Failure of prophylactic intravenous immunoglobulin to prevent sensitization to cryopreserved allograft tissue used in congenital cardiac surgery

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Objective: Cryopreserved allograft tissue used in the Norwood procedure for infants with hypoplastic left heart syndrome causes profound immunologic sensitization, which may complicate future transplantation. Intravenous immunoglobulin has been shown to reduce sensitization after it has developed, allowing successful transplantation. The purpose of this pilot trial was to determine whether intravenous immunoglobulin given before and after the procedure could prevent sensitization to cryopreserved allograft patches used in the initial repair of hypoplastic left heart syndrome.

Methods: Intravenous immunoglobulin (2 g/kg) was given preoperatively, 3 weeks postoperatively, and 4 months postoperatively to 7 infants undergoing the Norwood procedure. Panel-reactive antibodies were measured with flow cytometry preoperatively and at 1, 4, 6, and 12 months postoperatively and compared with values from a contemporary cohort of 12 infants undergoing the Norwood procedure who did not receive intravenous immunoglobulin.

Results: The groups were well matched for length and weight at time of surgery. Control infants were somewhat younger than the cohort receiving intravenous immunoglobulin (8 ± 5 vs 17 ± 14 days, P = .021). There were no differences in transfusion requirements. There was no difference in the degree of sensitization between control and intravenous immunoglobulin groups at 1 month (class I panel-reactive antibodies 20% ± 30% vs 4% ± 9%, P = .443, class II panel-reactive antibodies 17% ± 27% vs 20% ± 17%, P = .400), 4 months (class I panel-reactive antibodies 62% ± 40% vs 73% ± 41%, P = .813, class II panel-reactive antibodies 49% ± 42% vs 54% ± 41%, P = .706), and 12 months (class I panel-reactive antibodies 49% ± 42% vs 58% ± 39%, P = .686, class II panel-reactive antibodies 44% ± 36% vs 49% ± 42%, P = .651).

Conclusion: Despite studies showing intravenous immunoglobulin to reduce sensitization, we were unable to demonstrate that intravenous immunoglobulin prevented sensitization after exposure to allograft tissue in neonates undergoing congenital cardiac surgery.

It has been well documented that previous sensitization complicates solid organ transplantation. In cardiac transplantation, the presence of preformed anti-HLA antibodies, measured as panel reactive antibody (PRA), is associated with earlier and more frequent high-grade rejection, increased graft vasculopathy, and decreased survival. Importantly, Jacobs and colleagues recently reported that in pediatric transplantation (median age 130 days), a PRA level greater than 10% was associated with increased 30-day (25%) and long term (50%) mortality relative to those with a PRA level less than 10% (8% and 15%, respectively). Moreover,
Common causes of sensitization include previous transplantation, pregnancy, and allogeneic blood products. In addition, several case series as well as a prospective cohort study from our institution have demonstrated that the cryopreserved allograft tissue used in congenital cardiac surgery causes donor-specific sensitization, with class I and II PRA values approaching 100%. This is of particular concern for certain groups of infants, such as those with hypoplastic left heart syndrome (HLHS) undergoing the Norwood operation with cryopreserved allograft tissue. Although the results of this operation are improving, there remains concern that many of these children will eventually require cardiac transplantation.

Reasonable alternatives to cryopreserved allograft tissue are currently limited. Decellularization has been proposed to reduce the immunogenicity of allograft, and work in our laboratory has demonstrated that this does prevent sensitization in an animal model. Extensive testing, however, is required before this tissue is used in human beings. The use of typical immunosuppressive agents, although effective, is limited by long-term toxicity. Experience with kidney and cardiac transplantation has demonstrated that intravenous immunoglobulin (IVIG) can produce clinically significant and sustained reductions in anti-HLA antibody titers in some patients who have been previously sensitized, in turn allowing successful transplantation. Except for a recent trial of prophylactic IVIG in patients with left ventricular assist devices, there is a paucity of information regarding the use of IVIG to prevent sensitization in previously unsensitized individuals. Thus the purpose of this novel pilot study was to determine the efficacy of IVIG in preventing the development of anti-HLA antibodies in children undergoing repair of HLHS with allograft tissue.

