Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomised trial

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Summary

Background Couples with unexplained recurrent miscarriage may have an alloimmune abnormality that prevents the mother from developing immune responses essential for the survival of the genetically foreign conceptus. Immunisation with paternal mononuclear cells is used as a treatment for such alloimmune-mediated pregnancy losses. However, the published results on this treatment are conflicting. In this study (the Recurrent Miscarriage [REMIS] Study), we investigated whether paternal mononuclear cell immunisation improves the rate of successful pregnancies.

Methods Women who had had three or more spontaneous abortions of unknown cause were enrolled in a double-blind, multicentre, randomised clinical trial. 91 were assigned immunisation with paternal mononuclear cells (treatment) and 92 immunisation with sterile saline (control). The primary outcomes were the inability to achieve pregnancy within 12 months of randomisation, or a pregnancy which terminated before 28 weeks of gestation (failure); and pregnancy of 28 or more weeks of gestation (success). Two analyses were done: one included all women (intention to treat), and the other included only those who became pregnant.

Findings Two women in each group received no treatment, and eight (three treatment, five control) were censored after an interim analysis. In the analysis of all randomised women who completed the trial, the success rate was 31/86 (36%) in the treatment group and 41/85 (48%) in the control group (odds ratio 0·60 [95% CI 0·33–1·12], p=0·108). In the analysis of pregnant women only, the corresponding success rate was 31/68 (46%) and 41/63 (65%; odds ratio 0·45 [0·22–0·91], p=0·026). The results were unchanged after adjustment for maternal age, number of previous miscarriages, and whether or not the couple had had a previous viable pregnancy. Similar results were obtained in a subgroup analysis of 133 couples with no previous livebirth.

Interpretation Immunisation with paternal mononuclear cells does not improve pregnancy outcome in women with unexplained recurrent miscarriage. This therapy should not be offered as a treatment for pregnancy loss.

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Introduction

About 15% of clinically recognised pregnancies are spontaneously aborted; thus, miscarriage is the most common complication of human pregnancy. Although most miscarriages are sporadic, recurrent miscarriage (three or more spontaneous abortions) occurs in 0·5–1·0% of couples. In most women who experience recurrent miscarriage, no cause can be identified. Alloimmune mechanisms that prevent mothers from developing immunological responses essential for the survival of the semiallogeneic pregnancy have been proposed as the cause of some or all of these losses. On the basis of animal models of abortion and studies of human organ transplant survival, immunisation with paternal white cells was proposed as a treatment for alloimmune-mediated pregnancy loss. This immunotherapy is offered by many medical centres in the USA and elsewhere, although its efficacy remains controversial. Published trials and meta-analyses of published and unpublished studies have yielded conflicting results, indicating the need for large randomised trials. The purpose of this multicentre, randomised, double-blind trial, the Recurrent Miscarriage Study (REMIS), was to assess the efficacy of immunisation with paternal mononuclear cells as a treatment for unexplained recurrent miscarriage.

Methods

Patients

Patients were recruited at six centres between July, 1992, and December, 1997: University of Chicago, Chicago, IL; Washington University School of Medicine, St Louis, MO; University of Utah, Salt Lake City, UT; University of Pittsburgh, Pittsburgh, PA; Los Olivos Women’s Center, Los Gatos, CA, USA; and University of British Columbia, Vancouver, British Columbia, Canada. All study centres had approval from their institutional review boards, and informed consent was obtained from all patients. Since patients were recruited from a wide referral base, the number screened, and the number ineligible was not known.

