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Postnatal development of parthenogenetic ↔ fertilized mouse aggregation chimeras

A. PALDI[2], A. NAGY[2], M. MARKKULA[2,*], I. BARNA[1] and L. DEZSO[1]

[1]*Agricultural Biotechnology Center, H-2100 Godollo, Tessedik Samuel u. 4., Hungary*
[2]*Department of Behaviour Genetics, L. Eotvos University, H-2131 God, Javorka, S., u. 14., Hungary*

* Present address: University of Helsinki, Department of Virology, Hartmannin katu 3., 00290 Helsinki, Finland

Summary

Chimeras were made from parthenogenetic and fertilized cleavage-stage mouse embryos. The perinatal mortality was high. The parthenogenetic contributions to different tissues at birth ranged from 0 to 50 %. No selection of parthenogenetic cells was observed in the pigmentation of the coat, but this does not exclude that such selection could act in other tissues. The weight of chimeras at birth negatively correlated to the average contribution of the parthenogenetic part. The growth rate of chimeras was lower than that of nonchimeric animals.

The data presented demonstrate that, although parthenogenetic cells are not cell lethals and they can participate to some degree in normal development of most tissues, their extensive presence reduces the viability of chimeras and retards the postnatal development.

Key words: parthenogenesis, genomic imprinting, chimera, mouse, fertilization, postnatal development, aggregation chimera.

Introduction

Parthenogenetic mouse embryos do not develop to term when transferred to the uteri of pseudopregnant recipients (for detailed review see Kaufman, 1983). They cease development shortly after implantation (Kaufman & Gardner, 1974). Advanced limb-bud stage can be achieved only following ovariectomy of the recipients and 'delayed implantation' (Kaufman *et al.* 1977). Recently, nuclear transplantation experiments at the zygote stage have demonstrated that the inviability of parthenogenones is determined by pronuclei (Mann & Lovell-Badge, 1987) and the completion of mouse embryogenesis requires the presence of both the paternal and maternal genomes (Barton *et al.* 1984; Cattanach, 1986; Cattanach & Kirk, 1985; Surani *et al.* 1984; McGrath & Solter, 1984). Functional differences between the parental genomes are first observed at the pronuclear stage (Renard & Babinet, 1986; Mann, 1986) and persist during the preimplantation and postimplantation period as well (Surani *et al.* 1986; Nagy *et al.* 1987). The molecular mechanism for parental imprinting is unknown, but data on transgenic mice suggest that methylation of specific DNA sequences can satisfy criteria for an imprinting mechanism (Reik *et al.* 1987; Sapienza *et al.* 1987; Swain *et al.* 1987).

While neither diploid maternal (parthenogenone and gynogenone) nor diploid paternal (androgenone) genomes alone are able to support normal development, gynogenetic and parthenogenetic but not androgenetic embryos can be rescued successfully *via* production of aggregation chimeras (Surani *et al.* 1977; Stevens *et al.* 1978; Anderegg & Markert, 1986; Surani *et al.* 1987). Several of these chimeras develop into adults and, in some cases, parthenogenetic cells can give rise to fully functional ova. These results indicated that parthenogenetic cells are capable of differentiating into most somatic tissues and the germ cells in the presence of cells derived from normally fertilized embryos.

Androgenetic ↔ parthenogenetic chimeric embryos are not viable, indicating that the two parental genomes must be present within the same cell. Parthenogenetic cells in chimeric fetuses were found only in the embryo and the yolk sac while the trophoblast consisted almost entirely of androgenetic cells (Surani *et al.* 1987).

In our previous work, we investigated the fate of the parthenogenetic component in aggregation chimeras (Nagy *et al.* 1987). We observed a strong selection against parthenogenetic cells during fetal development. In the yolk sac, this selection was almost complete by term, but in the embryo the selective process was weaker.

In the present study, we found that the postnatal

development of parthenogenetic ↔ fertilized chimeras is greatly affected by the presence of parthenogenetic cells.

