Allogenic leukocyte immunization after five or more miscarriages

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Rather than investigate whether paternal leukocyte immunization improves the live birth rate in women with three or more abortions, we analysed the results of patients expected to have a poor outcome in a subsequent pregnancy if untreated, i.e. women with five or more abortions and no anti-paternal complement-dependent antibody (APCA) at initial testing. The analysis included the results of patients treated by us over the last 8 years and the results of randomized and non-randomized trials reported by the Recurrent Miscarriage Immunotherapy Trialists Group. Patients with a previous live birth were classified into two groups: secondary aborters if there was an initial live birth followed by miscarriages, or tertiary aborters if there were miscarriages followed by a live birth and at least three subsequent miscarriages. The results were evaluated separately for primary, secondary and tertiary aborters who demonstrated APCA activity as a result of immunization. The 107 primary aborters had double the live birth rate if immunized, with an overall benefit of 31%. The 45 tertiary aborters had an almost 3-fold increase in the live birth rate, with an absolute benefit of 50%. The number of patients needed to treat to achieve one extra live birth was three to four primary aborters or two tertiary aborters. Immunization had little beneficial effect in secondary aborters but was effective in preventing abortion in primary or tertiary aborters with five or more abortions.

Key words: habitual abortion/immunopotentiation/leukocyte immunization

Introduction

Immunopotentiation was first reported to prevent habitual abortion in 1981 (Beer et al., 1981; Taylor and Faulk, 1981). Despite 14 years of use, the efficacy remains in doubt. Comparative prospective trials have claimed this treatment to be beneficial (Mowbray et al., 1985), whereas others (Cauchi et al., 1991a; Ho et al., 1991) have disputed the efficacy of immunization. Immunologically mediated abortions have been poorly defined. At present no reliable laboratory test is available to diagnose immunologically mediated abortions, and no clinical criteria have been used to classify recurrently aborting women. Therefore treatment has been given to all women with three or more unexplained pregnancy losses. However, three abortions can occur by chance, and the subsequent live birth rate is ~50–60%. With such odds, it is extremely difficult to show that any form of treatment has a beneficial effect, and any trial would require large numbers of patients. The largest such trial is the worldwide meta-analysis on >400 patients from double-blind randomized studies [Recurrent Miscarriage Immunotherapy Trialists Group (RMITG), 1994], which showed an ~10% increased incidence of live births in immunized patients. It concluded that immunization might partially correct a widely prevalent condition or that immunization may be highly effective, but for only a small number of patients who have the condition.

We reasoned that in order to show whether immunization has a beneficial effect, it is necessary to define a group of patients who have a relatively poor prognosis. Various factors have been reported to affect the subsequent live birth rate, such as the number of previous miscarriages, the presence of a previous live birth, the time taken to conceive after immunization, patient age, the presence of anti-paternal complement-dependent antibody (APCA) and luteinizing hormone (LH) concentrations (Carp et al., 1990a,b, 1995; Cowchock et al., 1990; Cauchi et al., 1991b; RMITG, 1994). The most important predictive factor is the number of previous miscarriages. Each subsequent miscarriage lowers the live birth rate by 23% (RMITG, 1994). A patient with five or more abortions is much less likely to have a subsequent live birth than a patient with three miscarriages (Carp, 1993; Carp et al., 1993; Daya and Gunby, 1994; RMITG, 1994). A patient with APCA directed towards paternal human leukocyte antigens at initial testing has a high subsequent live birth rate (Carp, 1994; RMITG, 1994). We and others (Reznikoff-Etievant et al., 1988; Carp et al., 1990a,b; Carp, 1994; RMITG, 1994) have reported that treatment is more effective if APCA is produced as a result of immunization, and that immunization is effective in the primary aborter (who aborts all her pregnan-
Patients were classified as primary aborters if they had had no previous live births. The leukocytes were separated by Ficoll–Hypaque density gradient centrifugation. The ring of mononuclear cells at the Ficoll–serum interface was isolated and washed twice in Hartman’s solution. The final suspension was resuspended in 4 ml Hartman’s solution. Immunizations were repeated at 3–4 week intervals until APCA became apparent in the cross-match. Patients refrained from pregnancy until seroconversion to APCA positive occurred. The main difference between our regimen and those of others is that immunizations were boosted until APCA was detected in the maternal serum.