The eligibility criteria were: three or more previous miscarriages (not necessarily consecutive) that were not of chromosomally abnormal fetuses or ectopic pregnancies; no more than one liveborn child with the current partner; age 40 years or younger at the time of recruitment; not pregnant at the time of immunisation; no anti-HLA antibodies measured by a microcytotoxicity assay; no contraindications for immunisation with paternal mononuclear cells; and no identifiable cause for the previous miscarriages. The latter criterion was confirmed by means of: cytogenetic studies in both parents; luteal-phase serial progesterone measurements or in-phase endometrium; concentrations of thyrotropin in serum; intrauterine contour assessed by hysterosalpingography, sonohysterography, or hysteroscopy; and assays of antibodies to cardiolipin, which were measured in a single laboratory and lupus anticoagulant assessed by normal clotting times (within 2 SD of the mean) in sensitive, phospholipid-dependent clotting assays. Most centres used a sensitive partial thromboplastin time, and some centres used the dilute Russell’s viper venom time.
therapy was provided during the weekly visits by the patient's own physician. The nurse coordinators continued to contact each woman monthly during the remainder of the pregnancy. For women who progressed normally, umbilical cord blood and placental samples were collected at delivery for genetic studies. If a miscarriage occurred, every effort was made to collect the products of conception for cytogenetic and genetic studies. Pregnancy losses were considered to be pre-embryonic if they occurred before the documentation of fetal cardiac activity, embryonic if they occurred after establishment of fetal cardiac activity but before 10 weeks of gestation, and fetal if they occurred after establishment of fetal cardiac activity and after at least 10 weeks of gestation.21 No other concomitant therapies for recurrent miscarriage were used.

**Statistical analysis**

The primary analysis was by intention to treat, carried out on all randomised patients. Success was defined as a pregnancy that continued to at least 28 weeks of gestation, following the protocol of M owbray and colleagues.21 Treatment failures were women who did not become pregnant within 12 months of randomisation, and women who experienced a pregnancy loss before 28 weeks of gestation. A secondary analysis was done, including only women who became pregnant within 12 months of randomisation, with a failure defined as a pregnancy loss before 28 weeks of gestation. A subgroup analysis limited to women with no previous livebirth was also done.

Baseline differences between the treatment and control groups were analysed by means of Student's t test for continuous variables, and by χ² or Fisher's exact test for categorical variables. Success rates were compared by logistic-regression analysis to derive odds ratios, both unadjusted and adjusted, for three factors known to be associated with miscarriage (i.e., maternal age, number of previous miscarriages, and whether or not the patient had a previous liveborn child). The distributions of the number of months to a positive pregnancy test after randomisation, and the number of weeks to a miscarriage after pregnancy diagnosis were estimated by the Kaplan-Meier method, and were compared between the two groups by the log-rank test.

The study was designed to detect an increase in the rate of livebirths among pregnant women (specifically, a gestation of 28 weeks or more) from 60% in the control group to 80% in the treated group, assuming equal pregnancy rates of 60% in both groups (i.e., a difference in success rates of 12%). A target sample size of 262 women per group was necessary to have 80% power to detect a difference in pregnancy rates of 12% between the treatment and control groups, assuming equal pregnancy rates of 60% in both groups.

**Table 1: Demographic variables and pregnancy history by treatment group**

<table>
<thead>
<tr>
<th></th>
<th>Treatment group (n=89)</th>
<th>Control group (n=89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>32.7 (4.3; 23–41)</td>
<td>32.7 (4.4; 22–40)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>83 (93%)</td>
<td>78 (87%)</td>
</tr>
<tr>
<td>Other</td>
<td>6 (7%)</td>
<td>12 (13%)</td>
</tr>
<tr>
<td>Number of previous pregnancies*</td>
<td>4.9 (2.1; 1–14)</td>
<td>4.6 (1.6; 3–9)</td>
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<tr>
<td>Number of previous miscarriages*</td>
<td>4.3 (1.8; 3–13)</td>
<td>4.2 (1.4; 3–9)</td>
</tr>
<tr>
<td>Number of women with previous miscarriages</td>
<td>29 (33%)</td>
<td>17 (19%)</td>
</tr>
<tr>
<td>Number of women with previous ectopic pregnancies</td>
<td>9 (10%)</td>
<td>12 (13%)</td>
</tr>
<tr>
<td>Number of women with a previous chromosomally abnormal miscarried fetus</td>
<td>5 (6%)</td>
<td>7 (8%)</td>
</tr>
</tbody>
</table>

*Mean (SD; range). 1One woman was recruited at age 40 but randomised just after her 41st birthday.

