

Original Article

Plasmapheresis During Cardiopulmonary Bypass: A Proposed Treatment For Presensitized Cardiac Transplantation Patients

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ABSTRACT

Potential thoracic organ transplantation recipients who have positive cytotoxic antibody screens as quantified panel reactive antibodies (PRA) are at risk for immediate or long-term immunologic events that may affect the donor organ. The patient population at risk includes those who are supported with cardiac assist devices, multiparous women, and individuals receiving numerous homologous blood products. We treated three highly positive PRA patients with intraoperative plasmapheresis coupled to the cardiopulmonary bypass system to remove sufficient cytotoxic antibody. Upon the availability of donor hearts of an unknown HLA type, intraoperative plasmapheresis was performed using a Cobe Spectra Plasmapheresis system coupled to a Terumo CXSX18 oxygenator system. Three plasma volume exchanges of fresh frozen plasma (FFP) were performed while the patients were on cardiopulmonary bypass. One to one and one-half plasma volume exchange plasmaphereses were performed with a declining schedule for the next 30 days post-transplantation in combination with aggressive B-cell specific immunosuppressive therapy. The three patients are NYHA functional class I and free of rejection at 6 months post-transplantation. In conclusion, intraoperative plasmapheresis is effective and safe for the patient who would not be otherwise transplanted because of markedly elevated PRAs.

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INTRODUCTION

PRESENSITIZED PATIENT

Patients expressing cytotoxic antibodies to the human leukocyte antigen (HLA) and who are awaiting thoracic organ transplantation present unusual clinical difficulties. For this reason, we set forth to develop a method that removes the cytotoxic antibody intraoperatively by combining plasmapheresis with cardiopulmonary bypass (CPB) followed by aggressive post-transplantation plasmapheresis and immunosuppression.

INCIDENCE

The nondonor-specific panel reactive antibody assay (PRA) provides the initial screening assay for sensitization of the patient who may have anti-HLA antibodies. The estimated incidence of listed patients for cardiac transplantation who have positive PRA titers is 11–15% (1). Tsau et. al. reported that this rate of occurrence increases for patients supported with mechanical devices to 100% for females and 8% for males (2). Massad (3) reported at the Cleveland Clinic an over-all incidence of 66% of positive PRA patients supported with the mechanical left ventricular assist (LVAD) devices as compared to 15% in nondevice patients. Other causes of increased PRA titers are a history of multiple transfusions, previous allograft transplant, and multiparous females. As seen when crossing ABO blood types, the immediate problem with these sensitized patients is a potential hyperacute or accelerated antibody-mediated rejection subsequent to reperfusion of the transplanted heart.

OUTCOMES

Transplantation of patients with a PRA of greater than 10% poses a risk of hyperacute rejection, reduced graft, and patient survival (4–7). Furthermore, prospective donor–recipient cross match is usually not feasible for thoracic transplantation. The PRA may decrease over time (months) in the nondevice patient, but in the device-supported patient, the PRAs do not decrease significantly (3). Therefore, without an aggressive method to reduce the cytotoxic antibody concentration immediately before reperfusion of the transplanted heart and post-transplantation, these patients are not transplanted and thus maintained on the transplantation waiting list for extended periods of time. This report provides a protocol to identify the patients at risk, intraoperative and post-transplantation plasmapheresis techniques, and immunosuppressive therapies to support this class of patient.

MATERIALS AND METHODS

PANEL OF REACTIVE ANTIBODY

The PRA assay kit^a was used for screening for the cytotoxic antibody. In brief, lymphocytes with known antigens were in-

cubated with the patient's serum plus rabbit complement. If the serum antibodies bind specifically to those antigens present on the cell surface, cytolysis occurs. This test does not detect noncytotoxic antibodies; therefore, we used goat antihuman globulin (AHG) to augment cytotoxicity. The results in this study were reported as PRA; however, all were treated with AHG.

PLASMAPHERESIS

The volume exchanged was based on the body weight of each patient. The exchange rate was operated at the maximal rate of the plasmapheresis instrument; that is, 150 ml/min. The patient's plasma volume was exchanged with fresh frozen plasma (FFP) for the intraoperative and immediate postoperative periods because of exchanged volumes and the risk of postoperative bleeding. After 24 hours postoperatively, the exchange fluids were gradually adjusted with combinations of normal saline and 5% albumin.

