The independence of germ-cell genotype from somatic influence in chimaeric mice

By ANNE McLAREN*

A.R.C. Unit of Animal Genetics, Institute of Animal Genetics, West Mains Road, Edinburgh EH9 3JN

(Received 4 December 1974)

SUMMARY

Mouse chimaeras made by aggregating embryos homozygous for nine recessive genes with embryos carrying the corresponding dominants were mated with partners of the recessive strain. Progeny were either of the recessive type for all loci examined, or showed the dominant characters at all loci. Tail length in homozygous vestigial-tail progeny was unaffected by whether the fertilizing spermatozoon had undergone maturation in a chimaera or in a control vestigial-tail male. There was thus no indication that the germ cells had been in any way modified by their intimate association with germ cells and somatic tissue of contrasting genotype in the chimaeras.

1. INTRODUCTION

It is a fundamental tenet of Mendelian inheritance that the genetic content of germ cells is contaminated neither by the somatic tissue of the animal or plant in which they develop nor by their fellow germ cells.

Yet germ cells in mammals, as in many other groups, typically develop in very close association with one another and with the somatic cells of the gonad. During much of spermatogenesis, the germ cells are enveloped by the cytoplasm of the testicular Sertoli cells (Brökelmann, 1963; Nicander, 1967), and cytoplasmic connexions between spermatogonia, spermatocytes and spermatids have been demonstrated in several species of mammals (Nicander, 1967; Dym & Fawcett, 1971). In the ovary also the germ cells are linked together in groups by cytoplasmic bridges to form a syncytial organization (Gondos & Zamboni, 1969). Although mammalian oogenesis does not show the massive transfer of cytoplasm and RNA from nurse cells to oocytes characteristic of certain insect groups (e.g. MacGregor & Stebbings, 1970), the mammalian oocyte develops in intimate contact with the surrounding follicle cells, with the cell membranes associated in tight intercellular junctions (e.g. Zamboni, 1972). Mammalian oocytes are pinocytotically active (Anderson, 1972), and incorporate exogenous proteins and perhaps other macromolecules (Glass, 1961, 1970; Mancini et al. 1963).

There thus exists ample opportunity for informational macromolecules to enter mammalian germ cells. A low incidence of transformation or 'transgenosis' (a term that makes no assumptions about mechanism, see Doy, Gresshoff & Rolfe, 1973) of germ cells could not be detected in normal matings, since the diploid germ cells are of course identical in

* Present address: M.R.C. Mammalian Development Unit, Wolfson House, University College, 4, Stephenson Way, London NW1 2HE.
genetic constitution with each other and with the surrounding somatic tissue, while in
the haploid phase any modification would be confounded with other commonly occurring
causes of disturbed segregation. Some breeding experiments involving grafted ovaries
have been carried out in mice, but in the main these have involved either genetically
marked ovaries grafted into $F^1$ hosts, where a genetic modification in the grafted ovary
could not be distinguished from regeneration of host ovarian tissue (e.g. Russell & Hurst,
1945), or grafts into immunologically tolerant hosts, but in strain combinations where a
single transformational event would again have gone undetected (e.g. Jones & Krohn,
1960). In any case, the oocytes in an ovarian graft are of the same genetic constitution as
the surrounding follicle cells, so that any external influence would require to be mediated
by the body fluids.

