

HISTOINCOMPATIBILITY AND MATERNAL IMMUNOLOGICAL STATUS AS DETERMINANTS OF FETOPLACENTAL WEIGHT AND LITTER SIZE IN RODENTS*

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In mice it has been shown, though perhaps not unequivocally, that where differences at the major histocompatibility complex prevail, placental size or weight of allogeneic conceptuses is significantly influenced by the immunological reactive status of the mother with regard to the alien, paternally inherited tissue antigens of the fetuses (1-4).

In rats, mice, and hamsters we have previously reported that both solid tissue and monodisperse cellular allografts, such as those of skin and lymphoid cells or washed epididymal spermatozoa, when introduced into the lumen of an anatomically intact uterine horn, are highly effective in eliciting an immunologically specific hypertrophy of the draining para-aortic lymph nodes and a state of systemic transplantation immunity (5). Although naturally implanted allogeneic fetuses in the uterus are equally effective in eliciting regional lymphadenopathy on an immunogenetically specific basis, they neither provoke transplantation immunity, nor are they vulnerable to such an immunity specifically directed against them. In the rat it has also been shown that prior *local* sensitization of one uterine horn with intraluminal tissue or cellular allografts significantly improves its subsequent reproductive performance, as compared with that of the contralateral, nonimmunized horn, in terms of the number as well as the weights of the fetoplacental (fp)¹ units that implant and develop to term following mating with males against whose tissue alloantigens their sensitivity is directed (6).

The experiments to be reported on in this communication were designed to test the premise, based upon these and other observations, that in mice, hamsters, and rats histoincompatibility of conceptuses vis à vis their mother, and her immunological status with regard to alloantigens with which she is confronted, influence the weights of the fp units as well as the number of conceptuses that implant and are sustained to term. Attention was also paid to the influence of

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¹ Abbreviations used in this paper: BN, Brown Norway rats; FI, Fischer; fp unit, fetoplacental unit; LE, Lewis.

syngeneic and allogeneic pregnancies on the weight and cellularity of the maternal para-aortic lymph nodes and the spleen weight.

Principle of the Experiments

Baseline data were obtained by mating females of a selected strain with syngeneic males and killing them at a fixed time postconception. The para-aortic nodes draining the gravid uterine horns were dissected out, weighed, and the number of viable nucleate cells extricable from them determined. The spleen was also weighed. The number of viable fetuses present, the weights of the individual fp units and those of the fetuses and placentas separately were also recorded. The spleen weights and those of the para-aortic nodes were also determined from members of a panel of syngeneic virgin weight- and age-matched females.

Virgin females of the same strain which were either normal, specifically sensitized or immunologically tolerant with regard to the tissue antigens of an allogeneic strain were then mated with males of that strain and their fp units studied as above to provide data on the influence of maternal immunological status with regard to the implantation and growth of genetically alien fetuses.

Additional experiments will be described in the sections to which they pertain.

Materials and Methods

Experimental subjects were obtained from domestically maintained sublines of well-defined syngeneic strains and their appropriate F_1 hybrids: (a) sexually mature female mice weighing 20–23 g of the C57BL/6 ($H-2^b$), A ($H-2^k$), CBA ($H-2^k$), and C3H ($H-2^k$) strains. (b) Syrian hamsters weighing 75–90 g of the MHA and CB strains, which are believed to differ at the major histocompatibility complex and at only one or two minor loci in addition. (c) Sexually mature female rats weighing 150–175 g of the Fischer (FI) ($Ag-B^1$), Lewis (LE) ($Ag-B^1$), DA ($Ag-B^4$), and Brown-Norway (BN) ($Ag-B^9$) strains.

The day of mating was determined by detection of spermatozoa in vaginal washings and designated as day one of pregnancy. Pregnant mice were killed on day 16 of an 18- to 19-day gestation, hamsters on day 14 of a 16-day gestation and pregnant rats on day 18 of a 22-day gestation. Subjects in all experiments were matched for age, weight and endocrinological status.

Fetuses and Placentas. Fetuses and placentas were removed surgically and separated by cutting the umbilical cord at its placental insertion. Placentas were kept moist on a saline-saturated filter paper and weighed to the nearest 0.1 mg. The fetus and its membranes were weighed together in the same manner after incising the amniotic sac and draining its contents.

Maternal Lymphoid Organs. The bilateral para-aortic or lumbar lymph nodes, which drain the uterine horns and are located just superior to the bifurcation of the aorta, were dissected out, all adherent fat and connective tissue removed and then weighed to the nearest 0.1 mg. The maternal spleen was also excised and weighed. A measure of the lymphocyte population of these nodes was afforded by mincing them with the aid of fine, curved scissors and pressing them through a no. 60 stainless steel grid with Hanks' balanced salt solution to produce a monodisperse cell suspension which was counted in a standard hemocytometer.

In each experimental and control group representative f-p units, together with the intact uterine horn and para-aortic lymph nodes, were fixed in Bouin's solution, sectioned, and stained with hematoxylin and eosin, as well as with the periodic acid-Schiff (PAS) procedure, for histologic examination.

Sensitization of Animals. The methods employed for the preparation and transplantation of skin grafts, and for the preparation in Hanks' solution and subsequent intravenous or intraperitoneal administration of suspensions of bone marrow and lymphoid cells have been described in detail elsewhere (7). Sensitization of virgin subjects against transplantation alloantigens was accomplished by grafting them orthotopically on the lateral thoracic wall with full-thickness skin allografts, approximately 2–2.5 cm in diameter, from the donor strain followed, 3 wk later, by two weekly booster intraperitoneal injections of 40×10^4 viable lymph node cells of similar alien genetic provenance dispensed in 1.0 ml of Hanks' solution.

Preparation and Administration of Alloimmune Serum in Rats. This entailed sensitization of the future donor as described, and the withdrawal of blood by cardiac puncture for serum preparation a

week after the second booster injection. Aliquots of 0.5 ml of normal or immune serum were administered to pregnant hosts by the intracutaneous route on day 5 of gestation and every 3 days thereafter throughout pregnancy.

