Update on Treatment of Immunologic Abortion With Low-dose Intravenous Immunoglobulin

By Raphael B. Stricker, MD, and Edward E. Winger, MD

Problem
Recurrent spontaneous abortion associated with immunologic abnormalities has been termed immunologic abortion. Previously we showed that treatment with low-dose intravenous immunoglobulin (IVIG) appears to be beneficial for older women with immunologic abortion. We now report the results of IVIG treatment in a larger group of women with this disorder.

Method of Study
A total of 99 women were prospectively evaluated for immunologic abortion, which was defined as three or more miscarriages and the presence of specific immunologic abnormalities. Prior to the next conception, patients were treated with IVIG at a dose of 0.2 g/kg. Once conception was achieved, IVIG treatment was continued on a monthly basis through 26–30 weeks of pregnancy.

Results
The average age of the women was 37 years (range: 28–49), and the average number of miscarriages was 3.8 (range: 3–12). Of the 99 women, 72 received initial IVIG treatment, and 50 subsequently became pregnant. Of these women, 42 (84%) had a successful term pregnancy. Of the 27 women who refused IVIG therapy, 20 became pregnant and 18 (90%) miscarried. The difference in pregnancy success rate between the IVIG-treated and untreated groups was significant (P = 0.001). Four women had mild allergic reactions during IVIG infusion, and these reactions resolved when the IVIG brand was changed. Fetal abnormalities were not observed.

Conclusion
We conclude that low-dose IVIG therapy is safe and effective for older women with immunologic abortion.

Introduction
Recurrent spontaneous abortion (RSA) is a growing problem in our society, particularly among women over 30 years of age.1–3 In these women, RSA occurs with both natural and artificial fertilization techniques.4,5 There is increasing evidence that immunologic factors play an important role in RSA.6–11 These factors include various humoral abnormalities [antiphospholipid antibodies, antithyroid antibodies, antinuclear antibodies, antiovarian antibodies, and increased immunoglobulin (Ig)M levels] as well as cellular components (increased natural killer cells and decreased suppressor T cells). The immunologic factors may be associated with toxicity to the trophoblast, placenta or fetus, leading to recurrent pregnancy loss.12–16 The association of RSA with immunologic abnormalities has been termed immunologic abortion. Treatment of immunologic abortion has been controversial.1–3,17–29 Previously we showed that low-dose intravenous immunoglobulin (IVIG) therapy was beneficial for older women with immunologic abortion.29 We have now examined the efficacy of low-dose IVIG therapy in a larger group of women with this disorder.

Materials and Methods
Patient Selection
Ninety-nine women were prospectively enrolled in the study. Eighty-three of these women were described in a previous study.29 Entry criteria included a history of three or more unexplained miscarriages and aged >28 years. There was no upper age limit for the study participants. Women using both natural and in vitro fertilization (IVF) techniques were included in the study. Anatomic, infectious, and hormonal causes for RSA were excluded, and male factor was also ruled out.

Women were screened with a battery of immunologic tests including antiphospholipid antibody (IgG, IgA, and IgM), antimicrosomal antibody, antithyroglobulin antibody, antinuclear antibody, antiovarian antibody, and serum immunoglobulin levels, as previously described.29 CD4 and CD8 T-cell counts and CD3-negative/CD56-positive natural killer cell levels were determined using flow cytometry.29 An abnormal result of one or more of these tests was required for inclusion in the study. Antiphospholipid antibody testing was performed by Genetics & IVF Institute (Fairfax, VA, USA) or Reproductive Immunology Associates (Van Nuys, CA, USA). All other immunologic testing was performed by Immunodiagnostic Laboratories.
women with endometriosis were included in the study following appropriate treatment for this disease.

Treatment Protocol

Women were treated with IVIG (Venoglobulin-S; Alpha Therapeutic Corporation, Los Angeles, CA, USA or Gamimune-N; Bayer Biologics, West Haven, CT, USA) after informed consent was obtained. The consent form was approved by the Institutional Review Board of California Pacific Medical Center. IVIG therapy was initiated within 2 weeks prior to attempted conception either by natural means or IVF. The dose of IVIG was 0.2 g/kg per treatment, and the rate of infusion did not exceed 75 cc/hr. Once conception was achieved, IVIG was given every 4 weeks through 26–30 weeks of gestation. Thus on average, eight IVIG treatments were administered during a term pregnancy. Routine high-risk obstetric care including periodic uterine ultrasonography, amniocentesis, and fetal heart monitoring was provided in all cases. Patients were also monitored for side-effects of the IVIG therapy. Statistical analysis was performed using the unpaired Student’s t-test for parametric variables.

