Maternally Acquired Runt Disease

Immune lymphocytes from the maternal blood can traverse the placenta and cause runt disease in the progeny.

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In mammalian gestation the interaction of maternal immunocompetent cells with genetically alien fetal cells may occur (i) where maternal and fetal tissues confront one another at the chorio-decidual junction; (ii) in the maternal lymph nodes that drain the uterus and thus receive the leukocytic, erythrocytic, and trophoblastic cells of the fetus; and (iii) within the fetus, as a result of the incidental passage of maternal immunocytes across the placenta (*1–3*).

All previous attempts to demonstrate the vulnerability at implantation of histoincompatible blastocysts or of established embryos, to a specific state of hypersensitivity in the mother have failed (*1, 4*). Naturally implanted placental "grafts" with their dependent fetuses have proved resistant to experimentally induced transplantation immunity, although such immunity is certainly expressed with undiminished vigor in the endometrium (*2*). These empiric observations are consistent with evidence that the trophoblast, as a consequence of the unique surface properties of its cells (*5*), is an ineffective elicitor of the cellular component of transplantation immunity, is highly resistant to its forces, and is afforded some protection by "blocking antibodies" or by complexes of these antibodies with fetal antigenic material (*6*).

Transmission of cells from the mother to the fetus as a consequence of placental "porosity" is common in man (*3*) but apparently has not been observed in experimental animals (*1, 7*). Two possible consequences of the natural exchange of cells between the mother and her fetus during her pregnancy are: (i) the induction in the offspring of tolerance of maternal tissue antigens (*8*) and (ii) initiation of runt or graft-versus-host disease, an often fatal wasting syndrome that results from reactivity of maternal immunocytes against paternally inherited antigens of the immunologically immature fetus (*9, 10*). These two possibilities are not mutually exclusive. Attempts to obtain evidence of maternally induced tolerance of skin grafts in several species have been unsuccessful (*1, 11*). However, this lack of success may be ascribed in part to the fact that tolerance and chimerism with respect to alien lymphoid cells is not necessarily associated with tolerance of skin homografts (*12*). Careful clinical examination of infants failing to thrive has produced some excellent examples of runt disease (*13*).

Hitherto, in experimental animals runt disease has not been observed to afflict progeny of mothers specifically sensitized against the tissue antigens of the parental strain prior to mating. Recently, however, we reported that 57 percent of the offspring of female Fischer rats mated with males of the Fischer strain developed and succumbed to runt disease if their mothers' lymphohematopoietic tissue had been replaced by similar tissue of Lewis strain origin by treatment with cyclophosphamide and a bone marrow graft. Intraperitoneal injection of normal Fischer females 15 to 17 days pregnant by Fischer males, with lymphoid cells from specifically sensitized Lewis donors, likewise caused runt disease to develop in the progeny but left the mothers unscathed (*10*).

The experiments we report in this article represent a preliminary analysis of these unexpected observations and indicate the facility with which the placental barrier can be breached by harmful immunocytes from the maternal circulation in rats, mice, hamsters, guinea pigs, and rabbits. The results also provide indirect evidence of long persistence of allogeneic lymphoid cellular grafts transfused into pregnant females.

Adoptive Immunization with Allogeneic Lymphoid Cells

We found previously that there was no mortality beyond the fifth day of life among the Fischer offspring of a large number of normal, untreated Fischer mothers or of Fischer mothers treated with cyclophosphamide and whose lymphohematopoietic tissues had been reconstituted with bone marrow from (Fischer × Lewis)F_1 hybrid donors. However, if the expectant mothers were inoculated intraperitoneally with 100×10^6 lymphoid cells from allogeneic Lewis donors, previously sensitized against Fischer antigens (by means of a skin graft followed by an intraperitoneal injection of 100×10^6 splenic cells) 51 percent of their progeny developed fatal runt disease within 45 days of birth (*10*). We suggested that if this disease is indeed caused by graft-versus-host reactivity, its incidence should display some dependency upon the number of cells transferred, and be incitable only by transfer of "immune" cells genetically capable of attacking the fetuses. These predictions proved correct; we observed that transfer to gravid Fischer females that had been mated with Fischer males of (i) 200×10^6 lymphoid cells from Lewis rats specifically sensitized against Fischer antigens raised the incidence of fatal runt disease among the progeny to 91 percent, and (ii) 200×10^6 lymphoid cells from syngeneic, that is, Fischer, donors previously sensitized against the tissue antigens of an unrelated donor strain, DA, were completely innocuous to the offspring (Table 1, experiments 1 and 2). The same phenomenon occurred with other rat strain combinations as shown by the results of experiments 3 and 4 (Table 1).

