MATERNALLY INDUCED TRANSPLANTATION IMMUNITY, TOLERANCE, AND RUNT DISEASE IN RATS

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In studies on the biology of mammalian gestation, increasing attention is being focused upon the extent to which a natural, covert transplacental exchange of cells of various types, and possibly other isoantigenic material, takes place between mother and fetus, and its immunological consequences (1). The classic model here, of course, is Rh sensitization in man. With one exception (2), appropriately designed experiments in rodents have failed to establish that pregnancy can result in the induction of tolerance of maternal tissue antigens in the offspring, i.e., maternally induced tolerance (3). However, from the maternal viewpoint, it is well documented that sensitization against alien fetal isoantigens may occur as reflected by the appearance of humoral antibodies, but not by the acquisition of cellular immunity to solid tissue test homografts. Indeed, specific impairment of reactivity to test grafts, almost certainly a manifestation of immunological enhancement in most instances, has been established as a possible consequence (1, 4).

In 1965 Stastny (5) reported, on the basis of well-controlled experiments, that offspring of intrastrain mated (but outbred) Sprague-Dawley female rats, sensitized to Lewis strain tissue antigens by exposure to skin homografts during pregnancy, behaved as if they too were sensitized when challenged with skin grafts from Lewis donors. Likewise, young Sprague-Dawley rats born of mothers which had been (a) rendered tolerant of Lewis homografts by neonatal inoculation of Lewis spleen cells, and (b) subsequently reexposed to Lewis antigens during pregnancy by inoculation with a large dose of Lewis epidermal cells, rejected test skin grafts from this strain in an accelerated manner.

The subject matter of this communication comprises (a) an independent confirmation of Stastny's interesting and rather surprising findings; (b) their extension to an experimental situation involving two different isogenic strains of rat; and (c) presentation of various lines of indirect evidence which suggest very strongly that the observed altered immunologic status of offspring of mothers which have received living cellular homografts is due to maternal-fetal transmission of cells which, in some situations, may result in tolerance and cause homologous or runt disease.

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Materials and Methods

The subjects of the experiments were rats of the following denominations: Sprague-Dawley (George Holzman and Sons, Houston, Texas), an outbred strain, hereafter referred to as S-D; and domestically maintained sublines of the Lewis, Fischer, BN, and DA isogenic strains.

The median survival time (MST)[1] of skin grafts exchanged between a small group of randomly selected S-D individuals was approximately 16 days. The Ag-B genotypes and MST's of skin grafts exchanged between adult rats in the various donor/host strain combinations employed are as follows (6):

Lewis (Ag-B^1) → Fischer (Ag-B^1)	11.6 ± 0.3 days; SD, 1.04
Fischer (Ag-B^1) → Lewis (Ag-B^1)	7.6 ± 0.4 days; SD, 1.07
Lewis (Ag-B^1) → S-D (Ag-B?)	12.0 ± 0.44 days; SD, 1.1

Sensitization and Induction of Tolerance with Respect to Transplantation Antigens.—The methods employed for the preparation and transplantation of skin grafts, and for the preparation in Hanks' solution and intravenous or intraperitoneal administration of suspensions of bone marrow, lymphoid, and epidermal cells have been described in detail elsewhere (7).

Sensitization of adult rats was accomplished by grafting them bilaterally with full-thickness skin homografts, approximately 2.5 cm in diameter, from the donor strain, followed 3 wk later with a booster intraperitoneal injection of 40×10^6 viable lymph node cells of similar alien genetic constitution in 1.0 ml of Hanks' solution (Fig. 1).

Tolerance of Transplantation Antigens.—Tolerance was induced by intravenous injection of newborn hosts with a standard dosage of 40×10^6 homologous bone marrow cells in 0.1 ml of Hanks' solution. When approximately 60 days old the animals were challenged with skin grafts from the donor strain. Those whose grafts had remained in impeccable condition for an arbitrary 50 day period were classified as highly tolerant and employed for the experiments to be described.

Tolerant Bone Marrow Chimeras.—Chimeras were produced by the method of Santos and Owens (8). Young virgin adult rats were injected with cyclophosphamide (Cytoxan; Mead Johnson & Co., Evansville, Ind.) at a dosage of 225 mg/kg, via the intraperitoneal route, followed 18–24 hr later by an intravenous injection of 100×10^6 bone marrow cells from the appropriate donor strain (Fig. 2). They were also challenged with Lewis skin homografts 4 wk after reconstitution. This procedure is known to result in a much higher degree of cellular chimerism with respect to the host's lymphohematopoietic tissue system than intravenous injection of donor cells into otherwise untreated neonatal subjects. 6 wk after bone marrow transfusion these drug chimeras were mated with males of their own strain.

Determination of Altered Immunologic Reactivity of the Progeny.—Determination of reactivity was based upon challenging the progeny with ear skin homografts approximately 15 mm in diameter from the donor strain when they were 21 days old and determining the survival end points of the epidermis from its outward appearance (7). MST's were calculated according to Litchfield's (9) nomographic method. For most of the experiments controls comprised 21 day old offspring of normal mothers grafted with skin from the appropriate donor strain. Both the distribution of graft survival times and the MST could then be compared with those pertaining to the experimental series.

Preparation of S-D Anti-Lewis Serum.—Preparation of serum entailed sensitization of S-D rats as described, and giving them an additional intraperitoneal injection of 40×10^6 Lewis lymph node cells 1 wk after the previous one. A week later (i.e. 5 wk after the receipt of Lewis skin grafts) blood was withdrawn from the animals by cardiac puncture and serum prepared by the standard procedure.