The embryo aggregation chimaera provides a very different situation. Mouse embryos
of genetically contrasting type are aggregated in pairs, usually at the eight-cell stage;
provided both components survive embryogenesis, the composite develops into an
overly chimaeric but otherwise normal animal. Primordial germ cells of two genetic
types migrate from the yolk-sac wall into the genital ridges, the future gonads, which
themselves contain somatic tissue of two genetic types. Since there is no reason to believe
that there exists any cell recognition or assortative distribution between germ cell and
somatic tissue, female chimaeras will contain some oocytes surrounded by follicles con-
sisting wholly or partly of cells of the other component, and male chimaeras will contain
some spermatogonia enveloped by Sertoli cells of contrasting genotype. Some of the
consequences for gamete phenotype have already been explored. McLaren, Chandley &
Kofman-Alfaro (1972) found that $XX$ germ cells in an $XX/XY$ chimaeric testis entered
meiotic prophase before birth, at the usual time for female germ cells, but were unable to
continue normal development in the environment of the testis. Burgoyne (1973) reported
that head length and breadth and midpiece length of mouse spermatozoa developing in a
chimaeric testis behaved autonomously, though there were minor differences between the
dimensions of spermatozoa from chimaeras and control males, due perhaps to the different
uterine environment to which the chimaeras had been exposed.

The possible consequences of a genetically disparate somatic environment for the
遗传 (rather than phenotypic) potential of the gametes has received little attention.
Published data on the breeding performance of chimaeras is summarized in Table 5 of
McLaren (1972). Some of the studies involved aggregations between strains differing at
only one easily scorable locus (e.g. C3H→C57BL); in such cases any modification of germ
cells by somatic tissue could not be distinguished from temporal shifts in the proportions
of the two types of germ cell due to selection (Mintz, 1968). Some of the strain combina-
tions studied by Mullen & Whitten (1971) differed at two easily scorable loci (e.g. agouti
and albino), so that progeny derived from modified germ cells would in principle have
been detectable, but no details are given of how many progeny were bred from such
chimaeras.

In the present study, breeding tests were carried out on chimaeras derived from aggre-
gation of embryos differing at nine loci, in order to maximize the probability of detecting
any modifications of the gametes.

2. MATERIALS AND METHODS

The chimaeras formed part of a series obtained by embryo aggregation, as described
by Bowman & McLaren (1970). Each aggregated pair consisted of one embryo from a
multiple recessive stock homozygous for non-agouti, brown, dilute, pink-eye, chinchilla,
ved-2, short-ear, vestigial-tail and supernatant NADP IDH type $\alpha$, and one $F^1$ embryo
carrying the corresponding dominant alleles from crosses of C3H/Bi/McL males with
Table 1. The progeny of a series of overtly chimaeric mice of a multiple recessive–dominant strain combination, ‘back-crossed’ to the multiple recessive strain.

| Phenotype of young | Germ cell population | Sex of chimaera | No. of chimaeras | Birth* | 2-3* weeks | Id-1* |
|-------------------|----------------------|----------------|------------------|--------|------------|-------|
|                   |                      |                |                  | Female | Male       |       |
| Recessive         | Single               | ♀              | 3                | 81     | 94         | 10    | 185   | 136   | 96    |
| Recessive         | Single               | ♂              | 10               | 443    | 377        | 39    | 859   | 559   | 287   |
| Recessive         | Mixed                | ♂              | 1                | 20     | 28         | 0     | 48    | 31    | 0     |
| Total recessive   |                      |                |                  |        |            |       |       |       |       |
|                   |                      |                |                  | 544    | 499        | 49    | 1092  | 726   | 383   |
| Dominant          | Single               | ♀              | 1                | 54     | 54         | 6     | 114   | 90    | 0     |
| Dominant          | Single               | ♂              | 6                | 292    | 324        | 10    | 626   | 537   | 0     |
| Dominant          | Mixed                | ♂              | 1                | 10     | 9          | 0     | 19    | 18    | 0     |
| Total dominant    |                      |                |                  | 356    | 387        | 16    | 759   | 645   | 0     |

* At birth the young were classified for pink-eye and vestigial-tail; at 2-3 weeks the survivors were classified for dilute, brown, non-agouti, chinchilla, waved-2 and short-ear; after weaning the Id-1 type of some of the mice was determined.

either C57BL/McL females or CBA/Fa females. Observations on other aspects of the series are described by McLaren & Bowman (1969), McLaren (1972), Grünberg & McLaren (1972) and McLaren (1975).

