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# Systematic non-uniform distribution of parthenogenetic cells in adult mouse chimaeras

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## Summary

**Even though pure parthenogenetic mouse embryos die shortly after implantation, their cells are capable of participating in normal development of chimaeras when aggregated with fertilized embryos. Here we present data on parthenogenetic contribution to the oocyte populations measured by progeny tests in female chimaeras, and on distribution of parthenogenetic cells among the different organs by GPI typing. Systematic uneven distribution was detected. The highest level of participation was registered in the tissues of permanent cells (e.g. up to 63 % in female germline). On the other hand, parthenogenetic cells were absent in several tissues that have extensive capacity for postnatal growth or selfrenewal. This finding suggests that uneven selective processes operate against parthenogenetic cells within certain differentiation pathways during fetal and postnatal life, as has already been observed in the development of extraembryonal membranes. It is likely that more than one mechanism is responsible for these selections. Parthenogenetic cells may start to differentiate in all cell lineages, but they are not able to react normally at certain points in the developmental pathway, for example to induction signals and, therefore, the cells fail to complete the normal processes of development, or to the proliferation requirement so that the fertilized counterpart gradually takes over the cell lineage. Paternally derived gene(s) might have a unique role in the development of tissues lacking parthenogenetic contribution.**

Key words: parthenogenesis, imprinting, chimaeras, mouse, development.

## Introduction

Normal development in mammals requires contributions from both parental genomes (Surani *et al.* 1984; McGrath & Solter, 1984). Parental-specific modification (gametic imprinting) of chromosomes has been inferred on the basis of the apparent functional complementarity of the two parental genomes. This has been demonstrated in the development of extraembryonic membranes (Barton *et al.* 1984) and in later behavioural and anatomical characteristics of mouse (Cattanach & Kirk, 1985). Furthermore, the absence of one parental genome affects the spatial distribution of cells in chimaeric conceptuses; parthenogenetic and androgenetic cells are excluded from the trophoblastic and primitive ectoderm derivatives, respectively (Surani *et al.* 1987; Surani *et al.* 1988). In later stages of prenatal development, additional selection occurs against parthenogenetic cells in the chimaeric foetus (Nagy *et al.* 1987). To explore whether the development of specific tissues are affected we analysed the contribution of parthenogenetic cells to different organs of adult chimaeras. Deviation from the random distribution would reflect requirements for the paternal genomes to control certain developmental pathways.

## Materials and methods

### *Animals*

4- to 10-week-old females were injected with 5 i.u. pregnant mare's serum (Intervet), followed 48 h later by an injection of 5 i.u. human chorionic gonadotrophin (hCG) to induce superovulation. The (C57BL × CBA)$F_1$/Lati (referred to as $F_1$) animals were treated 16–18 h earlier than NMRI (outbred) females to synchronize the time of the 8-cell stage used for chimaera production between the parthenogenetically activated $F_1$ and fertilized NMRI × BALB/cLati embryos. Fertilized 8-cell embryos were ($F_1$ × $F_1$)$F_2$ and NMRI × BALB/c obtained on day 3 of pregnancy. (Day 1 designated as the day of the vaginal plug found in mated females.)

### *Parthenogenetic activation*

To obtain parthenogenetic embryos $F_1$ oocytes 17 h post hCG were incubated for 6 min in 7 % ethanol (Cuthbertson, 1983),

then transferred to M16 medium containing 5 $\mu g\,ml^{-1}$ cytochalasin B (Calbiochem). After 5–6 h the cumulus cells were removed by short treatment in hyaluronidase, then the eggs were washed in several drops of medium and cultured in M16 at 37°C in a humidified 5% $O_2$, 5% $CO_2$, 90% $N_2$ atmosphere up to 8-cell stage.

*Preparation of aggregation chimaeras*

To aggregate 8-cell-stage embryos, the zona pellucida was removed by acid Tyrode's solution (pH 2·1). Pairs of embryos to be aggregated were placed into a microwell made by pressing a cone-shaped darning needle into the bottom of plastic Petri dish through the paraffin oil-covered microdrops of M16. The wall of this small well kept the two embryos tightly together during handling and aggregation.