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[1] *Abbreviations used in this paper:* GVH, graft-*versus*-host; MST, median survival time.
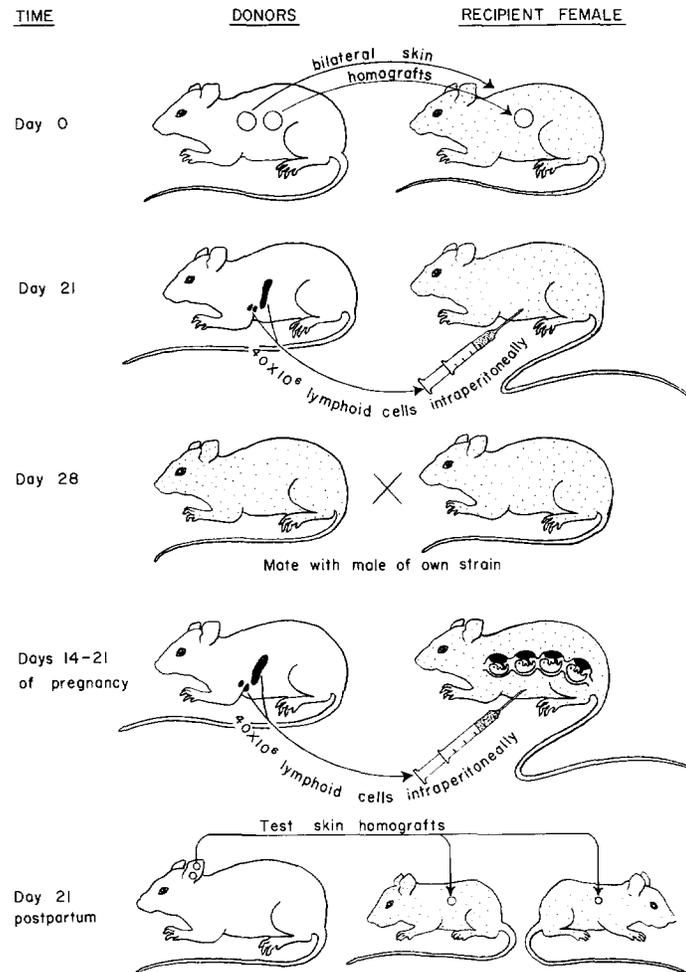
FIG. 1. Illustration of the protocol for sensitizing female rats with respect to the tissue antigens of an unrelated donor strain by inoculation of homologous lymphoid cells before and during gestation, and for detecting the influence of this treatment on the capacity of their progeny to reject skin homografts from the alien strain concerned.

RESULTS

Influence of State of Sensitivity or Tolerance with Respect to Lewis Tissue Antigens in S-D Females Mated with S-D Males on Reactivity of their Progeny to Lewis Skin Grafts.—To evaluate the influence of prior sensitization or the induction of tolerance in S-D females on the subsequent reactivity of their S-D offspring to Lewis strain skin homografts, the mothers were first sensitized or rendered tolerant of Lewis tissue antigens, as described above. They

were then time mated and injected intraperitoneally with 40 × 10^6 viable Lewis lymph node cells between the 14th and 21st days of gestation (see Fig. 1) In Stastny's experiments it will be recalled that this final booster inoculum was comprised of epidermal cells.

The offspring received test grafts of Lewis skin when they were 21 days old. Controls for these two series of experiments were provided by similarly grafting a comparable number of S-D weanlings of similar age born of normal, untreated mothers. The results of this experiment are summarized in Table I, experiments 1 a, b, and c. Comparison of the survival times and MST's of the three series of homografts indicates that being gestated by either a sensitized or a tolerant mother conferred what appears to be an immune status upon the offspring, the MST's of the two experimental series of grafts being 9.0 ± 0.7 and 9.6 ± 0.6 days, respectively, as compared with 15.0 ± 1.2 days for their controls. These findings are in good accord with Stastny's (5).

To determine whether injection of the tolerant rats with a booster inoculum of Lewis donor cells during gestation had made any significant contribution to the offspring's capacity to reject their test grafts in an accelerated manner, this treatment was withheld from one panel of pregnant S-D females, which subsequently produced a total of 21 offspring (see Table I, experiment 1 d). The observation that the MST of Lewis test grafts on the latter was 13.0 ± 1.3 days indicates that the tolerant status of the mothers did in fact heighten the reactivity of their progeny to grafts from the strain against which their mothers had been rendered specifically nonreactive. However, it is clear that the additional cellular inoculum administered during gestation augmented the capacity of tolerant mothers to influence the immune reactivity of their progeny.

That the apparent state of sensitivity acquired through gestation in a sensitized female is fairly long lasting is indicated by the observation that, even when grafting of the progeny was delayed until they were 70 days old, the MST of the Lewis skin grafts was still significantly shorter than that of control grafts transplanted to 70 day old offspring of normal mothers, 9.2 ± 1.0 days versus 13.0 ± 1.0 days, (see Table I, experiment 2).