Results

Patient characteristics are shown in Table I. The mean patient age was 37 years with a range of 28–49, and the median age was 37 years. The mean number of prior abortions was 3.8 with a range of 3–12, and the median number of abortions was 3. Among the women enrolled in the study, 83% had never had a successful pregnancy (primary recurrent abortion) while 17% had one prior successful pregnancy (secondary recurrent abortion). Twenty-four women (24%) used natural fertilization methods while 75 (76%) used IVF techniques.

Immunologic abnormalities in the study subjects are shown in Table II. The most common abnormality was the presence of antithyroid antibodies (54%), followed by antiphospholipid antibodies (34%), increased natural killer cells >12% of total lymphocytes (29%), antinuclear antibodies (26%), increased IgM level (20%), increased CD4/CD8 T-cell ratio (13%), and antiovarian antibodies (12%). In addition, IgA deficiency was found in two patients, and seven patients had endometriosis. In 67 of 99 patients (68%), more than one immunologic abnormality was detected. In particular, increased IgM levels were always associated with some other abnormality, particularly the presence of antiphospholipid antibodies and antithyroid antibodies. Patients with increased CD4/CD8 T-cell ratios had normal levels of CD4 T cells but decreased CD8 T cells. Further testing in these patients revealed low or absent suppressor/cytotoxic (CD57) CD8 T cells.

The pregnancy outcomes of the study are illustrated in Fig. 1. Of the 99 patients, 72 underwent IVIG therapy while 27 patients refused treatment. Of the 72 treated patients, 50 became pregnant and had evaluable pregnancy outcomes. There was no difference in mean age, number of prior abortions, use of IVF therapy or type of immunologic abnormalities between the women who became pregnant and those who did not (data not shown). Of the 50 pregnant women, 44 received IVIG therapy (or intended to receive it) for 26–30 weeks of gestation. Of these patients, 38 (86%) had a term pregnancy while six patients miscarried at 7–9 weeks of gestation. The karyotype of the abortus was not determined in these patients. Six patients discontinued IVIG therapy after 10–12 weeks of gestation, and four of these women (67%) had successful pregnancies. The other two patients discontinued treatment at 10 weeks and miscarried at 15–16 weeks of gestation. The karyotype of the abortus was normal in one of these women. Twin pregnancies occurred in six (14%) of the IVIG-treated women.

Of the 27 patients who refused IVIG therapy, 20 patients subsequently became pregnant and 18 (90%) had first-trimester miscarriages. The overall pregnancy success rate in the IVIG-treated group (84%) compared with the untreated group (10%) was statistically significant (P=0.001). There was no difference between the treated and untreated women in terms of mean age, number of prior abortions, use of IVF therapy or type of immunologic abnormalities (data not shown).

Table I Patient Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>99</td>
</tr>
<tr>
<td>Mean age, years (range)</td>
<td>37 (28–49)</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>37</td>
</tr>
<tr>
<td>Mean number of abortions, n (range)</td>
<td>3.8 (3–12)</td>
</tr>
<tr>
<td>Median number of abortions</td>
<td>3</td>
</tr>
<tr>
<td>Number of patients (%) with:</td>
<td></td>
</tr>
<tr>
<td>primary recurrent abortion</td>
<td>82 (83)</td>
</tr>
<tr>
<td>secondary recurrent abortion</td>
<td>17 (17)</td>
</tr>
<tr>
<td>In vitro fertilization (%)</td>
<td>75 (76)</td>
</tr>
<tr>
<td>No in vitro fertilization (%)</td>
<td>24 (24)</td>
</tr>
</tbody>
</table>
Side-effects of IVIG therapy are shown in Table III. Four patients (8%) had adverse reactions during the IVIG infusion. These reactions occurred toward the end of the infusion, and they were characterized by cold sensation followed by chills, nausea, and vomiting. Vital signs were stable during the reaction, which subsided within 20 min of stopping the IVIG infusion. These reactions were associated exclusively with Venoglobulin-S, and they did not occur when Gamimune-N was substituted for the other IVIG brand.

