Massive immunoglobulin treatment in women with four or more recurrent spontaneous primary abortions of unexplained aetiology

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Introduction
As a possible alternative treatment to leukocyte immunization in patients with recurrent spontaneous abortion (RSA) due to unexplained aetiology, the administration of i.v. immunoglobulin (IVIg) has been performed by many investigators. However, the conclusions drawn from prospective, randomized, double-blinded, placebo-controlled trials are still controversial. No significant effect of IVIg in women who have had three or more primary miscarriages was found by The German RSA/IVIG Group (1994), although a high success rate (74.1%) was observed. Christiansen et al. (1995) were also unable to show any significant effect (success rate, 52.9%) in women with secondary RSA and recurrent second trimester pregnancy losses. On the other hand, Coulam et al. (1995) have shown the efficacy of IVIg in primary and secondary RSA women who have two or more miscarriages, enhancing the percentage of live births (62%) when compared with a placebo group.

Other IVIg trials have been carried out with RSA patients who were expected to have a poorer outcome in a subsequent pregnancy if left untreated, i.e. women who had experienced four or more consecutive abortions. Christiansen et al. (1992) administered IVIg in 11 RSA patients with four or more abortions, and nine of the women delivered healthy infants. In another study concerning immunoglobulin treatment in 10 RSA patients with five or more abortions, immunoglobulin was effective in preventing abortion in five women (Carp et al., 1996). However, these studies included patients with secondary RSA, following a live birth.

In order to minimize the heterogeneity of subjects with regard to prior abortions, only primary RSA patients with four or more abortions due to unexplained aetiology were admitted to this study, and women with a history of delivering live births were strictly excluded. We tried a high dose treatment of immunoglobulin, which has never been attempted before for RSA with unexplained aetiology. A total of 100 g of immunoglobulin was infused i.v. over a period of 5 days.

Materials and methods
Subjects were nine women, undergoing 11 pregnancies, with a history of four or more consecutive recurrent spontaneous abortions (RSA) with unexplained aetiology and no live births. They visited Hokkaido University Hospital, Sapporo, Japan, during the period between January 1993 and December 1996, and were admitted to this study if they met the following requirements: (i) a history of at least four fetal losses due to unexplained aetiology and no live births, and (ii) most of the abortions were in the first trimester. IVIg was given after informed consent was obtained.

All patients were subjected to ultrasound and hysterosalpingo-graphical examinations to detect anatomical abnormalities of the genital tract and cervical incompetency. Blood analyses for syphilis, anti-nuclear antibody (ANA), anti-DNA antibody, lupus anticoagulant, anti-cardiolipin antibody, anti-cardiolipin β2-glycoprotein I complex antibody and activated partial thromboplastin time (APTT) were performed. When a biological false positive for the serological test for syphilis (BFP-STS), positive test for lupus anticoagulant, anti-cardiolipin antibody or anti-cardiolipin β2-glycoprotein I complex antibody was detected, anti-phospholipid antibody syndrome was diagnosed and the patients were excluded from this study. If ANA or anti-DNA antibody was present, further serological tests, including cell, rheumatic factor, anti-SSA(B) antibody, anti-RNP antibody, anti-Sm antibody were performed, and complements were measured for...
the exclusion of definite autoimmune diseases. Karyotypic analyses of all couples were also performed.

None of the patients had anatomical abnormalities of the genital tract, cervical incompetency, syphilis infection, anti-phospholipid antibody syndrome, or autoimmune disease. No cytogenetic aberrations were detected in any of the couples, thus, all patients with RSA were of unexplained aetiology. Most of the previous abortions occurred in the first trimester. None of the patients had immunoglobulin (Ig) deficiency. Table I shows the patient characteristics. Cases 1 and 3 are of the same patient and cases 2 and 4 are the same patient.

Massive immunoglobulin treatment and RSA

In the treatment and outcome of previous pregnancy, prefix superscript represents the order of previous pregnancy; 3 = third pregnancy; 4 and 5 indicate the fourth and fifth pregnancy. If chromosomal analysis of the abortus was performed the karyotype is indicated in parentheses. Cases 1 and 3 are the same patient and cases 2 and 4 are the same patient.

