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Report From a Consensus Conference on the Sensitized Patient Awaiting Heart Transplantation

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A consensus conference took place on April 8, 2008 to assess the current status of sensitization in the pre–heart transplant patient, the use and efficacy of desensitization therapies, and the outcome of desensitized patients after heart transplantation. The conference had 71 participants (transplant cardiologists, surgeons, immunologists and pathologists; see Appendix) representing 51 heart transplant centers from North America, Europe, Asia and Australia. Prior to the conference, survey data (regarding the sensitized patient) were submitted by 23 of the 51 centers participating in the conference (Table 1).

There are many unresolved issues in the management of the sensitized patient awaiting heart transplantation. Basic immunologic questions involve detection, specificity and quantitation of circulating antibodies. In addition, there are clinical questions, including:

- Which patients require desensitization therapy?
- What are the best therapies to lower circulating antibodies?
- Is the goal of desensitization therapy to achieve a negative prospective donor-specific crossmatch and/or to affect outcome after transplantation?
- In those desensitized patients who undergo heart transplantation, what post-operative immunosuppressive therapies can optimize outcome?

In what follows is a summary of the presentations given at the conference and the break-out sessions that followed. The information from this consensus conference reflects the current state of sensitized patients awaiting heart transplantation and will lead to further understanding, clarification, and treatment options for these patients.

Clinical Background

Patients awaiting heart transplantation may manifest circulating antibodies against human leukocyte antigens (HLA). This process by which antibodies are formed is called

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sensitization. Sensitization occurs from exposure to blood transfusions, pregnancy, previous organ transplant or the placement of a ventricular assist device. Identification of sensitized patients is a major concern because such patients are at increased risk of hyperacute rejection.

Several reports have demonstrated that pre-transplant sensitization also leads to decreased survival, increased rejection, and development of cardiac allograft vasculopathy (CAV) after heart transplantation. Initial studies have shown that panel-reactive antibody (PRA) tests >10% are associated with lower survival.[1–5] Some investigators have reported that a higher percentage of PRA-positive results are associated with poor outcome. A recent large registry has shown that PRA >25% is associated with poor survival after heart transplantation.[6]

The PRA test using the lymphocytotoxic assay identifies the presence of circulating anti-HLA antibody but not the specificity or strength of antibody. Results that reveal a high percentage of PRA reactivity refer to more individual anti-HLA antibodies being detected. However, in general, the more circulating antibodies detected, the more likely that some of these antibodies exist at high enough quantities to cause immunologic injury to the donor heart. In addition, patients who produce multiple anti-HLA antibodies prior to transplant appear to be more immunoresponsive, which may increase their ability to mount an immunologic response (rejection) against the donor heart after transplantation.[7] The clinical observations correlating high pre-transplant PRA results with lower survival and increased rejection after transplant corroborate these generalizations.[1–5]

There are other antibodies besides anti-HLA antibodies that may damage the donor heart.[8–10] These non-HLA antibodies that may have clinical relevance include autoantibodies (IgM non-HLA, vimentin and anti-heart antibodies) and antibodies to major histocompatibility complex Class I chain A (MICA), major histocompatibility complex Class I chain B (MICB) and undefined endothelial antigens. Antibodies to non-HLA antigens expressed on donor endothelial cells constitute the largest unknown group of potentially clinically relevant non-HLA antibodies. They may be polymorphic cell surface antigens or autoantigens exposed after damage to the endothelial cell.[10] The ability to test for non-HLA antibodies is far behind the refined and sensitive methods currently available to detect HLA antibodies. Further work is necessary to define the most important non-HLA antigens, because detection of non-HLA antibodies and their avoidance or removal is likely to lead to improved graft survival.