Evidence of Specificity of the Apparent Maternally Acquired Sensitivity.— An important question now to be resolved was whether the capacity to destroy Lewis skin grafts in an accelerated manner which young S-D rats acquire through gestation by sensitized or tolerant mothers is, indeed, an immunologically specific phenomenon, rather than the outcome of some kind of nonspecific heightening of the functioning of their immune response machinery. Two panels of S-D females were respectively rendered tolerant and immune with regard to tissue antigens of the unrelated Ag-B locus–incompatible BN donor strain. These treated females were then mated with S-D males, and again inoculated intraperitoneally with 40 × 10^6 lymph node cells from BN skin donors during gestation. Their progeny were challenged with Lewis test skin

TABLE I

Survival of Lewis Skin Grafts on S-D Progeny of S-D Mothers Previously Exposed to Lewis Tissue Antigens

Experiment	Treatment of mothers and immunologic status		Age of progeny when grafted	Grafts surviving* Days after transplantation											MST	SD
	Before conception	During pregnancy		0	6	8	10	12	14	16	18	20	22	24		
			(days)												(days)	
1 a	None (normal)	None (control)	21	37	37	36	32	20	11	9	8	4	2	0	15.0 ± 1.2	1.3
				100	*100*	*97*	*87*	*54*	*30*	*24*	*22*	*11*	*5*	*0*		
b	Sensitized, anti-Lewis	40 × 10⁶ Lewis node cells i.p. 14–21 days gestation	21	48	37	25	8	4	1	0					9.0 ± 0.7	1.3
				100	*92*	*56*	*18*	*9*	*2*	*0*						
c	Tolerant of Lewis antigens	40 × 10⁶ Lewis node cells i.p. 14–21 days gestation	21	36	36	24	18	6	2	2	0				9.6 ± 0.6	1.2
				100	*100*	*67*	*36*	*17*	*6*	*6*	*0*					
d	Tolerant of Lewis antigens	None	21	21	21	21	18	10	7	4	2	0			13.0 ± 1.3	1.2
				100	*100*	*100*	*86*	*48*	*33*	*19*	*10*	*0*				
2 a	None (normal)	None (controls)	70	24	24	23	21*	12	10	3	2	2	1	0	13.0 ± 1.11	1.3
				100	*100*	*96*	*87*	*50*	*42*	*13*	*8*	*8*	*4*	*0*		
b	Sensitized anti-Lewis	40 × 10⁶ Lewis node cells i.p. 14–21 days gestation	70	36	30	20	6	4	0						9.2 ± 1.0	1.3
				100	*83*	*56*	*17*	*11*	*0*							
3 a	Tolerant of BN antigens	40 × 10⁶ BN node cells i.p. 14–21 days gestation	21	24	24	24	24	20	12	4	0				15.0 ± 0.8	1.2
				100	*100*	*100*	*100*	*83*	*50*	*17*	*0*					
b	Sensitized, anti-BN	40 × 10⁶ BN node cells i.p. 14–21 days gestation	21	15	15	15	15	10	6	2	0				14.5 ± 1.3	1.2
				100	*100*	*100*	*100*	*67*	*40*	*13*	*0*					
4	None (normal)	None. Offspring received 0.2 ml SD anti-Lewis serum on days 1, 2, and 5	21	12	12	12	12	10	10	6	4	2	0		15.5 ± 1.7	1.2
				100	*100*	*100*	*100*	*89*	*89*	*50*	*33*	*17*	*0*			

* Figures in italics indicate percentages of surviving grafts.

grafts when 21 days of age. The finding that the survival times of both series of grafts (see Table I, experiment 3) were closely similar to those of the controls sustains the view that the phenomenon under study is an immunologic one.

Influence of Passive Immunization of Infant S-D Rats with S-D Anti-Lewis Serum on their Reactivity to Lewis Test Grafts.—In adult rats there is some evidence of an inconclusive nature that sensitivity to Ag-B–incompatible skin homografts is transmissible by means of immune serum (10, 11). Furthermore, it is well established that both actively and passively acquired isoantibodies are transmitted from mother to offspring in this species either before and/or

EXPERIMENT

Lewis strain rat

Adult Fischer ♀

② 50×10⁶ Lewis marrow cells

cyclophosphamide 225 mg/Kg
①

Test Skin Grafts

③ Chimeric Fischer ♀ reequipped with Lewis lymphohematopoietic tissue system

Fischer ♂

④ mate

X

⑤ Fischer fetuses gestated in Fischer ♀ whose peripheral leukocytes are of Lewis origin

50% of offspring develop fatal runt disease.
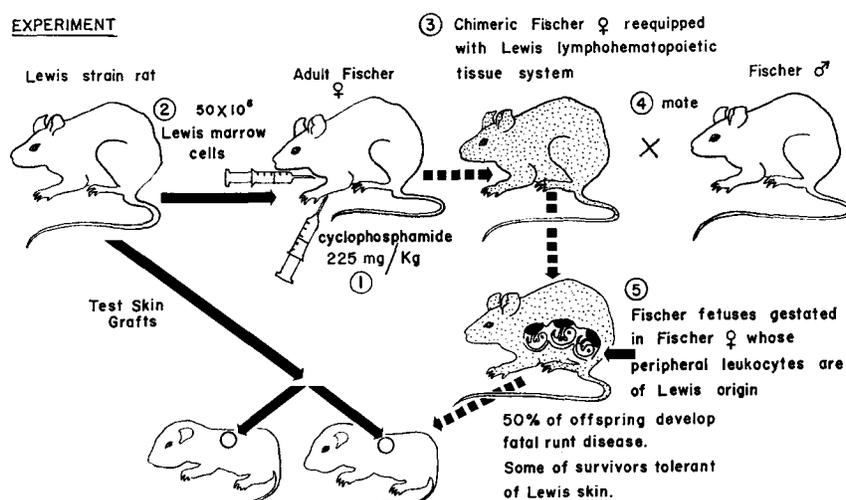Some of survivors tolerant of Lewis skin.

