Outcome of pregnancy in women with recurrent spontaneous abortion following immunotherapy with allogeneic lymphocytes

Suraksha Agrawal1,5, Raj Kishore1, A.Halder1, A.Sharma1, R.K. Sharma2, V. Das3, B.R.K.Shukla4 and S.S.Agarwal1

1Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute, Lucknow, 2Department of Nephrology, Sanjay Gandhi Post Graduate Institute, Lucknow, 3Department of Obstetrics and Gynaecology, KGMC, Lucknow and 4Department of Anthropology, University of Lucknow, India

5To whom correspondence should be addressed at: Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Post Box no. 375, Lucknow 226014, India

The efficacy of immunotherapy in the prevention of habitual abortion remains controversial. It has been suggested that the benefits are predominantly due to psychological factors. We have evaluated the success of pregnancy outcome following immunotherapy with allogeneic lymphocytes, in relation to the subsequent development of anti-paternal cytotoxic antibodies (APCA). It was observed that in women who developed an APCA titre of \( \geq 1:16 \), live births occurred in 16 out of 21 cases (76%), while only two out of seven (28%) women who failed to achieve an APCA titre of \( \geq 1:16 \) had successful pregnancies \( (P < 0.05) \). In eight women who had an APCA titre of 1:16 on initial screening, and were, therefore, excluded from the trial, successful pregnancy outcome was noted in 62.5% of cases. Although these results are based on a small sample and on an open, non-randomized trial, they show that the efficacy of immunotherapy is related to immune response to allogeneic lymphocytes, and is not simply a placebo effect. Measurement of APCA titre could serve as a marker for immunopotentiation.

Key words: anti-paternal cytotoxic antibody (APCA)/lymphocyte immunization/recurrent spontaneous abortion (RSA)

Introduction

Recurrent spontaneous abortion (RSA), defined as three or more consecutive abortions, occurs in up to 2% of couples in the reproductive age group (Mills et al., 1988; Carp et al., 1990a; Coulam, 1991). Although various genetic, anatomical, hormonal and infectious agents have been implicated as the causes of RSA (Stray-Pederson and Stray-Pederson, 1984; Carp et al., 1990a; Ecker et al., 1993), no cause has been identified in >50% of women with RSA (Beard, 1988; Carp et al., 1990b; Coulam et al., 1994a). It has been proposed by several investigators that failure of induction of the protective immune response could be responsible for habitual abortion in women with RSA without an identifiable cause (Mowbray et al., 1983; Beer, 1986; McIntyre et al., 1986; Gilman-Sachs et al., 1989; Cauchi et al., 1991; Clark and Daya, 1991; Carp et al., 1992). In animal models there is good evidence for partner-specific recurrent miscarriages. Failure of intrauterine induction of protective immunity can be overcome by more effective presentation of paternal antigens (i.e. by alloimmunization against specific antigens) or by other immunotherapeutic approaches (Chaouat and Iankar, 1988, Carp et al., 1990a, Clark, 1991; Coulam et al., 1994b). Embryo rejection in animal models appears to depend upon activated natural killer (NK) cells rather than on antigen specific effector cells. It has been shown in the animal model that granulocyte macrophage colony stimulating factor (GM-CSF) may prevent spontaneous abortion by prevention of NK cells and in humans CD 56+ lymphoid cells secrete a novel transforming growth factor, \( \beta_2 \), which has been shown to be immunosuppressive in nature (Clark et al., 1994a,b). Activation of the maternal immune system suppresses NK cells (Clark et al., 1991; Clark et al., 1994a). It has been reported that rejection mechanisms are, in fact, modifiable by alloimmunization (Mills et al., 1988; Labrere, 1989; Michel et al., 1989). It is on this premise that immunization with paternal lymphocytes has been used to prevent further miscarriages (Mowbray et al., 1985; Ho et al., 1991; Carp et al., 1992). Most immunotherapy trials have shown a success rate of \(~70\%\) (Mowbray et al., 1985; Clark, 1991).

However, these data have become controversial since the simultaneous success rate of pregnancy in the control group has ranged from 29 to 79\% (Cowchock et al., 1990; Coulam and Coulam, 1992; Christiansen et al., 1994). It has been reported by Christiansen et al. (1994) that active immunization does not provide any overall benefit in the group of women with miscarriage; however, it may improve the outcome with respect to the number of live births among women with primary recurrent abortion. These reported variations in results have been attributed to small sample size, heterogeneity of patient population and variability in treatment protocols (Carp et al., 1990a; Cowchock et al., 1990; Clark and Daya, 1991; Coulam and Clark, 1991; Carp et al., 1992; Hughes, 1992; Coulam, 1993; Fraser et al., 1993). However, the possibility of a placebo effect cannot be excluded (Coulam, 1991; Coulam and Clark, 1991).