Induction of Tolerance of Tissue Antigens. In rats tolerance was induced by intravenous injection of newborn hosts with a standard dosage of 40×10^6 semiallogeneic F₁ hybrid bone marrow cells in 0.1 ml of Hanks' solution. When 50–60 days old the animals were challenged with skin allografts from the donor strain. Hosts whose grafts remained in impeccable condition after an arbitrary 50-day period were classified as "highly tolerant" and employed for the experiments described.

In mice tolerance was induced by the intravenous injection of newborn subjects with a standard dosage of 20×10^6 semiallogeneic F₁ hybrid spleen cells in 0.1 ml of Hanks' solution. The animals were subsequently challenged with skin allografts from the donor strain, as above.

Procurement and Transfer of Rat Blastocysts into Foster Mothers. Rat blastocysts were obtained from females, on the morning of the fifth day following a successful normal mating, by flushing the freshly excised uteri with Hanks' solution equilibrated with 5% CO₂. Three blastocysts were taken up in a small volume of medium into a glass pasteur pipette drawn to 80–100 μ m internal diameter, according to the method of Rafferty (8). The blastocysts were immediately transferred to the recipient's uteri, three into each uterine horn following McLaren and Michie's procedure (9, 10). Animals serving as surrogate mothers had been "mated" with a mechanical vibrator 4 days previously (11). They were killed at 18 days gestation (day 1 = day of vibration) when the fp units, para-aortic nodes and spleens were excised and assayed as described above.

Statistical Analyses. Dunnett's (12) tests for multiple copies with a control were employed to compare the weights of the fp units in the various experiments conducted on rats. Comparisons of the weights of placentas and other organs in the remaining experiments were carried out with the aid of Duncan's (13) multiple range test.

Experiments and Observations on Mice (Table I)

Influence of the Presence of Immunogenetically Alien Fetuses on Maternal Lymphoid Organs. Comparison of the mean weights of the para-aortic lymph nodes, which drain the uterine horns, and the spleens of panels of normal, virgin strain A females and of strain A females bearing syngeneic and allogeneic [i.e., (A \times C57)F₁ hybrid] fetuses respectively (Table I, exp. 1–3) shows that the para-aortic nodes of strain A females bearing syngeneic fetuses were significantly enlarged compared with similar nodes from the panel of virgin females of this strain, and a significant degree of splenomegaly was also associated with syngeneic pregnancies ($P < 0.01$). The presence of allogeneic fetuses in the uteri stimulated a very striking hypertrophy of the draining nodes (by a factor of about 4) but exerted no influence upon the spleen weight.

The remarkable hypertrophy of the para-aortic nodes of (A \times C57)F₁ females sustaining F₁ A/C57 \times A backcross fetuses (Table I, exp. 6) may result from local graft-vs.-host reactivity on the part of fetal lymphocytes which crossed the placenta and settled in these nodes (6). Although these mothers were genetically incapable of reacting against any of the transplantation antigens of their fetuses, some of the latter were capable of reacting against certain maternal tissue antigens by virtue of their failure to inherit a complete set of their mother's histocompatibility genes.

CBA strain females pregnant by allogeneic C3H (but *H-2* locus compatible) males, as compared to CBA males, besides having a significant enlargement, albeit modest, of their para-aortic nodes displayed splenomegaly as well. (Table I, exp. 8 and 9).

Finally, in C3H females confronted by (C3H \times CBA)F₁ hybrid instead of by

TABLE I

Analysis of the Influence of Immunogenetic Disparity and Immunologic Status of Female Mice with Regard to the Tissue Antigens of their Conceptuses on the Weights of their Para-aortic Nodes and Spleens and upon the Weights and Numbers of their Fetoplacental Units

Exp.	Mother		Fetuses				Maternal lymphoid organs		
	Genotype	Immunologic status	Litters			Placental wt. mean \pm SE	Fetal wt. mean \pm SE	Para-aortic nodes mean \pm SE	Spleen mean \pm SE
			Genotype	No.	Mean size \pm SE				
1	A	Normal, virgin (8)	—	—	—	mg	mg	mg	mg
2		Normal	A	13	7.1 \pm 0.47	101.1 \pm 1.9	—	0.6 \pm 0.10	73.4 \pm 4.2
3		Normal	(A \times C57)F ₁	17	8.2 \pm 0.97	114.4 \pm 1.9	—	1.8 \pm 0.9	108.2 \pm 5.8
4		Immune: anti-C57	(A \times C57)F ₁	16	7.6 \pm 0.2	124.5 \pm 1.4	—	8.9 \pm 1.5	102.7 \pm 10.4
5		Tolerant of C57	(A \times C57)F ₁	6	6.0 \pm 1.53	103.6 \pm 3.5	—	—	—
6	(A \times C57)F ₁	Normal	F ₁ A/C57 \times A backcross	12	7.4 \pm 0.92	89.0 \pm 1.9	—	12.0 \pm 1.4	109.6 \pm 9.8
7	CBA	Normal, virgin (8)	—	—	—	—	—	3.0 \pm 1.1	—
8		Normal	CBA	15	6.2 \pm 1.7	113.0 \pm 2.6	394.0 \pm 6.5	3.6 \pm 0.6	113.0 \pm 6.8
9		Normal	(CBA \times C3H)F ₁	6	7.5 \pm 0.9	117.5 \pm 2.5	411.1 \pm 9.3	7.6 \pm 0.9	135.5 \pm 10.8
10		Immune: anti-C3H	(CBA \times C3H)F ₁	6	5.6 \pm 0.87	116.8 \pm 3.2	355.9 \pm 11.9	7.2 \pm 1.0	160.0 \pm 11.8
11		Tolerant of C3H	(CBA \times C3H)F ₁	13	5.2 \pm 1.8	111.1 \pm 2.1	453.1 \pm 7.6	3.3 \pm 0.6	107.2 \pm 7.3
12	C3H	Normal, virgin (8)	—	—	—	—	—	4.13 \pm 1.4	—
13		Normal	C3H	15	5.6 \pm 0.88	103.4 \pm 3.2	456.5 \pm 15.3	4.9 \pm 1.1	129.8 \pm 10.7
14		Normal	(C3H \times CBA)F ₁	11	5.8 \pm 0.67	121.0 \pm 2.1	465.7 \pm 10.1	4.3 \pm 0.7	162.3 \pm 7.5
15		Immune: anti-CBA	(C3H \times CBA)F ₁	6	7.0 \pm 0.48	117.3 \pm 2.8	487.3 \pm 13.7	7.6 \pm 1.1	163.7 \pm 10.7
16		Tolerant of CBA	(C3H \times CBA)F ₁	11	5.2 \pm 0.48	114.8 \pm 2.2	476.9 \pm 10.7	3.7 \pm 0.7	146.2 \pm 7.2

syngeneic fetuses there was a significant enlargement of their spleens but not of their para-aortic nodes (Table I, exp. 13 and 14).