FIG. 2. Illustration of the principle of the experiments designed to reveal the phenomenon of maternal induction of tolerance involving the production of chimeric females which are subsequently mated to males of their own strain; see text.

just after birth. It was possible, therefore, that the heightened reactivity of the progeny of the sensitized S-D females to Lewis skin homografts was due to a maternal-fetal transfer of humoral antibody of some kind. To evaluate this premise, 12 S-D rats born of normal mothers were injected intraperitoneally, on days 1, 2, and 5 after birth, with 0.2 ml of S-D anti-Lewis antiserum. The finding that the MST of Lewis grafts transplanted when these animals were 21 days old was 15.5 ± 1.7 days (Table I, experiment 4) makes it seem unlikely that antibodies were responsible for the phenomenon under investigation.

Influence of Pretreatment of Fischer Mothers with Lewis Cells on Immunologic Status of their Progeny.—Because of their genetic heterogeneity, S-D rats are very unfavorable subjects for further analysis of the apparent, transplacentally acquired sensitivity to homografts described. In the belief that an essential prerequisite for the phenomenon under study might be compatibility between

TABLE II

Survival of Lewis Skin Homografts on Young Fischer Rats Born of Mothers Exposed To Lewis Cells

Experiment		Treatment of mother before conception and immunologic status	Treatment of mother during pregnancy	Grafts surviving* Days after transplantation											MST	SD
				6	8	10	12	14	16	18	20	22	24	>26	(days)	
1	a	None (normal control)	None	37 *100*	32 *87*	5 *14*	0 *0*								10.2 ± 0.3	1.1
	b	Lewis skin grafts and i.p. injection of Lewis node cells (sensitized)	40 × 10⁶ Lewis node cells i.p. 5–7 days before parturition	82 *97*	71 *84*	50 *59*	42 *49*	26 *31*	13 *15*	5 *6*	4 *5*	2 *3*	2 *3*	0 *3*	10.8 ± 0.8	1.4
	c	i.v. injection of Lewis marrow cells at birth, followed by Lewis test graft (tolerant)	40 × 10⁶ Lewis node cells i.p. 5–7 days before parturition	32 *100*	27 *84*	14 *44*	9 *28*	6 *19*	2 *6*	1 *3*	0 *0*				10.5 ± 1.2	1.4
	d	Lewis skin grafts and i.p. injection of Lewis node cells (sensitized)	None	16 *100*	16 *100*	7 *44*	2 *13*	0 *0*							9.9 ± 1.1	1.2
	e	i.v. injection of Lewis marrow cells at birth followed by Lewis test graft (tolerant)	None	19 *100*	18 *95*	7 *37*	2 *11*	0 *0*							10.0 ± 1.1	1.2
2	a	None (normal)	i.p. injection of 100 × 10⁶ Lewis node cells 9–12 days antepartum	17 *100*	16 *94*	3 *18*	0 *0*								10.2 ± 0.3	1.1

TABLE II—Continued

	Immunization	Treatment											MST ± SE	
b	None (normal)	i.p. injection of 100 × 10⁶ Lewis node cells 5–7 days antepartum	15	15	14	12	10	7	3	1	1	0	15.0 ± 2.0	1.3
			100	*100*	*93*	*80*	*67*	*47*	*20*	*7*	*7*	*0*		
c	None (normal)	i.p. injection of 100 × 10⁶ Lewis node cells 2–4 days antepartum	17	12	11	8	6	3	2	1	1	0	12.0 ± 2.0	1.4
			100	*71*	*65*	*47*	*35*	*18*	*11*	*6*	*6*	*0*		
d	None (normal)	i.p. injection of 100 × 10⁶ Lewis node cells 0–24 hr antepartum	20	16	11	8	3	2	0				12.0 ± 1.6	1.3
			100	*80*	*55*	*45*	*15*	*10*	*0*					
e	None (normal)	i.p. injection of 100 × 10⁶ Lewis node cells 0–24 hr postpartum	34	34	21	1	0						10.5 ± 0.3	1.1
			100	*100*	*62*	*3*	*0*							
3 a	None (normal)	i.p. injection of 100 × 10⁶ Lewis epidermal cells 7 days antepartum	22	22	21	12	3	2	1	0			12.0 ± 0.6	1.2
			100	*100*	*96*	*55*	*14*	*9*	*5*	*0*				
b	None	i.p. injection of 100 × 10⁶ Lewis epidermal cells 4–5 days antepartum	34	30	9	5	2	0					10.8 ± 0.7	1.2
			100	*88*	*27*	*15*	*6*	*0*						
c	None (normal)	1 Lewis skin graft 10–12 days antepartum	35	31	22	5	2	1	0				11.2 ± 0.6	1.2
			100	*87*	*63*	*14*	*6*	*3*	*0*					

* Figures in italic type indicate percentages of surviving grafts.

the maternal and donor strains at the Ag-B locus (12), the influence of sub-
stituting inbred Fischer rats for the S-D animals was explored by repetition
of the key experiments described above.

