THE EFFECTS OF MATERNAL PRE-IMMUNIZATION ON PREGNANCY IN THE MOUSE

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Summary. Before mating with males of the A₂G or CBA inbred strains of mice, C57BL females were immunized against either the paternal strain antigens (allogeneic) or *Peromyscus* species antigens (xenogeneic). Evidence is presented which indicates that, contrary to earlier reports, pre-immunization has no effect on placental size, but may reduce foetal weight and litter size. The rôles of specific immunity and non-specific 'stress' are discussed.

INTRODUCTION

During the last 15 years, there have been conflicting reports concerning the effect on pregnancy of maternal pre-immunization against the paternal strain antigens. Several experiments have indicated that pre-immunization does not prejudice foetal survival (Mitchison, 1953; Heslop, Krohn & Sparrow, 1954; Medawar & Sparrow, 1956; Woodruff, 1957; Hasková, 1961), whereas Breyere & Sprenger (1969) and Currie (1969) have provided evidence to the contrary.

There have been suggestions that antigenic differences between the mother and her foetus have an influence on placental and foetal growth (Billington, 1964; James, 1965, 1967). Billington (1965) reported that trophoblastic invasion in an extra-uterine site tended to be greater when transplants were made between two inbred strains of mice than when they were made within strains. Clarke (1969), however, failed to detect any differences in trophoblastic invasion between isogeneic and allogeneic transfers using other strain combinations. Moreover, there was no effect of pre-immunization of the recipients against the donor-strain antigens. James (1967) found that placental and foetal weights in the mouse were increased by immunizing the female to paternal antigens and decreased by rendering her tolerant of them. The present experiments were carried out to re-examine the effect of maternal pre-immunization on placental and foetal weights and other aspects of pregnancy.

MATERIALS AND METHODS

The first experiment utilized the same strain combination as that used by James (1967). In the second experiment, a different paternal strain was used and an additional group of females was immunized xenogeneically to a *Peromyscus* species. This latter group was included to investigate the effect of a

general non-specific heightening of the immune response, and the effect of stress experienced by the females during the immunization procedure.

Experiment 1

C57BL female mice, aged approximately 6 weeks, were divided into three groups. The first group was immunized against A₂G male mice either with five spleen-cell injections (a half-spleen equivalent each), or five spleen-cell injections and two skin grafts, or two spleen-cell injections and two skin grafts. All treatments were given at fortnightly intervals. Full thickness skin grafts were applied using the method of Gottfried & Padnos (1959). The degree of immunity was measured by the human serum dextran technique (Gorer & Mikulska, 1954). The mice were then weighed, and mated to A₂G males. The second group was left untreated but also mated to A₂G males. The third group was untreated and mated to C57BL males.

A fourth group of C57BL female neonatal mice was injected with either 5 to 8 million A₂G spleen cells or with 16 million F₁ C57BL female × A₂G male spleen cells to render them tolerant to the paternal strain. Injections were made into the anterior facial vein within 24 hr of birth.

All mice were killed 17 days after the copulation plug was found. Embryos and placentae were removed from the uterus, blotted and weighed to the nearest milligram on a torsion balance. The size of a litter (live foetuses) and the number of deaths during early and middle pregnancy ('moles') were recorded. The reciprocal mating, A₂G females × C57BL males, was not studied.

Experiment 2

C57BL female mice, aged approximately 10 weeks, received three spleen-cell injections (a half-spleen equivalent each), and two skin grafts from either CBA males or from *Peromyscus polionotus polionotus* (the deer mouse). A control group was not immunized. All females were mated to CBA males and their embryos were recovered as previously described. This experiment was carried out in a different laboratory from that in which Exp. 1 was conducted and utilized a different population of mice.

