Intravenous Immunoglobulin Reduces Anti-HLA Alloreactivity and Shortens Waiting Time to Cardiac Transplantation in Highly Sensitized Left Ventricular Assist Device Recipients

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Background—Recipients of left ventricular assist devices (LVADs) develop prominent B-cell hyperreactivity. We investigated the influence of anti-HLA antibodies on waiting time to cardiac transplantation in LVAD recipients and compared the effects of 2 immunomodulatory regimens on anti-HLA serum reactivity.

Methods and Results—Fifty-five previously nonsensitized LVAD recipients of a TCI device implanted between 1990 and 1996 were studied. Patients with anti-HLA antibodies received monthly courses of either intravenous immunoglobulin (IVIg) or plasmapheresis, in conjunction with cyclophosphamide. The effects of these regimens on anti-HLA alloreactivity and waiting time to transplantation were then determined by Kaplan-Meier log-rank statistics, nonparametric Wilcoxon rank-sum test, and Student’s t test. Prolongation in transplant waiting time was related to serum IgG anti–HLA class I alloreactivity. Infusion of IVIg (2 g/kg) caused a mean reduction of 33% in anti–HLA class I alloreactivity within 1 week. Waiting time to transplantation was significantly reduced by IVIg therapy and subsequently approximated that in nonsensitized patients. Side effects of IVIg (2 g/kg) were minimal and related primarily to immune complex disease. Although plasmapheresis caused a similar reduction in alloreactivity to IVIg, this effect was achieved after longer treatment. Moreover, plasmapheresis was associated with an unacceptably high frequency of infectious complications. In patients resistant to low-dose (2 g/kg) IVIg therapy, high-dose (3 g/kg) IVIg was effective in reducing alloreactivity but was associated with a high incidence of reversible renal insufficiency.

Conclusions—These results indicate that IVIg is an effective and safe modality for sensitized recipients awaiting cardiac transplantation, reducing serum anti-HLA alloreactivity and shortening the duration to transplantation. The therapeutic and safety profile of IVIg would appear to be superior to plasmapheresis. (Circulation. 1999;100[suppl II]:II-229–II-235.)

Key Words: antibodies ■ immune system ■ transplantation ■ ventricular assist devices

Antibodies in the serum of a cardiac allograft recipient that are directed against donor HLA class I major histocompatibility complex (MHC) antigens constitutively expressed by allograft endothelium portend a significant risk for early graft failure (ie, within the first 24 to 48 hours) and poorer patient survival as a result of complement-mediated humoral rejection.1–3 Because T lymphocytes constitutively express MHC class I antigens, the presence of preformed lymphocytotoxic antibodies, particularly IgG isotype, detected in a routine T-cell cross-match is considered a contraindication to solid organ transplantation.1 To identify patients at high risk of having a positive donor-specific cross-match, cardiac transplantation candidates are screened for anti-HLA antibodies reactive with lymphocytes from a panel of volunteers representative of the major HLA allotypes, collectively referred to as measurements of panel-reactive antibodies (PRAs). Patients with high PRA levels are considered to be “sensitized” to various alloantigens and require donor-specific cross-matches before transplantation.

The proportion of highly sensitized patients on cardiac transplant waiting lists has been progressively expanding as a result of both widespread use of left ventricular assist devices (LVADs) and increasing numbers of patients undergoing retransplantation. LVAD recipients develop prominent B-cell activation, as evidenced by heightened production of anti–HLA class I and II antibodies.4–6 Although use of leukocyte-filtered platelets can partially reduce anti–HLA class I antibody production, B-cell hyperreactivity associated with LVAD implantation results from a multifactorial immunological dysregulatory process involving heightened T-cell apo-
ptosis, selective loss of Th1-type T cells, and unopposed production of Th2-type cytokines. As a consequence of circulating anti–HLA class I and II antibodies, LVAD recipients have repeated positive cross-match reactions, increased waiting time to cardiac transplantation, and heightened risk of cellular rejection after transplantation.

