Prevention of recurrent spontaneous abortions by leukocyte transfusions[1]

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Summary: One hundred and thirty-nine couples referred because of recurrent abortions with no obvious cause were assessed for genetic similarity using the HLA major histocompatibility system. Comparison with 103 fertile control couples demonstrated that a much higher proportion of couples in the abortion group shared two or more HLA antigens. Using this criterion, 44 wives out of the 139 couples referred, when compared with a child-bearing group, appeared to share a greater than expected number of histocompatibility antigens and were therefore considered suitable for treatment. Twenty-eight wives have received treatment with white cell infusions from erythrocyte-compatible donors and so far they have delivered 17 babies plus 2 second babies. Another 3 wives are pregnant beyond their previous dates for abortions (1 first and 2 second pregnancies). There have been 5 failures (4 first pregnancy and 1 second pregnancy); one of these was treated a second time and has now successfully delivered. Seven couples are awaiting conception. Of the patients who have become pregnant, 81.5% have had successful deliveries. No adverse transfusion reactions have been observed.

Introduction

Recurrent spontaneous abortion is thought to have many possible causes (Rock & Zacur 1983); there are, however, a certain number of cases in which no cause can be established. From prospective studies of urinary HCG (Miller *et al.* 1980), it appears that early pregnancy wastage is much more common that suspected previously, emphasizing the lack of knowledge about the pathophysiology of recurrent spontaneous abortion. During the past several years, research in reproductive biology has suggested that a normal pregnancy requires immunological recognition by the mother of trophoblast antigens, and the absence of such recognition is associated with pregnancy failure (Faulk & McIntyre 1981). This concept was extended by reports of shared histocompatibility antigens between mating couples who abort (Komlos & Halbrecht 1979). We have confirmed these findings and proposed a programme of immunotherapy for the prevention of recurrent spontaneous abortions, the preliminary results of which were published in 1981 (Taylor & Faulk 1981). These initial encouraging results have been sustained and extended in the present study.

Methods

Patients referred are only accepted after they have been thoroughly investigated for other possible causes of spontaneous abortion including chromosomal, infections, hormonal and anatomical problems. All patients had a minimum of three spontaneous abortions with the same partner, and no successful pregnancies. All were immunologically normal and, with the exception of one patient, gynaecologically normal in other respects. The one exception was a patient who had a repair to bicornuate uterus following 6 abortions and then had a further 4

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Table 1. Percentage of test and control couples sharing histocompatibility antigens

Number of HLA shared antigens	103 control couples	139 abortion couples
0	53.3	26.6
1	33.0	37.4
2	12.6	23.0
3	0.9	7.2
4	Nil	4.3
5	Nil	1.4

abortions. The couples were all HLA typed for A, B, C and Dr loci according to standard criteria (McIntyre 1976). Forty-four couples who shared more than 2 HLA antigens were offered treatment with infusions of white cells. Sharing of 2 HLA antigens was chosen as a criterion since twice as many patients shared 2 HLA antigens in the abortion group as compared with 103 matched fertile control couples (Table 1). Patients who were offered treatment were thoroughly counselled about the risk of white cell infusions, and 28 couples have so far accepted therapy and been treated.

White cell concentrations were prepared by using whole blood from 2–5 donors. Blood was taken into standard citrate/phosphate/dextrose (CPD) donor packs and tested for HBsAg by the Hepatest technique. The whole blood units were kept for at least three days in order to minimize the risk of cytomegalovirus infection. The buffy coats from each unit were removed, but the lymphocyte populations were not separated from other white cells, since to do this would increase the chances of accidentally infecting the white cell concentrates.

Patients and husbands were typed for ABO, Rhesus, Kell, Duffy, Lewis, Kidd and MNS blood groups. The erythrocyte groups of the donor units were selected in order not to stimulate antibodies in the mother, in particular antibodies directed against paternal antigens. Lymphocytes were assumed to be HLA incompatible with the mother though the individual packs were not HLA typed, since the wide distribution of HLA antigens makes it exceedingly unlikely that the donors would be HLA compatible with the recipients (Bodmer 1981). All red cells were cross-matched against maternal serum and the buffy coats removed in such a way as to reduce the number of erythrocytes and platelets transfused, and each preparation was packed to minimize the amount of donor plasma in the transfusion. The total volume of the concentrates was 250 ml, and the leukocyte-enriched preparations were infused over a period of one hour.

