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Developmental potential of parthenogenetic cells: role of genotype-specific modifiers

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Summary

The developmental potential of parthenogenetic cells derived from different mouse strains was investigated by examining their distribution in various tissues of adult aggregation chimeras. Using GPI-1 allozymes as marker, no striking differences were observed between chimeras whose parthenogenetic cells were derived from activated oocytes isolated from females of different genetic backgrounds, (C57BL/6×CBA/J) F_1, CFLP, 129, and SWR. In all the combinations tested, parthenogenetic cells were consistently absent from skeletal muscle, but there were varying contributions to most other tissues. These results suggest that the maternal duplication of chromosomes containing imprinted gene(s) responsible for the systematic elimination of parthenogenetic cells from skeletal muscle, are not subject to a pronounced influence of genotype-specific modifiers. However, the contribution of parthenogenetic cells to the brain does appear to be influenced by strain background, since a marked improvement in the survival of CFLP, 129 and perhaps SWR parthenogenetic cells in chimeric brains was observed compared with F2 cells.

Key words: parthenogenesis, chimeras, genomic imprinting, strain influence.

Introduction

Recent evidence suggests that some of the genes involved in development and differentiation of skeletal muscle may be subject to genomic imprinting, in that their expression is dependent on their parental origin. This is based on the observations in parthenogenetic↔fertilized chimeras (PFCs), in which a systematic and consistent elimination of parthenogenetic cells was found in skeletal muscle (Fundele *et al.* 1989; Nagy *et al.* 1989). This was not observed in fertilized↔fertilized chimeras (FFCs) of identical strain composition (Fundele *et al.* 1989; Nagy *et al.* 1989). A detailed study showed that the majority of parthenogenetic cells are eliminated from skeletal muscle between fetal days 13 and 15 (Fundele *et al.* 1990), which coincides with the time of fusion of myoblasts into myotubes in the mouse (Fürst *et al.* 1989). Only one definite exception has been described so far where parthenogenetic cells escaped elimination from skeletal muscle and contributed functionally to muscle differentiation, as judged from the fact that parthenogenetic myoblasts had fused with control cells. (Fundele *et al.* 1989).

Whereas a parthenogenetic genotype is associated with a deficiency especially in skeletal muscle formation, an opposite developmental capacity was recently described for androgenetic embryonic stem cells albeit in an ectopic site (Mann *et al.* 1990). Tumours obtained by subcutaneous injection of three different androgenetic stem cell lines were found to consist 'predominantly of striated muscle with isolated pockets of other differentiated cell types...' (Mann *et al.* 1990). However, in chimeras produced by injection of androgenetic ES cells into fertilized blastocysts, no obvious abnormalities of skeletal muscle were described and ES-cell-derived myogenic stem cells seem to have no proliferative advantage over fertilized cells, as judged from the GPI data provided by the authors (Mann *et al.* 1990).

The molecular mechanisms of genomic imprinting so far remain largely unknown, although three endogenous imprinted genes have now been identified (Barlow *et al.* 1991; DeChiara *et al.* 1990, 1991; Ferguson-Smith *et al.* 1991; Bartolomei *et al.* 1991). Several transgenes show differential methylation and/or expression depending on their parental origin (Reik *et al.* 1987; Sapienza *et al.* 1987; Swain *et al.* 1987; Hadchouel *et al.* 1987; DeLoia and Solter, 1990; Surani *et al.* 1990*a*). Furthermore, behaviour of transgenes may be influenced by strain-specific modifier genes (Sapienza *et al.* 1987, 1989; Allen *et al.* 1990; Surani *et al.* 1990*b*). This is

similar to the situation observed for several mutations in the mouse, e.g. *fused* and T^{hp} (Ruvinsky and Agulnik 1990; Johnson, 1974, 1975). In T^{hp} for instance, maternal transmission is associated with fetal lethality (Johnson, 1974) with exceedingly rare cases of surviving embryos (Winking, 1986), whereas paternal transmission results in a slightly abnormal phenotype at birth and a much lower incidence of fetal lethality (Johnson, 1974). The phenotype of viable paternally derived $T^{hp}/+$ mice is influenced by genetic background (Kintanar-Alton, 1982). Hence, genomic imprinting of transgenes and of endogenous genes, may be a special case of modifier control of gene expression (Allen *et al.* 1990; Surani *et al.* 1990*b*) and genetic background may regulate temporal and quantitative expression as well as number and identity of imprinted genes.