RESULTS

Experiment 1

The inbred controls had significantly smaller foetuses and placentae than the two outbred groups ($P < 0.001$; see Table 1); this finding is in agreement with McCarthy (1965) and McLaren (1965). No significant difference was found, however, between the immunized and non-immunized mothers in the weight of the hybrid 17½-day-old foetuses and placentae. Nevertheless, immunization appeared to have adverse effects on the outcome of a pregnancy. It significantly decreased the size of a litter ($P < 0.01$) and among those females whose litters contained early and middle deaths ('moles'), there were larger numbers of 'moles' within the immunized mothers ($P < 0.05$, Fisher's exact test). The total number of implantations in a litter (live embryos + 'moles') was significantly lower in immunized females than in non-immunized or in inbred controls ($P < 0.05$; see Table 2). The number of young in a litter is known to

TABLE 1
COMPARISON OF MEAN 17½-DAY FOETAL AND PLACENTAL WEIGHTS IN IMMUNIZED, NON-IMMUNIZED AND INBRED MOTHERS IN EXP. 1

Group	No. of litters	Mean litter size	Mean foetal wt (mg ± S.E.)	Adjusted* mean foetal wt (mg)	Mean placental wt (mg ± S.E.)	Adjusted* mean placental wt (mg)
Immunized C57BL × A ₂ G	25	4.1	876 ± 23.6	853	145.3 ± 4.6	140.6
Control non-immunized C57BL × A ₂ G	12	6.1	899 ± 10.3	899	140.3 ± 5.5	144.9
Inbred C57BL × C57BL	13	6.0	729 ± 16.9	730	111.6 ± 3.1	112.5

* Individual within-group regression coefficients used to adjust foetal and placental weight means to a constant litter size.

TABLE 2
COMPARISON OF NUMBER OF EARLY AND MIDDLE DEATHS OR 'MOLES' IN EXP. 1

Group	No. of litters*	Mean litter size	Females with three or more 'moles' / litter	Females with < three 'moles' / litter	Mean no. 'moles' / litter	Embryos + 'moles' / litter
Immunized C57BL × A ₂ G	34	4.2	11	7	3.4	5.8
Control non-immunized C57BL × A ₂ G	25	6.5	2	9	1.9	7.3
Inbred C57BL × C57BL	14	6.1	2	4	2.5	7.0

* Litter numbers larger than in Table 1 as not all litters used for 17½-day analysis.

TABLE 3
COMPARISON OF MEAN 17½-DAY FOETAL AND PLACENTAL WEIGHTS IN ALLOGENEICALLY IMMUNIZED, XENOGENEICALLY IMMUNIZED AND NON-IMMUNIZED MOTHERS IN EXP. 2

Group	No. of litters	Mean litter size	Mean foetal wt (mg ± S.E.)	Adjusted* mean foetal wt (mg)	Mean placental wt (mg ± S.E.)	Adjusted* mean placental wt (mg)
Allogeneically immunized C57BL × CBA	6	7.3	869 ± 7.3	869	122.7 ± 2.6	122.5
Xenogeneically immunized C57BL × CBA	8	7.2	890 ± 34.2	884	118.6 ± 4.0	117.5
Control C57BL × CBA	10	8.1	915 ± 15.6	918	119.3 ± 2.0	119.6

TABLE 4
COMPARISON OF NUMBER OF EARLY AND MIDDLE DEATHS OR 'MOLES' IN EXP. 2

Group	No. of litters	Mean litter size	Females with three or more 'moles' / litter	Females with < three 'moles' / litter	Mean no. 'moles' / litter	Embryos + 'moles' / litter
Allogeneically immunized C57BL × CBA	6	7.3	0	1	0.3	7.7
Xenogeneically immunized C57BL × CBA	8	7.2	1	4	1.1	8.4
Control C57BL × CBA	10	8.1	0	4	0.4	8.6

affect placental and foetal weights (Healy, McLaren & Michie, 1960). Regression analysis allows these weights to be corrected to a standard litter size (5.4 in this case). Regression of foetal weight on litter size ($b = -14.80$; $0.1 > P > 0.05$) is just below significance but regression of placental weight on litter size is significant ($b = -4.20$; $P = 0.01$). Although the correction for litter size reduces the mean foetal weight of the immunized group where litter sizes were small, the adjusted means of the control and immunized groups are not significantly different ($P > 0.05$). This result is not in agreement with James (1967) who found an increase in placental and foetal weight after maternal immunization. The data in Table 1 show, if anything, a decrease.