Recent studies have suggested that pooled human intravenous immunoglobulin (IVIg) is an effective modality to reduce allo sensitization. Postulated mechanisms include the presence in IVIg of anti-idiotypic antibodies, antibodies against membrane-associated immunological molecules such as CD4 or CD5, or soluble forms of HLA molecules. In this study, we initially investigated the effects of IVIg on serum reactivity to HLA class I molecules in LVAD recipients and compared these effects to plasmapheresis, an alternative modality for reduction of alloreactive antibodies. The results of our study demonstrate that treatment with IVIg is an effective and safe modality to reduce serum reactivity to HLA class I antigens, decreases the risk for positive cross-match reactions, and consequently shortens the waiting time of sensitized LVAD recipients to cardiac transplantation.

Methods

Patient Population and Study Design
The influence of anti-HLA antibodies on waiting time to cardiac transplantation was studied in 55 previously nonsensitized LVAD recipients of a TCI device implanted between 1990 and 1996. For each analysis examining the effects of anti-HLA antibodies directed against either HLA class I or II molecules on waiting time to transplantation, patients were divided into 2 groups on the basis of the presence or absence of antibody development. A treatment regimen consisting of 1 to 3 monthly courses of IVIg 2 g/kg administered in 4 divided daily doses was then administered to 16 sensitized patients with anti-HLA antibodies awaiting cardiac transplantation. In addition, 4 sensitized patients received 1 to 2 monthly courses of plasmapheresis administered 2 to 3 times per week. All patients additionally received cyclophosphamide monthly in a single infusion dose of 0.5 to 1.0 g/m² IV. The effects of these treatment regimens on anti-HLA alloreactivity and on waiting time to transplantation were then determined. The treatment protocols were approved by the Institutional Review Board, and patients were fully aware of all potential consequences of the regimens instituted.

Detection of Anti-HLA Antibodies
Sera were obtained from all patients at risk for sensitization on the day of initial listing as United Network of Organ Sharing status I for transplantation and then every 2 weeks until transplantation. Sera were screened for the presence of lymphocytotoxic antibodies against separated T and B lymphocytes obtained from a panel of 70 control individuals representative of the most frequently encountered HLA class I and II antigens in the general population. Anti-HLA IgG antibodies were considered positive if serum, in the presence of dithioerythritol (DTT), demonstrated complement-mediated lytic activity against >10% of the T-cell reference panel. Anti-HLA reactivity >20% was considered an absolute indication for requirement of a donor-specific cross-match before transplantation.

Determination of Anti-HLA Antibody Specificity for MHC Class I or II Antigens
Working definitions for IgG antibodies against HLA class I or II molecules were established in our laboratory by use of, as reference, sera from 28 cardiac transplantation patients with PRA values >10% and with anti–MHC class I and II specificities defined by standard tail analysis. Because MHC class I antigens are constitutively expressed by both T and B cells, IgG antibodies against HLA class I molecules were considered present in our working definition when DTT-treated serum reacted against >10% of both the T-cell reference panel and the B-cell panel. This working definition for IgG anti–HLA class I molecules correlated in 100% of cases (20 of 20) with patient sera having defined specificity for HLA class I antigens.

Concomitantly identify and discriminate IgG antibodies against HLA class II molecules in the presence of IgG anti–HLA class I molecules, we established an algorithm that used the ratio of serum reactivity to B versus T cells, because HLA class II antigens are constitutively expressed by B but not T cells. To confirm this working definition using sera with defined IgG anti–MHC class II specificities, a logistic regression analysis was performed by a maximum-likelihood procedure with Biological Management Database Program statistical software to calculate the predictive values of the IgG anti–HLA class II molecules for the ratios of B-cell serum reactivity to T-cell reactivity of 1.25, 1.50, 1.75, 2.00, and 3.00. Maximal sensitivity (91%) for identifying sera with reactivity against HLA class II antigens was obtained by use of a ratio of B-cell to T-cell serum reactivity of 2.00 (model coefficient, −3.48; SE, 1.19; P = 0.0002). Therefore, IgG antibodies against both MHC class I and II molecules were considered present if DTT-treated serum reacted against >10% of both the T- and B-cell reference panels and B-cell reactivity exceeded T-cell reactivity by ≥2-fold. IgG anti–HLA class II molecules were also considered present if DTT-treated serum reacted against >10% of the B- but not T-cell reference panel.