Materials and Methods

Study Design

IVIG 10% (2g/kg) was administered 1 day preoperatively and 3 weeks and 4 months postoperatively in children undergoing surgical repair of HLHS with cryopreserved allograft tissue. PRA values for class I and II antibodies were assessed with flow cytometry preoperatively and at 1, 4, 6, and 12 months postoperatively and compared with a historical control group who had undergone repair of HLHS without IVIG. The study was approved by the local institutional review board for human research, and written consent was obtained from parents on behalf of the patients. This study was funded by the Stollery Children’s Hospital Foundation at the University of Alberta. The funding agency played no role in designing the study; in collecting, analyzing, and interpreting the data; in writing the report; or in making the decision to submit for publication.

Study Cohort

Seven infants with HLHS undergoing first-stage palliation (Norwood procedure) with a cryopreserved allograft pulmonary artery patch were studied. Allograft tissue was provided by comprehensive tissue centers at two Canadian University Hospitals (University of Alberta Hospital, Edmonton, Alberta, Canada, and Sick Kids Hospital, Toronto, Ontario, Canada).

Control Cohort

Data from 12 infants with HLHS who had previously undergone the Norwood procedure with an allograft patch to reconstruct the aortic arch 12 to 18 months before this study were reviewed. These 12 infants had been included in a previously reported study, which had been approved by the local institutional review board. No significant changes had been made in operative or perioperative management during this short interval, and thus temporal bias is presumed to be limited.

Intravenous Immunoglobulin

IVIG 10% (Gammunex, caplyte and chromatography purified, 2 g/kg) was administered 1 day preoperatively and 3 weeks and 4 months postoperatively. Because of the volume-sensitive physiology of these children, IVIG was administered during hospitalization: preoperative, 3 weeks postoperative, and at the time of the bidirectional cavopulmonary anastomosis procedure (4 months). The first 2 doses are consistent with the 3-week half life of IVIG and previously described protocols, the timing of the third dose obviated a separate readmission specifically for the study. IVIG was infused according to established guidelines and given over the course of at least 8 hours.

Variables

Preoperative variables to ensure similarity between the two groups included age, sex, length, and weight. Perioperative factors included durations of crossclamping and cardiopulmonary bypass, use of hypothermic circulatory arrest, and blood product exposure (amount and type).

Donor and Recipient HLA Typing

Donor and recipient class I and II HLA typing was tested by molecular methodology. Recipient DNA was purified from whole acid-citrate-dextrose–stored blood. HLA A, B, and DR antigen typings were performed with the low-resolution Micro SSPTM DNA typing kit (One Lambda Inc, Canoga Park, Calif). Donor DNA was purified from bone marrow or aciid-citrate-dextrose–stored blood. HLA A, B, and DR antigen typings were performed with the low-resolution Micro SSPTM DNA typing kit (One Lambda Inc, Canoga Park, Calif). DNA fragments were separated by agarose gel electrophoresis. HLA antigens were determined through a combination of One Lambda DNA/LMT software analysis and manual interpretation of the electrophoresis results.
TABLE 1. Patient demographic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>No IVIG</th>
<th>IVIG used</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>12</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Age at surgery (d)</td>
<td>8.2 ± 4.8</td>
<td>17.1 ± 14.6</td>
<td>.021</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>75%</td>
<td>71%</td>
<td>&gt;.999</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>50.5 ± 2.4</td>
<td>51.3 ± 3.4</td>
<td>.608</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.4 ± 0.4</td>
<td>3.3 ± 0.5</td>
<td>.734</td>
</tr>
<tr>
<td>Crossclamping time (min)</td>
<td>42.6 ± 27.7</td>
<td>31.1 ± 17.9</td>
<td>.310</td>
</tr>
<tr>
<td>Cardiopulmonary bypass time (min)</td>
<td>127.2 ± 59.7</td>
<td>123.4 ± 40.0</td>
<td>.899</td>
</tr>
<tr>
<td>Total circulatory arrest time (min)</td>
<td>28.0 ± 10.6</td>
<td>18.4 ± 10.9</td>
<td>.062</td>
</tr>
<tr>
<td>Packed red blood cells (units)</td>
<td>12.3 ± 9.6</td>
<td>10.6 ± 5.0</td>
<td>.966</td>
</tr>
<tr>
<td>Platelets (units)</td>
<td>4.8 ± 7.5</td>
<td>3.7 ± 4.6</td>
<td>&gt;.999</td>
</tr>
<tr>
<td>Fresh-frozen plasma (units)</td>
<td>1.2 ± 1.2</td>
<td>1.6 ± 1.0</td>
<td>.565</td>
</tr>
<tr>
<td>Cryoprecipitate (units)</td>
<td>3.8 ± 4.3</td>
<td>3.9 ± 3.2</td>
<td>.567</td>
</tr>
</tbody>
</table>

All values are mean ± SD. IVIG, Intravenous immunoglobulin.