Table II shows the outcome following MIVIg treatment. The pregnancies of cases 1 and 2 resulted in missed abortions at 6 and 7 gestational weeks respectively. Mosaicism of normal karyotype (46XX, 20%) and double trisomy (80%, 48XX, +16, +20) in case 1, and tetraploidy (92XXXX) in case 2, were found by chromosomal analyses of the aborti. The other nine pregnancies resulted in live births (30–40 gestational weeks). Case 6 developed latent hyperthyroidism (i.e. thyroid stimulating hormone (TSH) concentration of <0.1 µIU/ml, and free T4 concentration of 2.01 ng/dl) after the last abortion; propylthiouracil (50 mg/day) was given during gestational weeks 5–9. In case 7, an abnormal elevation in serum fibrinogen degradation product (FDP) occurred without any evidence of pregnancy-induced hypertension; administration of heparin sodium (10–000 IU/day) was started at gestational week 23. The pregnancy resulted in a preterm Caesarean section due to fetal distress and intrauterine growth retardation. Cases 8 (gestational weeks 12–30) and 10 (gestational weeks 4–17) received aspirin (80 mg/day) because an increase in APTT was observed during these periods. During MIVIg treatments, maternal rash was observed in one patient (case 3) and the patient received prednisolone (10 mg/day). No transmission of infection, such as acquired immunodeficiency syndrome (AIDS) or hepatitis was observed. Although one preterm delivery and three small-for-date babies (cases 7, 8, and 10) were observed, no adverse effects were evident in any of the nine neonates. Excluding the two abortions with chromosome aberrations, MIVIg treatment was effective in all nine pregnancies in this study.

Results

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Maternal serum concentrations of IgG increased significantly ($P < 0.0001$), although transiently, after immunoglobulin treatment (mean ± SD, before: 1404 ± 281, 1 week after: 3639 ± 601, 2–3 months later: 1371 ± 272 mg/dl). The concentrations of IgM (256 ± 153, 246 ± 172, 248 ± 197 mg/
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Table II. Outcome of the treated pregnancies

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gestational weeks of infusion</th>
<th>Other treatment</th>
<th>Complications</th>
<th>Outcome</th>
<th>Gestational weeks</th>
<th>Birth weight (g)</th>
<th>Apgar score (1’, 3’, 5’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4–5</td>
<td></td>
<td></td>
<td>Missed Ab (46XX/48XX, +16, +20)</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
<td>Missed Ab (92XX)</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>PSL, 10 mg/day</td>
<td>TA, TPD</td>
<td>Caesarean section</td>
<td>37</td>
<td>2650 (8-9-9)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td></td>
<td>Breech presentation</td>
<td>Caesarean section</td>
<td>40</td>
<td>3280 (8-9-9)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4–5</td>
<td></td>
<td>TA, Malrotation</td>
<td>Caesarean section</td>
<td>38</td>
<td>2820 (8-9-9)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4–5</td>
<td>PTU, 50 mg/day</td>
<td>TPD, IUGR</td>
<td>Vaginal delivery</td>
<td>38</td>
<td>2930 (8-9-9)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Heparin 10 000 IU/day</td>
<td>Elevation of FDP</td>
<td>Caesarean section</td>
<td>30</td>
<td>960, SFD (7-8-9)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>Aspirin 70 mg/day</td>
<td>TA</td>
<td>Vaginal delivery</td>
<td>40</td>
<td>2470, SFD (8-9-9)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td></td>
<td>Vaginal delivery</td>
<td>Caesarean section</td>
<td>38</td>
<td>3270 (8-9-9)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4–5</td>
<td>Aspirin 80 mg/day</td>
<td>Vaginal delivery</td>
<td>Caesarean section</td>
<td>38</td>
<td>2370, SFD (8-9-9)</td>
<td></td>
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<tr>
<td>11</td>
<td>5</td>
<td>Breech presentation</td>
<td></td>
<td>Caesarean section</td>
<td>40</td>
<td>3270, SFD (8-9-9)</td>
<td></td>
</tr>
</tbody>
</table>

PSL = prednisolone; PTU = propylthiouracil; TA = threatened abortion; TPD = threatened premature delivery; IUGR = intrauterine growth retardation; FDP = fibrinogen degradation product; SFD = small-for-dates baby.

dl) and IgA (212 ± 81, 186 ± 64, 173 ± 35 mg/dl) were unchanged. The serum concentrations of C3 (68.0 ± 12.0, 62.5 ± 10.2, 82.8 ± 13.3 mg/ml) increased 2–3 months following infusion (P < 0.05). The serum concentrations of C4 (27.1 ± 5.1, 14.2 ± 4.1, 29.8 ± 7.9 mg/ml) decreased transiently (P < 0.005), but CH50 (35.2 ± 9.3, 37.0 ± 7.0, 37.5 ± 10.9 IU/ml) remained unchanged. Following infusion, log2ANA/10 (3.6 ± 9.9, 12.2 ± 13.0, 5.7 ± 15.1) and anti-DNA antibody (1.1 ± 0.0, 5.0 ± 0.6, 1.3 ± 1.1 IU/ml) increased transiently (P < 0.05).

Discussion

RSA remains an enigma and its main aetiologies are endocrinological, immunological and unexplained. The scientific basis for many traditionally accepted causes of RSA is weak, and a diagnosis of RSA after relatively few miscarriages could be attributed to the random occurrence of consecutive chromosomally abnormal conceptions. However, recent findings of cytokines and immuno-endocrine factors taking place at the feto-maternal interface may provide new pathophysiological mechanisms for immunologically-mediated RSA (Balasch et al., 1996; Christiansen, 1996; Lim et al., 1996).