Influence of Maternal Immunologic Status on the Placental Weights of Histo incompatible Fetuses. Comparison of the mean placental weights of (A \times C57)F₁ hybrid fetuses from panels of A strain females which, before conception, were unsensitized, sensitized and tolerant respectively with regard to tissue antigens of the C57 strain (see Table I, exp. 3-5) shows that: (a) the mean weight of hybrid placentas in normal females (114.4 \pm 1.9 mg) was significantly heavier than that of syngeneic placentas (101.1 \pm 1.9 mg P \leq 0.001); (b) heavier F₁ hybrid placentas developed in presensitized mothers (124.5 \pm 1.4 mg) than in normal, unsensitized mothers (P \leq 0.001); and, finally, (c) the mean weight of the hybrid placentas in the immunologically tolerant mothers, (103.6 \pm 3.5 mg) was closely similar to that of the syngeneic placentas (101.1 \pm 1.9 mg). The mean weight of the F₁ A/C57 \times A backcross placentas which developed in the genetically tolerant (F₁ hybrid) females (89.0 \pm 1.9 mg) was significantly less than that of the syngeneic A strain placentas (P \leq 0.001; see Table I, exp. 2 and 6).

Studies on the influence of the immunologic reactive status of CBA strain females towards their (CBA \times C3H)F₁ hybrid placentas (see Table I, exp. 7-11) gave no indications that presensitization caused heavier placentas to develop. Furthermore, with this particular maternal-fetal combination, hybrid placentas

were not significantly heavier than syngeneic placentas in similar normal females. The immunologically tolerant mothers produced placentas whose mean weight was essentially similar to that of syngeneic placentas.

The studies conducted on pregnant C3H strain females bearing F_1 fetuses with CBA alloantigens (Table I, exp. 12-16) showed that these allogeneic placentas in normal females are significantly heavier than syngeneic placentas, (mean weights 121.0 ± 2.1 mg vs. 103.4 ± 3.2 mg; $P < 0.01$) and that a state of immunologic tolerance in the mother results in a decrease in placental weight, though in this instance the placentas from the tolerant animals were still significantly heavier than syngeneic placentas. Presensitization of the mothers did not result in heavier F_1 hybrid placentas.

Influence of Immunologic Status of the Mother on Litter Size. Since, as indicated by the findings described, transplantation immunity influences the weights of allogeneic placentas in some genetic contexts, evidence was also sought whether it has any perceptible influence on the implantation and sustenance to term of immunogenetically alien conceptuses. Comparison of the mean litter sizes (i.e. number of viable fetuses present at autopsy on the 16th day postconception) hints that it does. For example, the F_1 hybrid litters gestated by normal A and CBA females were slightly larger than the syngeneic litters gestated by normal females of these strains. That this is not simply attributable to so-called hybrid vigor or heterosis on the part of the fetuses is suggested by the observation that the mean sizes of F_1 hybrid litters gestated by immunologically tolerant mothers were smaller than those gestated by normal mothers. With the C3H mothers it will be noted that sensitization was the only maneuver that caused any significant deviation of mean litter size from that of the syngeneic pregnancies (See Table I, exp. 13-16).

Experiments and Observations on Hamsters (Table II)

Unfortunately the paucity of syngeneic strains and the difficulty of producing tolerant hamsters restricted the scope of the experiments performed in this species. Confrontation of MHA strain females with $(MHA \times CB)F_1$ hybrid as compared to syngeneic conceptuses resulted in almost a threefold increase in the weights of both the maternal para-aortic nodes and the spleen (Table II, exp. 1-2). Gestation of F_1 MHA/CB \times CB backcross fetuses by $(MHA \times CB)F_1$ hybrid females also resulted in a considerable hypertrophy of the para-aortic nodes (Table II, exp. 4) which may reflect graft-vs.-host reactivity on the part of lymphocytes of fetal origin.

The placental weight studies conducted, though not entirely conclusive, suggest very strongly that histoincompatibility is a determinant of placental growth in this species too. The salient observations sustaining this belief are firstly, the significantly greater mean placental weight of F_1 hybrid as compared to the syngeneic placentas (340.7 ± 7.1 mg vs. 252.6 ± 10.0 mg; $P < 0.001$), and secondly, the inferior mean weight of the R_2 backcross placentae in F_1 hybrid mothers which was comparable with that of the syngeneic placentas in MHA mothers (258.7 ± 5.6 mg and 252.6 ± 10.0 mg respectively).

Especially interesting is the observation that the mean litter size of $(MHA \times$

TABLE II

Analysis of the Influence of Immunogenetic Disparity and Immunologic Status of Female Hamsters with Regard to the Tissue Antigens of their Conceptuses on the Weights of their Para-aortic Nodes and Spleens and upon the Weights and Numbers of their Fetoplacental Units

Exp.	Mother		Fetuses				Maternal lymphoid organs		
	Genotype	Immuno-logic status	Litters			Placental wt mean \pm SE	Fetal wt mean \pm SE	Para-aortic node wt mean \pm SE	Spleen wt mean \pm SE
			Genotype	No.	Mean size \pm SE				
1	MHA	Normal	MHA	6	7.3 \pm 0.56	252.6 \pm 10.0	1,236.7 \pm 53.2	3.7 \pm 0.5	106.2 \pm 12.8
2		Normal	(MHA \times CB) F_1	7	9.4 \pm 0.78	340.7 \pm 7.1	1,183.3 \pm 36.7	10.2 \pm 0.8	295.1 \pm 35.4
3		Immune-anti-CB	(MHA \times CB) F_1	8	11.4 \pm 0.63	303.8 \pm 5.9	1,078.5 \pm 28.3	—	—
4	(MHA \times CB) F_1	Normal	F_1 MHA/CB \times CB	6	10.5 \pm 0.38	258.7 \pm 5.6	1,219.7 \pm 59.9	7.8 \pm 1.02	119.0 \pm 15.6

CB) F_1 fetuses gestated by MHA mothers was significantly larger than that of MHA fetuses born by similar mothers (9.4 \pm 0.78 vs. 7.3 \pm 0.56) and that specifically sensitized MHA mothers bore even larger hybrid litters (11.4 \pm 0.63). No satisfactory explanation can be offered for the large mean litter size (10.5 \pm 0.38) of backcross fetuses.