Other side-effects included headache in 12% of patients. This symptom usually occurred several hours after the IVIG infusion, and no evidence was found for aseptic meningitis. Preterm labor occurred in 8% of patients and responded to conventional therapy. One patient who underwent IVF had both an intrauterine and an ectopic pregnancy. The ectopic site was resected, and the intrauterine pregnancy was successful. There was no evidence of intrauterine growth retardation or fetal abnormalities in any of the women treated with IVIG.

Discussion

Recurrent abortion is a growing problem in industrialized countries where women are delaying childbearing into their 30s and 40s. As gestational age increases, various immunologic abnormalities that interfere with successful pregnancy become common.30-32 These immunologic abnormalities appear to be caused by a shift in the immune response away from the so-called Th2 (humoral) pattern that promotes pregnancy toward the so-called Th1 (cellular) pattern that is deleterious to reproductive outcome.31,32 This shift may be an adjustment of the immune response from the ‘reproductive mode’ of younger women to the ‘pathogen-defense mode’ of older women.30 Because of the postulated immune rejection associated with RSA, the term ‘immunologic abortion’ has been used to describe women with RSA and various immunologic abnormalities. Although IVF treatment has been advocated for these women, the success rate with IVF has only been on the order of 16–24%.5,26 Limited success with IVF may be due in part to the same immunologic factors that interfere with natural pregnancy in these women.26

Treatment for immunologic abortion has been controversial. The initial association with the lupus anticoagulant syndrome and antiphospholipid antibody, which promotes vascular thrombosis, prompted the use of anticoagulant strategies using aspirin and heparin.17,18 Although this approach has been successful in about 50% of cases, significant bleeding occurs in about 16% of women, and fatal hemorrhage has been reported in at least one patient.19,20 Subsequent recognition of other immunologic factors prompted the use of immunomodulatory treatments for women with recurrent miscarriages. Corticosteroid

### Table II Immunologic Abnormalities in 99 Study Subjects

<table>
<thead>
<tr>
<th>Test result</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiphospholipid antibodies</td>
<td>34</td>
</tr>
<tr>
<td>Antithyroid antibodies</td>
<td>54</td>
</tr>
<tr>
<td>Antinuclear antibodies</td>
<td>26</td>
</tr>
<tr>
<td>Antiovarian antibodies</td>
<td>12</td>
</tr>
<tr>
<td>Increased natural killer cells</td>
<td>29</td>
</tr>
<tr>
<td>Increased IgM level</td>
<td>20</td>
</tr>
<tr>
<td>Increased CD4/CD8 T-cell ratio</td>
<td>13</td>
</tr>
<tr>
<td>IgA deficiency</td>
<td>2</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>7</td>
</tr>
</tbody>
</table>

Note that 67 of 99 patients (68%) had more than one immunologic abnormality. Ig, immunoglobulin.

### Table III Side-effects of Intravenous Immunoglobulin (IVIG) Therapy During 50 Pregnancies

<table>
<thead>
<tr>
<th>Side-effect</th>
<th>Positive, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion reactions</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Headache</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Preterm labor</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Intrauterine growth retardation</td>
<td>0</td>
</tr>
<tr>
<td>Fetal abnormalities</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 1 Pregnancy outcomes in 99 study subjects. Difference in pregnancy outcome between intravenous immunoglobulin (IVIG)-treated and untreated patients was significant ($P = 0.001$).

IVIG therapy

- IVIG x 26 - 30 Week
  - Term 38 (86%)
- IVIG x 10 - 12 Week
  - Term 4 (67%)

No IVIG therapy
- Term 2 (10%)
- P = 0.001
therapy has been shown to be ineffective for immunologic abortion, and this treatment is associated with numerous complications during pregnancy, especially preterm delivery.\textsuperscript{21} Allogeneic lymphocyte immunization (ALI) usually involving maternal immunization with paternal lymphocytes has been used successfully in some women with immunologic abortion.\textsuperscript{22,23} However the overall response rate has not been encouraging, and ALI has been associated with severe allergic reactions and painful scarring at the immunization site. The procedure is also non-standardized and labor-intensive.

IVIG therapy for immunologic abortion has also been controversial.\textsuperscript{29,33} Several studies have shown significant benefit of IVIG treatment in women with recurrent miscarriages, while other studies have failed to confirm this beneficial effect.\textsuperscript{29} A major yet often unrecognized problem with the latter studies involves poor patient selection, with deliberate exclusion of older women.\textsuperscript{29} The resultant comparison between younger women who have a high pregnancy success rate without any treatment has significantly biased the outcome of these studies against IVIG therapy.\textsuperscript{29,33} Other problems include irrational or excessive IVIG regimens and inadequate patient screening for immunologic abnormalities.\textsuperscript{29} These concerns have yet to be addressed in a large multicenter trial.