To determine whether the imprinted gene(s) involved in the development of parthenogenetic cells are influenced by genotype-specific modifiers in a major way, we have produced PFCs using several strain compositions. If such a modification of gene expression existed,we may predict substantial variation in the fate of parthenogenetic cells during development. Alternatively, it is possible that the development of parthengogenetic cells is largely independent of the genetic background. We suggest that the imprinting of the key endogenous genes involved in development are not affected by the genetic backgrounds tested.

Materials and methods

Animals

The following inbred and outbred strains were used in this study: SWR, 129 (both from Olac), CFLP (originally from Bantin and Kingman and bred in our own colony; Babraham), (C57BL/6×CBA/J) F_1 (parent strains from Bantin and Kingman) and transgenic strain 83 mice (Lo, 1986). 129, 83 and CFLP are homozygous for *Gpi-1*^{a}, whereas SWR and (C57BL/6×CBA/J) F_1 are *Gpi-1*^{b}.

Embryos

The embryological techniques for the isolation, handling and parthenogenetic activation of mouse embryos have been described in detail (Surani *et al.* 1987, 1988).

Aggregation and injection chimeras

Details of our embryo aggregation technique have been described previously by Fundele *et al.*(1989). The following combinations were used: parthenogenetic (pk) CFLP↔F_1×F_1 (F2), pkF2↔CFLP×CFLP (CFLP), pk129↔F2, pkSWR↔83×CFLP. Except for three pkF2↔CFLP chimeras, asynchronous aggregation chimeras were produced with the parthenogenetic embryo approximately 16 h more advanced than the fertilized embryo, to ensure a high contribution from parthenogenetic cells to the embryo proper (Surani *et al.* 1988). For the production of parthenogenetic→fertilized injection chimeras, ICMs were obtained from parthenogenetic blastocysts after culture of activated eggs for 5 days *in vitro*. ICMs were isolated by immunosurgery (Solter and Knowles, 1975). ICMs were transferred into microdrops of PB1 with 10% fetal calf serum containing 1 μg ml^{-1} nocodazole and 1 μg ml^{-1} cytochalasin B (Barton *et al.* 1987). ICMs were taken up into bevelled injection needles (outer diameter 28 μm) and transferred into injection drops made of PB1–FCS 10 containing 10 units ml^{-1} DNAase I. Whole ICMs were injected into d4 recipient blastocysts.

Analysis of chimeras

Chimeras were killed by exsanguination under avertin anaesthesia. Prior to exsanguination chimeras were injected with approximately 700 units of heparin. Tissues were dissected out and homogenized in approximately tenfold volumes of GPI electrophoresis buffer (Eicher and Washburn, 1978).

GPI-electrophoresis

Tissues of chimeras were first analyzed by electrophoresis on Helena Titan III plates (Eicher and Washburn, 1978). Semiquantitative determination of allozyme activities (Bücher *et al.* 1980; Fundele *et al.* 1985) was performed only on tissues with a parthenogenetic contribution.

Statistics

The Kruskal-Wallis test was applied to assess significance of differences between several groups. Independent values between two groups were then analysed using the Mann-Whitney U-test.

Results

A total of 23 adult chimeras were obtained for analysis. Eight chimeras, of which five have been described in a previous paper (Fundele *et al.* 1989), were of pkF2↔CFLP genotype, five were pk129↔F2, nine were pkCFLP↔F2 and one chimera was pkSWR↔83×CFLP. The contribution of parthenogenetic cells to various tissues of these chimeras is summarized in Table 1.

When all chimeras are considered together regardless of different genotypes, parthenogenetic cells were found in a few tissues only. In addition, parthenogenetic cells always contributed a minor proportion of cells, although in some tissues of some chimeras (see Table 1; Fig. 1), their contribution was significant.