Statistical Analyses
Kaplan-Meier log-rank statistics were used to study the effects of anti-HLA antibodies with specificities against HLA class I or II molecules on waiting time to cardiac transplantation. Similarly, Kaplan-Meier log-rank statistics were used to assess the effect of reduction in alloreactivity by IVIg on waiting time to cardiac transplantation. The Cox proportional-hazard model was used for multivariable analysis. A nonparametric Wilcoxon rank-sum test and Student’s t test were used to evaluate the effects of individual treatment courses and of repeated courses of either IVIg or plasmapheresis on reduction of serum alloreactivity. All data were analyzed with SAS system software (SAS Institute Inc.).

Results
Circulating IgG Antibodies Against Class I But Not Class II Molecules Increase Waiting Time to Cardiac Transplantation in LVAD Recipients
The first series of studies aimed to investigate the relationship between anti-HLA antibodies and waiting time to cardiac transplantation in 55 LVAD recipients. At our institution, a positive donor-specific cross-match, performed by screening recipient serum for complement-mediated lytic activity against donor T cells, is considered a contraindication to transplantation. Therefore, waiting time is a direct reflection of repeated instances of positive cross-matches between recipient serum and donor T cells.

Because resting T cells express HLA class I but not class II molecules, we investigated whether the presence of circulating alloreactive IgG antibodies directed against HLA class I antigens might be better predictors of positive donor-specific reactions and consequently of prolongation in waiting time to transplantation than IgG antibodies against HLA
class II antigens. As shown in Figure 1, the waiting time of LVAD recipients to transplantation was prolonged in the presence of IgG antibodies against HLA class I molecules compared with patients without these antibodies. The mean duration to transplantation in patients without IgG antibodies against HLA class I molecules (n = 18) was 3.1 months (range, 0.3 to 10.7 months), whereas in patients with IgG antibodies against HLA class I molecules (n = 37), it was increased to 7.1 months (range, 0.2 to 17.9 months; P = 0.001). In contrast, the presence of IgG antibodies against HLA class II molecules did not significantly increase the waiting time to cardiac transplantation (Figure 2). The mean duration to transplantation in patients without IgG antibodies against HLA class II molecules (n = 24) was 4.8 months (range, 0.5 to 17.9 months), which was not significantly different from the 5.2 months (range, 0.3 to 14.5 months) in patients with IgG antibodies against HLA class II molecules (n = 30; P = 0.21). These results indicated that prolongation of waiting time to cardiac transplantation in sensitized LVAD recipients was directly related to the presence of circulating alloreactive IgG antibodies against HLA class I molecules.

IVIg (2 g/kg) Reduces Reactivity of Circulating IgG Antibodies Against Allogeneic HLA Class I Molecules: Early Effect Sustained by Repeated Courses

We next evaluated the efficacy of monthly IVIg courses, at 2 g/kg, on reduction of reactivity of circulating IgG antibodies for allogeneic HLA class I molecules. Data were obtained from 16 patients who received 1 to 3 monthly courses of IVIg (total, 28 courses). Each course of IVIg was evaluated as an independent event, and the effects of each IVIg course on IgG anti–HLA class I antibodies during the ensuing 4 weeks were analyzed. Within 1 week of infusion of IVIg in 4 divided daily doses, the reactivity of circulating IgG antibodies for allogeneic HLA class I molecules was reduced by a mean of 33% (range, 14% to 52%; P = <0.01; Figure 3). This was the maximal level of reduction in alloreactivity during the 4 weeks after IVIg infusion, with IVIg efficacy progressively decreasing by the end of week 4 to a mean reduction in alloreactivity of 8±7%.

