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REVIEW

# Immunology of abortion

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## CAUSAL FACTORS IN ABORTION

Spontaneous abortion is the commonest complication of pregnancy (Stirrat, 1983). The percentage of pregnancies which terminate in spontaneous abortion is unclear. Roth (1963) calculated that 15% of diagnosed pregnancies resulted in abortion, while Miller *et al.* (1980) reported 43% and Edmonds *et al.* (1982) suggested as many as 60% of conceptuses are lost before 16 weeks gestation. There is evidence that abortion is a more common outcome of first (18%) than later pregnancies (Glass & Colbus, 1978).

Several reasons have been given for the failure of these pregnancies. Many of the very early losses may be due to genetic abnormalities (Miller *et al.*, 1980). The diagnosis of genetic abnormalities is not easy as only about 10–20% of products of abortion are suitable for study of chromosomal abnormalities. In a study of 360 abortions Ornoy *et al.* (1981) were only able to attempt culture in 95 cases and of these only 65 were successfully karyotyped. Of those karyotyped 41·5% exhibited chromosomal aberrations, or 10% of all the abortions. Other figures suggest that from 6–8% of all spontaneous abortions are due to genetic causes (Kardon *et al.*, 1981).

Other possible causes of abortion may be infections, anatomical or hormonal abnormalities. These probably account for only a small fraction of the total, the commonest cause of abortion is probably immunological. Johnson *et al.* (1985) suggests that the majority of abortions are due to the failure of the woman to make a recognition response to the pregnancy; thus she fails to circumvent allograft rejection and the fetus does not survive. Recurrent spontaneous abortion (RSA), that is three or more abortions, is even more likely to be immunological, as some of the infectious causes of abortion, such as toxoplasmosis, only cause abortion during the phase of active infection and subsequent pregnancies in immune women are unaffected.

## IMMUNOLOGICALLY-MEDIATED ABORTION

Taylor & Faulk (1981) have used four observations to support the idea that an ill defined immunological process is the common cause of recurrent abortion. They are as follows. (1) Aborting couples do not produce the inhibitors of cell-mediated immunity found in maternal blood during pregnancy. (2) Chronic aborting couples share more HLA antigens at the A and B loci than would be expected by chance. (3) Studies have revealed the absence of classical transplantation antigens and the presence of other alloantigens on human trophoblast, which could form a basis for immune reaction between mother and fetus. (4) Some trophoblast allotypic determinants are shared with lymphocytes and coded for in the HLA region. If HLA antigens are shared between husband and wife then it would be likely that trophoblast specific alloantigens (TLX antigens) were shared between blastocyst and mother. Thus, the TLX compatible embryo might not stimulate maternal protecting or blocking factors and the blastocyst would be rejected as are other tissues bearing foreign antigens implanted in the uterus (Beer, Billingham & Yang, 1972).

These ideas are somewhat controversial though experimental evidence exists supporting some of them. They are at variance with hypothesis of Medawar (1954). Medawar suggested that the

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reasons why the fetus was not rejected as a foreign graft were as follows. (1) The conceptus is not immunogenic and therefore does not evoke an immunological response. (2) Pregnancy altered the maternal immunological responses. (3) The uterus is an immunologically privileged site. (4) The placenta was an effective immunological barrier between the mother and the, as yet, immunologically incompetent fetus.

Since this hypothesis was proposed 30 years ago, an enormous amount of knowledge has accumulated, and many workers have shown that the conceptus is immunogenic and that it does evoke a response from the mother (Kirby, 1969; Raghupathy & Talwar, 1983). It is an alteration of this response that causes abortion (Johnson *et al.*, 1985). Several groups have shown that it is normal for the mother to make an immunological response to the fetus (Tongio, Berrebi & Mayer, 1972; Mowbray *et al.*, 1984; Beer & Billingham, 1977) and that the female, when pregnant, can respond to a wide variety of immunological stimuli (Gill & Repetti, 1979).

Beer & Billingham (1974) demonstrated that the antigens in the uterus would elicit an immune response. They placed skin grafts on the inter-uterine wall of the bicornuate murine uterus and injected leucocytes into the uterine wall. In both experiments they showed that the host was sensitized and would rapidly reject a subsequent skin graft. Thus the uterus is not a privileged site (Croy, Rossant & Clark, 1981; Beer & Billingham, 1974) and it is the trophoblast that appears to give immunological protection. This has been very elegantly demonstrated by the work of Croy *et al.* (1981) on the specific role of the trophoblast in the protection of the fetus. In experiments where the inner cell mass was replaced by a xenogeneic cell mass and the trophoblast was syngeneic with the mother, such an interspecies chimaera will be developed to parturition without evidence of rejection. In contrast, if the trophoblast is xenogeneic and the inner cell mass syngeneic the embryo is rapidly rejected (Croy *et al.*, 1981; Rossant, Mauro & Croy, 1982).