To determine whether the transfer of sensitized populations of allogeneic lymphoid cells was mandatory for the development of runt disease, lymphoid cells from normal Lewis donors were transferred to pregnant Fischer mothers carrying Fischer fetuses, and from normal Fischer donors into DA mothers

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with DA fetuses (Table 1, experiments 5 and 6). In experiment 5, where donors and hosts were compatible at the major histocompatibility locus, that is, the *Ag-B* locus, the incidence of the disease was negligible, but in experiment 6, where histoincompatibility at the *Ag-B* locus was involved, the incidence was 19 percent (*14*). Both of these results indicated the superior runt-inducing capacity of sensitized cells.

There were two possible explanations for the observed disparity in immunopathogenic potential between normal and "immune" transferred allogeneic lymphoid cells. Either immunologically activated cells might cross the placenta with greater facility than nonactivated cells, or immunologically activated cells might include a higher proportion of cells specifically capable of interacting with the transplantation antigens of the fetuses than an equivalent inoculum from a normal donor. We therefore attempted to discriminate between these possibilities. In experiment 7 (Table 1) lymphoid cells from Lewis donors which had previously been inoculated with complete Freund's adjuvant (CFA) as a nonspecific stimulator of immune reactivity were injected intraperitoneally into Fischer females bearing Fischer fetuses. The results (a 23 percent incidence of runt disease compared with 2 percent among the progeny of maternal recipients of cells from normal Lewis donors) although not decisive, suggested that sensitized cells are able to cross the placenta more easily than normal cells.

In the experiments described above, gravid female recipients must have transmitted to their fetuses alien lymphoid cells which the hosts, themselves, were capable of rejecting very rapidly [as inferred from the fate of skin grafts of similar genetic origin transplanted to such animals (*15*)]. Indeed the long-term, healthy status of these females, none of which was affected by clinically demonstrable graft-versus-host disease, must have depended upon their ability to destroy or otherwise inactivate the potentially harmful cells. In many experiments, females which had received "immune" allogeneic lymphocytes during pregnancy were remated after their first litters had been ravaged by runt disease. Contrary to expectation, although no further lymphocytes were inoculated, in the experiment in which Lewis cells sensitized against Fischer antigens were transferred, 5 percent of the members of second litters developed the disease. When Fischer cells sensitized against DA antigens were transferred, 55 percent of the members of second litters succumbed (see Table 1, experiments 2 and 4)—that is, a higher percentage of mortality than that which afflicted the first litters. With the same transfer of

Table 1. Adoptive immunization of female rats against their syngeneic fetuses by transfer of allogeneic lymphoid cells. The lymphoid cells, 200×10^6 cells in each experiment, were injected intraperitoneally to the pregnant females 2 to 6 days before parturition.

Exp. No.	Maternal strain	Cells transferred	No. of offspring alive (days after birth)				Cumulative mortality among offspring after day 5 (%)	
			0	5	20	45	First litter	Second litter
1	Fischer	Fischer cells sensitized against DA antigens*	47	47	47	47	0	
2	Fischer	Lewis cells sensitized against Fischer antigens	46	46	5	4	91	5
3	Lewis	Fischer cells sensitized against Lewis antigens	27	21	14	7	75	
4	DA	Fischer cells sensitized against DA antigens	67	67	45	33	51	55
5	Fischer	Lewis cells from normal donors*	45	45	44	44	2	
6	DA	Fischer cells from normal donors*	42	40	34	34	19	
7	Fischer	Lewis cells from donor sensitized to CFA†	35	35	33	27	23	

* Control experiments. † Complete Freund's adjuvant.

Table 2. Adoptive and active immunization of female Fischer rats against their alien F_1 fetuses. The lymphoid (node) cells were injected intraperitoneally 2 to 6 days before parturition.

Exp. No.	Cells transferred to mothers	Fetuses at risk	No. of offspring alive (days after birth)				Cumulative mortality among offspring after day 5 (%)	
			0	5	20	45	First litter	Second litter
1	200×10^6 Fischer cells sensitized against Lewis antigens	(Fischer × Lewis) F_1	37	29	23	19	50	
2	100×10^6 Fischer cells sensitized against DA antigens	(Fischer × DA) F_1	39	36	32	28	28	
3	200×10^6 Fischer cells sensitized against DA antigens	(Fischer × DA) F_1	37	37	2	1	97	4
4	DA skin graft (days before birth)							
	9 to 13		45	44	42	37	18	
	20		57	56	32	30	47	
	27 to 34		50	50	4	3	94	3
	42		25	25	25	25	0	
5	DA skin graft and 40×10^6 DA lymphoid cells 27 to 34 days before birth	(Fischer × DA) F_1	35	32	32	32	9	9
6	DA skin graft 21 to 34 days prior to birth of second litter		31	31	31	31	0	

Fischer cells sensitized against DA antigens, 43 percent of third litters were afflicted but all members of the fourth and fifth litters survived.