The various experiments performed, together with their results, are sum-
marized in Table II and Fig. 2. It will be noted that the MST of Lewis skin
grafts on a control panel of 37, 21 day old progeny of untreated Fischer mothers
was 10.2 ± 0.3 days, no graft surviving for as long as 12 days (Table II, ex-
periment 1 a). Judged in terms of MST's, the sojourn of Fischer fetuses in the
uteri of either sensitized or tolerant mothers was without any significant influ-
ence on their subsequent reactivity to Lewis grafts, irrespective of whether the
mothers had been inoculated with Lewis lymphoid cells during pregnancy or not
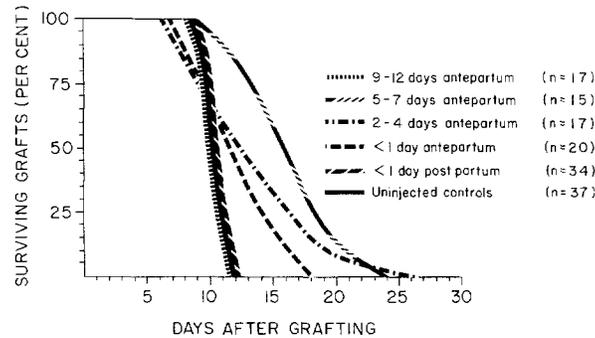
Fig. 3. Percentage of surviving Lewis skin homografts on 21 day old progeny of Fischer
females injected intraperitoneally with 100 × 10^6 Lewis lymphoid cells at various times dur-
ing pregnancy.

(Table II, experiments 1 a, b, c, and d). However, comparison of the distribu-
tion of the survival times of grafts on both series of experimental subjects,
whose mothers received donor cells during gestation, with that of the controls
(see experiments 1 a, b, and c) reveals that reinoculation of the pregnant rats
with Lewis cells did in fact result in a significant prolongation of survival of a
small proportion of the grafts, the influence being particularly striking in
animals gestated by sensitized mothers.

*Influence of Inoculation of Normal Pregnant Fischer Rats with Lewis Cells on
Subsequent Reactivity of their Offspring to Lewis Skin Grafts.* —In nearly all the
experiments so far described that resulted in altered reactivity of infant rats
born of tolerant and sensitized mothers, the only treatment shared in common
by the latter was intraperitoneal inoculation with 40 × 10^6 donor strain lymph-
oid cells during gestation. The experiment now to be described was designed
to determine whether this was, in fact, the only major relevant exposure of the
treated mothers to alien cells.

Normal, untreated virgin female Fischer rats were mated with males of their

own strain. Then, at various times during gestation and concluding 1 day postpartum, subgroups received a single intraperitoneal inoculation of 100 × 10^6 Lewis lymphoid cells. Again, the progeny received Lewis test skin grafts 21 days after birth. The results (Table II, experiments 2 a, b, c, d, and e and Fig. 3) show that whereas injection of the gravid females 9–12 days antepartum had no perceptible influence on the reactivity of their progeny, inoculation nearer to the time of delivery resulted not only in a prolongation of the MST, but also in a very significant extension of the life-span of some of the grafts. The effect was most striking after maternal inoculation 5–7 days antepartum, when the MST was 15 ± 2.0 days. It is important to note that whereas injection 24 hr before delivery still had a discernible effect, injection of the mothers 24 hr postpartum was ineffective. These time relationships are such as to make it very improbable that maternal-fetal transfer of antibody either before birth, or after birth via the milk, played any significant role in impairing the ability of the young rats to reject their test skin grafts.

Influence of Inoculating Pregnant Fischer Rats with Lewis Epidermal Cells or Skin Grafts on Reactivity of their Progeny to Lewis Grafts.—In Stastny's (5) experiments tolerant S-D females were injected with suspensions of Lewis epidermal cells during pregnancy. Since, in our experiments, for reasons of technical convenience, lymphoid cells were substituted for these, it now remained to compare the influence of epidermal cell inocula with that of lymphoid cells using the present strain combination and experimental protocol.

Two panels of normal virgin Fischer females were injected intraperitoneally with 100 × 10^6 Lewis epidermal cells 7 days and 4–5 days antepartum, respectively. An additional group of females received bilateral orthotopic Lewis skin grafts 10–12 days antepartum as an alternative means of exposing them to the alien "skin" cells.

Comparison of the results (Table II, experiment 3 a, b, and c) with the appropriate controls (experiment 1 a) shows that the behavior of each group of subjects to their Lewis test grafts underwent a slight modification as a consequence of the exposure of their mothers to the Lewis tissue antigens. However, the capacity of Lewis epidermal cells inoculated intraperitoneally into pregnant Fischer females to impair their offspring's reactivity to Lewis grafts is significantly inferior to that of Lewis lymphoid cells when both are administered at the optimum time, i.e., 5–7 days before parturition.

Influence of Cellular Status (Chimeric versus Normal) of Fischer Mothers on Survival and Immunologic Reactivity of their Progeny.—Many of the findings so far presented are consistent with the hypothesis that the specifically altered immunologic reactivity of the progeny of homologous cell–treated mothers is, in some way, due to a maternal-fetal transmission of alien cells.