Because IVIg has been shown to reduce circulating immunoglobulins by a variety of immunomodulatory mechanisms, we next sought to determine whether sequential courses of IVIg therapy demonstrated progressive augmentation in the effect on circulating anti–HLA antibodies. For this analysis, the maximal mean reduction in reactivity of circulating IgG antibodies with allogeneic HLA class I molecules was determined for each IVIg course, and the data for corresponding courses in each patient were then pooled. As shown in Figure 5, sequential courses of IVIg did not have an additive effect on reduction of reactivity of circulating IgG antibodies with allogeneic HLA class I molecules. Each course resulted in a similar level of reduction in alloreactivity compared with baseline, with mean decreases of 38±11%, 36±17%, and 35±24% accompanying first, second, and third courses of IVIg, respectively.

High-Dose (3 g/kg) IVIg Is Effective in Reducing Alloreactivity in Patients Resistant to Low-Dose (2 g/kg) IVIg Therapy

Of the 16 highly sensitized patients, 6 were found to be resistant to treatment with IVIg at 2 g/kg, with a mean
reduction of only 4% (range, 2% to 4%) in reactivity of circulating IgG antibodies with allogeneic HLA class I molecules per treatment course in this group. These patients were subsequently treated with 1 to 2 courses of high-dose IVIg therapy, 3 g/kg in 4 divided daily doses. In each patient treated, high-dose IVIg therapy reduced reactivity of circulating IgG antibodies with allogeneic HLA class I molecules. As shown in Figure 5, alloreactivity in this group was reduced by a mean of 20% (range, 16% to 24%) per treatment course ($P<0.05$).

**IVIg (2 g/kg) Has Faster Onset and Greater Efficacy in Reducing IgG Anti-HLA Class I Alloreactivity Than Plasmapheresis**

We next compared the effects of IVIg (2 g/kg) with plasmapheresis on the reduction of reactivity of circulating IgG antibodies with allogeneic HLA class I molecules in LVAD recipients. Four sensitized patients received 1 to 2 monthly courses of plasmapheresis, administered 2 to 3 times per week (total, 6 courses). Each monthly course of plasmapheresis was evaluated as an independent event, and the effects of each course on IgG anti–HLA class I antibodies during the ensuing 4 weeks were analyzed. As shown in Figure 6, reactivity of circulating IgG antibodies with allogeneic HLA class I molecules was not significantly reduced within the first 2 weeks of initiation of plasmapheresis. Maximal reduction in alloreactivity, 38±11%, occurred by week 4 of plasmapheresis. These results show that IVIg has earlier onset of action and greater efficacy in reducing IgG anti-HLA alloreactivity compared with plasmapheresis.

**IVIg Therapy Shortens the Waiting Time to Cardiac Transplantation in Sensitized Patients**

We next investigated whether treatment with IVIg (2 g/kg) to reduce alloreactivity in sensitized recipients affected waiting time to transplantation. The first 3 highly sensitized LVAD recipients to receive IVIg therapy had unsuccessfully been waiting for cardiac transplantation for a mean of 303±25 days before the onset of therapy as a result of repeated positive donor-specific cross-matches (mean, 33; range, 24 to 43). After initiation of IVIg therapy with or without additional immunodepletion with plasmapheresis, all patients obtained negative donor-specific cross-matches and were successfully transplanted in a mean duration of 99±8 days (Figure 7). On the basis of these results, a formal protocol was established to initiate monthly courses of IVIg therapy (2 g/kg) after initial detection of allosensitization. The duration from listing to cardiac transplantation was then compared between 28 sensitized patients who did not receive IVIg treatment and 16 sensitized patients who received 1 to 2 courses of IVIg (2 g/kg) after detection of anti–HLA class I IgG antibodies. None of these patients received additional plasmapheresis. Whereas the mean duration to cardiac transplantation was 7.1 months (range, 0.2 to 17.9 months) in patients with IgG antibodies against HLA class I molecules, this time was significantly reduced to 3.3 months (range, 0.3 to 6.2 months) in sensitized recipients receiving 1 to 2 courses of IVIg (2 g/kg) ($P<0.05$). No patient in either group was transplanted across a positive donor-specific IgG T-cell cross-match. This duration was similar to the waiting time to...
transplantation in 27 nonsensitized patients (3.1 months; range, 0.3 to 10.7 months).