Materials and methods

Animals

Unfertilized eggs were obtained from (C57BL×CBA)F_1/Lati (henceforth called BCF1) females after superovulation by *i.p.* injection of 5 i.u. of pregnant mare's serum gonadotrophin (Intervet) followed 48 h later by an injection of 5 i.u. of human chorionic gonadotrophin (hCG). Fertilized 8-cell NMRI× BALB/c and BALB/c embryos were obtained on day 3 of pregnancy after natural mating (day 1 designated as the day that the vaginal plug was found).

Parthenogenetic activation

Eggs surrounded by cumulus cells were recovered 16 h after hCG and incubated for 6 min in 7 % ethanol (Kaufman, 1982) in culture medium (Whitten, 1971), then transferred to medium containing 5 $\mu g\,ml^{-1}$ cytochalasin B (Calbiochem). After 4 h the cumulus cells were removed by incubation in 300 i.u. ml^{-1} hyaluronidase (bovine testis, type V, Sigma). Eggs with two pronuclei were cultured up to 8-cell stage in Whitten's medium at 37°C under 5 % CO_2/95 % air.

Preparation of aggregation chimeras

Two types of aggregates were produced: parthenogenetic BCF1 ↔ fertilized BALB/c and fertilized (BCF1×BCF1) ↔ BALB/c. The zona pellucida was removed by treatment with pronase (Mintz, 1971). Pairs of embryos were aggregated in microdrops and cultured overnight. Only those embryos that formed chimeras and developed into normal blastocysts were transferred into uterine horns of day-3 pseudopregnant BCF1 recipient females.

Analysis of GPI isoenzymes

Organs of newborn animals were homogenized and the GPI isoenzymes were separated by electrophoresis on polyacrylamide gel. The parthenogenetic contribution was assessed semiquantitatively from the intensity of staining of GPI-A and GPI-B isozyme bands in serial sample dilutions.

Results

Two sets of experiments were performed. In the first set, diploid parthenogenetic embryos (BCF1; GPI-B) were aggregated with fertilized embryos of NMR1 females mated with BALB/c males (GPI-A). 50 aggregates were transferred to six pseudopregnant recipients. Of the 19 pups born, nine died or were eaten by the mother before any chimerism could be detected. Of the ten live animals, four females and three males were identified as chimeric by the presence of pigmented patches in the coat and iris which could only have been derived from the parthenogenetic cell lineage. The three nonchimeric animals were males (Table 1).

Two pups that died soon after birth were dissected and their organ homogenates were analysed for GPI. Both animals were chimeras with a high contribution of the parthenogenetic component in all organs (Table 2; animals R80 and R81).

Animals that reached adulthood exhibited no viability problems and were fertile. The parthenogenetic contribution was estimated on the basis of extent of pigmented patches on the coat. The first chimeric animal contained about 40 % of parthenogenetic component, the second about 20 % and the third approximately 10 % (Fig. 1; from left to right). The larger the parthenogenetic contribution, the smaller the body size of these animals were. At the age of two months the chimeras with the largest, middle and smallest parthenogenetic part weighed 18 g, 24 g and 29 g, respectively, while the nonchimeric littermates weighed 35·3 g, on average.

At the time of preparation of the manuscript these animals were one year old. We did not observe any change in the pigmentation pattern of the coat. The GPI-B band characteristic for the parthenogenetic cell lineage could not be demonstrated in the blood of the three chimeric animals at this time. Other tissues were not analysed for GPI because we plan to use these animals for histological studies.

The chimeric females had been mated to albino males. They produced several litters. In addition to the

Table 1. *Development of parthenogenetic ↔ fertilized aggregation chimeras*

Chimera					
No.	Genotype	Number of aggregates transferred	Number of recipients	Number of live newborns	Number of chimeras
1.	BCF1 ↔ (NMRI×BALB/c)F_1	50	6	10	7
2.	BCF1 ↔ BALB/c	218	26	29	14

Table 2. *Contribution of parthenogenetic cell lineage to the organs of newborn chimeras*

Chimera (code)	Tissues examined (% of parthenogenetic contribution)									
	Brain	Liver	Lung	Kidney	Muscle	Skin	Spleen	Stomach	Intestine	Heart
R80	45	50	30	50	45	45	NE*	20	50	40
R81	40	35	40	35	10	35	NE	40	45	25
R93/1	10	20	15	10	10	10	10	10	10	15
R95/1	20	10	10	0	5	10	0	0	10	10
R95/2	40	40	40	15	40	25	25	10	25	30

*Not examined.