The patients' pulses and blood pressures were recorded during transfusions, and all patients were closely supervised. Before conception, each patient was given an infusion of white cells followed by another three weeks later. No further transfusions were given until the patients became pregnant, following which white cell infusions were given at three-weekly intervals starting at three weeks of pregnancy and continuing to 26 weeks gestation. This schedule was not followed in one case: this was a patient who clinically appeared to be aborting at the eight gestational week, so white cell infusions were given at weekly intervals from 8 to 12 weeks of her pregnancy. This increased frequency of treatment was successful, for she went on to deliver a normal baby at term. In those patients who wish to have a second pregnancy, it is our policy not to give further white cell transfusions, since protection once established should be specific and long-lasting.

Results

Data were compared from 139 couples who had suffered at least 3 consecutive spontaneous abortions with the same partner and from 103 child-bearing couples with at least two children and no history of previous spontaneous abortions. The percentage of couples who shared two

Table 2. Details of 17 patients with successful pregnancies

Age of wife	No. of previous abortions	HLA antigens shared by wife and husband	Outcome of pregnancy
34	3	A11, BW35 CW4, DRW3	Normal delivery, normal baby
32	3	A3, B7, DRW2	Normal delivery, normal baby, second baby
41	3	BW44, CW5	Caesarian section, normal baby
34	10	A2, A3, DRW4	Normal delivery, normal baby, second baby
35	3	B5, BW44, DRW1, DRW2	Normal delivery, normal baby
32	3	A1, B15	Normal delivery, normal baby, now 27 weeks pregnant
32	4	A2, B7, BW51, C7	Normal delivery, normal baby
38	4	A3, B7, CW7, DRW1	Normal delivery, normal baby
31	4	A19, B40, CW3	Normal delivery, normal baby
34	5	A1, CW3, DRW6	Abortion 12 weeks Second pregnancy = normal delivery, normal baby
30	3	A3, B27	Normal delivery, normal baby
39	4	A1, B12	Normal delivery, normal baby
36	2	A3, DRW1	Normal delivery, normal baby
37	4	B12, DRW2, DRW5	Normal delivery, normal baby
34	4	B27, CW2	Normal delivery normal baby
34	7	A2, B7	Normal delivery, normal baby
36	7	A2, B7	Premature delivery, normal baby

or more antigens was far greater in the abortion group and none of the couples in the control group shared 4 or 5 antigens (Table 1).

In order to eliminate any bias such as may be due to ethnic variation, the HLA types of the females in the couples of the abortion group were compared to the HLA types of their husbands as well as to the HLA types of a randomly selected male from the same group. The results of this method of comparison showed an average of 1.5 gene-product sharing between husband and wife in the aborting group and an average of 0.96 gene-product sharing between someone else's husband from the aborting group and the aborting wives, indicating a lack of such bias in our study population.

Out of the 139 couples referred because of recurrent spontaneous abortions with no apparent cause, 44 have been identified who when compared with the child-bearing control group appear to share a greater than expected number of histocompatibility antigens. Twenty-eight wives from this group have received treatment with white cell infusions and so far they have delivered 17 babies plus 2 second babies. Another 3 wives are pregnant beyond their previous dates for abortions (1 first pregnancy and 2 second pregnancies). There have been 5 failures (4 first pregnancy and 1 second pregnancy); one of these was treated a second time and has now successfully delivered. Seven couples are awaiting conception. Table 2 presents the age, HLA data and the results of the pregnancies of patients who have completed treatment.

In all but one of the couples accepted into the programme, gynaecological and other medical causes for their abortions had been excluded. In the patient who had had a bicornuate uterus repair after 6 spontaneous abortions, which was followed by a further 4 abortions, it was found that the wife shared 4 HLA antigens with her husband. She was successfully treated with white cell infusions and has now completed two successful pregnancies.

Of the 19 babies so far delivered there is no evidence of abnormality, including host-versus-graft reaction, at a follow up of four years.