Data and Statistical Analyses

All outcomes are expressed as mean ± SD. Comparisons between continuous data were made with the Mann–Whitney U test, and comparisons between nominal data were made with χ² or Fisher exact test as appropriate. All statistical analyses were performed with the Statistical Package for Social Sciences (version 13; SPSS Inc, Chicago, Ill).

Results

Patient demographic characteristics are summarized in Table 1. Except for the control group being somewhat younger (8.2 ± 4.8 vs 17.1 ± 14.6 days; P = .021), the two groups were well matched preoperatively. Most of the discrepancy in age resulted from a single infant in the IVIG group who had surgery delayed until 49 days. Cardiopulmonary bypass times were similar for the two groups (127.2 ± 59.7 vs 123.4 ± 40.0 minutes, P = .899), but the IVIG group required a marginally shorter circulatory arrest time (28.0 ± 10.6 vs 18.4 ± 10.9 minutes, P = .062). Both groups required similar amounts of blood products perioperatively, including packed red blood cells (12.3 ± 9.6 vs 10.6 ± 5.0 units, P = .966), platelets (4.8 ± 7.5 vs 3.7 ± 4.6 units, P > .999), fresh-frozen plasma (1.2 ± 1.2 vs 1.6 ± 1.0 units, P = .565), and cryoprecipitate (3.8 ± 4.3 vs 3.9 ± 3.2 units; P = .567).

The preoperative dose of IVIG was consistently given at 3.0 ± 2.4 days before surgery, the second dose at 22.4 ± 2.8 days after surgery, and the third dose during hospitalization for the bidirectional cavopulmonary anastomosis procedure (141.3 ± 28.3 days after initial surgery). IVIG was administered without adverse events.

Relative to control infants who did not receive IVIG, there were no significant differences in class I or II PRA values for those who did receive IVIG (Figures 1 and 2). Preoperatively (before the initial dose of IVIG), there were minor elevations in both class I and II antibodies, most likely reflecting maternally transmitted (passive) antibodies. One month postoperatively (1 week after the second dose of IVIG), there was evidence of a humoral immune response, with modest elevations in class I (19.7% ± 30.1% vs 4.5% ± 9.0%, P = .443) and class II (17.1% ± 27.5% vs 20.0% ± 17.1%, P = .400) PRA for the control and IVIG groups, respectively. Similarly, no significant differences were noted for the control and IVIG groups at 4 months (class I PRA 61.9% ± 39.9% vs 72.7% ± 41.1%, P = .813, class II PRA 49.3% ± 41.9% vs 53.8% ± 40.5%, P = .706) and 12 months (class I PRA 49% ± 42% vs 58% ± 39%, P = .686, class II PRA 44% ± 36% vs 49% ± 42%, P = .651). A more detailed review of the individual
PRA values at 4 months for recipients of IVIG revealed those with no response to IVIG, with PRA levels approaching 100%, and others with responses, with PRA levels ranging from 0% to 29% (Figure 3). Moreover, in a few patients, PRA appeared to decline after 4 months. HLA typing of donor and recipient confirmed that responders were mismatched for both class I and II antigens (Table 2).

Discussion
The Norwood operation has become the accepted standard of care for neonates with HLHS. Although alternatives have been reported, a cryopreserved allograft pulmonary artery patch has long been the preferred material to reconstruct the diminutive aorta in these infants. Despite previous beliefs that this tissue was immunoprivileged, recent investigations by our group and others have provided evidence that allograft tissues stimulate alloreactive immune responses. Human and animal studies have provided evidence for an intense T-lymphocyte response. In addition, others have demonstrated a humoral immune response in recipients of allograft tissue. The published frequency of development of anti-HLA antibodies (PRA) to cardiac valve allografts ranges from 78% to 100%, with PRA levels approaching 92%. In a prospective cohort study at our institution that compared PRA in infants receiving allograft tissue during the Norwood procedure with that in infants undergoing an arterial switch procedure and not receiving allograft tissue, we clearly demonstrated that children who received allograft tissue had PRA develop approaching 100%. Moreover, a substantial proportion of the antibodies were donor specific.