Experiments and Observations on Rats

Encouraged by the findings of the experiments conducted upon mice and hamsters, essentially similar experiments were performed on a more extensive scale, using rats of the Ag-B locus-incompatible FI and DA strains, the females deriving from the FI strain in the first series of experiments and the DA's in the second series.

Unlike the situation in mice and hamsters, in which placental and fetal weights bore no obvious relationship to one another, in all tests conducted on rats, it was observed that the ratio mean placental weight/mean fetal weight (see PW/FW in Tables III and IV) was approximately constant, usually falling within the range 0.33-0.37. This indicated the existence of a direct linear relationship between these parameters at least for fetuses of the particular age under study. For this reason, the mean weights of the fetoplacental (fp) units rather than the mean placental weights were made the basis of comparison in the various experiments to be reported on.

FI Females Exposed to DA Strain Tissue Antigens During Pregnancy (Table III)

INFLUENCE OF PREGNANCY ON MATERNAL LYMPHOID ORGANS. Syngeneic pregnancies stimulated a significant increase in both the weights and the cellularity of the draining para-aortic lymph nodes as well as an increase in spleen weight as compared with the status of these organs in virgin female rats of the same strain, age, and body weight. The presence of allogeneic (FI \times DA) F_1 fp units provoked a much more striking increase in weight and cellularity of the para-aortic nodes, but no further increase in spleen weight. (See Table III, exp. 1-3).

TABLE III
Analysis of the Influence of Immunogenetic Disparity and Immunologic Status of Female FI Rats with Regard to Tissue Antigens of their (FI × DA)F₁ Conceptuses on the Weight of their Para-aortic Lymph Nodes and Spleens and upon the Weights and Numbers of their Fetoplacental Units

Exp.	Mother		Litters				Fetuses			Maternal lymphoid organs			
	Genotype	Immunologic status	Genotype	No.	Mean size ± SE	Placental wt. mean ± SE	Fetal wt. mean ± SE	PW/FW*	FP unit wt. mean ± SD	Para-Aortic nodes		Spleen wt.	
										Mean wt. ± SE	Cell count × 10 ⁶ mean ± SE	Mean wt. ± SE	Spleen wt. mean ± SE
1	FI	Normal virgin (8)	—	—	—	—	—	—	—	—	—	—	—
2	FI	Normal	33	7.6±0.40	310.2±7.5	798.5±14.6	0.38	1,126.0±66.7	8.4±1.3	13.7±4.6	404.2±32.9	—	—
3	(FI × DA)F ₁	Normal	23	8.6±0.48	331.2±9.4	901.9±22.9	0.36	1,253.5±80.0	13.7±1.1	32.0±4.1	525.3±11.4	—	—
4	(FI × DA)F ₁	Immune anti-DA	8	9.0±0.47	332.5±16.6	943.1±30.4	0.35	1,302.0±122.3	23.3±2.7	56.3±5.4	535.4±22.4	—	—
5	FI	Immune anti-DA	6	9.2±0.73	276.9±18.2	750.4±33.1	0.38	1,029.4±71.3	—	—	—	—	—
6	(FI × DA)F ₁	Tolerant of DA	6	9.5±0.65	297.8±13.5	855.3±37.7	0.34	1,154.6±56.6	14.2±2.7	28.2±6.5	531.1±25.7	—	—
7	FI	Tolerant of DA	2	5.0±0.31	273.9±19.4	736.0±59.6	0.37	1,002.8±4.5	—	—	—	—	—
8	(FI × DA)F ₁	Normal; para-aortic nodes removed	8	7.6±0.99	313.1±11.9	852.2±29.9	0.36	1,120.8±52.7	—	—	—	—	—
9	(FI × DA)F ₁	Normal; sham operation	7	9.3±0.79	331.5±12.6	884.4±29.2	0.37	1,214.5±24.4	20.4±3.1	49.6±4.7	—	—	—
10	(FI × DA)F ₁	Normal splenectomized	7	9.5±0.57	314.4±11.7	874.0±38.0	0.34	1,185.2±69.5	18.4±7.6	55.4±7.9	—	—	—
11	(FI × DA)F ₁	Normal; given normal FI serum	7	10.7±0.42	325.1±21.5	897.8±48.5	0.36	1,225.9±105.5	17.8±1.5	48.4±6.6	505.9±82.5	—	—
12	(FI × DA)F ₁	Passively immunized with FI anti-DA serum	5	9.6±0.49	345.3±22.8	934.6±46.6	0.36	1,278.9±90.2	14.0±4.5	56.5±12.3	—	—	—
13	(FI × DA)F ₁	Normal	6	9.2±0.8	281.9±31.6	876.3±66.4	0.33	1,176.6±7.2	22.3±4.9	53.2±6.2	476.8±57.6	—	—
14	(FI × DA)F ₁	Normal	5	3.2±0.4	346.0±26.5	840.3±57.9	0.41	1,189.5±65.5	32.4±6.2	—	—	—	—
15	(FI × DA)F ₁	Normal	6	2.6±0.5	355±15.4	820.2±50.5	0.43	1,160.4±94.5	30.3±6.9	—	—	—	—

* Mean placental weight/Mean fetal weight.