We now present our experience with a group of women who were given similar supportive treatment, which demonstrates a higher success rate (76\%) in the adequately immunized group compared with those who either failed to develop anti-paternal cytotoxic antibodies (APCA) (23.7\%) or those who opted out of the trial (18.42\%).

Materials and methods

A total of 115 women with three or more consecutive abortions were seen at the Queen Mary Hospital and Genetics Clinic, Lucknow,
India, from 1988 to 1992. All underwent the following investigations: (i) karyotype of both the spouses; (ii) serology for toxoplasma; (iii) glucose tolerance test; (iv) hysterosalpingogram; (v) thyroid function test; and (vi) luteal phase plasma progesterone concentrations. Those who were found negative for the above tests were investigated for anti-paternal cell cytotoxic antibodies (APCA). Only those women who were negative for APCA and did not have an identifiable cause for RSA were considered eligible for immunotherapy with allogeneic lymphocytes. The details of the protocol approved by the ethical committee of the Institute were explained to all eligible couples and only those who gave written, informed consent were included in the study.

**APCA assay**
The presence of APCA was detected by a crossmatch between maternal serum and paternal peripheral blood leukocytes (PBL), using the extended National Institutes of Health (NIH) protocol and serum serially diluted to 1:64. Crossmatching was carried out at room temperature, 22°C, 4°C and 37°C against total mononuclear cells, T cells and B cells. A positive result was recorded when ≥50% cell death was observed at a serum dilution of 1:16 or greater.

**Immunization protocol**
The lymphocytes to be used for immunization were obtained from 10 ml of peripheral blood collected in heparin. All procedures were carried out under strict aseptic conditions employing plastic disposables and a vertical laminar flow hood. The mononuclear cells were isolated on a Ficoll-Hypaque density gradient. They were washed three times with Roswell Park Memorial Institute (RPMI) 1640 medium and were finally adjusted to 5×10^6 cells/ml. An aliquot of the final preparation was sent for microbiological testing. A total of 5×10^6 cells were injected intradermally, under medical supervision, at three separate sites in the forearm of the women. Immunization was repeated at four-weekly intervals up to a maximum of six times. Each immunization was followed by analysis for APCA at the time of the next immunization. Immunization was stopped when a titre of ≥ 1:16 was achieved for APCA. The husband was tested for Rhesus factor (Rh), hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) antibodies. A history of penicillin allergy was taken from all the recipients, since the medium in which the cells were finally suspended contained penicillin.

**Statistical tests**
The χ^2 test was used to compare the outcome in different groups. The SPSS/PCT statistical package for IBM PC was used to calculate logistic regression to consider the effect of serum titres other than 1:16 for statistical validity.

**Results**
No cause for habitual abortion was identified in 83 women with RSA. Eight of them were found to be already positive for APCA, and so were excluded from this trial (group 1). Of the remaining 75 women who were given information about the trial, 37 declined to enter the trial. Of these, 11 women were from out of station and could not be followed-up. The remaining 26 women were taken as one of the control groups (group 2). A total of 38 women volunteered for the study. Of these, seven dropped out of the trial after receiving between one and three immunizations (group 3); they were APCA negative at the last testing.

Nine women failed to develop an APCA titre of ≥ 1:16 (group 4). A total of 22 women developed an APCA titre of ≥ 1:16 after receiving between two and six immunizations (group 5). The number of immunizations required for the development of an APCA titre of ≥ 1:16 was variable (Table I). The control groups 3 and 4 were offered supportive therapy similar to that offered to the immunized group. The clinical characteristics of various groups included in the study are summarized in Table II.

Of the 22 women who developed an APCA titre of ≥ 1:16 (group 5), 21 became pregnant within 6 months of immunotherapy. The pregnancy was confirmed by a pregnancy test in each case. In this group, 16 women gave birth to a full term, healthy child, while five had five miscarriages. Thus, a success rate of 76% was observed in adequately immunized women. All eight women in group 1 conceived; five gave birth to a healthy child (62%) and three had a repeat abortion. Of the 26 women in group 2, 18 conceived during the observation period. Four of them delivered a healthy, full term child (22%) while 14 aborted in the current pregnancy as well. Four women in group 3 conceived, two gave birth to normal babies and two had repeat abortions. In group 4, seven women conceived; two gave birth to a healthy baby and five had abortions (Table III).

The difference in the outcome of pregnancy in the successfully immunized group (group 5) compared with that in the group which opted to remain out of the trial (group 2) was statistically significant (P <0.001). Within the immunized group, the rate of successful pregnancy in the women who did not develop an APCA titre of 1:16 (group 4) was significantly lower than that observed in group 5 (P <0.005). The significance of each APCA titre value was calculated by logistic regression and a titre value of 1:16 was taken as a cut-off value for all comparisons. Comparison of group 5 with group 1 (APCA positive at initial screening) did not reveal any significant difference (P >0.05). Group 3, consisting of seven women who dropped out of the study, has not been considered for comparison, because they did not complete the treatment (immunotherapy).