Treatment to reduce circulating antibodies prior to transplant has had mixed results. The use of plasmapheresis, intravenous immunoglobulin (IVIg), rituximab (anti–B-cell antibody) and high-dose cyclophosphamide successfully reduces circulating antibodies.[11–14] These therapies have allowed heart transplantation to proceed with a negative prospective donor-specific crossmatch and low risk of hyperacute rejection. However, it has not been established whether these successfully treated pre-transplant sensitized patients have acceptable outcome after heart transplantation.

Specific Background Topic Presentations

I. Detection of Circulating Antibodies: James George, PhD

Recent advances in screening for HLA antibodies have yielded solid-phase, multiplex testing platforms with better sensitivity and specificity than traditional cell-based assays. Today, crossmatching is often performed by flow cytometry, which yields fewer false-positive crossmatches than previously used methods.[15] Before the advent of newer solid-phase assays, the complement-dependent cytotoxicity-based (CDC) assay was commonly used. The addition of anti-human globulin (AHG) increased the sensitivity of CDC assays

and allowed for detection of cytotoxic-negative, absorption-positive HLA alloantibodies. However, because both IgG and IgM can bind complement, neither the CDC nor the AHG-CDC tests can distinguish between the immunoglobulin classes. The CDC also cannot distinguish between major histocompatibility (MHC) Class I or Class II antibodies. Another problem with the CDC assay is that large cell panels are needed to provide coverage for detecting the most common HLA antigens, and rare or unusual antigens are left out.[16]

The new solid-phase techniques can distinguish between IgM and IgG HLA and Class I and II antibodies. Single-antigen methods carry only one antigen per bead, and therefore unique identification of HLA specificities is possible. These new techniques include assays that utilize a multiplex platform and standard flow cytometry, such as the enzyme-linked immunosorbent assay (ELISA), Luminex and FlowPRA tests. The Luminex test allows for simultaneous detection of multiple antibodies, because up to 100 color-coded microspheres can be detected in a single well.[17] The FlowPRA test consists of a pool of microparticle beads coated with full HLA Class I or Class II phenotype derived from purified HLA-bearing cell lines.[18] The percentage of PRAs can be determined by calculating the percentage of beads that react positively with patient sera.

After determining the presence of HLA Class I or Class II antibodies, specificity assays are applied. Specificity tests for HLA antibodies based on flow cytometry use a panel of 55 HLA Class I beads and 32 HLA Class II beads. Because the HLA system is so polymorphic, multiplexing platforms have been developed for specificity testing that contain either HLA Class I or Class II proteins from platelets, or antigens from transformed cell lines that represent all major HLA antigens. However, for patients with multiple antibody specificities, single-antigen technology may be the best approach because it can define every antibody specificity for which there is a single-antigen bead designed.[19]

II. Role of Non-HLA Antibodies in Rejection of Allografted Hearts and Lungs: Marlene Rose, PhD

The impact of more sensitive methods of monitoring HLA antibodies means we are now in a much stronger position to assess the role of non-HLA antibodies in thoracic organ transplant rejection. Nowadays, hyperacute rejection is extremely rare. However, it is time to focus our attention on patients who do not survive beyond the first 30 days. A recent study of 565 adult heart transplant recipients, all of whom had had their pre-transplant sera analyzed for HLA antibodies, demonstrated that 15.6% of HLA antibody-negative patients lost their graft within 30 days of the transplant.[17] The most common cause of graft failure in the first 30 days is primary graft failure; this represents a mixed group of clinical and pathologic features. The disadvantage of the solid-phase assays is that they will not detect non-HLA antibodies that may be directed to antigens present on endothelial cells, cardiac myocytes or leukocytes from the donor. The non-HLA antibodies that may have clinical relevance include autoantibodies (IgM non-HLA, vimentin and anti-heart antibodies) and antibodies to MICA, MICB and undefined endothelial antigens.