These findings suggest that allogeneic lymphocytes are able to survive after transfer to some normal, unsensitized, pregnant female hosts for several weeks, regardless of their compatibility with the host cells at the *Ag-B* locus.

Adoptive Immunization by Syngeneic Cells

The design of the following experiments was that of a conventional adoptive transfer of sensitivity (Table 2, experiments 1 to 3). As a consequence of the inoculation of 200×10^6 lymphoid cells from Fischer donors sensitized against Lewis antigens into Fischer females bearing (Fischer $\times$ Lewis)F_1 hybrid fetuses, 50 percent of the progeny developed lethal runt disease. Likewise, when (Fischer $\times$ DA)F_1 fetuses gestated by Fischer females served as potential immunological targets, adoptive transfer of 100×10^6 lymphoid cells from Fischer donors sensitized against DA antigens resulted in a 28 percent incidence of runt disease and doubling the number of cells transferred raised this mortality to 97 percent. However, when females, which as a consequence of adoptive immunization with syngeneic cells had produced first hybrid litters that were ravaged by runt disease, were remated to produce more hybrids, few of the latter were affected by the disease.

Active Immunization

Our ability to transfer immunity to primiparous females against their F_1 hybrid fetuses encouraged us to investigate the further possibility of achieving the same results by actively immunizing female rats before they were mated. Accordingly, Fischer females mated with DA males received a single, orthotopic DA skin homograft, 1.5 centimeters in diameter, 7 to 14 days before conception (Table 2, experiment 4). This resulted in 94 percent of their offspring developing runt disease. To determine whether the timing of the sensitization of the females was an important factor, several groups of Fischer females were grafted with DA skin at various time intervals prior to or after mating. The results indicate that the most efficient way to cause runt disease among the progeny is to graft the skin a week before the females are mated so that maximum cellular immunity probably coincides with implantation. Skin grafted 22 days before conception proved ineffective in causing runt disease among the progeny.

When females which had been actively immunized at the optimum time to induce runt disease in their first litters were remated with males of the original paternal strain, the incidence of the disease among their second litters was negligible. One interpretation of the exemption from runt disease of offspring of these second litters, following adoptive or active immunization of their mothers, or the exemption of first litters born of mothers grafted with skin 20 days or more before conception, is that they were protected by "blocking" or "enhancing" isoantibodies (*6*) synthesized by the mothers. Such antibodies may have impaired the magnitude of the transplacental cellular traffic or may have competed with the cellular mediators of transplantation immunity for antigenic determinants on vulnerable target cells in the fetus because such isoantibodies can cross the placenta (*16*). Homologous lymphoid cells that are administered parenterally induce humoral immunity more effectively than orthotopic skin homografts. Thus it is not surprising that (i) when Fischer females bearing (Fischer $\times$ DA)F_1 fetuses received, in addition to a DA skin homograft at the optimum time prior to gestation, an intraperitoneal injection of 40×10^6 DA lymphoid cells, the incidence of lethal runt disease among the progeny was reduced to 9 percent (Table 2, experiment 5); and (ii) when Fischer mothers that had given birth to one litter of (Fischer $\times$ DA)F_1 progeny were grafted with DA skin 21 to 34 days prior to the birth of their second (Fischer $\times$ DA)F_1 hybrid litter, none of the second litter was affected by the disease (Table 2, experiment 6).

Maternally Induced Runting in Other Species

We have induced a greater than 50 percent incidence of runt disease in four other species. (i) In mice, by adoptive immunization of C57BL/6 females against their (C57BL/6 $\times$ A)F_1 fetuses with syngeneic cells, and of A strain females against their syngeneic fetuses by transfer of allogeneic C57BL/6 lymphoid cells sensitized against A strain antigens. (ii) In hamsters, by both adoptive and active immunization of MHA strain females against their (MHA $\times$ CB)F_1 fetuses. (iii) In guinea pigs, by active immunization of strain 13 mothers against their (13 $\times$ 2)F_1 fetuses. (iv) In rabbits, by grafting outbred females with small skin grafts from males with which they had recently mated. In all these species, as in rats, the timing of the immunization of the females in relation to conception is important.