TABLE IV
Analysis of the Influence of Immunogenetic Disparity and Immunologic Status of Female DA Rats with Regard to Tissue Antigens of their (DA × FI)F₁ Conceptuses on the Weight of their Para-aortic Lymph Nodes and Spleens and upon the Weights and Numbers of their Fetoplacental Units

Exp.	Mother		Litters				Fetuses			Maternal lymphoid organs			
	Genotype	Immunologic status	Genotype	No.	Mean size ± SE	Placental wt. mean ± SE	Fetal wt. mean ± SE	PW/- FW* ratio	FP unit wt. mean ± SD	Para-aortic nodes		Spleen wt.	
										Mean wt. ± SE	Cell count × 10 ⁶ mean ± SE	Mean wt. ± SE	Cell count × 10 ⁶ mean ± SE
1	DA	Normal virgin (8)	DA	10	6.0±0.8	274.8±14.7	814.8±28.4	—	—	11.3±3.4	28.9±13.3	292.3±25.8	—
2	Normal	Normal	(DA × FI)F ₁	7	6.1±0.9	328.4±17.2	864.3±39.4	0.33	1,087.8±78.1	21.3±2.5	60.0±9.1	368.7±78.9	—
3	Normal	Normal	(DA × FI)F ₁	9	5.2±0.4	339.6±12.4	942.9±39.6	0.36	1,275.2±100.3	22.2±4.8	40.6±6.8	364.4±32.9	—
4	Sensitized: anti-FI	Normal	(DA × FI)F ₁	5	6.8±0.9	320.8±13.5	374.3±73.4	0.36	1,190.4±140.5	22.1±4.2	74.6±8.2	376.2±23.4	—
5	Tolerant of FI	Normal	(DA × FI)F ₁	5	6.7±0.4	308.4±16.5	780.0±63.5	0.39	1,084.7±68.4	—	—	—	—
6	Normal; para-aortic nodes excised	Normal	(DA × FI)F ₁	6	6.8±0.5	325.7±13.4	958.0±38.7	0.33	1,140.3±64.9	17.5±7.2	63.8±16.3	343.8±21.2	—
7	Normal; given normal DA serum	Normal	(DA × FI)F ₁	9	6.8±0.3	339.2±16.8	925.7±56.5	0.36	1,291.9±80.0	20.5±5.9	74.0±21.1	372.4±50.4	—
8	Passively immunized with DA-anti-FI serum	Normal	(DA × FI)F ₁	7	8.3±0.3	285.1±16.0	865.9±29.8	0.32	1,142.7±77.3	22.3±1.8	55.0±2.8	476.8±57.3	—
9	(FI × DA)F ₁	Normal	F ₁ FI/DA × FI backcross	7	8.3±0.3	285.1±16.0	865.9±29.8	0.32	1,142.7±77.3	22.3±1.8	55.0±2.8	476.8±57.3	—

* Mean placental weight/Mean fetal weight.

In females tolerant of DA tissue antigens at the time of mating with males of this alien strain, the allogeneic fetuses were no more effective in stimulating hypertrophy of the para-aortic nodes than syngeneic fetuses, and the spleen weights of these tolerant animals were similar to those of females bearing syngeneic fetuses.

INFLUENCE OF MATERNAL IMMUNOLOGIC STATUS ON WEIGHTS OF FETOPLACENTAL UNITS. (FI × DA) F_1 hybrid fp units borne by immunologically normal FI females were significantly heavier than FI fp units borne by similar females ($P < 0.01$). Presensitization of the FI females against DA tissue antigens increased the mean weight of the (FI × DA) F_1 fp units from $1,253.5 \pm 80.0$ mg to $1,302.0 \pm 122.3$ mg ($P < 0.01$). (See Table III, exp. 1-4).

Additional experiments were performed to try and sustain the thesis that the superiority in weight of the F_1 hybrid fp unit over that of the FI fp unit borne by a normal FI mother, which was further increased by maternal sensitization, was immunologically based. All of these experiments involved procuring the implantation and development of hybrid conceptuses in mothers that were incapable of reacting against them immunologically.

The mean weight of (FI × DA) F_1 hybrid fp units borne by FI females that were tolerant of the DA strain tissue antigens— $1,154.6 \pm 55.6$ mg was significantly less than that of $1,253.5 \pm 80$ mg ($P < 0.01$) for similar hybrids borne by normal mothers; indeed it approached the mean weight of the syngeneic FI fp units $1,126.0 \pm 66.7$ mg (See Table III, exp. 2, 3, and 6).

Likewise, the mean weight of F_1 FI/DA × F_1 backcross fp units borne by genetically tolerant (FI × DA) F_1 mothers— $1,176.6 \pm 7.2$ mg—was comparable to that of the F_1 fp units in the immunologically tolerant mothers. (Table III, exp. 6 and 13). The most direct and discriminating experiment performed entailed procurement of (DA × FI) F_1 hybrid and DA blastocysts, transferring them to the uterine horns of two different panels of normal (FI × DA) F_1 hybrid females and comparing the mean weights of the 18-day fp units produced. These were closely similar— $1,189.5 \pm 65.5$ mg and $1,160.4 \pm 94.5$ mg—respectively (Table III, exp. 14 and 15) indicating that the primary responsibility for the superiority in mean fp weights in hybrid as compared to syngeneic pregnancies must stem from maternal reactivity or other influence, rather than from genetically determined, intrinsic differences in growth potential in the two types of conceptuses.

The results of two different experiments attest to the specificity of the putative, immunogenetically based determination of fp weight. The mean weight of FI fp units from FI females which had been sensitized against DA strain antigens— $1,029.4 \pm 71.3$ mg, did not differ significantly from that of FI fp units from normal FI mothers $1,126.0 \pm 66.7$ mg (see Table III, exp. 2 and 5). FI fp units from FI females that were tolerant of DA antigens had a mean weight of $1,002.8 \pm 4.5$ mg (see Table III, exp. 7) which, again, is close to that of the aforementioned controls.

Since the para-aortic nodes appeared to be an important, if not the principal seat of the maternal response incited by genetically alien fetuses in the uterus, it follows that if this response contributes to the determination of fp weight, excision of these nodes from normal females before pregnancy should result in

smaller fp units. This prediction was borne out by the observation that the mean weight of (FI × DA) F_1 hybrid fp units borne by FI females lymphadenectomized a month before mating was $1,120.8 \pm 52.7$ mg, whereas that of similar fp units gestated by sham-operated females was $1,214.5 \pm 24.4$ mg ($P < 0.01$) (See Table III, exp. 8 and 9).