IgM non-HLA—IgM non-HLA antibodies are IgM cytotoxic antibodies that react with all leukocytes on a panel including the patients' own leukocytes. A large, retrospective, single-center study of 616 adult heart transplant patients from our center has demonstrated that patients transplanted in the presence of these antibodies have a 1-year survival of 55.9% compared with 75.8% of antibody-negative patients transplanted in the same era ($p = 0.006$, unpublished data). The patient's demise occurs in the first few months after transplantation. Unfortunately, the antigen specificity is unknown, although it may be a carbohydrate antigen.

Antibodies to vimentin and cardiac proteins—Both vimentin and cardiac protein antibodies constitute autoantibodies. Approximately 30% of heart transplant and kidney transplant recipients make de novo anti-vimentin antibodies after transplantation. Anti-vimentin antibodies are made significantly earlier than anti-HLA, and are probably produced as a result of antigens exposed on the surface of damaged or activated cells. Production of these antibodies may only reflect tissue damage, but experimental studies[20] have suggested that they actively participate in rejection, by activating vimentin-positive neutrophils or platelets. Many heart transplant recipients have anti-heart antibodies as a result of their pre-transplant cardiac pathology, and these antibodies may contribute to the rejection of their new graft.

MICA and MICB—Both MICA and MICB are polymorphic antigens, expressed on the surface of epithelial cells; however, their distribution on endothelial cells is not yet established. Studies from our group and others[21] have shown that about 20% of patients have anti-MICA antibodies prior to transplantation. Zou et al demonstrated that pre-transplant MICA antibodies are associated with poorer 1-year survival.[22] However, studies have not yet demonstrated that MICA antibodies lead to rejection episodes after heart transplantation, even when they have been shown to be against mismatched donor MICA. More work needs to be done in this area.

Endothelial antigens—Antibodies to non-HLA antigens expressed on donor endothelial cells constitute the largest unknown group of potentially clinically relevant non-HLA antibodies. They may be polymorphic cell surface antigens or autoantigens exposed as a result of damage to the endothelial cell. Ideally, one should test patient serum by flow cytometry against donor endothelial cells, but this is not practical. Research using methods of purifying donor-derived endothelial cell precursors is currently being undertaken to address this problem.

Summary—The ability to test for non-HLA antibodies is far behind the refined and sensitive methods currently available to detect HLA antibodies. Further work is necessary to define the most important non-HLA antigens. Detection of non-HLA antibodies and their avoidance or removal is likely to lead to improved graft survival.

III. Alloantibodies in Thoracic Organ Transplantation: Are All Antibodies Bad? Adriana Zeevi, PhD

Antibody-mediated rejection (AMR) is associated with worse survival and predisposes patients to vasculopathy. In 2004, under the direction of the ISHLT, a multidisciplinary task force reviewed the biopsy grading system and established criteria for the pathologic diagnosis of AMR.[7] Kfoury and colleagues defined patterns of AMR and cellular rejection based on biopsy diagnosis taken in the first 6 to 12 weeks post-transplant.[23,24] Patients defined as antibody-mediated rejectors, based on three or more AMR episodes, had a significant increase risk for cardiovascular mortality and a 9-fold increase in CAV.[23,24] Isotype switching from IgM to IgG Class II HLA antibody in cardiac recipients was associated with increased risk of recurrent rejection, progression to CAV, and poor long-term allograft survival.[25] In contrast, a lack of isotype switching and persistent IgM production was associated with decreased acute cellular rejection and protection from CAV.[25]

Implementation of sensitive and specific solid-phase antibody detection methods improved the ability to detect pre-formed antibodies and to introduce the virtual crossmatch as a screening tool for sensitized patients.[26] However, with these improved methodologies new questions were raised regarding the clinical significance of all the HLA antibodies detected

(Class I vs Class II) and the importance of titer and specificity (donor-specific antibodies [DSA] vs non-DSA). Further-more, most of the solid-phase assays do not discriminate between complement-activating and non–complement-activating antibodies, and the role of non–complement-activating antibodies in clinical transplantation is controversial. Recent experimental study in a cardiac mouse model of AMR suggested that non-complement antibodies can synergize with low levels of complement antibodies to induce graft damage and AMR.[27]