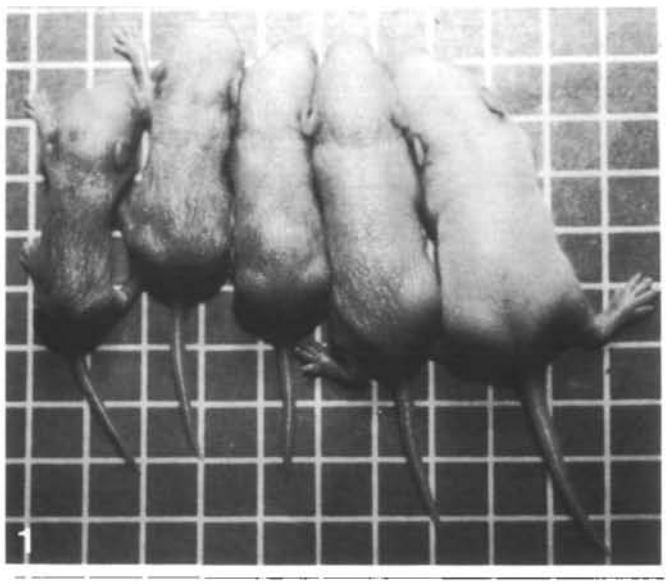

Fig. 1. Animals few days after birth from the first set of experiments. Estimated parthenogenetic contributions (left to right, respectively): 40 %; 20 %; 10 %; 0 % and 0 %. (Parthenogenetic contribution was assessed from the coat pigmentation.) Note the differences in body size.

albinos, all the females also produced some pigmented young. The more pigmented the chimeric females were, the more pigmented were the pups they produced. The pigmented pups could only have been developed from ova of parthenogenetic origin. All the young were of normal size irrespective of whether they developed from ova of parthenogenetic or normal origin (not shown).

We performed a second set of experiments to confirm the initial observation on the size differences of chimeras. In order to have genetically well-characterized animals, normally fertilized BALB/c embryos were used for chimera production instead of (NMRI× BALB/c) embryos. 218 aggregates were transferred to 26 recipients and the pups were delivered by Caesarian section on day 20 of pregnancy. The perinatal mortality in this experiment was also high. Of the 29 live pups, 14 were identified as chimeras by the GPI analysis of tissue homogenates and/or blood.

Here again, there was a negative correlation between the birthweight of chimeras and the relative parthenogenetic contribution (Fig. 2). Chimeras were usually smaller than their normal littermates. Although the correlation was significant ($P < 0{\cdot}05$), the correlation coefficient was relatively low ($R = -0{\cdot}47$). Many factors (individual variations of recipients, number of resorbtions in the uterus, litter size, etc ...) affect the birthweight. The presence of parthenogenetic cell lineage in the animal represents only one of these factors; this might be the explanation for the relatively low value of the correlation coefficient observed. Also the size differences between chimeras and nonchimeras persisted during the postnatal development. At the

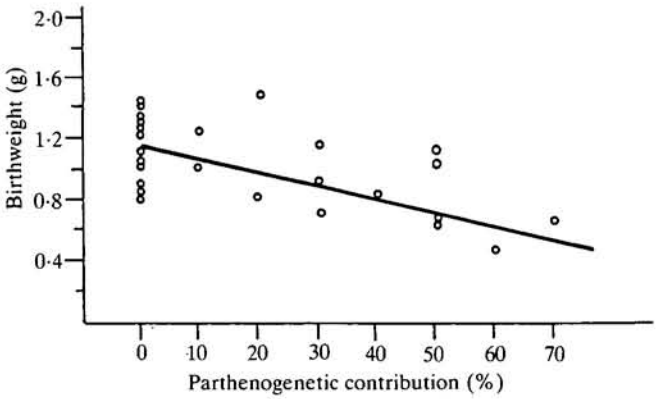

Fig. 2. Negative correlation between the birthweight and parthenogenetic contribution to chimeras.

time of preparation of this manuscript, one female with about 15 % of parthenogenetic part weighed 10·8 g while her nonchimeric female littermate weighed 14·1 g.