CARDIOPULMONARY BYPASS

The cardiopulmonary bypass was configured in the usual fashion with a Terumo CXSX18 oxygenator.^b Once full bypass was established, the plasmapheresis system^c was attached to the oxygenator, as illustrated in Figure 1. In brief, the blood for the plasmapheresis system was withdrawn from the luer connection on the venous return port and plasmapheresis return was into the luer connection at the top of the venous reservoir. With this configuration, the volumes withdrawn were equal to the replacement volumes. Plasmapheresis flow rates of 150 ml/min were achieved to provide a three volume (6 to 12 liters of FFP) exchange based on the patient's body weight (40 ml/kg). The computed volume exchange was based on 90% plasma volume exchange of the patient. Because of the exchange of the patient's plasma with FFP containing calcium chelating anticoagulant (citrate), the patient's calcium was closely monitored. Furthermore, because of the fluid exchange during bypass, the heparin, calcium chloride, anesthetics, and volumes were monitored, and levels were corrected at 15-min intervals.

POST-TRANSPLANTATION

Post-transplantation, all three patients were maintained on triple immunosuppressive therapy including prednisone, cyclosporin, and cyclophosphamide. Heart biopsies to assess for allograft rejection were performed on a weekly basis during the first month post-transplantation.

RESULTS

Table 1 demonstrates that the risk factors for these three patients were transfusions, pregnancy, previous surgery, and/or

a Lambda Cell Tray # LCT-30ABC, One Lambda, Inc., Canoga Park, CA

b Terumo, Inc. Somerset, NJ

c Cobe Cardiovascular, Inc., Arvada, CO

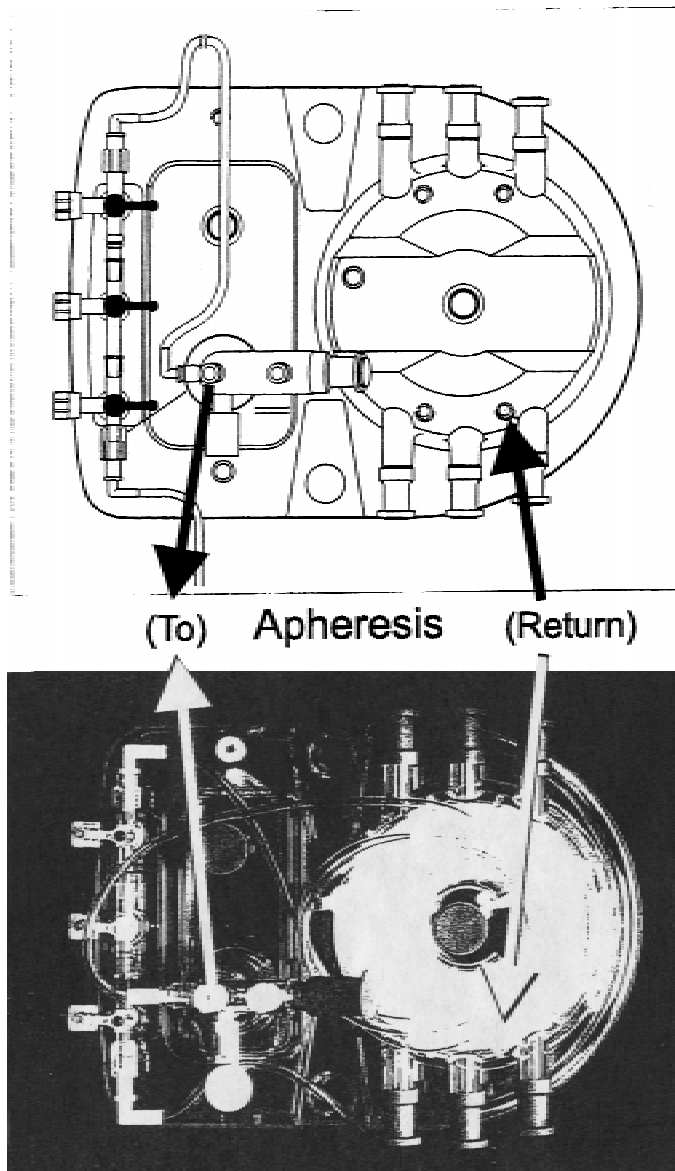


Figure 1: The connections to withdraw the patient's blood for the plasmapheresis instrument was via the venous return port of the venous reservoir, and the exchange fluid was returned through the Luer connection on the venous reservoir.