The role of HLA-C and HLA-DP mismatches in allograft survival and their consideration in virtual crossmatch is still under investigation. The expression of HLA-C on cells is about 10% that of the other Class I HLA alleles, HLA-A and HLA-B. Although anti–HLA-C antibodies in sera of highly sensitized patients are present, the probability of the single HLA-C mismatch in heart recipients is very low. Similarly, DP-reactive antibodies have been associated with positive B-cell crossmatch in renal transplant recipients who were zero HLA antigen mismatched (i.e., matched for A, B and DR).[28] Anti–HLA-DP antibodies were more common in patients with a history of rejection, and therefore these antibodies may have a greater impact in retransplantation evaluations.

Absence of antibody-mediated injury and continued graft function despite the presence of circulating DSA and C4d deposition in the graft may be considered accommodation. Many potential mechanisms may participate in this phenomenon, including the induction of complement-regulatory proteins. In a murine heart transplant model of AMR, blocking the fifth complement component with anti-C5 monoclonal antibody prevented rejection in combination with cyclosporine and cyclophosphamide. Permanent graft survival was achieved with normal histology despite the presence of systemic and intragraft DSA.[29]

The presence of any anti-HLA antibodies is not an absolute barrier to transplantation: it provides risk stratification and may assist in the determination of the optimal immunosuppressive protocol. More studies are needed to better define histologic, immunologic and serologic changes in AMR. C4d deposition and solid-phase techniques for antibody detection should aid in better defining the onset of process of AMR prior to allograft dysfunction.

IV. Antibody Studies: Elaine F. Reed, PhD

Recent studies implicate HLA antibodies in regulating endothelial cell survival and proliferation by binding to Class I and Class II molecules on the surface of the cell and transducing intracellular signals. Anti-HLA antibodies exhibit two primary effector functions: stimulation of cell proliferation and upregulation of cell survival pathways. The intracellular events appear to be influenced by the specificity and concentration of the anti-HLA antibody and the degree of molecular aggregation. High-titered anti–Class I antibodies stimulate growth factor–mediated cell proliferation, whereas low-titered antibodies activate the phosphoinositide 3-kinase (PI3K)/Akt pathway and promote expression of cell survival proteins. These observations suggest that low-titered anti-HLA antibodies could be “good” and benefit the graft by promoting graft accommodation. Conversely, antibodies can have a “bad” or “ugly” effect on graft survival by stimulating cell proliferation that can ultimately result in the development of CAV.

The “good”—Ligation of HLA Class I molecules by anti-HLA antibodies triggers a series of intracellular signaling cascades within endothelial cells. The signaling events include phosphorylation of Src, p125 focal adhesion kinase (FAK) and paxillin.[30–32] PI3K and Akt are important downstream targets of Class I–mediated FAK phosphorylation and their kinase activity promotes cell survival by regulating levels of the anti-apoptotic proteins Bcl-2 and Bcl-xL.[33] Exposure of endothelial cells to anti–Class I antibodies resulted in

elevated levels of Bcl-2 and Bcl-xL expression. Maximum Class I–mediated increases in Bcl-2 and Bcl-xL protein expression were observed when endothelial cells were exposed to low concentrations of anti–Class I antibodies.[33] A similar effect of antibody concentration on Class I–mediated phosphorylation of Akt was seen, with the highest level of Akt phosphorylation achieved when cells were treated with low concentrations of antibody. Akt stimulates cell survival by phosphorylating members of the death apparatus, such as Bad, and preventing its interactions with Bcl-2 and Bcl-xL at the mitochondrial membrane.[34] These findings are reminiscent of studies in xenogeneic and ABO-incompatible transplantation that showed increased expression of Bcl-xL, Bcl-2, A-20 and HO-1 on the graft endothelium and protection from apoptosis after exposure to antibodies.[35,36] This phenomenon of resistance to the effects of anti-graft antibodies has been termed graft accommodation.[35,36] Our data are also consistent with studies by Salama et al and Narayanan et al showing that endothelial cells treated with sub-saturating concentrations of anti-HLA antibodies had increased expression of Bcl-2 and Bcl-xL and were rendered refractory to endothelial cell activation and became resistant to complement-mediated lysis.[37,38]