To explore this possibility more critically required the production of Fischer females whose lymphohematopoietic tissue system, including their peripheral leukocytes, had undergone almost total replacement by Lewis cells. This need

TABLE III

Influence of Cellular Status (Chimeric Versus Normal) of Fischer Mothers on Survival and Immunologic Reactivity of Their Progeny

Experiment	Status of Fischer mothers at mating	Mated with male	Inoculated during pregnancy with	No. of litters studied	No. of animals surviving on day										Mortality after day 5	Survival times of donor strain skin grafts on 21 day old progeny
					0	5	10	15	20	25	30	35	40	45	%	(days)
1 a	Normal (control)	Fischer	—	6	54	54	54	54	54	54	54	54	54	54	0	Lewis grafts: 10.2 ± 0.3 (SD 1.4)
b	Chimera (Lewis bone marrow)	Fischer	—	8	66	66	62	55	49	44	42	35	28	28	57	9, 9 × 11, 5 × 13, 2 × 15 >25+, >28+, >31+, >35+, >37+
2	Chimera (Fischer bone marrow) (control)	Fischer	.	8	66	60	60	60	59	59	59	59	59	59	2	Lewis: 6 × 10–11
3	Chimera (Lewis × Fischer) F₁ marrow	Fischer	—	4	22	22	22	22	22	22	22	22	22	22	0	Lewis grafts: 13.5 ± 0.6 (SD 1.1)
4 a	Sensitized against Lewis	Lewis	—	10	85	85	85	85	84	80	78	78	78	78	8.3	
b		Fischer	—	6	42	42	42	42	42	42	42	42	42	42	0	

TABLE III—*Continued*

		Normal	Fischer	100 × 10⁶ Lewis anti-Fischer node cells	6	41	41	41	41	41	35	32	27	23	23	21	51	
5		Normal																
6	a	Chimera (Lewis bone marrow)	DA	—	7	59	56	56	56	56	56	56	56	56	56	56	0	$12.6 + 0.35$ (SD 1.12) 3 × 11, 5 × 12, 6 × 13, 5 × 14, 4 × 15, 16
	b	Normal (control)	DA	—	4	20	20	20	20	20	20	20	20	20	20	20	0	8.4 ± 0.7 (SD 1.2) All grafts rejected before 11th day
7		Chimera-Lewis mother with Fischer marrow	Lewis	—	2	14	12	12	12	12	12	12	12	12	12	12	0	16.0 ± 2.9 (SD 1.5) 3 × 13, 14, 15, 2 × 16, 4 × 17, 18

was met by treatment of young adult virgin females with cyclophosphamide followed by inoculation of Lewis marrow cells (8). When these animals had regained weight and health, and had received a Lewis skin homograft to confirm their tolerant status, they were mated with Fischer males. Since as few as 250,000 Lewis marrow cells injected intravenously into neonatal Fischer hosts represents a highly effective tolerogenic stimulus (6), it was hoped that sufficient Lewis leukocytes would gain access to the Fischer fetuses gestated by these highly chimeric Fischer mothers to produce high degrees of tolerance of the antigens concerned (Fig. 2). No booster inoculum of Lewis cells was administered to these animals during gestation. It was not anticipated that runt or homologous disease would be a complicating issue since, in the rat, as in the mouse, when donor and host are compatible at the major histocompatibility locus overt manifestations of graft-*versus*-host (GVH) reactivity do not develop unless lymphoid cells are transferred from specifically sensitized donors (13, 14).

Although most of the cyclophosphamide-induced Fischer chimeras gave birth to healthy litters of normal size, a high proportion (57%) of these infants subsequently developed and succumbed within 10–35 days to a wasting syndrome that had all the hallmarks of runt disease as described in this species after the injection of mature lymphoid cells into neonatal Ag-B–incompatible hosts (15) (Table III, experiment 1 b).

When they had attained an age of 21 days and looked healthy enough to withstand the grafting operation, 22 of the survivors received Lewis skin grafts. Ten of these animals gave no evidence of being tolerant, and seven were feebly tolerant; none of these animals was affected by runt disease. However in five animals in this group the test grafts were still in impeccable condition when their hosts succumbed to runt disease 25–27 days after the grafting operation.

To evaluate the unlikely possibility that drug treatment of the mothers before conception was in some way responsible for the observed mortality in the progeny, a group of Fischer females was treated with cyclophosphamide and then infused with isologous, i.e. Fischer, bone marrow cells. When these animals were mated with Fischer males there was only a single death among a total of 60 progeny after the 5th day of life and this could not be ascribed to runt disease (Table III, experiment 2). Further confirmation that the antecedent drug treatment of the females had no adverse influence on their progeny, and additional evidence in support of the view that GVH reactivity was responsible for the deaths of the offspring observed in experiment 1 b (Table III) were forthcoming. It was found that cyclophosphamide-treated Fischer females reconstituted with bone marrow cells from (Fischer $\times$ Lewis)F_1 donors, and subsequently mated with Fischer males, gave birth to healthy litters which continued to thrive (Table III, experiment 3). When a panel of these animals was challenged with Lewis grafts, the majority displayed a slight but significant impairment of their reactivity as compared with the controls (see Table III, experiments 1 a and 3).

It was observed that most of the Fischer females whose bone marrow had been replaced with that from Lewis donors eventually developed what appeared to be a chronic form of GVH disease, progressively losing weight. The fact that Fischer females chimeric with respect to marrow from isologous or (Fischer × Lewis)F_1 donors remained perfectly healthy lends considerable weight to this interpretation. If this is correct, it follows that the lymphocytic population in Fischer females reconstituted with Lewis bone marrow eventually comprised a "sensitized," rather than a normal one, which would be consistent with the development of runt or GVH disease in the progeny.

Further Definition of the Conditions Necessary for the Occurrence of Maternally Induced Runt Disease.—The experiments now to be described were all performed further to define the conditions under which maternally induced runt disease can be procured.

(*a*) A panel of Fischer females was sensitized against Lewis tissue antigens. Some of the members were then mated with Lewis males and the remainder with Fischer males (to provide controls) and the fate of their progeny carefully followed (Table III, experiments 4 a and b). Whereas there was no mortality among the Fischer offspring as expected, a small proportion (7/85) of the (Lewis × Fischer)F_1 progeny died after presenting the typical runting syndrome.