an implanted artificial heart device. All patients demonstrated an immediate decrease in the PRA, but patient 3 had a persistent increase in PRAs subsequent to the intraoperative plasmapheresis (Table 2). Patients 1 and 2 had sustained, nondetectable PRAs, and all three had no rejections throughout their follow-up period of more than 1 year. All patients are an NYHA Functional Class I at the time of this report. Table 3 shows that patients 1 and 2 had four to five plasmapheresis procedures subsequent to the two initial procedures. These procedures were performed daily after the day of transplantation. However, patient 3 had a persistent 100% PRA, which neces-

Table 1: Patient Demographics and Risk Factors

| | Age (yr) | WT (kg) | Gender | Risk Factors |
|-----------|----------|---------|--------|--|
| Patient 1 | 42 | 74 | Female | 2 Previous open-heart procedures 4 Transfusions Artificial heart |
| Patient 2 | 39 | 48 | Female | 3 Pregnancies 4 Transfusions |
| Patient 3 | 58 | 105 | Male | 1 Previous open-heart surgery 6 Transfusions |

Three patients were accepted for allograft heart transplantation because of end-stage heart disease who demonstrated risk factors related to production of anti-HLA cytotoxic antibody production.

Table 2: Panel of Reactive Antibodies

| | PRA (%) | | Rejections | |
|-----------|---------|----------------|------------|---|
| | Pre-op | Post-op (24 h) | 6 months | |
| Patient 1 | 93 | 4 | 0 | 0 |
| Patient 2 | 70 | 0 | 0 | 0 |
| Patient 3 | 100 | 5 | 100 | 0 |

The PRA assay results of the three study patients before and after plasmapheresis. Through clinical evaluation, cardiac biopsy, and ECHO analysis, these patients did not exhibit allograft rejection.

Table 3: Intra- and Postoperative Plasmapheresis

| | Vol Ex/Vol (L) during CPB | Vol Ex/Vol (L) 24 h post-tx | Vol Ex/Vol (L)/# after 24 h |
|-----------|---------------------------|-----------------------------|-----------------------------|
| Patient 1 | 3/9 FFP | 1.5/3 FFP | 1.5/3 alb/4 |
| Patient 2 | 3/6 FFP | 1.5/3 FFP + alb | 1.0/2 alb/3 |
| Patient 3 | 3/12 FFP | 1.5/6 alb | 1.5/6 alb/12 |

These patients had plasmapheresis with FFP during the first 24 hours of the transplantation procedure and plasmapheresis with albumin subsequently. Not shown, Patient 3 also underwent six photopheresis procedures because of persistently elevated PRA values. Vol Ex = volume exchanged, Vol (L) = volume liters # = numbers of exchanges, FFP = fresh frozen plasma, alb = albumin.

sitated 12 plasmapheresis procedures, followed by six photopheresis procedures.

DISCUSSION

PLASMAPHERESIS

We have demonstrated that coupling plasmapheresis with cardiopulmonary bypass provides a means to remove cytotoxic antibodies in the PRA positive patient. In two of the three patients, the therapeutic effect was sustained when coupled with aggressive B-cell immunosuppressive therapy. These pa-

tients were hemodynamically unstable, which necessitated their need for a heart transplantation procedure. Therefore, the plasmapheresis during CPB provided a high flow exchange when it would not be possible in these hemodynamically unstable patients. The volume exchange of two to three times took about 1 hour, with flow rates at 150 ml/min, resulting in 100 ml plasma exchange/min. One major problem with performing plasmapheresis during CPB is removal of a number of drug therapies; namely, heparin, anesthetics, aprotinin, steroids, and ionized calcium. To summarize, plasmapheresis during CPB provides easy access connections to cardiopulmonary bypass and stable management of volumes, but requires that particular attention is given to serum factors that were removed by the plasmapheresis.

