FACTORS AFFECTING THE GROWTH OF TROPHOBLAST TRANSPLANTED TO THE TESTIS

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Despite the probable existence of a protective layer around trophoblastic cells (Bradbury, Billington & Kirby, 1965; Currie & Bagshawe, 1968), there is evidence suggesting that antigenic diversity between mother and foetus has immunological consequences (Billington, 1964; McLaren, 1965; James, 1965). Billington (1965) reported that trophoblast transplanted to the testis of mice genetically dissimilar from the donor was more invasive than trophoblast transplanted to genetically similar mice. The present work was designed to investigate such an effect of genetic difference and, in particular, to see whether it was due: (1) to a response by the testis to a foreign trophoblast, (2) to a response by the trophoblast to a foreign testis, or (3) to a combination of both. In order to distinguish between these possibilities, transplants were carried out in various combinations between two strains of mice and their F_1 hybrid.

The initial series of experiments (1 to 4) was designed to investigate the first possibility and to determine if prior immunization of the recipient altered the degree of invasion. The recipient mice were all CBA males aged between 10 and 20 weeks. Half of them were grafted with two full thickness skin grafts from C57 B1 males with an 11-day interval between grafts (Gottfried & Padros, 1959). Transplantation was made to the right testis, using $7\frac{1}{2}$ -day-old ectoplacental cones of two kinds, either CBA or hybrid F_1 (C57 B1 $\sigma \times$ CBA φ) (for technique, see Kirby, 1962). The ectoplacental cones obtained from any one female were distributed equally amongst the grafted and ungrafted mice as far as this was possible. Recipient animals were killed 8 days after transplantation. The degree of invasion was estimated in two ways: from the difference in weight between the treated right testis and the control left testis, as in Billington (1965), and by dissecting out and weighing the trophoblastic nodules (see Table 1).

No significant differences were found between these first four experiments. This result differs from that of Billington (1965), who reported that Balb/C trophoblast grew to a larger size in C57 B1 testis than in Balb/C testis. Furthermore, in these experiments, prior immunization of recipients against donor antigens did not significantly affect trophoblastic invasion. Assuming that placental size depends on the extent of invasion by the trophoblast, this result is not in agreement with the finding of James (1965) who reported that prior immunization to maternal antigens resulted in a significant increase in placental size.

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During transplantation it was noticed that ectoplacental cones varied considerably in size, both within individual females and, in particular, between different females. In Exps. 5 to 10, cones were therefore measured before transplantation. As 7½-day ectoplacental cones are roughly triangular in shape, the length from the tip of the triangle to the base was measured as representing the overall size.

The second series of experiments (5 to 8) was designed to investigate the possibility that the trophoblast responds to foreign antigens present in the testis. Recipient mice were either hybrid (CBA ♂ × C57 B1 ♀) or C57 B1 males and were injected with either CBA or hybrid 7½-day ectoplacental cones.

TABLE 1
GROWTH OF TROPHOBLAST TRANSPLANTED TO THE TESTIS

Series	Donor	Recipient	No. of mice (no. of invasions)	Mean wt of experimental — control testis ± S.E. (mg)	Mean wt of dissected trophoblast ± S.E. (mg)
1	Hybrid (CBA♀ × C57 B1♂)	CBA	14(13)	18.0 ± 8.7	17.6 ± 4.4
2	Hybrid	CBA immunized	15(12)	20.8 ± 6.9	18.4 ± 6.1
3	CBA	CBA	14(12)	23.4 ± 4.9	24.5 ± 4.6
4	CBA	CBA immunized	13(11)	14.6 ± 2.3	16.6 ± 3.1
5	CBA	Hybrid	16(14)	8.8 ± 2.5	11.3 ± 4.0
6	CBA	C57 B1	13(11)	9.4 ± 1.9	4.7 ± 1.3
7	Hybrid	Hybrid	12(10)	14.0 ± 5.1	12.6 ± 3.6
8	Hybrid	C57 B1	14(10)	12.2 ± 4.5	11.8 ± 3.9
9	Hybrid	CBA	14(14)	29.9 ± 6.7	29.6 ± 5.4
10	Hybrid	CBA immunized	13(13)	27.6 ± 4.8	30.5 ± 5.7

No significant differences were found between these four experiments (5 to 8) (see Table). There is, thus, no evidence in these experiments that trophoblastic growth is enhanced by the presence of foreign antigens in the testis.

Transplantation of hybrid donor tissue into non-immunized and pre-immunized CBA recipients was then repeated (Exps. 9 and 10). Results (see Table 1) supported the previous finding that trophoblastic invasion is unaltered by prior immunization of the recipient.

A significant positive correlation was found between ectoplacental cone size and the degree of trophoblast invasion (Spearman's Rank Correlation Method, $P = 0.001$). Measurements of cones varied from 1.6 to 8.8 mm, with a mean of 4.0 mm. As the mean ectoplacental cone size in each series of transfers was similar, correction of the data to a standard 5 mm cone size had little effect on mean trophoblast weight. The exception was in Exp. 9 where the mean trophoblastic weight was slightly reduced.

Billington (personal communication) did not measure ectoplacental cone size or distribute cones obtained from one female randomly between the different series of transplantation. Although he made several inter-strain transfers, those of Balb/C into C57 B1 testis provided the only significant example of increased

invasion in the presence of genetic diversity (Billington, 1965). The results of these experiments suggest that the ectoplacental cones from the two Balb/C females used could have been larger than average.

A second factor contributing to the variability in trophoblastic growth was the different capacity of the three recipient testes (CBA, CBA $\times$ C57 B1 and C57 B1) to support growth of both CBA and hybrid donor tissue ($P < 0.05$). CBA testes provided the most favourable environment, and C57 B1 the least favourable. A similar analysis comparing the ability of the two donor tissues (CBA and hybrid) to proliferate in all testes was not significant ($P > 0.5$).

These results are interpreted as suggesting that factors other than immunological influences are determining the growth of 7½-day-old ectoplacental cones transplanted to the testis. The size of donor tissue transplanted and the capacity of the recipient tissue to support growth influence the degree of invasion obtained.

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