Patients were classified as primary aborters if they had had no previous live births, or secondary aborters if they had experienced a live birth followed by abortions. We also classified a third group of patients, termed tertiary aborters (Carp, 1994), who had experienced abortions followed by a live birth and at least three subsequent abortions. These patients have not been defined previously as a distinct group, but had been included as secondary aborters.

Before inclusion in the Tel Aviv register, objective evidence of pregnancy was required. This might have included original laboratory reports of a positive human chorionic gonadotrophin test, histological confirmation after curettage, ultrasound reports of a pregnancy sac, etc. The patient’s word alone or the referring letter of the patient’s physician was not considered to be sufficient unless objective evidence was available. The international register was accepted at face value.

The computer was programmed to compare the subsequent live birth rate in patients who had had three abortions with patients who had experienced five or more abortions. An analysis was then performed on the results of immunized and control (non-immunized) patients with five or more previous miscarriages and no detectable APCA at initial testing. Controls were included irrespective of seroconversion because this information was not usually available in control patients. The results of the immunized patients were only included if seroconversion to APCA positive was induced by immunization. The results of patients who did not seroconvert were not included in the analysis. Inclusion of the results was conditional on there being no karyotypic or uterine abnormalities, or other presumptive causes of abortion such as antiphospholipid antibodies.

Materials and methods

Patients

The original registers, as described above, included patients with three or more consecutive pregnancy losses under 20 weeks. The register of patients from the Sheba Medical Center (Tel Hashomer, Israel) comprised 489 patients treated by us between 1987 and 1994. It consisted of patients immunized with paternal leukocytes or patients considered suitable for immunization but who decided to forgo immunization for various reasons. The international register, which originally included patients from our centre, was modified by excluding these patients (because they were included in our register). The international register was then divided into two groups: patients from randomized studies and those from non-randomized studies. The randomized register comprised patients reported by nine centres. The non-randomized register was drawn from seven centres (one centre, Taiwan, reported randomized and non-randomized patients). The method of data collection for the international register has been fully described previously (RMITG, 1994). Briefly, centres practicing paternal leukocyte or other forms of immunopotentiation for recurrent miscarriage submitted data forms with 140 variables for each patient, including history, details of the investigation and abortions. As expected, the patients with five or more abortions had a lower live birth rate than those with three abortions in each of the three registers. When the non-immunized patients from all three registers were taken together as a whole, it was seen that the live birth rate fell from 63% for patients with three abortions to 34% for patients with five or more abortions. Taken separately, the live birth rate fell from 54 to 30% in the Tel Aviv patients, from 60 to 29% in the randomized patients.

### Table I. Details of patients in the registers

<table>
<thead>
<tr>
<th>Order of abortion*</th>
<th>Tel Aviv register</th>
<th>Randomized register</th>
<th>Non-randomized register</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>284 (24)</td>
<td>393 (19)</td>
<td>798 (21)</td>
</tr>
<tr>
<td>Secondary</td>
<td>155 (29)</td>
<td>94 (14)</td>
<td>103 (19)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>50 (76)</td>
<td>18 (50)</td>
<td>25 (56)</td>
</tr>
<tr>
<td>Total</td>
<td>489 (31)</td>
<td>505 (19)</td>
<td>926 (19)</td>
</tr>
</tbody>
</table>

Values in parentheses are the proportion of patients with five or more abortions.

*Primary = no previous live births; secondary = live birth followed by abortions; tertiary = abortions followed by a live birth followed by at least three abortions.

The regimen of immunization varied between centres, but most used the method described by Mowbray et al. (1985). The regimen of immunization and APCA testing used at the Sheba Medical Center has been fully described elsewhere (Carp et al., 1990a,b). Briefly, immunizations were prepared from 100 ml samples of heparinized blood. The leukocytes were separated by Ficoll–Hypaque density gradient centrifugation. The ring of mononuclear cells at the Ficoll–serum interface was isolated and washed twice in Hartman’s solution. The final suspension was resuspended in 4 ml Hartman’s solution. Immunizations were repeated at 3–4 week intervals until APCA became apparent in the cross-match. Patients refrained from pregnancy until seroconversion to APCA positive occurred. The main difference between our regimen and those of others is that immunizations were boosted until APCA was detected in the maternal serum.