(*b*) A group of normal Fischer females bearing fetuses sired by Fischer males was injected intraperitoneally with 100×10^6 lymph node cells from Lewis donors presensitized against Fischer antigens (Table III, experiment 5). The result was striking and totally unexpected: none of the mothers were affected by this treatment but 51% of the 41 offspring that were perfectly healthy 5 days after birth later succumbed to runt disease.

It is interesting to note that of the 41 offspring in this experiment that were healthy on the 5th day postpartum 26 were females and 15 were males, suggesting that the experimental treatment of the mother had altered the sex ratio from its normal value of unity in this species. Evidently the selective force continued to operate since of these 26 females 10 (38%) subsequently succumbed to runt disease, whereas of the 15 males, 10 (67%) suffered a similar fate. The differential susceptibility of males is in accord with evidence that in rats males are more easily rendered tolerant than females, and in general, males of all species are more susceptible to harmful agents than females (16).

(*c*) In nearly all the experiments described so far Ag-B locus–compatible Fischer fetuses comprised the "target" organisms for the putative attack by alien cells gaining access to them from the maternal circulation. To determine whether introduction of incompatibility at the Ag-B locus would influence the susceptibility of fetuses, Fischer females with a Lewis-type hematopoietic tissue system were mated with DA males (Table III, experiment 6 a). In this situation the F_1 hybrid offspring are antigenic to both Lewis and Fischer immunocompetent cells. None of 56 offspring, belonging to seven litters, which were healthy 5 days after birth, ever looked sick or died during the following

40 day period. 24 of these offspring were challenged with grafts of Lewis skin when they were 21 days old. Comparison of the survival times of these grafts with those of similar grafts on (Fischer $\times$ DA)F_1 offspring born of normal Fischer mothers revealed that chimerism on the part of the mother did result in feeble degrees of immunological unresponsiveness in most of the offspring.

(d) Finally, our basic experiment in which Fischer females with a reconstituted Lewis-type lymphohematopoietic tissue system were mated with Fischer males was repeated on a small scale with the roles of the two strains reversed, i.e., chimeric Lewis females served as the mothers and Fischer donors provided the putative "attacking" cells (Table III, experiment 7). None of 12 offspring that were healthy on the 5th day postpartum died during the following 40 days. It will be noted however, that gestation of these animals by Lewis/Fischer chimeric females did result in impairment of their capacity to reject Fischer grafts.

The only factual information which may have some bearing upon this differential susceptibility of Fischer infants to runt disease is: (i) The tempo of reactivity of Lewis rats to grafts of Fischer skin is greater than that of Fischer rats to Lewis skin. (ii) Over lower dosage ranges neonatal Fischer rats are slightly more tolerance-responsive to Lewis cells than are neonatal Lewis rats to Fischer cellular inocula (6).

DISCUSSION

The experimental findings reported are essentially that in certain genetic contexts inoculation of adult female rats with homologous lymphohematopoietic cells before and/or during gestation may affect their syngeneic progeny in such a manner that the latter may (a) behave as if specifically sensitized to test skin homografts from the alien donor strain; (b) display impaired reactivity to donor strain test grafts, suggestive of maternally acquired tolerance; and (c) succumb to what is almost certainly GVH or runt disease. The simplest general hypothesis capable of accounting for these seemingly disparate results of diverse exposures of pregnant females to viable foreign cells is that these various treatments resulted in the transmission from mother to fetus of immunologically significant numbers of alien leukocytic cells, probably lymphocytes. This covert, natural "second passage" of the grafted cells may have occurred during gestation and/or during the traumatic upheaval of parturition.

It is well documented that both neonatal mice and rats can respond to inoculation of relatively small numbers of homologous lymphohematopoietic tissue cells by becoming sensitized and to large numbers by becoming tolerant (17–19). Thus depending upon the actual number and timing of the postulated maternal-fetal transmission of cells and the genetic relationship between donor and recipient, one might anticipate that some offspring would become sensitized and some tolerant.

In the light of the complete failure of nearly all prior attempts to prejudice the gestation of an F_1 hybrid fetus in a presensitized mother (1), the develop-

ment of the runting syndrome in a high proportion of Fischer progeny of (a) Fischer mothers chimeric with Lewis bone marrow or (b) of normal Fischer mothers which received an inoculum of Lewis anti-Fischer cells during pregnancy is surprising. In the first instance, the development of this syndrome was not expected since a wealth of empirical evidence sustains the generally accepted view that the *sine qua non* for this type of GVH reactivity is that donor and host of the putative attacking cells must differ at the major histocompatibility locus for the species concerned, unless the donor has been specifically presensitized (14). Furthermore, it is generally believed that alien immunocompetent cells inoculated into neonatal hosts of a potentially reactive strain must establish some degree of tolerance or anergy to enable them to survive long enough to produce overt GVH disease (20). These observations are particularly important since they constitute the most compelling evidence that transmission of alien donor cells from mother to fetus is the basis of the phenomenon reported.

It is worth stressing that the behavior of test skin homografts provides no reliable indication of the fate of other types of tissue or cellular homograft of similar genetic origin, especially where donor and host differ with regard to alleles at the major histocompatibility locus. For example, rats neonatally injected with homologous lymphoid cells may remain tolerant with respect to them as well as to auxillary heart homografts and yet reject skin homografts of similar genetic provenance (21), and animals that are chimeric with respect to bone marrow ingredients may reject skin grafts having the same genetic constitution as the long-accepted marrow graft. Furthermore, heart and renal grafts exchanged between Ag-B locus–compatible Lewis and Fischer rats enjoy anomalously long survival times as compared with skin (22). Equally relevant is the evidence that lymphocytes of fetal origin are sometimes demonstrable in the blood of normal women long after parturition (23, 24).