Although the mean spleen weight of normal FI females bearing (FI × DA) F_1 fp units was closely similar to that of FI females bearing FI units (535.4 ± 22.4 mg vs. 525.3 ± 11.4 mg), indicating that this organ probably was not stimulated significantly by fetal alloantigens, for completeness' sake the influence of removal of this organ on the size of subsequent F_1 hybrid fetuses was evaluated. The finding that the mean weight of (FI × DA) F_1 fp units borne by splenectomized mothers— $1,185.2 \pm 69.5$ mg—was very similar to that of comparable fetuses born of sham-operated mothers— $1,214.5 \pm 24.4$ mg indicates that this organ plays no significant role in the determination of fp size (See Table III, exp. 9 and 10).

Finally, to determine whether humoral antibody plays a role in mediating the immunogenetic determination of the size of the fp units, two panels of FI females gestating (FI × DA) F_1 fetuses were treated with: (a) Normal FI serum (controls) and (b) FI anti-DA tissue serum respectively (Table III, exp. 11 and 12). The mean fp weights in these two series were $1,225.9 \pm 105.5$ mg and $1,278.9 \pm 90.2$ mg respectively, affording no evidence that alloantibody from sensitized donors had exerted any significant influence on the weight of the fp units.

INFLUENCE OF IMMUNOGENETIC FACTORS ON IMPLANTATION. The mean sizes of the various panels of litters present in their mothers' uteri on the 18th day postconception are also presented in Table III. These show that genetically alien hybrid litters in general were significantly larger than syngeneic litters ($P < 0.05$) but provide no evidence that the immunologic status of the mother, immune or sensitized vis à vis the alien antigens of her fetuses, exerted any significant influence on the number which implant and develop successfully. However, mothers whose para-aortic nodes had been removed before mating gestated significantly smaller F_1 hybrid litters than normally reactive females.

DA Females Exposed to FI Strain Tissue Antigens During Pregnancy (Table IV). The results to be presented in this section were obtained by repeating the various experiments described in the previous section using DA strain females carrying (DA × FI) F_1 conceptuses as subjects.

INFLUENCE OF PREGNANCY ON MATERNAL LYMPHOID ORGANS. Again, pregnancies by syngeneic males provoked a very considerable increase in both the weight and cellularity of the draining para-aortic lymph nodes (by a factor of about 2), and a less dramatic but still highly significant degree of splenomegaly as compared with the situation in virgin DA females. However, with this combination, confrontation of immunologically virgin DA females with allogeneic (DA × FI) F_1 hybrid fetuses proved to be no more effective in stimulating hypertrophy of these lymphoid organs than syngeneic conceptuses. (Table IV, exp. 1–3).

INFLUENCE OF MATERNAL IMMUNOLOGIC STATUS ON WEIGHTS OF FETOPLACENTAL UNITS. (DA × FI) F_1 fp units in normal DA mothers were significantly heavier than syngeneic fp units in similar mothers (mean weights $1,183.6 \pm 36.9$ mg vs.

1,087.8 \pm 78.1 mg, ($P < 0.01$) and specifically presensitized females gestated even heavier hybrid fp units (mean weight 1,275.2 \pm 100.3 mg (Table IV, exp. 2-4), though the increase here was not statistically significant. The mean weight of the F₁ hybrid fp units borne by the specifically tolerant mothers was almost identical with that of normally reactive mothers (1,190.4 \pm 140.5 mg vs. 1,183.6 \pm 36.9 mg).

The thesis that maternal reactivity to allogeneic transplantation antigens is a determinant of the weight of the fp unit received further support from the following observations: (a) Excision of the para-aortic nodes from DA females before mating them with FI males resulted in fp units whose mean weight was almost identical with that of DA fp units borne of normal DA mothers (1,084.7 \pm 68.4 mg vs. 1,087.8 \pm 78.1 mg) (Table IV, exp. 2 and 6). (b) Passive immunization of normal DA females with DA anti-Fischer serum resulted in a significant *increase* in the weight of (DA \times FI)F₁ hybrid fp units over that of similar fp units gestated by normal DA females given normal DA rat serum (1,291.9 \pm 80.0 mg vs. 1,140.3 \pm 64.9 mg, $P < 0.01$). It will be noted that the mean weight of the F₁ FI/DA \times FI backcross fp units—(1,142.7 \pm 77.3 mg)—was not significantly less than that of (DA \times FI)F₁ hybrid fp units (1,183.6 \pm 36.9 mg) gestated by normal DA mothers.

Histological Observations on Placentae and Para-Aortic Lymph Nodes. Microscopic examination of histological cross-sections of syngeneic and allogeneic placentae of rats confirmed that the larger placentae seen in the allogeneic matings were due to an increase in all placental tissue components as illustrated in Fig. 1. Specifically, there was no consistent difference in the degree of maternal decidual proliferation, trophoblastic invasion, quantity of fibrinoid or morphologically distinct changes at the choriodecidual junction. The para-aortic nodes of virgin female rats did not differ histologically from other nonstimulated nodes of the subject. The para-aortic nodes from animals bearing syngeneic pregnancies were slightly enlarged and contained many prominent germinal centers (Fig. 1, A1). By contrast the para-aortic nodes from females bearing allogeneic conceptuses were more markedly enlarged, far more diffusely cellular in the paracortical areas, and contained fewer obvious germinal centers. (See Fig. 1, B1).