Plasmapheresis has been shown to be efficacious if performed preoperatively (8). It has been shown that recipient plasmapheresis is efficacious if performed as soon as notification of donor organ acceptance. A 1.5 volume exchange has been reported as a target exchange for preoperative procedures. Robinson (8) and we found that a 3.0 volume exchange during cardiopulmonary bypass could be performed because of the higher flow rates achieved when connected to the heart-lung machine.

Post-transplantation plasmapheresis frequency and volumes should be governed by the PRA results subsequent to each plasmapheresis procedure. A left-subclavian dual-lumen catheter is necessary for postoperative plasmapheresis. This technique leaves the right jugular available for performing heart biopsies post-transplantation.

Without aggressive post-transplantation plasmapheresis and B-cell specific immunosuppressive therapy, this procedure may provide a short-lived effect because of potential B-cell response to the allograft. Figure 2 illustrates a flow sheet of pre- and postoperative procedures recommended to be performed to identify the patients at risk and to institute appropriate therapeutic steps. The issue of cost effectiveness when adapting the scheme displayed in Figure 2 is not in question. We estimate that the cost for a patient in end-stage heart failure is greater than \$3000 per day while being maintained on a dobutamine infusion or on a LVAD support device. These Status 1 patients may be in the hospital for an extended period before transplantation while waiting for the natural decay of the antibody titers. In summary, the Figure 2 scheme provides a cost-effective method and the flexibility for transplant team to perform the transplant procedure at availability of the best suited donor heart.

Potential Outcomes for Patients with Elevated PRAs: Allograft recipients who have preformed antibodies to major histocompatibility complex (MHC) determinants or develop antibodies post-transplantation have a long-term higher incidence of rejection, graft loss, vascular disease, and lower survival (4-7,9). It is unclear whether this association is an etiologic one or whether the presence of these antibodies solely identifies individuals with more pronounced alloimmunologic responses.

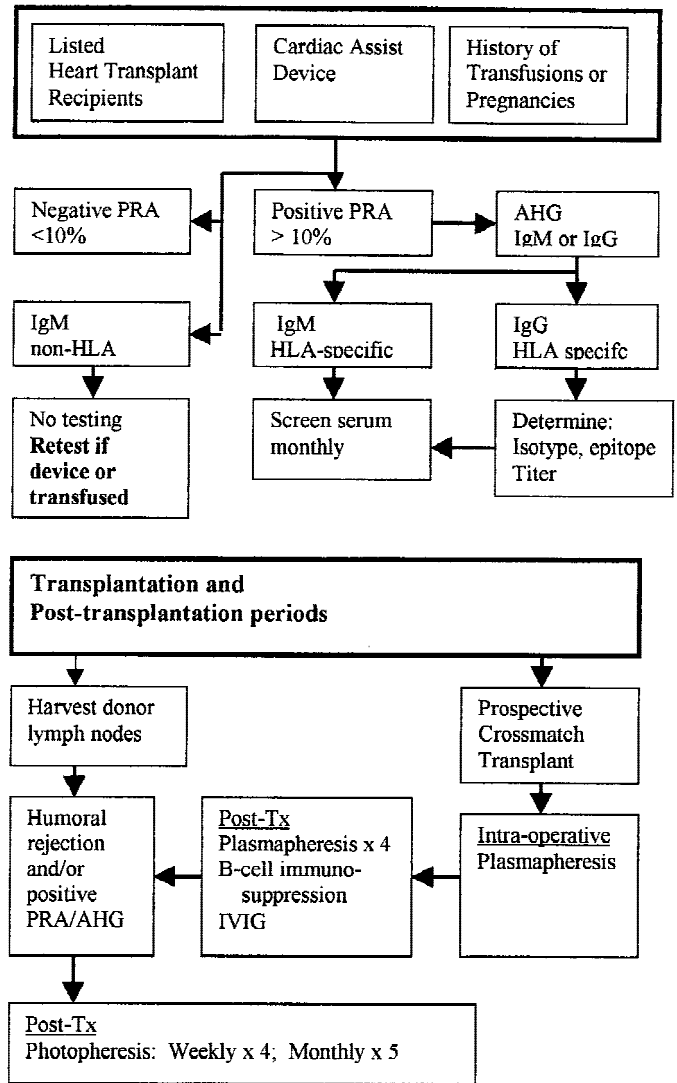


Figure 2: The algorithm demonstrates a means for the risk analysis and treatment of PRA positive patients.