**Table 2: Study outcomes by treatment group**
to detect a difference of this magnitude, with a two-sided significance test at \(a=0.05\). Interim analyses were planned after every 50 outcomes, with an O’Brien-Fleming monitoring boundary.24 However, the sample size was smaller than planned when the last participant was randomised in December, 1997, and only three interim analyses and one final analysis were done. All results were reviewed by an independent data and safety-monitoring committee. After the third interim analysis in February, 1998, the committee recommended that no further 6-month reimmunisations be given. The reason for this decision was that the miscarriage rate in the treatment group was higher than that in controls. Consequently, three women in the treatment group and five in the control group who did not become pregnant at 6 months were not reimmunised. For the intention-to-treat analysis, outcomes from these eight patients were “censored” at 6 months—ie, no follow-up information beyond 6 months was used in the calculation.

Results

183 women were randomly assigned treatment or placebo (figure 1). Two in each group were disqualified: before immunisation, results of screening indicated that one was pregnant and one had a blood-group incompatibility; two other couples decided not to take part after randomisation (but before immunisation), one because the husband was positive for cytomegalovirus antibodies, and one for personal reasons. 131 (73·2%) of the 179 patients immunised with paternal mononuclear cells (treatment—solid line) and patients immunised with sterile saline (control—dotted line) became pregnant within 12 months of randomisation, 40 (22·3%) did not, and for eight the result was indeterminate. Among the 131 pregnant women, 72 (55%) delivered and 59 (45%) had miscarriages. Among the 59 pregnancy failures, five were ectopic, 31 were pre-embryonic, 17 were embryonic, and six were fetal.

The distribution of demographic and pregnancy history variables in the treatment and control groups is shown in table 1. The groups were similar except that a higher proportion of women in the treatment group had had a previous livebirth (p=0.054). This variable, along with maternal age, and number of previous losses, were included as covariates in subsequent analysis.

**Figure 2: Time from pregnancy to miscarriage among patients immunised with paternal mononuclear cells (treatment—solid line) and patients immunised with sterile saline (control—dotted line)**

In the intention-to-treat analysis, the success rate was 36% in the treatment group and 48% in the control group (table 2; odds ratio 0·60 [95% CI 0·33–1·12], p=0·108). The corresponding analysis adjusted for maternal age, number of previous miscarriages, and previous livebirth gave a similar odds ratio (0·54 [0·28–1·02], p=0·056). None of the covariate effects reached statistical significance, although a previous livebirth was associated with greater odds of success, of marginal significance (2·05 [0·96–4·35], p=0·062). Kaplan-Meier-estimated pregnancy rates did not differ significantly between the groups: 78% in the treatment group and 72% in the control group (log rank p=0·232).

In the analyses that included pregnant women only, the success rate was 46% in the treatment group and 65% in the control group (odds ratio 0·45 [0·22–0·91], p=0·026). The corresponding analysis adjusted for covariates again gave a similar odds ratio (0·40 [0·19–0·84], p=0·015). In this analysis, the number of previous pregnancy losses had a significant effect on success rate (odds ratio 0·75 per additional loss [0·58–0·99], p=0·040). The treatment effects were also similar among the participating centres (data not shown).

Analyses were repeated for couples with primary recurrent miscarriage—ie, couples without a previous livebirth. The results again favoured the control group, with success rates in the intention-to-treat analysis of 18/59 (30%) in the treated group and 32/70 (46%) in the controls (odds ratio 0·52 [0·25–1·08], adjusted p=0·082). Among pregnant patients, success rates were 18/46 (39%) and 32/51 (63%) in the treatment and control groups, respectively (0·37 [0·16–0·86], adjusted p=0·021). In the treatment group, 26% of patients developed HLA antibodies after immunisation. There was no evident association between success rate and HLA-antibody status (31% and 30% for patients with and without HLA antibodies after immunisation, respectively, p=1·0).