The 22 overt chimaeras comprised 4 females and 18 males. The unequal sex ratio reflects the fact that the $XX<->XY$ chimaeras developed as males (McLaren, 1975). At 6 weeks of age, each chimaeric female was mated to one male, and each chimaeric male to two females, from the multiple recessive stock. Control matings were made up in parallel, with multiple recessive animals of approximately the same age as the chimaeras mated to contemporaries (litter-mates where possible) of the animals used in the chimaera matings.

Young were classified at birth for pink-eye and vestigial-tail, and at 2-3 weeks for non-agouti, brown, dilute, chinchilla, waved-2 and short-ear. Liver biopsies were done on some of the ‘recessive’ young at 3-6 weeks of age, and their Id-1 type determined by starch electrophoresis. Since the $F_1$ component was heterozygous at the Id-1 locus, Id-1 type of the ‘dominant’ young was not determined.

At 15 days of age the young of chimaera and control matings had their tails measured to the nearest millimetre. In homozygous vestigial-tail animals, in which the tails are short and curly, an outline of the tail was traced on paper and the length of the midline measured with a ‘Curvimetre’ recording map measurer. Litter means were used for statistical analysis.

3. RESULTS AND DISCUSSION

With the exception of one male, all the chimaeras bred (Table 1). Seven (1 ♀, 6 ♂) produced progeny of dominant phenotype only, 13 (3 ♀, 10 ♂) of recessive phenotype only, and one male produced both types of progeny, showing that both components of the chimaera had formed functional germ cells. In no case did any of the progeny show segregation at any of the loci examined: young either resembled the multiple recessive strain at all loci or carried all the corresponding dominant alleles.

The expression of the genes in the progeny of chimaeras appeared qualitatively similar to that seen in control matings. A quantitative assessment was made on tail length in homozygous vestigial-tail offspring. The young of female chimaeras were not used, to avoid the complication of possible maternal effects on gene expression during embryonic development. The 10 male chimaeras that produced recessive young were each mated to
two recessive females, and 10 control recessive males were also each mated to two recessive females. There were thus 20 chimaera/control paired matings available; of these, 14 produced young for paired comparisons. Tail measurements were carried out on 222 young of chimaeric males, from 44 litters, giving a mean tail length of $3.93 \pm 1.70$ mm, and on 206 young of control males, from 39 litters, giving a mean of $3.44 \pm 1.52$ mm, calculated from litter means. When the chimaera/control paired matings were weighted according to the number of litters born to each, the (chimaera-control) weighted mean difference in tail length came to $0.23 \pm 1.83$ mm; that is, there was no significant difference between chimaeras and controls with respect to the tail length of their progeny.

For the nine loci examined there is thus no evidence that any allele was transformed into its homologue under the influence of germ cells or somatic tissue of contrasting genotype, nor is there any indication (for the one locus examined quantitatively, namely vestigial tail) that the expression of the paternal gene was affected by its sojourn in a chimaeric testis. The same loci proved equally refractory to transgenosis when embryos were exposed to DNA of contrasting genotype during cleavage (Snow & McLaren, 1974). Unless the loci selected are very unrepresentative of the genome, we may conclude that the genetic content of mouse germ cells is not subject to contamination by the cellular environment.

I am grateful to the Ford Foundation for financial support.

REFERENCES

Anderson, E. (1972). The localization of acid phosphatase and the uptake of horseradish peroxidase in the oocyte and follicle cells of mammals. In Oogenesis (ed. J. D. Biggers and A. W. Schuetz), pp. 87-117. London: Butterworth.

Bowman, P. & McLaren, A. (1970). Viability and growth of mouse embryos after in vitro culture and fusion. Journal of Embryology and Experimental Morphology 23, 693-704.