## ALLOANTIGENS INVOLVED IN RSA

Evidence from a number of authors suggests that MHC and ABO systems provide the genetic basis of some recurrent abortions (Szulman, 1973; Gill & Repetti, 1979). Komlos (1977) reports a correlation between class I HLA sharing and the incidence of abortion. Taylor & Faulk (1981) and Beer *et al.* (1981) also suggest that sharing of HLA antigens between couples predisposes them to recurrent abortion. From animal studies it seems that a species may need to possess an extreme degree of genetic polymorphism or genetic diversity of the MHC system for successful reproduction. Some rodent lines demonstrate an 80% loss when inbreeding takes place (Gill, 1983), and some species cannot be inbred at all, suggesting that a degree of heterozygosity is necessary for successful reproduction (Gill & Repetti, 1979). These studies would seem to demonstrate that there is intense selection pressure operating during gestation against individuals that are homozygous with their mother with regard to the MHC system. There may be decreased reproductive efficiency when MHC homozygous young are gestated in homozygous mothers. These two factors may result in the selective elimination of MHC homozygotes either *in utero* or early after birth as a result of maternal responses to fetal antigens not associated with the MHC system (Palm, 1971).

In human studies Beer *et al.* (1981) has also found a significant degree of sharing of HLA antigens in couples experiencing spontaneous abortion of unknown aetiology, and a marked alteration in the mixed lymphocyte reaction (MLR) between partners when compared with MHC typed third party stimulators and responders. This would suggest an abnormal response of the mother to her partner. Several groups (Faulk & McIntyre, 1981; Gerencer *et al.*, 1978; Komlos *et al.*, 1977; Beer *et al.*, 1981) support this idea and suggest the sharing of HLA antigens between couples is not uniquely associated with spontaneous abortion; recurrent abnormal pregnancies of unknown aetiology, neural tube defects and pre-eclamptic toxaemia have also been reported in these couples (Gill & Repetti, 1979). All these authors suggest that sharing of a factor is further substantiated by the fact that if either partner takes another partner they are likely to have exclusively normal pregnancies. This need not necessarily be true; the antigenic interaction between the couples that cause or prevent abortion is accepted, that this is of shared antigens is unproven.

Other groups studying recurrent spontaneous abortion have not found increased sharing of

class I HLA antigens (Oksenberg *et al.*, 1983; Lauritsen, Kristensen & Grunnet, 1976; Mowbray *et al.*, 1983; Johnson *et al.*, 1985). Lauritsen showed in a carefully selected group of couples all of which had had karyotypically normal spontaneous abortions, that there was no significant sharing of class I HLA antigens between couples, but that this group of mothers did have a significantly depressed cellular response to their husbands' cells but not to other men. This would indicate that these women are not pan-non-responders but that the defect is specific to the pairing. Oksenberg *et al.* (1983) reversed the experiment and found that the husbands of a group of recurrent spontaneous aborters exhibited a significant and specifically low MLC response to their wives but not controls. This situation might obtain if class II antigens were shared between the couples, thus both might show decreased reactivity to the other. In our large study of 240 RSA couples we have found that, although Class I sharing was not higher than expected, highly statistically significant DR antigen sharing occurred in almost all couples (Underwood, Kent & Mowbray, unpublished observations).

In a recent study Johnson *et al.* (1985) showed that in a group of 20 women with recurrent spontaneous abortion there was no increased sharing of HLA-A and -B antigens although there was an increased frequency of homozygosity at the B locus. This last result needs confirmation in a much larger group—it may well be chance. A proportion of them showed impairment of the MLR to the husband. The striking finding was that these women had a low prevalence of serum antibodies to cytomegalovirus but not to Herpes simplex virus or Epstein–Barr virus. He suggests that these women demonstrate some selective immunological unresponsiveness and that there is a necessity for a form of allosensitisation to insure a successful pregnancy.