Although the results of the Norwood operation for HLHS are steadily improving, it is possible that many of these children will eventually require cardiac transplantation. It has been documented that the presence of antibodies in the serum of the allograft recipient significantly increases the risk of early allograft failure and poorer patient survival as a result of humoral rejection. Notable is a review by the United Network of Organ Sharing Registry of 14,535 heart transplants performed between 1987 and 1996; it demonstrated that an elevated PRA at transplantation significantly increased the relative risk of graft failure ($P = 0.0001$). Moreover, a PRA value greater than 60% was found to be associated with a 2.242 relative risk of graft failure. Similar findings have recently been reported in pediatric heart transplantation. Consequently, the presence of anti-HLA antibodies limits the ability to find a T-cell crossmatch–negative donor. Waiting times for a suitable allograft are thus considerably longer, and the mortality whilst waiting for a donor is high.

Despite extensive investigation, suitable alternatives to allograft tissue have yet to be identified. Glutaraldehyde-treated xenograft tissue tends to undergo rapid calcification and fail even more rapidly than allograft tissue, especially in children. Moreover, no material comes close to the handling properties of allograft tissue, for instance when attempting to reconstruct the aortic arch of a newborn infant. This latter concern is particularly true of synthetic material (eg, polytetrafluoroethylene and Dacron polyester fabric). Thus allograft tissue continues to play an essential role in congenital cardiac surgery. Numerous potential methods exist or are being investigated to reduce the immunogenicity of allograft tissues. Tissue matching (donor-recipient HLA match-
ing) would be a complex and expensive process. Decellularization techniques are currently being explored in a number of laboratories, including our own12; however, their use requires extensive additional investigation.

Altering the host with typical immunosuppressive agents (eg, cyclosporine [INN ciclosporin], mycophenolate mofetil) is effective but is limited by these agents’ well-documented short- and long-term toxicities.14 A recent study by Shaddy and associates13 demonstrated that mycophenolate mofetil (600 mg/m2 per dose) twice daily for 3 months substantially reduces anti–class I (but not anti–class II) antibodies. One patient withdrew after 2 weeks because of a sinus infection that was successfully treated with oral antibiotics, and 3 patients had a transient adverse effect of postoperative vomiting. Of note, this study used leukocyte-depleted blood products, (both filtered and irradiated products) which may have impacted the degree of antibody response. Blood in our study was not routinely irradiated. Long-term use of these agents in children is difficult to justify, despite efficacy.

IVIG is an alternative immunomodulatory agent demonstrated to be safe. Experience with kidney and cardiac transplantation has demonstrated that this agent can produce clinically significant and sustained reductions in anti-HLA antibody titers in individuals who have been previously sensitized, in turn allowing successful transplantation.15-18 A recent article by Glotz and colleagues18 reported the successful desensitization in 13 of 15 patients (87%) in a pilot trial with 3 monthly courses of 2 g/kg body weight IVIG. These 13 patients underwent immediate kidney transplantation, with loss of only 1 of the kidneys to rejection. In addition, the National Institutes of Health–sponsored IG02 trial randomly assigned 101 adult patients with end-stage renal disease who had a PRA level greater than 50% to receive either IVIG (2g/kg monthly for 4 months) or placebo.16 IVIG therapy was associated with a modest improvement in transplantation rates (35% vs 20%, $P = .069$), reduced time to transplantation ($P = .05$), and decreased mortality (8% vs 16%, $P = .22$). The numerous mechanisms by which IVIG is thought to exerts its immunomodulatory effects are beyond the scope of this article and have been summarized in a number of recent reviews.23-26

In this study we tested the hypothesis that IVIG could reduce development of sensitization in previously unsensitized individuals. Despite the extensive aforementioned documentation of the effectiveness of IVIG in reducing alloreactive antibody levels, this pilot study did not demonstrate any benefit in giving IVIG before and after exposure to allogeneic material. In this study, there was no difference between PRA values of patients receiving IVIG and those of patients not receiving IVIG. The lack of response to IVIG may be dose related; however, we used a dose (2 g/kg) that is standard in most successful desensitization protocols, including the National Institutes of Health IG02 trial.15-18 Our findings are consistent with a recent trial of IVIG to prevent sensitization in ventricular assist device recipients.20 In that trial, IVIG (10 g/d for 3 days) had no effect on either the mean PRA or the number of individuals becoming sensitized during mechanical support. It is important to note that our study did not use additional desensitization techniques, including plasmapheresis and other immunosuppressive agents as noted in many previous case series.