Complication of Therapy for Reduction of Allosensitization in Patients Awaiting Cardiac Transplantation

The Table summarizes the complications associated with IVIg and plasmapheresis therapies in sensitized patients awaiting cardiac transplantation. IVIg therapy (2 g/kg) was associated with clinical manifestations of immune complex disease in 4 of 27 monthly courses (15%), as evidenced by fevers, arthralgias, and maculopapular rashes. Only 1 of 27 courses was associated with systemic infection, *Staphylococcus aureus* sepsis. High-dose IVIg therapy (3 g/kg) was associated with reversible renal insufficiency (defined as >50% increase in serum creatinine level) in 4 of 6 courses. All cases resolved spontaneously over the ensuing 3 weeks after infusion. Renal insufficiency was not observed in any courses of low-dose IVIg. Systemic infection accompanied 3 of 6 courses (50%) of plasmapheresis (2 cases of *Staphylococcus aureus* sepsis, 1 case of *Acinetobacter* sepsis). In addition, 2 of 6 courses of plasmapheresis were associated with systemic anaphylaxis, as defined by hypotension requiring pressor support. Together, these results suggest that low-dose (2 g/kg) IVIg has a better safety profile than plasmapheresis in this group of patients.

Discussion

Preexisting recipient serum reactivity against donor HLA class I antigens constitutively expressed by cardiac allograft endothelium can cause complement-mediated humoral rejection and early graft failure.1–3 To identify patients whose sera contain antibodies against allogeneic HLA class I molecules, cardiac transplantation candidates are screened for anti-HLA antibodies reactive with lymphocytes from a panel of volunteers representative of the major HLA allotypes, collectively referred to as measurements of PRA. At our institution and many other cardiac transplant centers, sera from patients with high PRA levels are subsequently screened for donor-specific T-cell alloreactivity before donor allograft selection, because T lymphocytes constitutively express HLA class I antigens.1 Individuals whose sera repeatedly test positive in donor-specific cross-match assays will obviously have longer waiting times to transplantation. Moreover, the requirement for a cross-match precludes distant organ procurement for sensitized patients because of the requirement for short ischemic times in cardiac transplantation. The complications of long-term LVAD use, together with the effects of the underlying disease state, make such prolongation of the waiting time to transplantation a significant risk factor for morbidity and mortality of patients on cardiac transplant waiting lists.

In this study, we initially showed that prolongation in the waiting time to cardiac transplantation in LVAD recipients was directly related to the presence of circulating IgG antibodies against allogeneic HLA class I molecules. Infusion of IVIg (2 g/kg) in 4 divided daily doses caused within 1 week a mean reduction of 33% in the reactivity of circulating IgG antibodies for allogeneic HLA class I molecules. More importantly, the waiting time to transplantation in sensitized LVAD recipients was significantly reduced by IVIg therapy and subsequently approximated that in nonsensitized patients. Side effects of IVIg (2 g/kg) were minimal and related primarily to immune complex disease. Although plasmapheresis caused a similar reduction in alloreactivity to IVIg, this effect was achieved after a longer duration of treatment, presumably because of IgG equilibration between the intravascular and extravascular spaces. Because plasmapheresis removes only intravascular IgG, replenishment of the removed IgG occurs rapidly as a result of diffusion from the extravascular to the intravascular space. Thus, to achieve a prolonged steady state of low circulating IgG levels, multiple courses of plasmapheresis are necessary. Moreover, plasmapheresis was associated with an unacceptably high frequency of infectious complications. Together, these results indicate that IVIg is an efficacious and safe modality for sensitized recipients awaiting cardiac transplantation, reducing serum anti-HLA alloreactivity and shortening the duration to transplantation. The therapeutic and safety profiles of IVIg would appear to be superior to those of plasmapheresis.