In the light of such observations it is not unreasonable to postulate that Lewis strain lymphocytes transferred to normal pregnant Fischer females may survive long enough in their hosts' blood stream to gain access to their fetuses, and it is perhaps not inconceivable that Lewis lymphoid cells injected into pregnant S-D or Fischer females previously sensitized, by means of Lewis skin homografts and inoculation of Lewis lymphoid cells, may also enjoy a few days' survival in the circulation of their hosts.

To explain the slight prolongations of survival of donor test skin grafts on a few of the progeny of mothers injected during pregnancy with suspensions of epidermal cells presents more difficulties since it is almost inconceivable that these could cross the placenta in an intact, viable form. The presence of contaminating donor mononuclear leukocytic 'passenger' cells (25) in the relatively high dosage epidermal cell inocula is one possibility.

Palm (26) has reported that a typical (maternally induced) immunological runting syndrome affects a small proportion of the progeny of DA × (BN/ DA)F$_1$ crosses and, on the basis of various lines of indirect evidence, has sug-

gested that the immune interaction involves non–Ag-B incompatibilities between mother and offspring. Her findings suggest that backcross progeny that are Ag-B incompatible with their mothers, but which should nevertheless differ from their mothers at the pertinent non–Ag-B loci as do the Ag-B–compatible homozygotes, enjoy a greater resistance to attack. Our own data, especially those presented in Table III, experiment 6, sustain this thesis.

In the course of raising (Fischer $\times$ Lewis)F_1 hybrids for another study using discarded Fischer females that had been sensitized against Lewis antigens as breeders, a high mortality was noted among the progeny, many being affected by a wasting syndrome. Unfortunately, specific records were not kept. When evidence of maternally acquired runting was forthcoming from other experiments, the influence of mating Fischer females, presensitized against Lewis antigens, with Lewis males was therefore investigated on a controlled basis. Only a few of the progeny (8%) succumbed to runt disease (see Table III, experiment 4 a). This apparent variability in result may be due to the influence of the variable but undefined environmental factor(s) invoked by Palm (26) to account for seasonal variation in her findings.

If, as we have argued, the altered immunological reactivity of the progeny observed in many of the present experiments described is ascribable to the transmission of alien cells from maternal to fetal circulations, then three important questions remain unanswered: (a) Why are cells that are alien to the mothers better able to gain access to the fetuses than a mother's own cells in a normal pregnancy? (b) Why do sensitized lymphoid cells appear to be capable of crossing the placenta more readily than unsensitized cells? and (c) What natural mechanisms protect fetuses in most experimental and natural genetic contexts from large-scale and potentially harmful infiltration by maternal cells?

SUMMARY

A previous report that the offspring of outbred Sprague-Dawley rats, born of mothers presensitized or tolerant with respect to tissue antigens of the Lewis strain, and reinoculated with Lewis cells during their pregnancy, reject test grafts of Lewis skin in an accelerated manner has been confirmed. This "maternally induced" alteration in reactivity of the progeny has been found to be long lasting, immunologically specific, and probably not due to transfer of humoral antibody. It has been established that the reexposure of the mothers to donor cellular antigen during pregnancy augmented the influence of the prior states of tolerance or sensitivity.

To obviate the complications inherent in working with the outbred Sprague-Dawley rats, the key experiments summarized above were repeated with isogenic Fischer rats as parents and Lewis rats as the tissue donors as before. With this combination it was found that a state of prior sensitization or tolerance in the mothers resulted in the apparent induction of tolerance in some of their progeny. Reinoculation of either the tolerant or sensitized mothers during pregnancy slightly increased the incidence and degree of impairment of

their offsprings' capacity to reject Lewis skin grafts. A single intraperitoneal injection of 100 × 10^6 million Lewis lymphoid cells into normal Fischer rats in the 14th–16th day of pregnancy also weakened the reactivity of their progeny to Lewis test grafts.

Further to test the premise that this weakened reactivity might be due to maternal induction of tolerance, by antenatal transmission of alien cells, the lymphohematopoietic tissue system of adult Fischer females was replaced by that from Lewis donors with the aid of cyclophosphamide. It was anticipated that when these animals were mated with Fischer males, sufficient Lewis leukocytes might cross the placentas to induce high degrees of tolerance. Although normal sized healthy litters were born, about 50% of the infants succumbed to graft-*versus*-host (GVH) or runt disease within 40 days, many of them giving evidence of being tolerant of Lewis grafts. The mothers, too, developed chronic GVH disease. The offspring of Fischer females made chimeric with cells from (Fischer × Lewis)F_1 hybrid donors, as well as their mothers, remained healthy.

Intraperitoneal injection of normal Fischer females, in the 15th–17th day of pregnancy, with 100 million lymphoid cells from specifically sensitized Lewis rats, also caused fatal runt disease to develop in about 50% of their offspring, but left the mothers unscathed.

Taken together, these various findings indicate that in some genetic contexts at least the extent of the natural surreptitious transplacental cellular traffic can be considerably augmented experimentally, though how this comes about and why lymphocytic cells that are foreign to the mother can apparently gain access to fetuses more readily than her own cells remain to be determined.

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