When chimeras consisting of parthenogenetic cells of

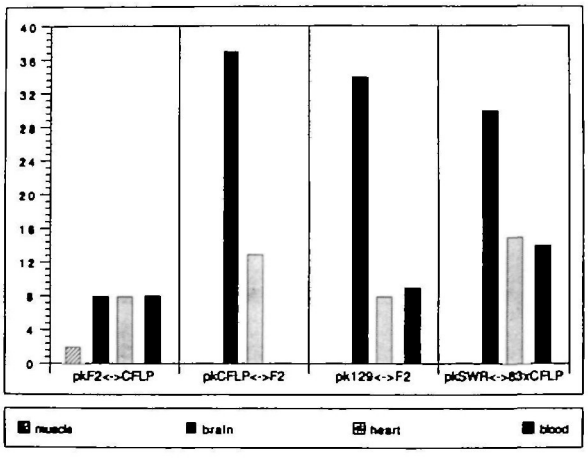

Fig. 1. Summary of parthenogenetic contribution, in percentage of total GPI-1 activity, to four tissues of parthenogenetic↔fertilized chimeras of different genotype.

Table 1. *Contribution of parthenogenetic cells in percentage of total GP1-1 activity in tissues of postnatal parthenogenetic↔fertilized chimeras*

No. of chimera	pkF2↔CFLP									pkCFLP↔F2										pk129↔F2						pkSWR↔ 83×CFLP
	1	2	3	4	5	6*	7*	8*	x̄±S.E.M.	9	10	11	12	13	14	15	16	17	x̄±S.E.M.	18	19	20	21	22	x̄±S.E.M.	23
Brain	23	11	0	0	1	7	9	11	8±3	50	53	58	45	53	18	30	4	19	37±4	35	41	26	36	33	34±2	30
Muscle	13	0	0	0	0	0	0	0	2±2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart	22	16	0	0	0	7	8	10	8±3	29	34	0	15	27	5	7	0	0	13±4	0	19	21	0	0	8±3	15
Kidneys	13/21	9/13	3/0	0/3	0/0	0/0	0/5	3/4	5±3	28/n.d.	17/21	20/21	0/0	15/13	0/0	26/28	0/0	0/0	11±3	0/0	9/11	10/5	0/0	0/0	4±2	15
Spleen	43	13	0	0	0	0	0	16	9±4	0	0	0	8	0	0	0	0	0	1±2	0	n.d.	21	0	0	5±3	0
Blood	35	9	0	12	0	0	0	7	8±3	0	0	0	0	0	0	0	0	0	0	n.d.	21	13	0	0	9±3	14
Thymus	39	5	0	0	0	6	0	22	9±4	0	0	22	17	0	0	0	0	0	4±3	0	n.d.	0	0	0	0	0
Pancreas	0	0	0	0	0	0	0	9	1±2	17	0	0	0	0	0	0	0	0	2±2	0	9	0	0	0	2±2	0
Liver	0	1	0	0	8	0	0	2	1±2	0	0	0	7	0	0	0	0	0	1±2	0	0	0	0	0	0	0
Stomach	n.d.	n.d.	n.d.	n.d.	n.d.	+	12	n.d.		n.d.	13	0	19	26	0	8	0	25	11±3	0	16	20	0	0	7±3	14
Duodenum	29	30	5	0	33	+	+	+	19±4	18	n.d.	0	32	15	0	9	0	15	11±3	0	n.d.	14	0	0	3±3	+
Colon	19	9	0	0	n.d.	0	10	15	8±3	19	0	0	36	22	0	15	0	0	10±4	0	15	0	0	0	3±3	0
Lung	0	11	0	0	0	0/0	0/n.d.	1/4	2±2	24/8	5/7	0/0	13/17	0/0	0/0	0/0	0/0	0/0	4±3	0/0	18/19	0/0	0/0	0/0	4±3	0
Bladder	12	11	0	5	0	0	4	5	5±2	0	0	0	0	0	0	0	0	0	0	0	13	6	0	0	4±2	0
x̄±S.E.M.	19±4	10±3	1±1	1±2	3±3	2±2	3±2	9±3		14±4	10±4	8±4	13±4	10±4	1±2	8±3	1±1	2±2		2±3	15±3	9±3	2±3			

* PFCs 6, 7 and 8 only were derived from synchronous aggregations using day 3 parthenogenetic and day 3 fertilized embryos.
+=qualitative analysis of tissues only with pK derived GPI-1 present.