The criterion for treating RSA has usually been three or more unexplained pregnancy losses. In this instance, the subsequent live birth rate is ~54–75% in untreated women (Scott et al., 1987; Regan et al., 1988; Parazzini et al., 1988; Stirrat, 1990). In order to elucidate the efficacy of immunoglobulin treatment for RSA, we analysed a group of relatively homogeneous patients who experienced four or more consecutive abortions, with no history of a live birth. In five out of nine patients in this study, at least one karyotypically normal abortion had been confirmed prior to immunoglobulin treatment. In addition, paternal leukocyte vaccination (LV)-treated previous pregnancies in three patients had resulted in spontaneous abortions with normal karyotypes of the abortuses. Thus, our subjects were expected to have a poor prognosis. As a result, by MIVIg treatment nine out of 11 pregnancies resulted in live births, and the other two missed abortions had chromosomal abnormalities in which immunoglobulin could not be expected to alter the outcome.

In previous trials of immunoglobulin treatment for RSA with unexplained aetiology, the regimens of immunoglobulin infusion were as follows: (i) 400 mg/kg body weight in the follicular phase of the cycle, and another dose when pregnancy is confirmed (Carp et al., 1996); (ii) 30 g/person when pregnancy is confirmed, and 20 g/person every 3 weeks until 25 weeks gestation (German RSA/IVIG Group, 1994); (iii) 500 mg/kg body weight in the follicular phase of the cycle, and 500 mg/kg body weight every 4 weeks until 30 weeks gestation (Coulam et al., 1995); (iv) 30–40 g/person in gestational weeks 5 and 6, 20–30 g/person in gestational weeks 7 and 8 and every 2 weeks during gestational weeks 10–26, and 25–35 g/person every 2 weeks during gestational weeks 26–34 (Christiansen et al., 1995). When compared with the above regimens used in the previous studies, our administration protocol is different. The massive dose (20 g/day for 5 consecutive days, a total of 100 g) of immunoglobulin was infused during gestational weeks 4–7, mainly during gestational weeks 4–5, i.e. prior to the gestational age when the previous abortions occurred.

It is well known that MIVIg treatment has been effective for auto-immune thrombocytopenia in pregnant and non-pregnant patients (Wenske et al., 1983; Yamada and Fujimoto, 1995). The mechanism of the anti-thrombocytopenic effect is assumed to be through blockade of Fc receptors in an immune complex destruction site and down-regulation of anti-platelet antibody production. Regarding the mechanism of the possible anti-abortive effect of i.v. immunoglobulin infusion, the same or similar immune modulation can be postulated. The immune modulation by IVIg, including passive transfer of blocking or anti-idiotypic antibody (Brand et al., 1988), blockade of Fc receptor (Kimberly et al., 1987), enhancement of suppressor T cell function (Delfraissy et al., 1985), and down-regulation of B cell function (Nydegger, 1991), have been proposed as the mechanisms responsible for anti-abortive activity. Recently, it has been found that peripheral blood natural killer (NK) cells (CD56+ and CD56+/16+) and NK cell activity are effectively
suppressed after IVlg therapy, and down-regulation of NK cells and NK cell activity in women with RSA has been associated with a favourable pregnancy outcome (Kwak et al., 1996; Ruiz et al., 1996). If the immune modulation through infused immunoglobulin is critical and necessary for prevention of abortion, the massive dose may be more efficacious because serum concentrations of immunoglobulin are extremely high and the efficacy of MIVlg has been broadly accepted in pregnancies complicated by auto-immune thrombocytopenia. Additionally, starting MIVlg treatment at an early gestational age may be very important, since a previous study (German RSA/IVIG Group, 1994) has shown that the anti-abortive effect was significantly increased with early commencement of the infusion. In the present study, the IgG concentration increased up to 3639 ± 601 mg/dl by 1 week following the commencement. The increase of C3 2–3 months after IVlg treatment seems to be a physiological increase during pregnancy in accordance with a previous study (Schena et al., 1982). Although transient increases of ANA and anti-DNA antibody were observed, no symptoms concerning autoimmune diseases were found in each case entered in this study.

The scale of the present study is too small to make a conclusion on the efficacy of massive immunoglobulin treatment for RSA. The group of patients was never homogeneous with respect to other treatments given. However, excluding the two abortions with chromosome aberrations, MIVlg was effective in all nine pregnancies of women who had a history of four or more primary abortions with unexplained aetiology. Hence the efficacy of this treatment regimen (20 g/day, 5 days administered in the first trimester, mainly in gestational weeks 4–5) should be further evaluated in a multicentric, placebo-controlled randomized study employing a larger number of homogeneous patients at a high risk of first trimester abortion.


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References