of the cell types in these two layers shows clearly that the expression of the *rd* gene is independent of the genotype of overlying cells of the pigment epithelium. This is further confirmed by occurrence of chimaeras in which the pigment epithelium was chimaeric but the layer of visual cells was identical with the + + phenotype.

The question arises whether the *rdrd* cells, in the above mentioned types of chimaeras, are altered in their phenotypic expression by surrounding + +

cells or whether their absence is due to preferential selection of + + cells in the layer of visual cells. In those instances in which phenotypic alteration in chimaeric tissues has been reported, such as in experiments with *se* and *vt* mutants (McLaren & Bowman, 1969) and the dy^{2J} mutant (Peterson, 1974), chimaeric distribution was infrequent and tissues concerned tended to resemble one of the genotypes. In contrast, chimaeric distribution in the layer of visual cells was recorded in 55 % of the animals and furthermore, extreme variation in the relative proportions of + + and *rdrd* cells could be readily observed among the chimaeric individuals. Therefore it is more likely that the phenotypic expression of the *rdrd* cells is not affected by neighbouring + + cells, although conclusive evidence from identification of the cells by other criteria attributable to the genotype is lacking.

The outer layer of the optic cup, which develops into the pigment epithelium, remains a single row of cells, whereas the inner layer grows and differentiates into the stratified neural retina of which the visual cell layer alone consists of 8–10 rows of cells. It seems possible, therefore, that selection during the extended period of mitosis in the neural retina favours accumulation of + + cells in the visual cell layer. Instances of tissue-specific selection in chimaeric mice, favouring cells from one of the donor strains, have been recorded in several studies (Mintz, 1970; Moore & Mintz, 1972; Tuffrey, Barnes, Evans & Ford, 1973; West & McLaren, 1976). In the present study, individuals with chimaeric pigment epithelium and a completely normal (+ +) neural retina were seen in both series of chimaeras; but individuals with chimaeric pigment epithelium and a completely *rdrd* neural retina were never encountered. Thus + + cells appear to be selected irrespective of the mouse strain suggesting that the activity of the locus or of factors closely linked to it may affect the growth rate of the presumptive visual cells.

We thank Camilla Verhamme and Richard Hawkins for technical assistance, Paula Delfos and Mr. W. van den Oudenalder for the illustrations, Dr Peter Schenck for discussion and Professor J. Moll for reading the manuscript.

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(Received 6 April 1976, revised 29 June 1976)