Table 2. *Contribution of parthenogenetic cells in percentage of total GP1-1 activity to tissues of fetal day 19 asynchronous pkCFLP↔F2, pkF2→CFLP, and asynchronous pkF2↔CFLP chimeras*

	pkCFLP↔F2								pkF2→CFLP									pkF2↔CFLP*				
	1	2	3	4	5	6	7	x̄±S.E.M.	8	9	10	11	12	13	14	15	x̄±S.E.M.	16	17	18	19	x̄±S.E.M.
Brain	56	43	37	36	67	76	48	52±4	7	49	9	9	7	12	16	0	14±4	33	23	35	36	32±2
Muscle	3±2	0	0	0	0	4±4	0	1±1	0	17±2	0	0	0	0	0	0	2±2	0	0	0	0	0
Heart	26	28	27	17	44	48	21	30±3	7	27	6	2	4	10	14	4	9±3	41	27	31	19	30±3
Kidney	25	34	21	31	33	36	39	31±3	0	22	0	0	0	13	10	0	6±3	18	27	20	19	21±2
Blood	26	32	19	27	47	50	19	31±4	6	40	16	19	19	16	28	0	18±4	21	25	30	n.d.	25±2
Liver	23	7	0	22	29	28	14	18±3	0	18	0	0	3	3	6	0	4±2	18	10	10	13	13±2
Pancreas	14	0	0	16	n.d.	24	16	12±3	0	9	0	0	0	0	0	0	1±2	0	0	0	0	0
Colon	42	20	27	26	48	58	15	33±4	0	24	0	5	0	5	8	0	5±3	22	6	13	18	15±3
Bodyweight [mg]	733	850	961	902	607	503	1037		1187	483	1650	1548	1365	1516	1260	1611						

* Results on the four pkF2↔CFLP chimeras taken from Fundele *et al.* (1990).

different genotypes are compared, the following similarities become apparent. One of the most consistent observations was that the parthenogenetic cells appeared frequently and at comparatively high levels in the brain. Of the 23 chimeras described in Table 1, only two were exceptions to this rule and did not have parthenogenetic cells in the brain as judged by GPI-1 activity. Selection against parthenogenetic cells was also not very pronounced in the heart and the areas of digestive tract that were analyzed in this study (stomach, duodenum, colon). In all strain combinations used, PFCs were smaller than their normal litter-mates, which were also transferred as parthenogenetic↔ fertilized aggregations but had excluded the parthenogenetic cells from the conceptus. This was observed for example in pkCFLP↔F2, chimeras (Table 2; Fig. 2). As CFLP mice are usually larger than F2 this shows that the reduced size of PFCs is caused by the presence of parthenogenetic cells and the size is not dictated by the strain composition used in chimeras (Gardner *et al.* 1990). The size reduction of PFCs compared to normal litter-mates sometimes became more pronounced during postnatal development. For instance, PFC 13 (Table 1) weighed 4.2 g when 9 days old. By day 19, when it was killed, its weight was down to 3.3 g. On dissection, signs of gastric and perhaps intestinal bleeding were observed in PFCs 12 and 13. We also examined skeletal elements in these chimeras but no obvious morphological abnormalities were observed after skeletal staining with alizarin red and alcian blue (McLeod, 1980) (see Fig. 2).

While the low selective pressure on parthenogenetic cells in the brain was apparent in all types of aggregation chimeras, some difference in the contribution from these cells may be due to genotype-specific factors. The two chimeras without parthenogenetic contribution to the brain were of pkF2↔CFLP genotype. In addition, parthenogenetic contribution to the brain was considerably higher in pkCFLP↔F2 (37±4%; n=9) and pk129↔F2 (34±2%; n=5) chimeras compared to pkF2↔CFLP chimeras (8±3%; n=8). Using the Kruskal-Wallis test we found that the differences between the pkF2 group and the pk129/pkCFLP groups are statistically significant at the 0.1% level (P=0.003). Excluding the three synchronous aggregation chimeras in the pkF2↔CFLP group from the statistical analysis, thus obtaining a homogeneous pkF2 group, the differences between the pkF2 and the pk129/pkCFLP are still significant at the 5% level (P=0.016). When tissue distribution of parthenogenetic cells in a given chimera was compared, the levels of contribution in brains of pk129↔F2 and pkCFLP↔F2 were higher than those observed in other tissues. This was not generally the case in pkF2↔CFLP chimeras, as shown in Table 1.

