Isoimmunization associated with cesarean section in the mouse

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MATERNAL isoimmunization associated with pregnancy in the mouse has been a subject of increased interest, partly because of its analogy to pregnancy isoimmunization in the human.[1-3] We have previously shown that outcrossed female mice develop antibodies which react with paternal antigens carried by the offspring.[3] Reported here is the further observation that delivery by cesarean section markedly increases the intensity and frequency of maternal isoimmunization in the mouse.

Method

Fifty-four 6-week-old C57Bl/6J (H-2b antigen) females were mated to DBA/2J (H-2d antigen) males of similar age; 2 females were caged with 1 male. Two series of animals were observed, 1 group for 13 months, the other for 7. The normal length of gestation is 18 to 20 days for these strains of mice.

Pregnancy was terminated on days 17 or 18 by cesarean section as follows: a small uterine incision was made over the rump of each fetus through which the intact amniotic sac and its contents were delivered. The procedure was eventually simplified to the extent that no effort was made to achieve asepsis, uterine closure, or hemostasis. The peritoneum and abdominal wall incisions were closed with a single layer of No. 2-0 silk.

Anti H-2d hemagglutination titers were determined as previously described[1] during the first and second postpartum weeks and at periodic intervals thereafter. Sera with positive titers were re-examined with 1:1500 mercaptoethanol added to the developing agent (polyvinylpyrrolidone).

The control group of animals, which were delivered vaginally, consisted of 60 C57Bl/6J females caged as above with DBA/2J males. As soon as an animal was delivered, the newborn mice were removed from the cage. Anti H-2d titers were done on the third and tenth postpartum days and at periodic intervals thereafter.

To collect the equivalent of the amniotic fluid and debris that normally would be spilled into the peritoneal cavity during a cesarean section, the uterus was suspended over a petri dish before the incision was made. The spillage was aspirated into a 1 ml. syringe through a No. 25 needle, every effort being made to remove any visible solid material. All the "spillage" from 1 animal was injected intraperitoneally into a single virgin female of the same strain (C57Bl). H-2 titers in these virgin females were determined 2 weeks after the injection.

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Results

A total of 46 female mice had at least one cesarean section. With increasing numbers of abdominal deliveries, the frequency of positive titers markedly increased (Table I). Only 4 of 46 were positive after one cesarean section, whereas all 5 animals that had had four cesarean sections had positive postpartum titers.

As shown in Table II, there is an apparent correlation between postcesarean section fertility and immunization, those animals that display positive postpartum titers being more likely to have future deliveries than those that have negative titers. Whether this correlation is caused by an effect of maternal immunization upon fertility, or simply represents two parameters of the surgical morbidity and complications rates, remains to be seen.

Since high anti H-2 titers are classically induced in the mouse by the intraperitoneal injection of cells and cellular debris, the effect of "spillage" of amniotic fluid (and other debris associated with the products of conception) into the peritoneal cavity during abdominal delivery of offspring was tested. Data in Table III indicate that 3 of 5 virgin females receiving intraperitoneal injections of such fluid became immunized after a single injection. Amniotic fluid, which in the mouse occasionally contains passively transferred maternal H-2 antibodies, also was found to be able to evoke H-2 antibodies.

Postpartum anti H-2d titers after vaginal delivery ranged from 1:80 to 1:640 and with mercaptoethanol the highest titers were only 1:20. Postcesarean section titers ranged from 1:2560 to 1:10,240 and with mercaptoethanol from 1:1280 to 1:5120. Whereas the control animals demonstrated weak titers which were not detectable in the presence of mercaptoethanol, indicating these antibodies to be mainly macroglobulin, "19S" antibody, the abdominal-delivery group had high titers which were stable to mercaptoethanol, suggesting that the antibody response was caused by the low molecular weight "7S" gamma globulin. In this laboratory, mouse H-2 antibody response to spleen injection is likewise the latter (low molecular weight) type of antibody.

Table I. Postpartum anti H-2d titers 1 to 2 weeks post partum

No. of cesarean sections per animal	*No. of animals*	*% of animals with postpartum anti H-2d titers*
1	46	9
2	18	44
3	8	87
4	5	100

Table II. Fertility according to postpartum titers in animals delivered by cesarean section

Postpartum titers	*No. of animals*	*No. of animals with subsequent pregnancies*
Negative	53	17 (32%)
Positive	19	14 (73%)

Table III. Titers 2 weeks after injection of various substances into peritoneal cavity

Material	*No. of animals injected*	*No. with H-2d titer*
Amniotic fluid (H2b/H2d)	17	10
Debris from cesarean section (H2b/H2d)	6	3
DBA spleen (H2d/H2d)	40	10

Comments

It has been shown previously that postpartum anti H-2 antibodies associated with vaginal delivery of F_1 offspring of C57Bl/6J females mated to DBA/2J male mice are weak and sporadic in appearance.[3] Data presented here demonstrate that abdominal delivery of offspring from the same cross markedly increases the intensity and frequency of maternal isoimmunization.

The latter observation may be explained by the demonstration that uterine spillage associated with cesarean section is sufficient alone to cause strong immunization against

H-2 antigen in the mouse. Indeed, the response to a single challenge with uterine spillage is comparable to that observed after several injections of spleen at doses used to hyperimmunize animals.

Little information is available which would suggest that cesarean section in the human produces an immunization comparable to that in the mouse. The H-2 is perhaps the strongest mouse histocompatibility antigen known. It is found on most mouse tissue, and antisera directed against it can agglutinate both erythrocytes and leukocytes. Whether a comparable antigen exists in man is not known. Human leukocyte agglutinins (which may be analogous to the mouse H-2 isoagglutinins) have been shown to rise as a result of pregnancy in the human, but the relationship of cesarean section has not been studied.

Comparisons between H-2 and Rh have sometimes been made, as both are determined at a complex genetic locus, with a series of cross-reacting alleles; antibodies to both are induced by outcrossed pregnancies. However, while demonstrating that serum antibodies pass into the fetuses of H-2 immunized female mice, we have been unable to show any evidence of erythroblastosis fetalis as it occurs in the human, even in mothers hyperimmune to H-2, which limits the usefulness of an analogy of Rh to H-2 in this system.

Aside from the possible effects of abdominal delivery upon immunity, the whole question of why the uterine spillage or amniotic fluid is a strong antigen in the peritoneal cavity and weak in the uterine cavity is obviously of great interest.

Summary and conclusions

1. Anti H-2 hemagglutination titers were determined in C57Bl/6J mice delivered by cesarean section which had been mated to DBA/2J males.

2. Uterine fluid was collected at the time of cesarean section and injected into virgin females of the same strain.

3. Abdominal delivery increased the intensity and frequency of pregnancy-induced antibodies to paternal type H-2 antigens.

4. The "uterine spillage" associated with abdominal delivery is alone sufficient to explain the strong immunization noted against H-2 antigen in these animals.

5. The virgin females injected with uterine spillage or amniotic fluid developed strong immunization against the H-2 antigen.

6. There is an apparent association between postcesarean section fertility and the presence of postpartum antibodies. The nature of this association is unclear.

REFERENCES

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