## ROLE OF ANTIBODY IN PREVENTING ABORTION

Studies on the production of anti-paternal lymphocytotoxic antibody in normal pregnancy (Tongio *et al.*, 1972; Vives, Gelabert & Castillo, 1976; Gelabert *et al.*, 1981) show that in normal women antibody may be present after one pregnancy and with successive pregnancies the antibody is more marked. The time of testing of these women for antibody is important; Vives *et al.* (1976) have shown the antibody is boosted in the first trimester and falls towards term. It rises again to a peak in the immediate post-natal period falling again with time. Taylor & Hancock (1975) and Kolb, Chaouat & Chassour (1984) showed that the cytotoxic effect of maternal lymphocytes on cultured trophoblast was completely prevented by the presence of maternal serum. If the IgG fraction was removed from the serum the protective effect was significantly reduced. This supports the idea that the presence of anti-paternal antibody in maternal serum has a protective effect, although it is the serum which has produced the effect, not necessarily the antibody measured (Beard *et al.*, 1984). The ability of sera of pregnant women to inhibit cellular responses against the husband may be due to the non-specific inhibitors of pregnancy (Revillard *et al.*, 1973) as well as specific antibodies. Not only antibodies directed against maternal antigens may be effective—suppression of the response by anti-idiotypic antibodies may also occur. Thus maternal serum can potentially contain this kind of suppressive antibody and yet not have detectable anti-paternal antibody. Currently we would favour the possibility that modulation of HLA antigen expression on the trophoblast by antibody may be the important factor in normal allogeneic pregnancies, rather than a cellular immunity blocking antibody. It is obvious however that both do occur together in normal pregnancy, and further work is needed to establish the role of either of them. Study of which antibodies are absent in RSA might be a useful way of finding out which antibodies are important. We have found however (Mowbray *et al.*, 1983) that the correlation between the two antibodies is very high. Thus in recurrent abortion neither antibody is found commonly, but where one is found, so is the other. This approach has not then been very useful.

We suggest that the lack of maternal cytotoxic antibody to paternal HLA antigens is the dominant feature of women with RSA. In our large series of couples (more than 240) we have found that only 12% of women with RSA and no live children have demonstrable cytotoxic antibody to their husbands' lymphocytes. Of women with RSA who had had one or more live children only 23% had demonstrable antibody. The study of Johnson *et al.* (1985) supports this, as only 10% of the 20 couples that he studied had anti-paternal lymphocytotoxic antibody.

Sera of multiparous women inhibit the MLR (Revillard *et al.*, 1973; Faulk *et al.*, 1974; Gatti, Yunis & Good, 1973) specifically. In pregnancy sera there are also non-specific blocking factors. The specific blocking action can be removed by absorption with paternal lymphocytes or pooled platelets indicating that the blocking factor is probably an antibody to class I HLA antigens (Revillard *et al.*, 1976). Blockade of MIF production by such sera has been shown by Rocklin, Zuckermann & Alpert (1973). This factor has not been found in sera of women with RSA (Rocklin *et al.*, 1976). The inhibitor of MIF production in normal pregnancy sera was found to be specific to the response of the woman to her partner. Serum from other randomly chosen multigravid women would not block the production of MIF by the maternal lymphocytes in response to paternal alloantigens (Rocklin, Kitzmiller & Kaye, 1979). This is what might be expected if the suppression were caused by private anti-idiotypic antibodies, rather than anti-paternal HLA antibodies.

Rocklin *et al.* (1976) also showed that one woman who had had three abortions and subsequently had a normal pregnancy by the same husband produced the blocking factor during her successful pregnancy. This further supports the view that there are antibodies or blocking factors in maternal serum that have a specific protective effect.

In Rocklin's series of spontaneous aborting couples there was no sharing of class I antigens over expectation, showing that the absence of the blocking factor was not due to class I compatibility. He suggests that there might be 'abnormal' trophoblast antigens that do not evoke blocking factor production but do induce a cellular response.

Youtananukorn, Matangkasombut & Osathanondh (1974) showed that lymphocytes of all of 41 post-partum women reacted with pooled placental antigens. He also found that the autologous plasma of a pregnant woman would block reaction of her lymphocytes with antigens extracted from her own placenta, but did not block the reaction of her lymphocyte with PPD. The blocking factor was not found in the first trimester but in some women was evident in the fourth month and it was present in all of the women tested in the fifth month.

It thus seems that generally, whatever technique is used to look for antibody to paternal antigens in pregnancy, in RSA it is rare to find the antibodies found in normal pregnancy. Although there is an overlap in detecting antibody such that several methods may for example detect IgG anti-class I antibodies or those which inhibit transformation, lack of one or more may predispose to the rejection of the fetus and subsequent abortion (Rocklin *et al.*, 1976). Faulk & McIntyre (1983) develop this idea further suggesting two basic trophoblastic antigens, $TA_1$ and $TA_2$ exist. Antibodies to $TA_2$ blocks recognition to $TA_1$ allowing for normal pregnancy to proceed. If $TA_2$ antibodies fail to form recognition of $TA_1$ takes place and pathological condition develops manifested as toxaemia or abortion. They have suggested that similar blocking factors may play a role in tumour growth. Antibodies to either $TA_1$ and $TA_2$ will block the MLR, as do those to the trophoblast–lymphocyte cross-reaction (TLX) antigens described by Beer *et al.* (1972). Faulk & McIntyre (1983) suggest that some failed pregnancies may be due to sharing of TLX antigens between husband and wife; TLX thus having functions similar to transplantation antigens and indeed represented on the surface of lymphocytes. They claim that these antigens inititate a response invoking the maternal recognition which is necessary to support the placenta. The finding of these trophoblast antigens on the maternal–fetal interface further suggests that they may be important in the maternal acceptance or rejection of the allogeneic trophoblast.