Discussion

Data from our group and others (Beer *et al.* 1981, McIntyre & Faulk 1982*a*, Uander & Olding 1983) have supported a concept that many aborting couples share more than the expected number of HLA antigens. This information has been collected by comparison of HLA gene

frequencies (Uander & Olding 1983) and by matching each abortion couple with either a control child-bearing couple (McIntyre & Faulk 1982*a*) or other aborting couples in whom reasons for abortion are known (Beer *et al.* 1981). The phenomenon of HLA sharing has attracted attention from many quarters (Smith 1982, Gill 1983), prompting us to make two points about this finding. First, HLA sharing is only one of four laboratory criteria which can be used to define these patients, the others being (1) diminished allogeneic responses by maternal lymphocytes to paternal lymphocytes but not to third-party lymphocytes in mixed lymphocyte culture (MLC) reaction (Beer *et al.* 1981, McIntyre & Faulk 1983*a*); (2) the presence of a blocking factor in the wife's non-pregnant plasma (but not serum) which inhibits stimulation of her cells by the husbands lymphocytes (but not third-party lymphocytes) in the MLC reaction (McIntyre & Faulk 1983*a, b*); and (3) the absence of specific migration-inhibition-factor blocking activity from maternal serum during pregnancy (Rocklin *et al.* 1976). Secondly, HLA sharing in aborting couples is an epiphenomenon: it is not the HLA sharing that is important but rather the sharing of trophoblast antigens which are in turn probably associated with the major histocompatibility complex (MHC) (McIntyre & Faulk 1982*b*).

Some abortion-prone couples do not share HLA, but mixed lymphocyte culture testing of these husband/wife combinations has revealed that the wife often responds less well to her husband's lymphocytes than to cells from a third-party control (McIntyre & Faulk 1983*a*). Whether this subgroup of patients should be grouped with aborters who normally recognize their husbands' cells is a question that has yet to be answered, but at present HLA sharing is a useful marker to help establish clearer definitions of the patient population.

Another useful aspect of HLA typing has arisen from our studies of the aborting patient's medical history. Women who have never had a child are both clinically and immunologically different from spontaneous aborters who have previously had successful pregnancies or late-pregnancy losses with their present husbands. The former group, which we refer to as 'primary' aborters (McIntyre & Faulk 1984), tend to abort early, manifest no detectable antipaternal immunity, and share a statistically significant number of HLA antigens with their husbands (Table 1). They are distinct from women who suffer repeated miscarriages 'secondary' to having had children or late-trimester pregnancy losses, because the latter group do not share HLA antigens with their spouses but do manifest high-titre antipaternal lymphocytotoxins in their blood (McIntyre *et al.* 1984).

Whilst the HLA system has been employed as a method of identifying genetic similarity (McIntyre & Faulk 1984), the absence of HLA antigens from syncytiotrophoblast (Faulk & Temple 1976, Sunderland *et al.* 1981, Galbraith *et al.* 1981), which provides the first interface between the new fetal material and the mother (Faulk *et al.* 1982), makes it unlikely that the HLA antigens themselves are responsible for recurrent abortions. It has been demonstrated that some of the antigens from syncytiotrophoblast are cross-reactive with human lymphocytes and show allotypic variation (McIntyre & Faulk 1982*b*). Further analysis by cytotoxicity of allotypy has shown three groups of antigens designated TLX1, 2 and 3 (Faulk & McIntyre 1983, McIntyre *et al.* 1983). Compatability of TLX antigens between mating couples thus results in TLX sharing between wife and blastocyst, resulting in failed maternal recognition and blatocyst rejection, like any other foreign tissue from the uterus.

We believe that leukocyte transfusions from third-party donors to a wife who clinically aborts with the same husband provide a barrage of TLX incompatibility antigens that stimulate her to mount protecting responses, such as blocking factors (Jeannet *et al.* 1977) and suppressor cells (Chaouat & Voisin 1980), which disallow rejection of an implanted blastocyst by uterine immune cells. The basis of such protection must depend upon cross-reactivity with paternal allotype as a result of the large number of donors and frequency of leukocyte transfusions used in our programme of therapy.

With regard to the babies' health, we have scrupulously avoided forming antibodies in the mother to red cell antigens which could possibly result in haemolytic disease of the newborn. In particular we have avoided using paternal antigens, which may sensitize the mother to fetal allotypes inherited from the father.

The results of this study show a final success rate of approximately 81.5% in patients who between them had had 106 abortions (i.e. 3.6 abortions per patient). The maximum expectation of a spontaneously successful pregnancy following 4 abortions, according to six different authors (Hawkins 1974), is a mean value of 61%. A better immunogen for these patients would be a trophoblast antigen prepared from syncytiotrophoblast, for this membrane does not contain HLA or other known MHC antigens (Faulk *et al.* 1982), and its use would not carry theoretical objections such as graft-versus-host reactions caused by hyperimmunization with HLA incompatible leukocytes. For the moment, the well established technology of white cell transfusions provides a practical alternative means of treatment.

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