<table>
<thead>
<tr>
<th>Case</th>
<th>Antigen mismatches*</th>
<th>PRA at 4 mo</th>
<th>Antibody specificities</th>
<th>Donor-specific antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Class I</td>
<td>Class II</td>
<td>Class I (%)</td>
<td>Class II (%)</td>
</tr>
<tr>
<td>1</td>
<td>A1 A24 B8</td>
<td>DR17 DR15 DR52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>A3 B7 B62</td>
<td>DR11 DR16 DR51 DR52</td>
<td>98</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>A1 A30 B18 B63</td>
<td>DR1 DR13</td>
<td>96</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>98</td>
<td>93</td>
</tr>
<tr>
<td>5</td>
<td>A2 B44 B62</td>
<td>DR4</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>46</td>
<td>27</td>
</tr>
<tr>
<td>7t</td>
<td>A32 B27</td>
<td>DR17 DR103</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

PRA, Panel-reactive antibody; NA, not applicable (donor or recipient typing was not performed). *Antigen mismatches represent donor antigens at which recipient was not matched. †Withdrew from study before 4 months.
The dosing regimen took into consideration the half-life of IVIG (3 weeks) and the need to administer the agent under closely monitored conditions in highly volume-sensitive infants. This led to the choice of dosing times: preoperative (before exposure to allograft tissue), 3 weeks (infant still in hospital), and 4 months (infant returns for bidirectional cavopulmonary anastomosis). The Stollery Children’s Hospital is a tertiary referral center for pediatric heart surgery and receives patients from an extremely large geographic area (Western Canada); thus having infants return for more frequent dosing would not have been possible and would have limited enrollment. Consequently, the major limitation of this study was the need to administer IVIG while the patient was in the hospital and the resultant inability to administer IVIG at 2 and 3 months after surgery. This period is the critical window of antibody development, when the child is most likely acquiring memory B cells to the allograft tissue. Thus in effect we were only testing the efficacy of the first two doses of IVIG, with the impact of the third dose at 4 months questionable. Regardless, Glotz and colleagues reported a mean reduction of 33% in anti-HLA class I reactivity within 1 week of 2 g/kg IVIG. Moreover, Glotz and colleagues demonstrated that the maximal reduction in alloreactivity occurs within 1 week of IVIG therapy and that sequential doses of IVIG did not have an additive effect on reduction of circulating anti-HLA class I IgG antibodies. Similarly, in the IG02 trial, in which IVIG was administered at 2 g/kg monthly for 4 months, most of the reduction in PRA was seen within the first month. The findings of these studies contrast sharply with ours, in which there was no response to IVIG seen within the first month of therapy. There are a number of limitations to this study. Small sample size limits statistical calculations. Review of the scatterplots and comparison with control patients who did not receive IVIG, however, demonstrates a lack of response to IVIG. There is also increasing discussion that the PRA may not be the most suitable test to determine sensitization. Antibody specificity and titers may be a more sensitive method to determine the degree of sensitization. Alternatively, use of less sensitive methods than flow PRA, such as complement-dependent cytotoxicity and antiglobulins-enhanced complement-dependent cytotoxicity, might have helped determine the impact on antibody titers. If the antibodies we detect after IVIG therapy are low titer, they may be more amenable to subsequent modulation therapies at the time of transplantation. Additionally, longer follow-up may reveal a delayed effect of IVIG after 12 months. Finally, if heart transplantation is required in the future, determination of crossmatch results may be revealing.

In conclusion, this novel study was unable to demonstrate that IVIG prevents sensitization after exposure to allograft tissue in neonates undergoing congenital cardiac surgery. These findings were seen despite studies in adults, which have demonstrated that high-dose (2 g/kg) IVIG reduces sensitization. The ultimate test will be long-term follow-up and determination of whether early treatment with IVIG improves future transplantability.

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References


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