*Dissection of organs and analysis of parthenogenetic contribution*

The parthenogenetic contribution to the female germline of chimaeras was tested by mating with albino males. Oocytes of parthenogenetic origin carry the pigmented phenotype, while the fertilized part determines albino. Thus the frequency of pigmented offspring is equivalent to the frequency of oocytes ovulated from the parthenogenetic counterpart. Contribution to the coat pigmentation was estimated from the percentage of pigmented hairs in a representative random sample. In other tissues, the participation was measured at 12 (P1–P4) and 6 (P5–P8, F1–F6) months of age by semiquantitative typing of glucose phosphate isomerase-1 (GPI-1) by polyacrylamide gel electrophoresis, since the two components were homozygous for different isozymal variants (A and B). Before dissection of organs, we perfused the deeply anesthetized animals with physiological solution by means of a thin plastic tube introduced into the aorta through the heart. Except for the lungs, this procedure removed the majority of 'contaminating' blood from the tissues.

## Results

*Development of aggregation chimaeras*

In previous studies of adult parthenogenetic↔fertilized chimaeras, no extensive analysis of the contribution of parthenogenetic cells was carried out (Surani *et al.* 1977; Otani *et al.* 1987), because of low developmental potency and high perinatal mortality of these chimaeras (Otani *et al.* 1987; Anderegg & Markert, 1986). We have found (Markkula & Nagy, unpublished observation) that the combination of parthenogenetic $F_1$ and fertilized NMRI × BALB/c embryos at the 8-cell stage results in reasonable prenatal and postnatal survival.

Of the 72 parthenogenetic↔fertilized aggregates transferred, 25 developed to term and 20 reached maturity. Of the 20 adults, 12 proved to be chimaeras. Since we did not preselect for the GPI-1 genotype in the outbred NMRI strain, unintentionally the fertilized counterpart of four chimaeras were heterozygous, so they had to be excluded from GPI typing. However, the three females of these four were used – along with other females – to determine the parthenogenetic contribution to the oocytes by test-cross.

The controls were produced by aggregating fertilized embryos of the same genotypes used for parthenogenetic↔fertilized chimaeras.

*Parthenogenetic contribution to the organs*

Analysis using GPI isoenzymes revealed that the average contribution of the parthenogenetic component to adult chimaeras was much lower than that of the same genotype to fertilized↔fertilized controls (Table 1). Further, in these controls, the two genotypes were detected in all organs, without any distinctive coloniz-

**Table 1.** *Percentage contribution of parthenogenetic cells to the parthenogenetic↔fertilized and that of the same but fertilized genotype to control fertilized↔fertilized chimaeras*

| | Parthenogenetic↔Fertilized | | | | | | | | Fertilized↔Fertilized | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | F1 | F2 | F3 | F4 | F5 | F6 |
| Sex | f | m | m | f | f | m | f | f | m | m | m | m | m | f |
| Forebrain | 30 | | | | 20 | 10 | 5 | 0 | | | 40 | 50 | 20 | 7 |
| Cerebellum | 5 | 20 | 5 | 20 | 10 | 15 | 10 | 0 | 60 | 40 | 50 | 50 | 20 | 5 |
| Heart | 5 | 20 | 5 | 20 | 5 | 5 | 5 | 0 | 60 | 40 | 50 | 50 | 30 | 10 |
| Coat | 15 | 10 | 10 | 10 | 10 | 5 | 5 | 5 | 60 | 20 | 50 | 50 | 30 | 25 |
| Ovary | 5 | | | 20 | 5 | | 0 | 0 | | | | | | <5 |
| Testis | | 20 | 5 | | | 0 | | | 50 | 60 | 60 | 30 | 50 | |
| Uterus | <5 | | | 20 | 0 | | 0 | 0 | | | | | | 10 |
| Lung | <5 | 10 | <5 | 10 | 0 | 0 | 0 | 0 | 50 | 40 | 60 | 50 | 15 | 5 |
| Appendix | 5 | 10 | <5 | 5 | | 0 | 0 | 0 | 60 | 50 | 30 | | 10 | 5 |
| Kidney | <5 | 10 | <5 | 20 | 0 | 0 | 5 | 0 | 50 | 20 | 60 | 30 | 20 | 5 |
| Spleen | | | | | 10 | 10 | 5 | 0 | | | 55 | 50 | 30 | 30 |
| Stomach, pancreas | 0 | 5 | 0 | 5 | | 0 | 0 | 0 | 50 | 20 | 40 | 50 | 10 | 20 |
| Abdominal muscles | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 30 | 45 | 50 | 5 | 5 |
| Diaphragm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 50 | 30 | 50 | 5 | <5 |
| Pectoral muscle | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 30 | 45 | 30 | 10 | <5 |
| Tongue | | | | | 0 | 0 | 0 | 0 | | | 40 | 50 | 25 | <5 |
| Liver | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 50 | 55 | 80 | 20 | 30 |
| Adrenal gland | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 40 | | 50 | 30 | <5 |
| Blood | 0 | <5 | 0 | 0 | 0 | 0 | 0 | 0 | | | 45 | 50 | 10 | 5 |
| Bladder | | | | | 0 | 0 | 0 | 0 | | | 40 | 40 | 20 | <5 |
| Salivary gland | | | | | 0 | 0 | 0 | 0 | | | 70 | 60 | | 15 |