Discussion

In three different species—mice, hamsters, and rats—it has been found that gestation of allogeneic as compared with syngeneic fetuses usually incites a striking degree of hypertrophy and increase in cellularity of the draining, para-aortic lymph nodes of the female. Exposure of the latter to experimental cellular or tissue allografts via the intrauterine route provokes a similar response from these particular lymphoid organs which is also on an immunogenetically similar basis. However, intrauterine presentation of similar alloantigens in these two entirely different forms, conceptuses vs. grafts, has quite disparate immunologic consequences: pregnancy does not normally heighten the reactivity of the female to test allografts of skin or other tissue, i.e. evoke a state of transplantation immunity, whereas experimental intrauterine

allotransplantation consistently does. Nevertheless, cogent evidence now exists that allogeneic pregnancies do indeed lead to the production of specific lymphocytic effector cells by the females concerned, but these cells are incapable of expressing their reactivity because of the operation of both specific (blocking antibodies) and nonspecific (hormones and other agents) restraining factors within the placenta (14-19). It need hardly be emphasized that neither experimentally procured presensitization nor a mother's natural covert and restrained response to her allogeneic conceptuses poses any threat to their well-being. On the contrary, they appear to afford them a small but significant selective advantage over syngeneic conceptuses, operating at two different periods of their early life—at the time of implantation and subsequent to parturition. The observed capacity of syngeneic conceptuses to incite a modest though significant hypertrophy of the maternal para-aortic lymph nodes may reflect stimulation by autoantigens associated with trophoblast, and/or fetal or developmental antigens (20-22).

Maternal reactivity to the alien tissue antigens of their fetuses has been shown to be a significant determinant of the size or weight of their placentas and, in the rats, of the entire fp units. With some, though not all, fetal-maternal genetic combinations in the species studied, F_1 hybrid fetuses had larger placentas than syngeneic placentas; specific presensitization of the females usually resulted in significantly heavier F_1 placentas, and specifically tolerant females gestated hybrid fetuses with smaller placentas than normal females.

Additional support for the premise that maternal reactivity against fetal alloantigens in some way promotes the growth of genetically disparate fp units comes from the finding that virgin females, whose para-aortic nodes had been excised several weeks before interstrain mating, gestated smaller fp units than sham-operated females. Splenectomy was without effect. That humoral alloantibodies are probably the principal effectors of this histoincompatibility-based influence of placental size is suggested by the results of tests in which F_1 females bearing $(FI \times DA)F_1$ fetuses were passively immunized with FI anti-DA antiserum.

The long-recognized weight disparity between F_1 hybrid and homozygous fp units is usually attributed to heterosis or hybrid vigor. However, the present finding that the weights of DA and $(DA \times FI)F_1$ fp units which, through blastocyst transfer, had been caused to develop in the uteri of normal $(FI \times DA)F_1$ hybrid females, were closely similar, suggests that maternal reactivity against fetal alloantigens may in fact be a much more important determinant of fetoplacental weight than heterosis.

Since transplantation antigens are expressed by preimplantation zygotes (20) and specific *local* sensitization of rats' uteri significantly increases the mean litter sizes resulting from interstrain matings (6), evidence was sought in the present study for an influence of maternal reactivity against the conceptuses' alloantigens on litter size. In the three species studied suggestive evidence has been forthcoming for such an influence. Furthermore, in the hamster, presensitization of the females against paternal tissue antigens of their future conceptuses resulted in significantly larger litters than those produced by normal females.

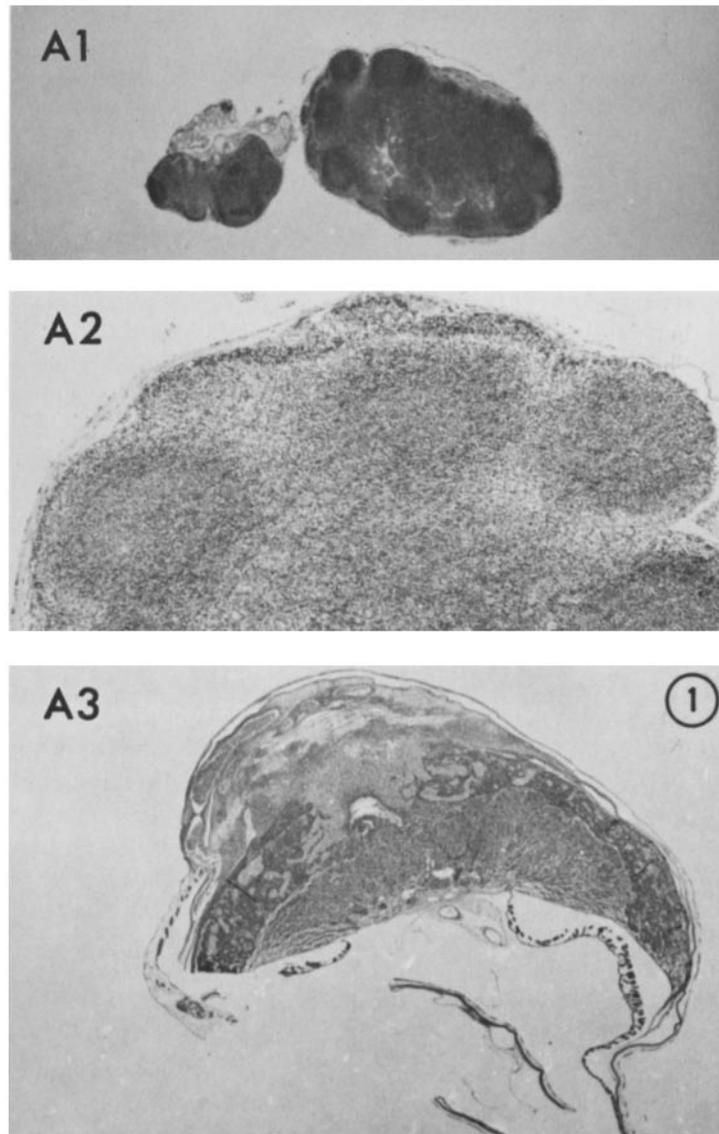
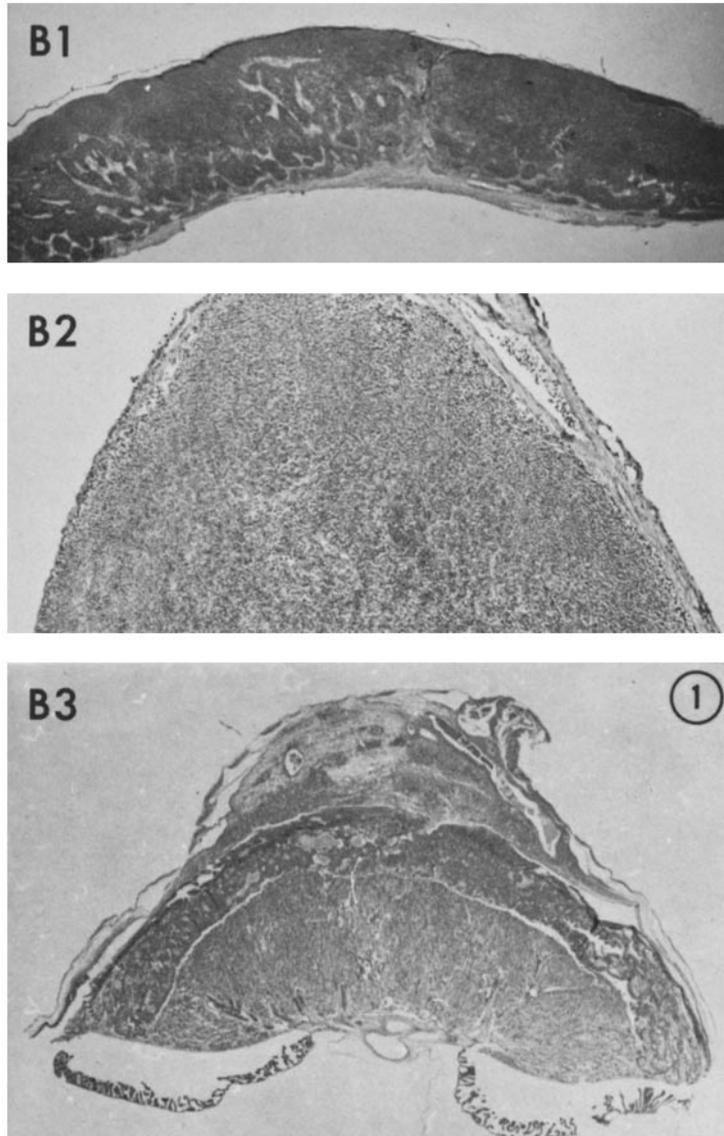