The "bad"—The mammalian target-of-rapamycin (mTOR)/S6 kinase/S6RP pathway has emerged as a major effector of cell growth and proliferation via the regulation of protein synthesis. Class I ligation on the surface of endothelial cells leads to activation of mTORC1, resulting in phosphorylation of S6 kinase and S6RP.[39] Knockdown of mTOR inhibited Class I–induced proliferative responses, demonstrating a role of mTOR in regulating Class I–mediated cell protein synthesis and proliferation. Using siRNA knockdown of Rictor, we identified mTORC1 as an upstream regulator of MHC Class I–induced proliferation. Exposure of endothelial cells to rapamycin blocked Class I–mediated cell proliferation. However, long-term exposure of endothelial cells (EC) to rapamycin also blocked MHC Class I–induced phosphorylation of Akt Ser473 and expression of pro-survival protein Bcl-2. This indicates that the mTOR inhibitor rapamycin may be effective in mitigating anti-HLA antibody-mediated activation of the mTOR/S6K/S6RP pathway. However, blocking mTOR with rapamycin blocks Class I–mediated activation of Akt at Ser473 and upregulation of Bcl-2.

The "ugly"—The most severe consequence of antibody binding to Class I molecules on endothelial cells is the initiation of intracellular signals that synergize with growth factor receptors to stimulate cell proliferation.[40–43] The primary mechanism through which anti-HLA Class I antibodies stimulate cell proliferation is by upregulating expression of fibroblast growth factor receptors and increasing fibroblast growth factor ligand binding.[42] Antibody ligation of Class I molecules in endothelial cells stimulates the redistribution of fibroblast growth factor receptors from intracellular stores to the plasma membrane in a dose-dependent fashion with the highest dose of antibody promoting the greatest degree of fibroblast growth factor receptor expression and cell proliferation. Antibody binding to HLA-A and -B locus molecules and signal transduction is dependent both on the antibody concentration and the level of HLA antigen expression.

Clinical implications—Our results indicate that anti-HLA antibodies will likely play an important role in influencing transplant outcome depending upon their concentration. High concentrations of anti–Class I antibodies could have a detrimental effect on graft survival by inducing expression of fibroblast growth factor receptors and cell proliferation, and promoting development of CAV. Lower concentrations of anti-HLA antibodies may play an important role in promoting graft accommodation by activating the PI3K/Akt cascade, upregulating expression of anti-apoptotic proteins, and conferring endothelial cell resistance to injury.

V. Defining Unacceptable Antigens and Use of Virtual Crossmatch in Thoracic Transplantation: Nancy Reinsmoen, PhD

For patients who have developed anti-HLA antibodies through pregnancy, transfusion and prior transplants, the wait times for transplantation are significantly longer than for patients who are not sensitized. For sensitized patients on the thoracic organ wait lists, these increased wait times have resulted in an increased rate of death while on the wait list. Prospective crossmatches were required to identify compatible donors for these patients. This approach required the predominant use of donor organs procured locally. In 2001, the virtual crossmatch was implemented at Duke University Medical Center (DUMC) to aid in selecting compatible donors for these sensitized patients. Flow cytometry–based single-antigen bead assays allowed for the clear identification of antibody specificities present. Thus, donors with these antigens could be avoided and a compatible donor could be selected without the need for a prospective crossmatch. With this approach, the percentage of sensitized lung patients transplanted at DUMC increased from 8% in 2001 to 26% in 2007. The donor organs procured outside our organ procurement organization (OPO) rose from 30% to 78% during that period. This increase in the donor pool and the elimination of the prospective crossmatch has resulted in decreased wait times and a decreased incidence of death on the wait list for sensitized patients, comparable to that of non-sensitized patients.[44]