Quantification of Sensitization: The nondonor-specific PRA provides the initial screening assay for sensitization of the patient who may have anti-HLA antibodies. An AHG PRA assay was used as a secondary assay to differentiate between IgM and IgG and to identify low complement binding IgG antibodies, as reported by Rodey (10). It is important to underscore the limitations of this assay, given that the PRA and AHG only characterize antibody reactivity to T-cell HLA antigens, specifically major histocompatibility complex (MHC) Class I A, B, and C and not those antibodies reacting against HLA antigens associated with the B-cell MHC Class II (DR, DP,DQ).

Antibody Titers: Antibody titers would define quantitatively the humoral immunologic risk. The PRA and AHG are qualitative assays; therefore, they do not determine the concentration of antibody. Thus, it is suggested that for PRA-positive

patients, the titer, the isotype, and subtype for the HLA reactive antibodies be determined; thereby, permitting a more specific assessment of the therapeutic effects.

THERAPEUTIC APPROACHES

Blood Product Transfusion: There should be an aggressive attempt to prevent exposure through blood product transfusion in potential transplantation recipients. The use of cell saving devices, AMICAR, aprotinin, and leukodepleted blood products is absolutely necessary. The three patients presented in this study all had histories of homologous blood transfusions, supporting this point.

Donor-Specific Lymphocyte Cross Match (DSXM): The sensitized patient (PRA >10%) may require a prospective DSXM. That is, the identification of the target antigen in the positive patient is required information for a prospective cross match between the donor and recipient. This recommendation is based on the renal transplantation experience, wherein sensitized recipients had a lower graft survival without the DSXM. However, in thoracic organ transplantation, there is usually insufficient time and number of available donors for DSXM.

IVIG (Intravenous Immunoglobulin): IVIG (20 gm of 5%) is used to reduce the antibody-mediated responses by obstructing the Fc receptor on the B-cells and diminishing the antibody-mediated rejection. Robinson (8) recommended administering IVIG after plasmapheresis and before reperfusion of the donor heart at the time of transplantation. The question to be answered in future studies is whether IVIG may be effective as a single therapy in suppression of this humoral immune response. Additional questions are: should IVIG be given after each plasmapheresis and to what degree does IVIG interfere with specific donor-directed antibody responses? IVIG also seems to interfere with the PRA assay and, therefore, limits the clinician's ability to assess the efficacy of this and other therapeutic treatments. In contrast, attempting to decrease a positive PRA that occurred following the implantation of a Novacor, McIntyre (11) administered IVIG at 3-week intervals. The PRA became negative, and the patient received a donor heart. In summary, a dramatic increase in alloantibody activity was promptly reversed with IVIG, however, its effect on the PRA remains an important unsolved issue.

Photopheresis: Photopheresis is an extracorporeal therapy developed for several T cell-mediated disorders, including transplantation (12,13). The principle of photopheresis is the elimination of the specific pathogenic clones of T-cell. Photopheresis is based on the combined effect of 8-methoxypsoralen (8-MOP) plus ultraviolet-A light (UVA) on autologous alloreactive lymphocytes. It seems that the reinfused photoactivated lymphocytes act as antigens to initiate a specific immune response against alloreactive lymphocytes. Weiland (14) reported that 8-MOP administered PO (0.6 to 1.0 mg/kg) 90 min before the photopheresis achieved a target blood level of 50–100 ng/ml. After 8-MOP treatment, about 5×10^5 mononuclear cells were collected and treated with ultraviolet light

for 1.5 hours and were reinfused. This treatment protocol provided normalization of cardiac allograft function with high-grade rejection. A number of reports support this therapy in renal allograft rejection and in cardiac rejection (15,16).

MAINTENANCE IMMUNOSUPPRESSIVE THERAPY FOR THE SENSITIZED PATIENT

Sensitized patients or those with intractable humoral rejection should be placed on an alternate immunosuppressive therapy regime that has a combined effect on both the T- and B-lymphocyte function.