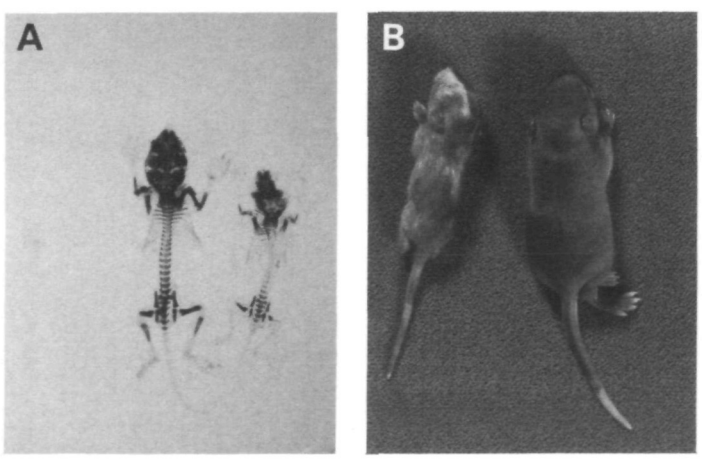

Fig. 2. Comparison between a pkCFLP↔F2 chimera and a normal F2 littermate on day 9 *post partum*. A shows a skeletal stain (McLeod, 1980) of the two mice; the PFC is on the right. B shows the high contribution of parthenogenetic albino cells to the coat of the chimeric mouse on the left. The parthenogenetic contribution to tissues of this chimera is given in Table 1 (PFC9).

Parthenogenetic cells seemed to be systematically eliminated from skeletal muscle in all types of chimeras analysed. Only one pkF2↔CFLP chimera (Chimera 1) showed detectable parthenogenetically derived GPI-1 activity in skeletal muscle. All the other adult chimeras had apparently excluded parthenogenetic myoblasts or parthenogenetic nuclei from myotubes during myogenesis; this was observed even in chimeras that had a significant parthenogenetic contribution to the majority of tissues.

Systematic elimination of parthenogenetic cells from the skeletal muscle and low selective pressure in the brain were also observed in fetal day 19 pkF2↔CFLP, pkF2→CFLP and pkCFLP↔F2 chimeras analyzed in this study (Table 2). High levels of CFLP parthenogenetic cell contribution were observed in chimeras; the contribution to the brain of fetal chimera 6 was 62% and 82% respectively, in the two brain samples analysed. Two pkCFLP↔F2 chimeras showed GPI-1A activity in one skeletal muscle sample, out of three taken. However, no GPI-1AB heterodimers were observed indicating either the presence of parthenogenetic cells without fusion of myoblasts to form myotubes or a large patch-size with parthenogenetic myoblasts fusing predominantly with each other. In contrast, the one pkF2→CFLP chimera with high levels of GPI-1B and therefore a high contribution of parthenogenetic cells in all three muscle samples, did show heterodimeric GPI-1AB bands. The combined data indicate very low levels of parthenogenetic cells in skeletal muscle which occasionally lead to fusion of normal and parthenogenetic myoblasts giving rise to the heterodimers.

Discussion

Our study shows that, in chimeras, parthenogenetic cells are at a strong selective disadvantage compared with the cells derived from normal fertilized zygotes. This selection against parthenogenetic cells in PFCs is a general feature and occurs regardless of strain background. Hence, the overall decreased proliferative

capacities of parthenogenetic cells, or the inability of conceptuses with high parthenogenetic contribution to survive have to be attributed to some key imprinted gene(s). This parental imprint is not apparently modified by the genetic background of animals. Similarly, the identical phenotypes of parthenogenetic or androgenetic embryos from different strains of mice support the idea that some aspects of imprinting are not affected by the genotype-specific modifiers. (Surani and Barton, 1983; Solter, 1988; Surani *et al.* 1990*b*).