In patients resistant to low-dose (2 g/kg) IVIg therapy, high-dose (3 g/kg) IVIg was effective in reducing alloreactivity. However, a high incidence of reversible renal insufficiency accompanied high-dose IVIg. Reversible renal insufficiency is a well-recognized complication of IVIg and appears to result from osmotic damage to the renal tubules and interstitium by carbohydrates in the IVIg preparation.23–25 The histopathology shows severe tubular vacuolization with cellular swelling and preservation of the brush border. There is no evidence of inflammatory cell or immune complex–mediated injury in this process, and renal insufficiency is reversible after cessation of treatment. Although this was also the case in our patients, 1 required temporary hemodialysis. Because preexisting renal insufficiency is a risk factor for renal complications associated with IVIg use, LVAD recipients receiving IVIg should be carefully monitored for this complication, particularly when receiving high-dose therapy.

The effect of each IVIg infusion on anti-HLA antibodies was transient, and serial IVIg infusions were required for maintenance of reduction in alloreactivity. These results argue against an immunomodulatory mechanism of action, such as reduction in helper CD4 T-cell activity16,17,26 or suppression of T-cell alloreactivity by inhibition of cytokine production,27 both of which would cause sustained alterations in immune function. More likely, the mechanism may involve a direct effect of IVIg on circulating IgG anti-HLA immuno-
gobulins. Because naturally occurring anti-idiotypic antibodies to HLA improve graft survival in sensitized renal allograft recipients,28,29 a similar effect may occur after exposure of serum from sensitized LVAD recipients to anti-idiotypic antibodies in pooled human IVIg.13–15 Moreover, IVIg appears to stimulate the production of anti-idiotypic IgM blocking antibodies in recipient serum.9 Alternatively, transient reduction in anti-HLA serum reactivity may be related to the presence in the IVIg preparation of soluble HLA class I molecules, which may bind circulating anti-HLA antibodies,18,19 or of non–complement-fixing antibodies against HLA class I molecules, which may compete with recipient alloreactive antibodies for HLA binding.30 Such antibodies have been demonstrated in IVIg preparations to react with nonpolymorphic determinants in the alpha-helical region of HLA class I molecules.

We emphasize the need to carefully screen all patients at risk for sensitization before transplantation and to identify the presence, isotype, and specificity of anti-HLA antibodies that portend heightened risk for adverse posttransplant outcomes. We advocate that before transplantation all patients should be specifically screened for the presence of antibodies against both MHC class I and II antigens and that immunosuppressive strategies be instituted in these patients before transplantation.

<table>
<thead>
<tr>
<th>Side Effects of Immunoglobulin Depletion Therapy</th>
<th>IVIg</th>
<th>Plasmapheresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious, n/total</td>
<td>1/27</td>
<td>3/6</td>
</tr>
<tr>
<td>Type</td>
<td>Staphylococcus aureus sepsis</td>
<td>Staphylococcus aureus and Acinetobacter sepsis</td>
</tr>
<tr>
<td>Immunological, n/total</td>
<td>4/27</td>
<td>2/6</td>
</tr>
<tr>
<td>Type</td>
<td>Immune complex disease (fever, rash, arthralgia)</td>
<td>Anaphylactic reactions</td>
</tr>
<tr>
<td>Reversible renal insufficiency (≥50% increase in serum creatinine), n/total</td>
<td>0/21 low-dose (2 g/kg)</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>4/6 high-dose (3 g/kg)</td>
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We also have observed similar results using this regimen in cardiac transplant recipients sensitized by means other than LVAD implantation and currently treat all sensitized patients in a similar manner. The long-term efficacy and safety of IV cyclophosphamide used before and after transplantation in sensitized LVAD recipients remain to be fully evaluated.

References


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