Cyclosporine vs. FK506: Cyclosporine (CSA) and FK506 are equal in their ability to suppress the B-lymphocyte function. This effect is assumed to be through the indirect influence B-cells function *in vivo* by inhibition of T-cell helper function. FK506 is 100 times more potent than CSA and may have a slightly increased therapeutic index than that of CSA and, therefore, may provide a small margin of B-cell inhibition, as compared with CSA.

Azathioprine vs. Cyclophosphamide: Cyclophosphamide, a nitrogen mustard, is a potent immunosuppressive agent with inhibitory effects on both T- and B-lymphocytes. Thus, cyclophosphamide has been reported to be effective in preventing cellular and/or humoral rejection in cardiac transplant recipients with recurrent or recalcitrant rejection (17). Cyclophosphamide has been substituted for azathioprine with a reduction in rejection frequency, and cyclosporine levels were unchanged with the normal tapering of prednisolone (17). Moreover, Weyand (18) reported that high-risk patients with positive PRAs should be treated with 300 mg/day for 1 week and tapered to 100 mg/day without changing their triple drug therapy dosages. Clinical data have demonstrated that there is a 4–7 day delay before the therapeutic effect on B-cell proliferation with cyclophosphamide (19).

Rapamycin: Rapamycin immunosuppressive site of activity is distinctively different from CSA or FK506. Rapamycin seems to inhibit the IL-2-mediated signal transduction pathways and, as a result, prevent T-cell cell cycle progression (20). As with CSA and FK506, rapamycin inhibits Ca^{++} -dependent pathways; however, rapamycin's effect is directly on the B-cell function. Rapamycin, therefore, affects both B-cell and T-cell functions (21).

Mycophenolic Acid (CellCept): Mycophenolate mofetil, the morpholinoethylester of mycophenolic acid (MPA), inhibits specifically the T- and B-lymphocyte *de novo* pathway for purine synthesis. This pathway inhibition interferes with T- and B-lymphocyte activation and proliferation, the glycosylation of adhesion molecules, and the production of antibodies and possibly cytokines (22). Not only does it specifically inhibit T lymphocyte responses, MPA is a superior inhibitor of antibody formation through inhibition of IL-1 and IL-6 production by macrophages, as compared with other therapeutics. Perhaps the more important feature of MPA is its ability to inhibit proliferation of human arterial smooth muscles at clini-

cally attainable blood concentrations. This smooth muscle inhibitory activity is not seen with cyclosporine, FK506, or azathioprine. Halloran et al. (23) reported, from their analysis of a large pooled database of Phase III CellCept studies ($n = 1493$), that for patients with PRAs $> 20\%$, their first biopsy results were positive for 56% of those treated with azathioprine, 54% for those treated with 2 gm/day of CellCept, and 40% for those treated with 3 gm/day of CellCept. These patients were not treated with plasmapheresis and were not given IVIG or cyclophosphamide. The Halloran analysis does indicate that, with PRA patients, the 3-gram dose may be preferred.

CONCLUSION

We have applied a therapeutic approach to treat patients with elevated PRAs with intraoperative plasmapheresis coupled with cardiopulmonary bypass. The technique has shown an immediate decrease in the PRA and with sustained effect in two of three patients. The plasmapheresis procedures will not achieve their full effect without a concomitant and aggressive B-cell immunosuppressive regime. Therefore, this report provides a comprehensive algorithm for the clinician and perfusionist with a method to treat these patients in a cost-effective and efficacious manner.