Conclusions

Without altering the structural integrity of the placenta by irradiation or drugs, we have shown that it is possible to immunize females both adoptively and actively against the paternally inherited transplantation antigens of their fetuses. Such immunization causes a high incidence of runt disease among the litters. Although the putative chimeric status of the affected offspring has yet to be confirmed, the results of our experiments support the thesis that runt disease is caused by the activities of "unwanted" immigrant lymphocytes from the maternal circulation. Our results suggest that immunologically activated cells are more likely to cross the placenta than normal cells and that this greater mobility may not be related to the immunologic specificity of the activated cells.

Two factors may have contributed to the apparent failure of numerous previous attempts to demonstrate the capacity of transplantation immunity to affect the well-being of a fetus or, more correctly, its placenta, in the way that might be expected of a homograft. (i) Investigators were preoccupied with obtaining a classic type of rejection, in utero, analogous to the rejection of an orthotopic skin homograft. The birth of consistently healthy-looking litters, interpreted as a failure of the experiment, convinced the investigators of the efficacy of nature's solution of the homograft problem and there was no reason for them to suspect its possible limitations. Observation of the litters for several weeks might have uncovered the phenomenon of maternally induced runt disease. (ii) Most investigators resorted to hyperimmunization of the mothers. This would have facilitated the synthesis of protective isoantibodies capable of interfering with the expression of the potentially harmful cellular immune response (*6*).

Ever since the abnormalities of runt disease were first described they have repeatedly been compared to those observed in patients with certain lymphomas (*17*). Various theories have been propounded as to how maternally transmitted graft-versus-host reactivity might lead to the development of these tumors. In mice it has been established that graft-versus-host reactivity may result in a high incidence of lymphomas (*18*). Recent analysis indicates that this graft-versus-host reactivity unmasks and activates normally latent and undemonstrable oncogenic viruses (*19*). The work we describe in this article may have some relevance to the possible clinical significance of transplacental cellular mobility in man. We suggest that the relatively high incidence of lymphomas in children might also be, in part at least, due to unmasking of oncogenic viruses by subclinical graft-versus-host reactivity mediated by immunocompetent cells of maternal origin. The statistical evidence that male infants are at greater risk than females (*20*) is concordant with our observation that maternally induced runts include a significantly higher proportion of males than females (*10*).

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Gene Flow and Population Differentiation

Studies of clines suggest that differentiation along environmental gradients may be independent of gene flow.

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Since the time of Darwin and Wallace there has been considerable interest in how species come to be different in different parts of their geographic ranges. Geographic isolation and spatial differences in environmental factors are thought to lead to the observed geographic differentiation within species, and may finally lead to speciation, when sexual and geographic isolation become complete (*1–3*). Differentiation into species is usually assumed to be impossible without barriers because gene flow is supposed to "swamp out" any differences evolved in response to local environmental factors (*1–9*).

Ehrlich and Raven (*10*), and the proponents of sympatric speciation (*11*) take exception to the view of the dedifferentiating effect of gene flow, and recent experiments (*12, 13*) and theoretical studies (*14, 15*) suggest that gene flow may not have as great an effect as has been postulated. The possibility of parapatric divergence is less commonly discussed (*1, 3, 16, 17*) and is usually assumed to have the same problems that are inherent in sympatric speciation, in particular the difficulty of accounting for the evolution of sexual isolation in the face of considerable gene flow (*1*). The crucial question is how much does gene flow actually retard the development of geographic differentiation within a species (*2*). In this article I present experimental and theoretical evidence suggesting that the effect of gene flow may be small.

Huxley (*17*) defined a cline as a gradient in a measurable character. Relative to the dispersal rate of a species, the slope of a cline between regions is indicative of the extent to which the inhabitants have differentiated. A steep cline means sharp differentiation, as in the pelage colors of the deermouse, *Peromyscus maniculatus* (*18*), and gentle clines mean indistinct divergence between areas, as in the plumages of many duck species (*19*). The basis of subspeciation and speciation is geographic variation in gene frequencies. For a polymorphic character (*20*) a cline is a temporally stable gradient of geotype or gene frequencies.

In spite of the number of clines that have been described (*1, 17, 21, 22*), there is a dearth of natural systems for which all the necessary ecological information has been recorded for each morph along a cline. Therefore I chose to study a model system that could be investigated both experimentally and theoretically. I discuss here a hypotheti-

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