The distributions of time from pregnancy to miscarriage in the treatment and control groups are shown in figure 2. The mean duration of gestation at the time of miscarriage was 8·9 weeks (SD 4·5) and 6·2 weeks (1·5) in the treatment and control groups, respectively (p=0·002). The duration of gestation at which miscarriage occurred was before 10 weeks in all patients in the control group, whereas six (16%) of 37

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Ectopic</th>
<th>Pre-embryonic</th>
<th>Embryonic</th>
<th>Fetal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group (n=68 pregnancies)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total failed pregnancies</td>
<td>11 (16%)</td>
<td>18 (26%)</td>
<td>12 (18%)</td>
<td>6 (9%)</td>
</tr>
<tr>
<td>Number for which cytogenetic studies were available</td>
<td>0</td>
<td>4</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Number of normal karyotypes (46,XX/46,XY)</td>
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<td>2/0</td>
<td>0/4</td>
<td>3/1</td>
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<tr>
<td>Abnormal karyotypes</td>
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<td>47,XX+20</td>
<td>45,X</td>
<td>-</td>
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<tr>
<td>47,XX+16</td>
<td>45,X</td>
<td>47,XX+14</td>
<td>47,XX+16</td>
<td>69,XXX</td>
</tr>
<tr>
<td>Control group (n=63 pregnancies)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total failed pregnancies</td>
<td>4 (6%)</td>
<td>13 (21%)</td>
<td>5 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>Number for which cytogenetic studies were available</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
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<tr>
<td>Number of normal karyotypes (46,XX/46,XY)</td>
<td>0/1</td>
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<td>2/0</td>
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<tr>
<td>Abnormal karyotypes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Developmental stages and abnormalities in failed pregnancies
losses in the treatment group occurred after 10 weeks of gestation. The timing of pregnancy loss by stage of development is shown in table 3. Ectopic, pre-embryonic, and embryonic loss rates did not differ significantly between the treatment and control groups (p=0·320, 0·564, and 0·162, respectively), but the rate of fetal loss was significantly greater in the treatment group (p=0·036).

Chromosome studies in the products of conception were successful for 21 of the 59 abortuses. Seven (33%) of the 21 abortuses on which cytogenetic studies were done had normal karyotypes; all were in the treatment group (table 3). Four additional fetuses in the treatment group had abnormalities: two had a cystic hygroma; one had an omphalocele, a tracheo-oesophageal fistula, duodenal atresia, and a single umbilical artery; and one had an abnormal triple screen (α-fetoprotein, unconjugated oestriol, and human chorionic gonadotropin).

There were no pregnancy losses or fetal deaths after 28 weeks of gestation. There were no differences with respect to delivery statistics between infants born to mothers in the treatment group (31 liveborn) and those born to mothers in the control group (41 liveborn). The mean gestational ages at delivery were 39·2 weeks (SD 1·7; range 35·6–44·4) in the treatment group and 39·4 weeks (1·3; 36·4–41·0) in the control group (p=0·698). The mean birthweights in the two groups were 3395 g (SD 623; 2241–4592) and 3353 g (491; 2381–4479), respectively (p=0·755). Sex ratio (M/F) was 0·82 in the treatment group and 0·86 in the control group (p=1·0).

Discussion

In the REMIS study, the pregnancy success rate was higher in the control group than in patients immunised with paternal mononuclear cells, irrespective of whether all randomised patients or only patients achieving a pregnancy were considered, or whether or not patients with previous liveborn children were excluded. In addition, outcomes were similar among patients in the treatment group irrespective of whether they developed HLA antibodies after immunisation. Thus, we found no evidence of benefit from immunisation with paternal mononuclear cells for prevention of recurrent miscarriage. Furthermore, higher rates of pregnancy loss among patients immunised with paternal cells than those immunised with saline suggests that immunotherapy with paternal mononuclear cells may increase the rate of clinically recognised pregnancy losses.