The computer was programmed to compare the subsequent live birth rate in patients who had had three abortions with patients who had experienced five or more abortions. An analysis was then performed on the results of immunized and control (non-immunized) patients with five or more previous miscarriages and no detectable APCA at initial testing. Controls were included irrespective of seroconversion because this information was not usually available in control patients. The results of the immunized patients were only included if seroconversion to APCA positive was induced by immunization. The results of patients who did not seroconvert were not included in the analysis. Inclusion of the results was conditional on there being no karyotypic or uterine abnormalities, or other presumptive causes of abortion such as antiphospholipid antibodies.

Statistical analysis

Data were analysed by Fisher’s exact test. Significance was assumed at P < 0.05. The relative risk of a live birth was also determined with 95% confidence limits (Fleiss, 1981). It was calculated as the number of live births as a proportion of the total number of patients immunized, relative to the proportion of live births in non-immunized patients. The 95% confidence intervals were calculated and expressed on a log scale.

Results

The total number of patients in each register is summarized in Table I. It can be seen that our Tel Aviv register had a higher proportion of patients with five or more abortions than the other two registers.

Figure 1 shows that the subsequent live birth rate in non-immunized patients was related to the number of previous abortions. As expected, the patients with five or more abortions had a lower live birth rate than those with three abortions in each of the three registers. When the non-immunized patients from all three registers were taken together as a whole, it was seen that the live birth rate fell from 63% for patients with three abortions to 34% for patients with five or more abortions. Taken separately, the live birth rate fell from 54 to 30% in the Tel Aviv patients, from 60 to 29% in the randomized patients.
and from 74 to 48% in the non-randomized patients (without any correction for other predictive factors).

Details of the patients with five or more abortions who were included in the analysis are summarized in Table II. There were 96 patients with five or more abortions in the randomized studies reported in the international register; only 42 (44%) could have their results analysed. The other 54 patients either had no information available as to whether they seroconverted to APCA positive after immunization or remained seronegative. The non-randomized studies comprised 201 patients; only 39 (19.4%) could have their results analysed. In our 148 patients, 110 with five or more abortions had their results analysed (74%). It can be seen that patient age and the number of abortions were similar in the immunized and control patients.

Table III relates the results of patients undergoing immunization to those of control patients. A higher live birth rate could be seen in primary and tertiary aborters who were immunized. This difference was statistically significant ($P = 0.0015$ and 0.002 for primary and tertiary aborters respectively). Immunization had no apparent effect on the subsequent live birth rate in secondary aborters. In fact, the non-immunized secondary aborters fared slightly better than the immunized patients, but this difference was not statistically significant. In the randomized trials, the 25% benefit seen in primary aborters was not statistically significant because of the small sample size. When the randomized and non-randomized trials were taken together, there was a 24% benefit seen in immunized patients. This difference was statistically significant ($P = 0.01$, Fisher’s exact test). When the results from our centre were added, the data reached a higher level of statistical significance ($P = 0.0015$). Figure 2 summarizes the relative risk in each of the subgroups. Again, the significant difference in live birth rates between the immunized and control groups for primary and tertiary aborters, but not for secondary aborters, was reflected. The relative risks were 2.04 [confidence interval (CI) 1.24–3.58] and 2.92 (CI

Table II. Details of patients with five or more abortions included in the study

<table>
<thead>
<tr>
<th></th>
<th>Tel Aviv register</th>
<th>Randomized register</th>
<th>Non-randomized register</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunized</td>
<td>Controls</td>
<td>Immunized</td>
</tr>
<tr>
<td>No. of patients</td>
<td>75</td>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td>Mean ± SD age (years)</td>
<td>33.26 ± 5.25</td>
<td>32.26 ± 4.69</td>
<td>31.47 ± 4.26</td>
</tr>
<tr>
<td>Age (range) (years)</td>
<td>23–43</td>
<td>23–43</td>
<td>24–45</td>
</tr>
<tr>
<td>Mean ± SD no. of abortions</td>
<td>6.19 ± 1.49</td>
<td>6.26 ± 1.57</td>
<td>5.91 ± 1.30</td>
</tr>
<tr>
<td>No. of abortions (range)</td>
<td>5–11</td>
<td>5–12</td>
<td>5–9</td>
</tr>
<tr>
<td>Mean ± SD time taken to conceive (months)</td>
<td>7.53 ± 8.54</td>
<td>7.83 ± 8.56</td>
<td>5.57 ± 5.85</td>
</tr>
<tr>
<td>Time taken to conceive (range) (months)</td>
<td>1–47</td>
<td>1–46</td>
<td>1–26</td>
</tr>
</tbody>
</table>

