COMMUNICATIONS IN BRIEF

Further studies on the mechanism of pregnancy-induced isoimmunization in mice*

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ABDOMINAL delivery in mice markedly increases the frequency and intensity of maternal isoimmunization to paternal H-2 antigens. We have previously shown that "uterine spillage" associated with such deliveries is sufficient to explain this immunization response. There also appeared to be a positive correlation between fertility and anti H-2 postpartum titers in this group of mice.[3] Because of the analogy between H-2 in mice and Rh in humans (both are erythrocyte antigens determined at a complex genetic locus with a series of cross-reacting alleles and antibodies to both are induced by incompatible pregnancies), study of pregnancy induced H-2 isoimmunization in mice has been continued.

Najarian and Dixon[7] have noted that maternal administration of hyaluronidase in rabbits doubles the number of maternal red cells that normally cross the placenta. Reported here are further studies of the effects of abdominal delivery on isoimmunization and fertility, of the provocative effect of amniotic fluid injections on H-2 titers of pregnant and nonpregnant females and of hyaluronidase administration effects in pregnancy-induced isoimmunizations.

Abdominal delivery. Thirty-six 6-week-old C57B1/6J (H-2b) females were mated to DBA/2J (H-2d) males. Pregnancies were terminated on gestation days 17 and 18 as previously described.

Amniotic fluid injections. Amniotic fluid (H-2b/H-2d) was obtained from C57B1 females mated to DBA males, gestation days 14 to 16. All of the amniotic fluid from one female was injected intraperitoneally into C57B1 (H-2b) females mated to C57B1 males (experimental group) within one or 2 days after the appearance of a vaginal plug. Similar H-2b/H-2d amniotic fluid was also injected into a group of C57B1 nonpregnant females (control group). H-2d titers were performed as previously described at 3 or 4 weeks (third and tenth postpartum days) following amniotic fluid injections.

Hyaluronidase. Hyaluronidase in doses varying between 3.75 and 15 international turbidity units was injected intraperitoneally on gestation day 10 to 12 into 31 C57B1 females mated to DBA males. Thirty-eight similarly mated females served as controls. Titers were recorded 3 and 10 days postpartum over the course of three pregnancies.

Cesarean section. There was no difference in subsequent fertility between those females with positive postpartum anti H-2 titers and those females with negative titers. Primipara mice with positive postpartum anti H-2 titers subsequently delivered an average of 10.8 offspring as compared with 12.1 for the primipara with negative titers, a nonsignificant difference.

Amniotic fluid injections. In the experimental group (true pregnant females) only 2 out of 26 responded to injection of H-2b/H-2d amniotic fluid with positive H-2d titers whereas in 35

Table I. H-2d antibody titers in C57B1 females 3 and 4 weeks after H-2d/H-2b amniotic fluid injections

Group	No. of mice	Mice with positive titers*	
		No.	%
Controls (nonpregnant)	35	12	34
Experimental			
A) False pregnancy	23	3	13
B) True pregnancy	26	2	8

*A positive titer represents clumping in at least the fourth dilution tube, in the H-2 hemagglutination assay as previously described.

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controls (nonpregnant females), 13 responded with titers, a significant difference at the 0.05 level (Table I). False pregnant females, i.e., those originally considered to be pregnant because of vaginal plug findings but subsequently never delivering any recognizable fetal tissue, also showed fewer positive H-2d titers than the controls, P = 0.10.

Hyaluronidase. Twelve of our 38 noninjected females demonstrated positive H-2d titers on at least one occasion during three pregnancies, while of 31 females receiving varying amounts of hyaluronidase during pregnancy, 17 had anti-H-2d titers. This difference is not significant (p = 0.20).

The subject of transplantation and pregnancy has been reviewed recently by Billingham.[2] Data have been presented from various species suggestive of altered maternal response to skin grafting, but no clear concept is available of the responsible mechanisms.[2] Andersen and associates[1] have evidence that in the rabbit the placental site plays a role in inhibiting a general immune response.[4] Simmons and Russell have suggested that in mice, the trophoblast forms an immunologic buffer between mother and fetus.[8] Kirby[4] has demonstrated that, for the mouse at least, trophoblast cells are not recognized as "foreign" by hosts because of the surrounding fibrinoid layers. Mitchell,[6] in discussing various hypotheses for the success of the fetus and placenta as homografts, suggests that pregnancy itself reduces the immunologic capability of the female. While Medawar[5] has demonstrated that this is unlikely in mice, because of our previous findings of increased anti H-2 response with abdominal deliveries, it appeared worthwhile to test whether isologous amniotic fluid would elicit the same H-2 response in the pregnant as the nonpregnant animal.

Because of the short gestational period in mice and the possible pregnancy associated changes in blood volume and antibody catabolism, conclusions concerning the immune response based solely on differences in serum antibody titers in pregnant mice are hazardous. As with Rh isoimmunization, pregnancy related H-2 isoimmunization is a variable phenomenon and, in our experience, at times unpredictable. Whether "false pregnancies" represent true pregnancies with early absorption of fetuses, perhaps related to the trauma of injection, is unknown. The inclusion of the "false pregnancy" group with the experimental group would still give results of borderline significance (p = 0.03) as regards the question whether pregnancy reduces the antibody response of female mice to isologous fetal cells (amniotic fluid debris).

If maternal H-2 isoimmunization is related to passage of fetal antigens to the mother, hyaluronidase therapy might well increase the incidence of such sensitization because of reduced placental barrier permeability. Najarian and Dixon[7] have demonstrated that pregnant rabbits given hyaluronidase have decreased ability to reject grafts of their offspring. A similar altered pregnancy H-2 titer response was not found in the present study in females given hyaluronidase.

The present study failed to substantiate our previous suggestion that there was a correlation in abdominally delivered multiparous mice between fertility and the presence of postpartum H-2 antibodies. The surgical mortality rate was one third of that of the earlier study, suggesting that the previous observations were related to the general health of the females and not to any ability to respond to a specific antigen.

These data suggest that the immunologic response may be reduced during pregnancy and that hyaluronidase does not increase the incidence of pregnancy H-2 isoimmunization in mice.

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Reconstruction of deficient anal sphincter in cases of old complete perineal tear

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In cases of old complete perineal tears, the torn sphincter and muscle tends to retract,