**Table 2.** *Composition of the litters of parthenogenetic↔fertilized chimaeras tested by mating with albino males. P and A stand for the number of pigmented and albino offspring in the particular litters, respectively*

| I.D. of chimaera | Litter | | | | | | | | | | Rate of pigmented offspring |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | | 2nd | | 3rd | | 4th | | 5th | | |
| | P | A | P | A | P | A | P | A | P | A | P/P + A (%) |
| P1 | 3 | 4 | 5 | 1 | 5 | 3 | 4 | 4 | 5 | 1 | 22/35 (63 %) |
| P4 | 3 | 3 | 3 | 7 | 4 | 7 | 1 | 12 | 2 | 0 | 13/42 (31 %) |
| P5 | 2 | 9 | 1 | 11 | 2 | 8 | 1 | 13 | 1 | 7 | 7/55 (13 %) |
| P7 | 2 | 7 | 3 | 10 | 8 | 7 | 6 | 7 | 8 | 8 | 27/66 (41 %) |
| P8 | 0 | 14 | 0 | 14 | 0 | 14 | 0 | 9 | 0 | 12 | 0/63 (0 %) |
| P9 | 2 | 9 | 0 | 11 | 2 | 10 | 1 | 8 | 3 | 7 | 8/53 (15 %) |
| P10 | 1 | 7 | 0 | 10 | 0 | 9 | 0 | 2 | 0 | 4 | 1/33 (3 %) |
| P11 | 1 | 5 | 0 | 13 | 0 | 9 | 2 | 6 | 0 | 7 | 3/43 (7 %) |
| Proportion of offspring derived from oocytes of parthenogenetic origin in the first five litters (P/P + A): | 14/72 (19 %) | | 12/89 (13 %) | | 21/88 (24 %) | | 15/76 (20 %) | | 19/65 (29 %) | | |
| | | | | | | | | | | | Total: 81/390 (21 %) |

ation by either genotype. By contrast, the parthenogenetic cells were found preferentially in specific tissues:

1. The largest participation and consistent presence of parthenogenetic cells were registered in the forebrain, cerebellum, heart and pigmentation of coat.
2. Lower and sporadic contributions were also found in ovary, testis, uterus, lung, appendix, kidney, spleen and stomach.
3. Parthenogenetic cells were systematically absent in skeletal muscles, liver, adrenal gland, blood, bladder and salivary gland.

*Parthenogenetic contribution to the oocyte populations and to the pigmented cells*

Parthenogenetic cells were capable of extensive contribution to the female germline (Table 2). Furthermore, the contribution of parthenogenetic cells to this germline is the greatest (up to 63 %, compare the marginal percentages of Table 2 to that of Table 1). The variation of average contributions between the litter numbers was within the sampling error. Consequently, there was no selection against parthenogenetic oocytes with age. This was also true for the melanocytes of mature hair follicles and pigmented epithelial cells of the retina. There was no visible change in the extent of pigmentation in the coat and in the eyes.