Table III. Pregnancy outcome in patients with five or more miscarriages, divided into groups according to the order of abortions (see Table I)

<table>
<thead>
<tr>
<th></th>
<th>Tel Aviv register</th>
<th>Randomized register</th>
<th>Non-randomized register</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunized</td>
<td>Controls</td>
<td>Immunized</td>
<td>Controls</td>
</tr>
<tr>
<td>Primary aborters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live births</td>
<td>20 (55)</td>
<td>2 (17)</td>
<td>5 (55)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>12</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Secondary aborters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live births</td>
<td>9 (47)</td>
<td>7 (64)</td>
<td>0</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>11</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Tertiary aborters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live births</td>
<td>16 (80)</td>
<td>2 (17)</td>
<td>0</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>12</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

Values in parentheses are the proportion of live births.

* $P = 0.0015$.

Not significant.

$P = 0.002$. 

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The relative risk of a live birth after immunization, with 95% confidence intervals (log scale), for each subgroup. The significant beneficial effect of immunization in primary and tertiary aborters (Table III) is reflected in high relative risk values, whereas no such effect is seen in secondary aborters in whom no significant benefit of immunization was identified (Table III).

1.37–7.04) for primary and tertiary aborters respectively. The relative risk was also calculated for the randomized and non-randomized trials in the international register and found to be 1.94 (CI 1.12–3.35).

Discussion

All papers on this topic published to date have asked whether immunization improves the live birth rate in patients with three or more unexplained abortions. Using these criteria, the treatment effect is small and open to various interpretations. Therefore we chose to analyse the effect in patients with five or more abortions who were expected to have a poor prognosis. Women with five abortions, no APCA at initial testing and who were induced to produce APCA as a result of immunization is only produced at or near term in successful pregnancies. Hence, there may be factors other than those operating in primary and tertiary aborters causing abortion in at least some of these patients.

Patients with five or more abortions have been poorly described in the literature. Kwak et al. (1992) described the incidence of antiphospholipid antibodies and antinuclear antibody according to gravidity. Kilpatrick and Liston (1993) described 26 patients with five or more abortions, but did not relate this group to the outcome of leukocyte immunization. They did, however, calculate that the chance of five abortions occurring by chance was only 5%, as opposed to 48% in women with three or more abortions. Christiansen et al. (1994) described the human leukocyte antigen haplotypes in women with four or more abortions. Most epidemiological studies of recurrent miscarriage do not mention these patients as a separate group. No study has described the outcomes of subsequent pregnancies or the results of therapeutic trials. This is probably because these patients constitute only 20–25% of all recurrently miscarrying women. Even the international register contains only 81 patients with five or more abortions. The fact that our register contains a higher proportion of patients with five or more miscarriages than the other three registers may indicate that we have a population at a higher risk for subsequent abortions than most other centres, or that our patients do not easily give up hope. As these patients have a poorer prognosis than women with three abortions (Figure 1), they probably require treatment more than their counterparts with three miscarriages. We reasoned that such women are more likely to have a single cause for their abortions. Our preliminary results (Carp, 1993; Carp et al., 1993) and those of Daya and Gunby (1994) indicated that such women are more likely to respond to immunotherapy. As far as we are aware, no other form of treatment doubles or triples the live birth rate in patients with such a poor prognosis.

However, even this group, although selected for its poor prognosis, is not homogeneous, because various predictive factors affect the outcome of subsequent pregnancies in addition to immunization. These criteria include primary, secondary or tertiary aborter status and APCA. The tertiary aborter has been described previously only briefly (Carp, 1994). All other reports include these patients as secondary aborters. We classified tertiary aborters (those who have a live birth in the middle of a series of abortions) as a group separate from those women who start their obstetric careers with a live birth. In both this study and our previous study (Carp, 1994) we found that these patients responded differently to immunization than other secondary aborters; hence we felt justified in classifying them as a distinct group.