Brökelmann, J. (1963). Fine structure of germ cells and Sertoli cells during the cycle of the seminiferous epithelium in the rat. Zeitschrift für Zellforschung und Mikroskopische Anatomie 59, 820-850.

Burgoyne, P. S. (1973). The genetic control of germ cell differentiation in mice. Ph.D. Thesis, University of Edinburgh.

Doy, C. H., Gresshoff, P. M. & Rolfe, B. G. (1973). Biological and molecular evidence for the transgenosis of genes from bacteria to plant cells. Proceedings of the National Academy of Sciences, U.S.A. 70, 723-726.

Dym, M. & Fawcett, D. W. (1971). Further observations on the numbers of spermatogonia, spermatocytes, and spermatids connected by intercellular bridges in the mammalian testis. Biology of Reproduction 4, 196-215.

Glass, L. E. (1961). Localization of autologous and heterologous serum antigens in the mouse ovary. Developmental Biology 3, 787-804.

Glass, L. E. (1970). Translocation of macromolecules. In Cell Differentiation (ed. O. A. Schjeide and J. de Vellis), pp. 201-223. New York: Van Nostrand–Reinhold.

Gondos, B. & Zambroni, L. (1969). Ovarian development: The functional importance of germ cell interconnections. Fertility and Sterility 20, 176-189.

Grünewerg, H. & McLaren, A. (1972). The skeletal phenotype of some mouse chimaeras. Proceedings of the Royal Society, London B 182, 9-23.

Jones, E. C. & Krohn, P. L. (1960). Orthotopic ovarian transplantation in mice. Journal of Endocrinology 20, 135-146.

MacGregor, H. C. & Stebbings, H. (1970). A massive system of microtubules associated with cytoplasmic movement in telotrophic ovaries. Journal of Cell Science 6, 431-449.

Mancini, R. E., Vilar, O., Heinrich, J. J., Davidson, O. W. & Alvarez, B. (1963). Transference of circulating labeled serum proteins to the follicle of the rat ovary. J. Histochem. Cytochem. 11, 80-88.
McLaren, A. (1972). Germ cell differentiation in artificial chimaeras of mice. In The Genetics of the Spermatozoon (ed. R. A. Beatty and S. Gluecksohn-Waelsch), pp. 313–324. Edinburgh, New York.

McLaren, A. (1975). Sex chimaerism and germ cell distribution in a series of chimaeric mice. Journal of Embryology and Experimental Morphology 33, in press.

McLaren, A. & Bowman, P. (1969). Mouse chimaeras derived from fusion of embryos differing by nine genetic factors. Nature 224, 238–240.

McLaren, A., Chandley, A. C. & Kofman-Alfaro, S. (1972). A study of meiotic germ cells in the gonads of foetal mouse chimaeras. Journal of Embryology and Experimental Morphology 27, 515–524.

Mintz, B. (1968). Hermaphroditism, sex chromosomal mosaicism and germ cell selection in allophenic mice. Journal of Animal Science 27, 51–60.

Mullen, R. J. & Whitten, W. K. (1971). Relationship of genotype and degree of chimerism in coat color to sex ratios and gametogenesis in chimeric mice. Journal of Experimental Zoology 178, 165–176.

Nicander, L. (1967). An electron microscopical study of cell contacts in the seminiferous tubules of some mammals. Zeitschrift für Zellforschung und Mikroskopische Anatomie 83, 375–397.

Russell, W. L. & Hurst, J. G. (1945). Pure strain mice born to hybrid mothers following ovarian transplantation. Proceedings of the National Academy of Sciences, U.S.A. 31, 267–273.

Snow, M. H. L. & McLaren, A. (1974). The effect of exogenous DNA upon cleaving mouse embryos. Experimental Cell Research 86, 1–8.

Zamboni, L. (1972). Comparative studies on the ultrastructure of mammalian oocytes. In Oogenesis (ed. J. D. Biggers and A. W. Schuetz), pp. 5–45. London: Butterworth.