## Discussion

We have analysed the contribution of parthenogenetic cells to different organs and cell types of aggregation chimaeras. Three groups of tissues could be distinguished. A consistent and relatively large participation of parthenogenetic cells was observed in the group of tissues that consisted of non-dividing cells or tissues renewed on a clonal basis (pigment). The initial contribution could change only if the parthenogenetic cells degenerated more rapidly than the fertilized ones. The extensive contributions at 6 or 12 months of age and the unchanging pigmentation patterns suggest that there is no significant difference between parthenogenetic and fertilized cells in these tissues. The oocytes, which are definitely permanent, fit into this group well. This is the line where the largest parthenogenetic contribution was found. We are also certain about the functional normality of these parthenogenetic cells, since the offspring from them were normal. It is worth noting that cell types and tissues of this group segregate from the germ layers at very early stages of development.

Clearly, in organs of the second group, there are differentiation pathways in which parthenogenetic cells can survive as well as fertilized cells. However, the low and sporadic contributions suggest that parthenogenetic cells may be at a disadvantage in some cell lineages within these organs. Analysis using *in situ* markers will be necessary to determine the functional normality or abnormality of these cells, and their exact tissue distribution. However, it seems that the presence of some parthenogenetic cells does not disturb the function of these organs.

Concerning the third group we cannot determine from this analysis whether parthenogenetic cells failed to contribute to the formation of these tissues or were selected out in postnatal life. Some support for the latter possibility is provided by our observation of occasional extensive contribution of parthenogenetic cells to liver and blood in newborns (Páldi *et al.* 1989). Similar results were observed by Otani *et al.* (1987), who found cells with two maternal genomes present in the liver, but absent from the skeletal muscle and blood in the one adult chimaera they examined. We detected (Páldi *et al.* 1989) the GPI-B isoenzyme of parthenogenetic cells in the muscle homogenate of some chimaera newborns, however, we could not find any trace of the GPI-A/B hybrid enzyme in those cases. Only the presence of this hybrid could prove that functional parthenogenetic myoblast can develop and fuse, together with the normal myoblast (GPI-A), to form muscle cells. Probably, the detected parthenogenetic

contribution came from 'contaminating' other cells, e.g. blood.

In the group of organs that lack parthenogenetic contribution, the urinary bladder and salivary gland are the only organs with low mitotic rate, the others consist of tissues with extensive capacity for selfrenewal. If the proliferative capacity of parthenogenetic blood, liver and interrenal cells is impaired, they will gradually be lost from these tissues during adult life. The lack of parthenogenetic contribution to the skeletal muscles does not mean that parthenogenetic cells are not able to differentiate into myoblasts. If they can, certainly they are unable to fuse into the definitive myotubes. Their disability should affect the postnatal growth rate of chimaeras. Such an effect was observed: the larger the contribution of the parthenogenetic counterpart to coat pigmentation, the slower the growth of the chimaeras (Páldi *et al.* 1989).

These results suggest that presence of paternally derived gene(s) is required for the normal function and/or development of cell lineages of tissues such as red blood cells, hepatocytes, skeletal muscle cells, interrenal cells, etc. On the other hand, there is no evidence of a requirement for paternally derived genes during oogenesis.

On the basis of results presented here, it is apparent that parthenogenetic cells show restricted developmental potency within certain differentiation pathways during fetal and postnatal life, as was already observed in the development of extraembryonal membranes. Exclusion of parthenogenetic cells from trophoblastic cell lineages in parthenogenetic ↔ fertilized (Nagy *et al.* 1987; Clarke *et al.* 1988) and parthenogenetic ↔ androgenetic (Surani *et al.* 1988) chimaeras does not mean that parthenogenetic cells are absolutely unable to form trophoblast, since pure parthenogenetic embryos occasionally develop to midsomite stages (Kaufman *et al.* 1977) certainly having extraembryonal membranes, even trophoblastic cells. However, these tissues have deteriorated. It is proposed that parthenogenetic cells start to differentiate in all cell lineages, but at certain pathways they are not able to react normally, for example to the induction signals or to the proliferation requirements, and fail to complete the normal processes of development and differentiation.

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