The higher rate of miscarriage in the treatment group was associated with losses occurring later in gestation. Indeed, all losses occurring after 9 weeks of gestation were in the treatment group (figure 2). All chromosomal and other abnormalities also occurred in the treatment group. However, this finding probably reflects the greater chance of identifying fetal tissues in the products of conception later in gestation, and the larger number of later pregnancy losses in the treatment group. For example, in this study, chromosome analyses were successful in five (16%) of 31 pre-embryos, 11 (65%) of 17 embryos, and four (67%) of six fetuses (table 3). Extrapolation of epidemiological studies of chromosome abnormalities in abortuses suggests that a significant proportion of the earlier losses were chromosomally abnormal. However, without knowledge of the chromosomal status of all abortuses we cannot confine our analyses to chromosomally normal pregnancies.

Nevertheless, given the almost identical maternal age distribution among the treatment groups (table 1), the occurrence of chromosome abnormalities should have been randomly distributed among the groups.

The results of this study differ from those of the smaller clinical trial by Mowbray and colleagues. Although we followed an almost identical protocol, there were some differences between the studies. First, the previous study used maternal mononuclear cells from 10 mL blood as the control treatment, whereas we used sterile saline. Because of the lower than expected pregnancy success rate in the control group in that study (37%), we chose to use saline, as in the trial of Cauchi and colleagues. Second, Mowbray and colleagues' trial did not provide first-trimester supportive therapy. The use of saline as a placebo and the provision of supportive therapy in the first trimester could account for the greater success rate in our control group than in Mowbray and colleagues' study (65 vs 37%, respectively, among pregnant women), although the success rate in our control group is similar to success rates reported in epidemiological and cohort studies of women with recurrent miscarriage.

However, these differences in our protocols are unlikely to account for the disparity in success rates in the treatment groups between the two studies (46 vs 77%, respectively, among pregnant patients). On the other hand, differences in experimental design and analysis could explain some of the differences between our studies. Mowbray and colleagues used a fully sequential design (stopping early) and did not analyse by intention to treat, as we did, which may have biased the outcome if either pregnancy rates or rates of preclinical pregnancy losses differed between the two groups. Also, when 18 more patients in Mowbray and colleagues' study (analysed by Jeng and colleagues) were followed up after their trial had stopped, the treatment effect was lower, and the differences between treated patients and controls were not significant.

In one meta-analysis, which included published and unpublished data from the study of Mowbray and colleagues and from several additional randomised trials, a small but significant effect in favour of leucocyte immunotherapy was found. In this analysis, the success rates in treated women ranged from 62% to 77% (pooled sample, 68·4%). Results were reported on a relative-risk scale—ie, as the ratio of livebirth rates in treated and control groups. In that paper, two analysis teams worked independently and arrived at estimated ratios of 1·16 (95% CI 1·01–1·34) and 1·21 (1·04–1·37), respectively. In a 1995 meta-analysis including updated data from these trials, the effect of immunotherapy did not reach significance (livebirth rate ratio 1·12 [0·97–1·31]). If the results from our trial are added to these data, the estimated livebirth rate ratio falls to 1·04 (0·91–1·20). A test for heterogeneity of the results across the trials was not significant (p=0·320).

In this study, immunisation with paternal mononuclear cells did not improve pregnancy outcome in women with recurrent miscarriage. Despite a history of unexplained recurrent miscarriage, nearly 65% of control patients who became pregnant had a successful pregnancy. We found a higher rate of miscarriage and a greater gestational age at the time of loss in immunised women who became pregnant. Because of the lack of benefit, we recommend against this intervention as a treatment for unexplained recurrent miscarriage.
Contributors
Carole Ober, Theodore Karrison, Randall Odem, Randall Barnes, Ware Branch, Mary Stephenson, Beverly Baron, James Scott, and James Schreiber were involved in the study design, execution, data analysis, and writing of this work. Mary Ann Walker coordinated the day-to-day activities, collected data on patients, and contributed to the preparation of the paper.

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References