Animal studies to evaluate the importance of these antibodies or suppressor factors have not been easy since abortion in small animals is uncommon. Clark, McDermott & Sczewzuk (1980) described a mouse model, CBA/J females mated with DBA/2J, which showed a high reabsorption rate of fetuses. They found that a non-specific suppressor factor was absent from the lymph nodes draining the uterus of the mice with the high incidence of fetal loss. The factor was present in mice who had normal pregnancies. Chaouat, Kiger & Wegmann (1983) vaccinated the CBA/J females with allogeneic spleen cells from DBA/2J males. They used mice from a number of sources to insure that the effect was not confined to one inbred strain. There was little effect from the DBA/2J spleen cells but when spleen cells from BALB/c males were used, the reabsorption rate was reduced from 23% to 5%. Also they were able to demonstrate the generation of a MLR suppressive factor in the serum of CBA/J females which had been injected with BALB/c spleen cells but not in those injected with DBA/2J cells.

## TROPHOBLAST ANTIGENS

There is now accumulating considerable evidence of the nature of the antigenic molecules on trophoblast (Johnson *et al.*, 1981). This has been very largely aided by the ability to produce pure trophoblast microvilli in quantity (Smith, Brush & Luckett, 1974). It appears that the molecule which carries the TLX antigens on trophoblast is a 34K protein without $\beta_2$-microglobulin. Although it does react with several monoclonal antibodies raised against trophoblast products (Johnson *et al.*, 1981; Whyte & Loke, 1979), it does not react with W6/32 or 2A1, the monoclonal antibodies which react with the 45K heavy chain of the class I molecules. Johnson and his collaborators have shown that the 34K chain is located near the HLA B gene locus. Gill has shown in the rat that a monoclonal raised against the rat trophoblast antigen equivalent to the 34K protein can be used to isolate from rat lymphocytes a 45K chain associated with $\beta_2$-microglobulin. This suggests that there may be considerable homologies between the trophoblast alloantigenic molecule and the HLA class I molecule. This does not directly help in understanding the role of immunity in preventing abortion, but modulation of the trophoblast class I antigens by maternal antigens seems a reasonable possibility. All workers agree that it is not possible to detect class I molecules on sections of normal placenta (Faulk & Johnson, 1980; Faulk, Sanderson & Temple, 1977; Goodfellow *et al.*, 1976), but Chatterjee-Hasrouni & Lala (1979) and Wegmann (1981) have shown that if the intact cells are exposed to labelled anti-class I antibody and binding studied very promptly antigenic sites on the trophoblast are detectable. This would support the idea that modulation of antigens on the trophoblast may be occurring, perhaps leading to the production of a different class I heavy chain in the membrane.

Whatever is eventually shown to be the mechanism, the trophoblast cell is unique in two respects. It is the only nucleated cell in the body known not to express class I antigens, and the only one to be bathed in antibody to the antigens which it does not express.

## TENTATIVE CONCLUSIONS

Although there is no clear indication of which factors are important in allowing allogeneic pregnancies to proceed to term, there are now enough factors established which modify the maternal response to the fetus that the real effector is likely to lie among them. The older ideas of a suppressed mother and the uterus as a privileged site do not longer seem tenable.

The factors which appear at present important are summarised thus.

(1) The normal trophoblast does not have detectable HLA antigens on it.
(2) The normal maternal response to an allogeneic pregnancy is to make antibodies to paternal HLA antigens expressed on fetal cells (other than trophoblast).
(3) The antibodies detected can suppress cellular reactivity of the mother to paternal HLA antigens, as measured by inhibition of MLR, lymphokine production and cellular cytotoxicity.
(4) Although there are some general inhibitors of MLR in pregnancy, the maternal immune response is in general enhanced rather than suppressed in normal pregnancy.
(5) Failure of the protective effector mechanism results at least in spontaneous abortion, and may possibly be associated with pre-eclampsia and retarded fetal growth.

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