FIG. 1. Histology of a para-aortic lymph node draining the uterus, and of a placenta from a female Fischer rat gestating 18 day, syngeneic fetuses (A1-A3) and of similar organs from a Fischer rat gestating allogeneic (F1 \times DA)F₁ hybrid fetuses (B1-B3). Hematoxylin and eosin. (A1) The para-aortic node draining the uterus bearing syngeneic fetuses is modestly enlarged and contains conspicuous germinal centers. \times 20. (A2) Same as above. \times 400. (A3) Sagittal section through placenta of syngeneic fetuses. \times 20. (B1) Para-aortic node draining a uterus bearing allogeneic fetuses. This node is conspicuously enlarged compared with that draining the syngeneic fetuses (A1) and there is a tremendous overall increase in cellularity especially in the paracortical areas. \times 20. (B2) Same as above. \times 400. (B3) Sagittal section through placenta of an allogeneic fetus. Both the placenta and the maternal decidua tissue are enlarged as compared with those of the syngeneic fetus shown in A3. \times 20.



(Fig. 1 concluded)

Comparing the results of the present study with those of our previous study on the rat (6), it is obvious that *local* presensitization of the uterus exerts a much more striking influence on the reproductive performance than does systemic immunization of the mother, suggesting that a cellular rather than a humoral immunologic response is in some way a contributory factor in implantation.

Reproductive biologists are well aware that an intense local inflammatory response occurs in the endometrium at the site of implantation of the fertilized egg (23). This response, which is reminiscent of a delayed hypersensitivity

reaction, is believed to stimulate local angiogenesis which facilitates transportation to the site of nutrients and various blood cellular elements. Immunogenetic disparity between conceptus and mother and the corresponding reactivity of the latter may contribute to the magnitude of this response and so help promote both the implantation of the fertilized ova and the growth rate of the fp units. No satisfactory explanation can be offered at present for the influence of alloantibody on placental growth.

In 1966, Clarke and Kirby (24) postulated that some kind of immune interaction between females and their histoincompatible offspring, leading to increased birth weight, might favor the survival of the offspring and so help maintain the complex histocompatibility gene polymorphisms found in mammals. Subsequently, on the basis of rather tenuous evidence, Kirby (25) suggested that blastocysts that are genetically dissimilar to the mother may implant more readily than genetically similar blastocysts. Our present findings are in accord with both of these premises. It is ironical that the complex genetic polymorphism which exerts such a frustrating influence on therapeutic allografts, favors Nature's allografts, i.e. conceptuses in the uterus.

Summary

Studies conducted upon inbred strains of mice, hamsters and rats have shown that following the interstrain matings the now familiar covert reactivity of pregnant females to the alloantigens of their conceptuses may benefit the latter in two ways; firstly, it exerts a significant influence upon placental weight, and indirectly upon the birth weight of the fetus—allogeneic placentas tending to be heavier than syngeneic placentas, and mothers specifically presensitized against alien paternal tissue antigens gestate fetuses with heavier placentas than normal females. Specifically tolerant mothers, on the other hand, produce smaller, F_1 hybrid, fetoplacental (fp) units.

The classic notion that the disparity between the birth weights of F_1 hybrid and homozygous offspring is due to hybrid vigor has been challenged by the finding that DA and $(DA \times FI)F_1$ hybrid blastocysts transferred to the uteri of genetically tolerant $(DA \times FI)F_1$ hybrid rats produce fp units of similar weight. Maternal immunological reactivity against the fetus qua allograft may make a significant contribution here.

Additional support for the premise that maternal reactivity against fetal alloantigens in some way promotes the growth of the fp unit was afforded by the finding that excision of the para-aortic lymph nodes (which drain the uterine horns) from females before interstrain matings resulted in smaller fp units than in females subjected to sham operations. The finding with one rat strain combination that passive immunization of females with serum against their F_1 hybrid conceptuses promoted the growth of the latter suggests that a humoral rather than a cellular immunity may be involved.

Secondly, in the three species studied, it was observed that genetic disparity between a conceptus and its mother significantly improved its chances of implantation and development to term.

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