In some tissues of PFCs, a selection against parthenogenetic cells is to a large extent not influenced by strain background. This is seen most convincingly in skeletal muscle where parthenogenetic cells or nuclei are consistently absent. A trivial explanation for this observation might be that the strain combinations selected by us might have caused an *a priori* restricted allocation of parthenogenetic cells to myogenic stem cells, with a secondary reduction due to uniparental genotype later in development. This was in fact recently proposed by Mann and co-workers (1990) to explain the exclusion of parthenogenetic cells from the skeletal muscle of fetal pkF2↔CFLP chimeras (Fundele *et al.* 1990). In the case of both pkF2↔CFLP and pkCFLP↔F2 chimeras however, this explanation is not convincing because in the skeletal muscle of CFLP↔F2 control chimeras, no preferential allocation of F2 or CFLP cells was observed (Fundele *et al.* 1989). Furthermore, the identical results obtained with reciprocal aggregations strongly argues against a strain-specific allocation of cells to this tissue. As no FFCs were produced with SWR↔83×CFLP and 129↔F2 aggregations, a strain-specific restricted allocation cannot be entirely excluded in these cases. However, since a number of different strains used to produce aggregation chimeras, yielded identical results, this prediction seems unlikely. In addition, a stringent selection of parthenogenetic cells in skeletal muscle has been observed using *in situ* hybridization analysis of sections obtained from pk(83×F_1)↔CFLP and pk83↔CFLP chimeras (Fundele *et al.* unpublished).

Our overall results can be explained by proposing the presence of imprinted gene(s) which are not subject to modifier control. Some of these genes may be expressed during myogenesis, so that with the parthenogenetic genotype, there is probably a loss of function while with the androgenetic genotype there is gain of function (Mann *et al.* 1990). An analysis of expression of genes involved in the development of muscle, such as MyoD (Davis *et al.* 1987), myogenin (Wright *et al.* 1989; Edmondson and Olson, 1989), myf-5 (Braun *et al.* 1989) and MRF-4/herculin/myf-6 (Rhodes and Konieczny, 1989; Braun *et al.* 1990; Miner and Wold, 1990) may be informative.

It is difficult at present to reach a definitive conclusion regarding the fate of parthenogenetic cells in most other tissues due to the small number of different strain compositions analyzed, and because of the irregular occurrence of parthenogenetic cells in these tissues. However, a comparison of day 19 fetal chimeras shows, that at this stage the selection pattern is very similar in pkCFLP↔F2, pkF2→CFLP and pkF2↔CFLP chimeras (Table 2). In most cases, skeletal muscle, liver and pancreas show the lowest levels of parthenogenetic contribution compared with the other tissues. Hence, the early onset of selection observed in these tissues of pkF2↔CFLP chimeras (Fundele *et al.* 1990), probably occurs in pkCFLP↔F2 chimeras, too.

In contrast to skeletal muscle, a correlation between strain background and selection against parthenogenetic cells seems to exist in brain. Parthenogenetic cells survive in the brains in most chimeras well into adulthood, independently of strain background (see Table 1; Fundele *et al.* 1989; Nagy *et al.* 1989; Fundele *et al.* 1990). However, a degree of selection does take place because parthenogenetic cells always constitute a minor proportion of the overall cell population. The influence of preferential allocation of cells of particular genetic background to this tissue can again be excluded in the case of the reciprocal aggregations pkCFLP↔F2 and pkF2↔CFLP as F2 and CFLP cells do equally well in brain of F2↔CFLP control chimeras (Fundele *et al.* 1989) and it is very unlikely in the case of pk129↔F2 and pkSWR↔83×CFLP aggregations. The degree of selection against parthenogenetic cells observed in the brain is strongly influenced by the genetic background. Hence, pkF2↔CFLP chimeras had significantly lower levels of parthenogenetic contribution in the brain compared with the chimeras where parthenogenetic cells were derived from 129, CFLP and SWR embryos. This is also demonstrated by examining chimeras 18, 21 and 22, which were negative for parthenogenetic cells in most tissues, except for the very high contribution to the brain. Hence, these results argue that an imprinted gene relevant to the developing brain is likely to be controlled by a polymorphic modifier gene.

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