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REFERENCES

- Schafer AJ, Hasert K, Opelz G. Collaborative transplant study crossmatch and antibody project. *Transplant Proc.* 1985;17:2469–71.
- Tsau PH, Arabia FA, Toporoff B, et al. Positive panel reactive antibody titers in patients bridged to transplantation with mechanical assist device. *ASAIO J.* 1997; 44:M634–M637.
- Massad MG, McCarthy PM, Smedira NG, et al. Does successful bridging with the implantable left ventricular assist device affect cardiac transplantation outcome? *J Thorac Cardiovasc Surg.* 1996;112:1275–81.
- Loh E, Bergin JD, Couper GS, et al. Role of panel-reactive antibody cross-reactivity in predicting survival after orthotopic heart transplantation. *J Heart Lung Transplant.* 1994; 13:194–201.
- Kerman RH, Orosz CG, Lorber MI. Clinical relevance of anti-HLA antibodies pre- and post-transplant. *Am J Med Sci.* 1997;313:275–78.
- Kerman RH, Kimball P, Scheinen S, et al. The relationship among donor–recipient HLA mismatches, rejection, and death from coronary artery disease in cardiac transplant recipients. *Transplantation.* 1994;57:884–88.
- Kobashigawa JA, Sabad A, Drinkwater D, et al. Pretransplant panel reactive-antibody screens. Are they truly a marker for poor outcome after cardiac transplantation? *Circulation.* 1996;94:II294–97.
- Robinson JA, Radvany RM, Mullen MG, et al. Plasmapheresis followed by intravenous immunoglobulin in pre-sensitized patients awaiting thoracic organ transplantation. *Therap Apheresis.* 1997;1:147–51.
- Smith JD, Danskine AJ, Laylor RM, et al. The effect of panel reactive antibodies and the donor specific cross-match on graft survival after heart and heart-lung transplantation. *Transplant Immunol.* 1993;1:60–65.
- Rodey GE, Revels K, Fuller TC. Epitope specificity of HLA class I alloantibodies: II. Stability of cross-reactive group antibody patterns over extended time periods. *Transplantation.* 1997;63:885–93.
- McIntyre JA, Higgins N, Britton R, et al. Utilization of intravenous immunoglobulin to ameliorate alloantibodies in a highly sensitized patient with a cardiac assist device awaiting heart transplantation. Fluorescence-activated cell sorter analysis. *Transplantation.* 1996;62:691–93.
- Wolfe JT, Lessin SR, Singh AH, et al. Review of immunomodulation by photopheresis: Treatment of cutaneous T-cell lymphoma, autoimmune disease, and allograft rejection. *Artificial Organs.* 1994;18:888–97.
- Barr ML. Immunomodulation in transplantation with photopheresis. *Artificial Organs.* 1996;20:971–73.
- Wieland M, Thiede VL, Strauss RG, et al. Treatment of severe cardiac allograft rejection with extracorporeal photochemotherapy. *J Clin Apheresis.* 1994;9:171–75.
- Wolfe JT, Tomaszewski JE, Grossman RA. Reversal of acute renal allograft rejection by extracorporeal photopheresis: A case presentation and review of the literature. *J Clin Apheresis.* 1996;11:36–41.
- Dall'Amico R, Montini G, Murer L. Benefits of photopheresis in the treatment of heart transplant patients with multiple/refractory rejection. *Transplant Proc.* 1997;29: 609–11.
- Wagoner LE, Taylor DO, Olsen SL, et al. Cyclophosphamide in cardiac transplant recipients with frequent rejection: A 6-year retrospective review. *Clinic Transplant.* 1996;10:437–43.
- Weyand M, Frye K, Fahrenkamp A, et al. Cyclophosphamide as an adjunct to maintenance immunosuppression in cardiac transplantation. *Transplant Proc.* 1995;27:1967–68.
- Starzl TE, Halgrimson CG, Penn I, et al. Cyclophosphamide and human organ transplantation. *Lancet.* 1971;2: 70–74.
- Aagaard-Tillery KM, Jelinek DF. Inhibition of human B

- lymphocyte cell cycle progression and differentiation by rapamycin. *Cell Immunol.* 1994;154:493–507.
21. Karras JG, Wang Z, Huo L et al. Signal transducer and activator of transcription-3 (STAT3) is constitutively activated in normal, self-renewing B-1 cells but only inducibly expressed in conventional B lymphocytes. *J Exp Med.* 1997;185:1035–42.
 22. Tomlanovich SJ, Mycophenolate Mofetil Renal Refractory Rejection Study Group. Rescue therapy with mycophenolate mofetil. *Transplant Proc.* 1996;28:34–36.
 23. Halloran P, Mathew T, Tomlanovich S, et al. The International Mycophenolate Mofetil Renal Transplant Study Groups. Mycophenolate mofetil in renal allograft recipients: A pooled efficacy analysis of three randomized, double-blind, clinical studies in prevention of rejection. *Transplantation.* 1997;63:39–47.