The present report extends and confirms the results of our previous study of low-dose IVIG therapy for women with immunologic abortion.\textsuperscript{29} Our study included only women over 28 years of age; 98% of the women were over 30, and 35% were over 40. Each woman had experienced at least three miscarriages, and every subject was screened for immunologic abnormalities. Antithyroid antibodies were again found to be the most common immunologic abnormality in these patients, followed by antiphospholipid antibodies and increased natural killer cells. Furthermore, 68% of the women were found to have more than one abnormal immunologic test. In contrast, the incidence of immunologic abnormalities in women with normal reproductive outcomes is reportedly <10%.\textsuperscript{7,8,11} The presence of multiple immunologic aberrations in our patients reinforces the concept of immunologic abortion and the need for rational immunomodulatory treatment for this disorder.

Previous IVIG regimens for immunologic abortion have often used excessive amounts of IVIG in nonphysiologic treatment intervals.\textsuperscript{2,4,28} Since the half-life of IVIG is about 23 days, treatment every 4 weeks should be adequate for immune modulation. Furthermore, the immunomodulatory effect of IVIG appears to be qualitative rather than quantitative.\textsuperscript{34-37} Although the standard dose of IVIG used in many protocols is 0.4 g/kg per treatment, we and others have postulated that a lower dose of IVIG (0.2 g/kg per treatment) should be effective in modulating the ‘Th1 to Th2 switch’ necessary for successful pregnancy.\textsuperscript{25,29,36,37} Although the exact mechanism of IVIG therapy is still not understood, modulation of lymphocyte reactivity and cytokine production is probably at the core of the immune response to IVIG.\textsuperscript{34-37} Thus, the low dose of IVIG used in our patients should be adequate for immune modulation in pregnancy. The response to this treatment appeared to be significant, as illustrated in Fig. 1.

IVIG therapy was continued through the end of the second trimester in most patients. The rationale for this length of treatment is based on studies showing a 25% abortion rate in the second trimester for women with immunologic abnormalities.\textsuperscript{7,8} Indeed, in the women who discontinued IVIG after the first trimester, two of six (33%) miscarried. As IVIG is relatively expensive, shorter treatment courses for immunologic abortion would certainly be attractive. Although our study was too small to evaluate this issue, the high success rate with longer treatment suggests that the 6-month regimen should remain the standard, particularly in older women with limited pregnancy potential, pending larger trials of a short-course IVIG protocol.

In general, IVIG was well tolerated. A stereotypical infusion reaction was seen in 8% of patients. This reaction could be avoided by changing the brand of IVIG, suggesting that it was probably due to a brand-specific preservative in the IVIG preparation. None of the patients discontinued IVIG therapy because of this side-effect. Significant toxicity to mother or fetus was not seen in our study. Although renal insufficiency caused by a sugar stabilizer has been associated with high-dose IVIG therapy\textsuperscript{38} this complication did not occur with the low-dose IVIG regimen used in the study. IVIG was always administered by slow infusion, and rate-related reactions to the IVIG were not encountered.

In the present study, 29% of patients failed to become pregnant after testing for immunologic abnormalities. It is possible that other factors contributed to failure of conception in these women, and the variability of fertilization success underscores the difficulty in evaluating IVIG therapy in this older female population. The possibility that different IVIG strategies in conjunction with IVF treatment may be more useful in these women also merits consideration.\textsuperscript{29} Our study represents a cohort-controlled trial that was prospective but not randomized. It has been shown that cohort-controlled trials do not produce a bias toward a treatment effect when compared with
randomized controlled trials and the results of randomized and non-randomized studies appear to be similar.\cite{40,42} Given the chronic shortages of IVIG products, the expense of IVIG therapy and the reluctance of women to be randomized to placebo treatment during pregnancy, it is uncertain whether appropriately randomized IVIG trials can be implemented. Our results require confirmation in larger groups of women with immunologic abortion.

In conclusion, the present study extends our previous observation that low-dose IVIG therapy is safe and effective for older women with immunologic abortion. Pending the results of larger controlled clinical trials, monthly administration of low-dose IVIG through the end of the second trimester of pregnancy appears to be the optimal treatment regimen for these patients.

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References

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