The use of APCA as a marker may be controversial. It is only present in ~20% of normal parous women’s serum and is only produced at or near term in successful pregnancies (Regan, 1988). In addition it is present in 20% of habitually aborting women (Carp et al., 1988; Smith and Cowchock, 1988). However, the international meta-analysis (RMITG, 1994) and we (Carp et al., 1990b) have reported that patients with APCA at initial testing have a 71% subsequent live birth rate without treatment. Consequently treatment is unnecessary, and these patients were excluded from the analysis. Their inclusion would have skewed the results, thus raising the live birth rate of the control patients. Serocoversion to APCA positive after immunization may also be controversial because APCA is not a prerequisite for a successful pregnancy. Other parameters may be better markers, e.g. mixed lymphocyte reactivity, flow cytometry or even the human embryonic antigen associated with spontaneous abortion (Shiraishi et al., 1995). However, the results of these other parameters were not available from the registers, making their effect impossible to analyse. Meanwhile, APCA remains the only parameter to be widely reported as associated with an improved outcome after immunization (Reznikoff-Etievant et al., 1988; Carp et al., 1990a; Carp, 1994; RMITG, 1994; Agrawal et al., 1995). Hence we felt justified in including this parameter despite reports of a worse prognosis in patients who seroconverted (Smith and Cowchock, 1988).
It was difficult to make the test group more homogeneous because we could not control for the other factors that influence the response to immunization, such as maternal age, time taken to conceive and follicular phase LH concentrations (Carp et al., 1995). Only four patients were reported to have a raised LH concentration in the international register, and the number of patients with a normal LH concentration was not quoted. We did not correct the figures for maternal age, because the patient with five or more abortions is more likely to be in the higher age group. However, Table II shows that the mean age and the age range were similar in all groups. We did not correct for the time taken to conceive because this cannot be foreseen prior to treatment and cannot therefore influence the decision as to whether to treat. The time taken to conceive was longer in our patients than in the two other registers (Table II). However, this was not reflected in the outcomes of the subsequent pregnancies. The results of our patients were certainly not worse than in the other groups.

Originally we intended analysing the randomized trials alone. However, this was not feasible because very few patients had complete details available as to whether they seroconverted. It is indicated in the primary and tertiary aborters. When the patients from our register were added, the results reached a higher level of significance \((P = 0.01)\). When the patients from our register were added, the results reached a higher level of significance \((P = 0.0015)\). Because these results included non-randomized trials, it is possible that there is some bias. However, the 25% benefit seen in primary aborters in the randomized trials was not altered substantially by adding those patients from the non-randomized trials and those included in our register (Table III), which justified our approach in pooling the data.

To rule out any bias from our centre, the relative risk was calculated for the international register as a whole (randomized and non-randomized) and found to be 1.94. The addition of our patients raised the relative risk to 2.5. Hence we believe it to be essential that when the results of more trials are added to the international register, they must include information on seroconversion.

It is therefore necessary to ask: what is the place of paternal leukocyte immunization in 1996? Because of the hitherto unproven efficacy of immunization, some workers have abandoned immunization altogether. Similarly, physicians with little experience of recurrent miscarriage have not referred patients or have even discouraged them from undergoing immunization. This approach has undoubtedly caused patients who could benefit from immunization to suffer many more miscarriages. Patients with 10 miscarriages have been denied treatment on the basis that the patient with three miscarriages has a good chance of a live birth in her fourth pregnancy. It may be more valid to continue treating patients and to attempt to more accurately define the population of women who can benefit. The hitherto used criteria of three or more unexplained miscarriages up to 20 weeks requires urgent revision. The patients, type of abortion and laboratory markers of immunization need to be defined more strictly. Assessors of future results will then be able to ask whether there is a subgroup of patients who benefit from immunotherapy. Prospective randomized trials can then be carried out in strictly defined and matched populations with a poor prognosis. Only such a trial will be able to define accurately the place of immunopotentiation (or any other form of treatment) in recurrent miscarriage. If we continue to ask whether immunotherapy increases the chance of a live birth in women with three or more abortions, we will only be able to conclude that immunotherapy is not a panacea. At present it seems that the patient most likely to benefit is the primary or tertiary aborter who is APCA negative. The patient whose immune parameters (such as APCA) change after immunization is more likely to benefit. However, booster immunizations may be necessary to induce seroconversion. It should also be borne in mind that allogenic immunization is the only form of treatment that has been assessed in these very high risk patients, and in our view it is indicated in the primary and tertiary aborters.

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References


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