**Discussion**
This study highlights the benefit of immunotherapy with allogeneic lymphocytes in women with primary RSA (i.e.
Table II. Clinical characteristics of successfully immunized and control groups at the time of inclusion in the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>Successfully immunized group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>group 1</td>
<td>group 2</td>
</tr>
<tr>
<td>n = 8</td>
<td>n = 26</td>
<td>n = 7</td>
</tr>
<tr>
<td>Age</td>
<td>23.8 ± 0.9</td>
<td>26.8 ± 1.0</td>
</tr>
<tr>
<td>Previous abortions (first trimester)</td>
<td>3.2 ± 0.8</td>
<td>3.09 ± 0.4</td>
</tr>
<tr>
<td>Live births</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Family history of RSA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other treatments</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prior blood transfusions</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Socio-economic status</td>
<td>higher</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>lower</td>
<td>3</td>
</tr>
<tr>
<td>Time from last abortion (months)</td>
<td>2.0 ± 0.6</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>Partner</td>
<td>Single</td>
<td>Single</td>
</tr>
</tbody>
</table>

There was no statistically significant difference in any of the parameters between different groups.

Higher income group >4000 rupees per month; middle income group 1500-4000 rupees per month; lower income group <1500 rupees per month.

Table III. Pregnancy outcome in women with recurrent spontaneous abortion (RSA) undergoing immunotherapy with allogeneic lymphocytes and non-immunized control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Confirmed pregnancy</th>
<th>Subsequent abortion</th>
<th>Live births</th>
<th>Success proportion*</th>
<th>Significance compared with group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-immunized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. APCA +ve at initial screen</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>0.62</td>
<td>NS</td>
</tr>
<tr>
<td>2. Declined to enter trial but available for follow up</td>
<td>26</td>
<td>18</td>
<td>14</td>
<td>4</td>
<td>0.22</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>3. Failed to develop APCA titre of ≥1:16</td>
<td>9</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>0.28</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>4. Developed APCA titre &gt; 1:16</td>
<td>22</td>
<td>21</td>
<td>5</td>
<td>16</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Immunized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Calculated as live births/confirmed pregnancies.
NS = not significant.

where no cause was demonstrable) who successfully converted from APCA negative to APCA positive status with a titre of ≥ 1:16 following immunization. The effects of age, parity and interval between immunotherapy and pregnancy were evaluated by logistic regression, but none of these factors had any effect on the outcome of pregnancy in the treated group (Table III). However, the data set is too small to calculate the effect of these factors by multivariate analysis. The success rate of 76% in the immunized women is in accordance with the results of other investigators in this field (Carp et al., 1990a; Coulam et al., 1994a; Illeni et al., 1994), and re-evaluated with reference to APCA status. Besides the above, other variables such as a trend towards fewer prior abortions, the interval between immunotherapy and pregnancy and the age at index pregnancy could also be important determinants. More studies on larger numbers and multi-variate analysis is required.

Interactions between the fetoplacental unit and maternal immune system are not very well characterized. While the development of APCA may be taken as an appropriate marker for maternal recognition of fetoparental antigens, its absence is of questionable significance as only 20-50% of multiparous women develop cytotoxic antibodies against their husband’s lymphocytes (Dudley and Branch, 1989). Similarly, the role of blocking antibodies is not very well established. Thus, more information is required about the cellular immune responses.
Immunotherapy in recurrent spontaneous abortion


Stray-Pederson, B. and Stray-Pederson, S. (1984) Etiologic factors and precision of the methodology used for measuring various factors like APCA and MLR-bf. The need for a well matched, randomized, placebo controlled, multi-centre trial is obvious (Coulam et al., 1994a).

The importance of psychotherapy (Stray-Pederson and Stray-Pederson, 1988) associated with immunotherapy cannot be excluded. In this context, the women in this study who have elected to undergo the trial, and to continue on to six immunizations even if the first three have been ineffective, could be considered to be a group of self-selected ‘believers’. However, the relationship of APCA titres = 1:16 with the pregnancy outcome seen in the present study is a point against the impact of psychological factors alone in determining the outcome of immunotherapy for prevention of recurrent abortion. Further studies, therefore, should incorporate appropriate immunological studies, besides being double blind and randomized. Although in our limited experience we have not come across any side effect of immunotherapy, a careful evaluation of all possible side effects (Takakuwa et al., 1989, 1990; Coulam and Clark, 1991; Kats et al., 1992) is essential to establish the utility and safety of the procedure in clinical practice.

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