The degree of immunity of immunized females as measured by the human serum dextran technique was extremely variable and could not be correlated with the mode of immunization. Haemagglutination titres ranged from 1/16 to 1/16,384. Litter sizes, foetal and placental weights and the number of 'moles' in a litter could not be correlated with the rejection times of the second immunizing skin grafts (6.0 ± 2 days), or with the titres of circulating antibody.

Of the forty-eight C57BL female mice that were injected with A₂G male spleen cells, all but two developed runt disease. Varying degrees of the typical symptoms (cessation of growth and development, the presence of diarrhoea, sparse hair from about 2 weeks of age) were followed at varying intervals by death. The two survivors received skin grafts at 8 weeks but normal rejection times (10 ± 2 days) showed the absence of tolerance. The sixty-four neonates receiving F₁ (C57BL female $\times$ A₂G male) spleen cells in an attempt to overcome runt disease also showed no tolerance. Ten individuals grafted with A₂G male skin at maturity showed rejection times that were normal.

Experiment 2

The results (Tables 3 and 4) were essentially similar to those of the first experiment. Analysis showed that the mean F₁ foetal weights from mothers specifically pre-immunized to the paternal antigens were, in this case, significantly smaller than those from control non-immunized mothers ($P < 0.02$). Females immunized against the non-specific antigens of *Peromyscus polionotus polionotus* gave a mean foetal weight intermediate between the allogeneically immunized and non-immunized groups, but was not significantly different from either of them. Placental weights were similar in all three groups. Although the litter sizes and the total number of implantations were lower in the two immunized groups, the differences were not significant. The overall number of 'moles' was too small for a valid comparison to be made (Table 4). As in Exp. 1, the regression of foetal weight on litter size was not significant ($b = -10.73$; $P > 0.05$) and the regression of placental weight was significant ($b = -2.1$; $P < 0.05$). Correction of foetal and placental weights to a standard litter size of 7.5 reduced the significance of the difference in foetal weight between specifically immunized and non-immunized females ($P = 0.05$).

DISCUSSION

The results of the two experiments indicate that maternal immunization to

specific paternal antigens does not enhance placental and foetal growth. Indeed, both experiments show no effect on placental growth but a reduced foetal growth after allogeneic immunization, although this was significant only in Exp. 2. These results therefore conflict with those of James (1965, 1967), who found an increase in placental and foetal weight after maternal immunization. Both of the present experiments suggest that immunization reduces litter size, although the effect was significant only in Exp. 1. This was not apparent in the experiments of James, who did not include litters containing less than five young, and litters containing 'moles'. The present findings are in agreement with those of Breyere & Sprenger (1969) who reported a decreased litter size at birth in C57BL females specifically immunized against DBA/2 sarcoma, and mated to DBA/2 males but no decrease when C57BL females were immunized non-specifically to C₃H adenocarcinoma. The present findings in Exp. 2 also indicate that non-specific xenogeneic immunization does not reduce foetal weight significantly, but some effect of stress or the raising of a general immune response on foetal weight and litter size cannot as yet be excluded.

Attempts to induce tolerance to A₂G spleen cells in C57BL female neonates failed because of the occurrence of fatal runt disease (Exp. 1). James (personal communication) found only a 5% incidence of runt disease in this strain combination using similar doses of spleen cells.

Billingham & Brent (1959) found the A/C57BL strain combination the least liable to produce tolerance of many strains tested. They obtained low tolerance in the A into C57BL combination and in the reciprocal combination (C57BL into A), they found 100% fatal runt disease. The A₂G strain was derived about fifty generations ago from the A strain and is unlikely to have acquired many changes at the histocompatibility loci during this time. This has been confirmed by Davies (personal communication) who has typed the histocompatibility loci of the A₂G strain and found them similar to the A strain.

C57BL neonates injected with F₁ (C57BL females × A₂G males) spleen cells should not be capable of responding to the antigens of the A₂G strain. Nevertheless, difficulty was experienced in bringing these mice through weaning. Billingham & Brent (1959) also report reactions to F₁ cells in the C57/A strain combination.

No satisfactory explanation can be found to account for the difference in the incidence of runt disease between the animals in this study and those of James (1967). The possibility exists that the significantly smaller foetuses and placentae which James obtained from female mice rendered 'tolerant' to the paternal antigens could have been due in part to subclinical runt disease.

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