In control experiments, (BCF1×BCF1) ↔ BALB/c aggregates were produced. Here the (BCF1×BCF1) component tended to outgrow the BALB/c part. Of the 26 newborns, only 4 were chimeras with the contribution of the BALB/c component ranging from 20 % to 70 %. Chimeras had higher birthweight (1·4 g, S.E. = 0·050) than the nonchimeric animals (1·079 g, S.E. = 0·045).

Three parthenogenetic ↔ fertilized chimeras were killed after birth and their organ homogenates were typed for GPI. The parthenogenetic cell lineage contributed to all organs analysed about the same extent (Table 2). The remaining 11 newborn chimeras were fixed for later histological studies.

Discussion

Our data fit well with those published earlier. Diploid parthenogenetic embryos obtained by ethanol activation are able to participate in the differentiation of tissues in chimeras during the fetal development. Because of the high intrauterine mortality (Nagy *et al.* 1987), the rate of development to term after transfer of parthenogenetic ↔ fertilized aggregates was relatively low as compared to fertilized ↔ fertilized chimeras. The perinatal mortality of these animals is also high (Anderegg & Markert, 1986 and this study), but some animals reach adulthood.

Extensive participation of the parthenogenetic cell lineage has been demonstrated in all organs of newborn chimeras analysed for the distribution of GPI isoenzyme. However, this does not mean automatically that parthenogenetic cells can differentiate into all kind of tissues, because each organ is composed of different types of differentiated cells.

No parthenogenetic part was present in the blood of the three adult chimeras. Unfortunately, we do not know whether parthenogenetic cells were initially present among the progenetors of blood cells of these

animals at birth. The lack of parthenogenetic cells in some tissues could be explained in terms of germline-specific imprinting of the genome. Because of the absence of complementary preprogrammed paternal genome, parthenogenetic cells might be defective in some pathways of differentiation. It is known that cell allocation during the fetal development is influenced by the differential imprinting of parental genomes: spatial distribution of parthenogenetic cells is restricted to the embryo and the mesodermal part of the visceral yolk sac while androgenetic cells are confined to the extra-embryonic tissues (Surani *et al.* 1987, 1988). In addition, a strong selection acts against parthenogenetic cells in the chimeric embryo itself during the second part of gestation (Nagy *et al.* 1987). No such selection was observed in the present study during the postnatal development, at least in some tissues like the melanocytes, germ line and retinal pigment epithelium.

We observed a negative correlation between the body weight and the extent of parthenogenetic contribution in chimeras at birth and during the adult life as well. It remains to be established whether the smaller size of the mice with the large parthenogenetic component is due to lower cell number or smaller cell size of the parthenogenetic part. On the basis of studies on chimeras between strains of mice with large and small size, it was concluded that the growth is a function of genotype of the cells in the body (Falconer *et al.* 1978). It is clear that the reduced growth of our chimeras is also related to the cellular composition of their body as a whole; in other terms, the observed size effect in our experiments may be attributed to the lack of appropriately preprogrammed paternal genome in a proportion of cells of the body, thus it can be considered as a postnatal manifestation of the genomic imprinting phenomenon. Similar effects on the size and growth rate of animals were observed in the study of Robertsonian translocation involving the proximal region of chromosome 11 in the mouse. Maternal disomy/paternal nullisomy caused lower birthweight and reduced growth rate while the paternal disomy/maternal nullisomy resulted in opposite phenotype (Cattanach & Kirk, 1985).

The combined data of experiments on parthenogenetic fertilized chimeras indicate that the functional differences between male and female genomes persist during fetal development and the postnatal life. It seems reasonable to say that, although parthenogenetic cells are not cell lethals and they can participate to some degree in normal differentiation of certain tissues, their extensive presence reduce the viability of chimeric animals and retard their postnatal development.

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