Successful implementation of the virtual crossmatch requires several strategies. First, the level of immunologic risk must be identified and the ability to manage high-risk patients must be determined by each transplant program. Based on our previous studies,[45] we worked under the premise that all donor antigenspecific antibodies have consequence, including those negative by cytotoxicity but positive by flow cytometry techniques. We also correlated the level of binding observed in the single-antigen bead assay with the level of binding determined to result in a positive flow cytometry crossmatch. Recently, we have implemented a quantitative approach to single-antigen bead, Luminex-based assays that allows for comparison from one test to the next. Ongoing education sessions for the transplant team and the immunology laboratory team members is essential because a high level of HLA knowledge is required as well as the timing and urgency of results needed. Effective and continual communication among the team members is needed to identify the high-risk patients and to follow post-transplant antibody courses. Alert indicators must be established to identify when immediate communication is required. It is essential that the deceased donors be accurately typed for all relevant HLA antigens. If the patient has antibodies to HLA-C, -DRw51/52/53, -DQ and/or possibly -DP, this additional HLA information needs to be transmitted to the transplant team to allow for proper selection of compatible donors. However, the virtual crossmatch is only as accurate as the last serum sample tested. Thus, it is vital that the laboratory be informed of all sensitizing events, such as transfusions, infections and the placement of assist devices, that can result in changes of the antibody status. In conclusion, selecting compatible donors by identifying unacceptable antigens in the donor pool and the use of the virtual crossmatch for sensitized patients has resulted in a significantly increased number of sensitized patients being successfully transplanted.

VI. Circulating Antibodies in Pediatrics: Lori West, MD, DPhil

Cryopreserved allograft tissue is often used for potential heart transplant infants with hypoplastic left heart syndrome. However, this tissue is associated with significant donor-specific immunologic sensitization due to increased Class I and Class II HLA alloantibody response and broad panel reactivity.[46–48] Mechanical circulatory support is also becoming more common in pediatric patients awaiting transplants. High pre-transplant PRA levels in children are associated with significantly higher mortality rates post-transplant, despite increased immunosuppression.[49]

Due to the difficulty of obtaining a prospective negative crossmatch for pre-sensitized pediatric heart transplant candidates, the outcome of these patients without negative prospective crossmatches has been evaluated.[50,51] In both studies, plasmapheresis was initiated in patients upon identification of a potential donor. Post-transplant plasmapheresis ± IVIg was used in addition to induction therapy with cyclophosphamide or rituximab. The pre-sensitized pediatric patients without prospective crossmatch had comparable short-term survival to that of unsensitized patients, but had a higher frequency of early post-transplant rejection, often with hemodynamic compromise. The long-term effect on CAV and mortality is still unknown.

VII. Circulating Antibodies and Ventricular Assist Devices: Mandeep Mehra, MD

Left ventricular assist devices (LVADs) are responsible for sensitization through upregulation of the immune system and an increased antibody production,[52,53] due to their specific physical properties, their blood-contacting surface, and the frequent need for blood product support. However, the true impact of LVAD sensitization on outcome after heart transplantation is controversial.[54–56] Contemporary evidence suggests an approximate 30% incidence of antibody production (PRA >10%) after LVAD placement.[57,58] Different devices have reported different incidence rates for sensitization. Most studies in the USA with the HeartMate I LVAD have reported sensitization rates ranging from 40% to 66%.[55,59,60] The reason for the high rate of sensitization for the HeartMate I LVAD is that the texture of the inner surface allows for the formation of a pseudo-intima that contains T cells and dendritic cells, which activates and upregulates both T- and B-cell populations. The Novacor devices have shown an 18% sensitization incidence,[61] whereas the Thoratec device had a higher rate of sensitization than either of the aforementioned devices.[62] Axial flow pump LVAD devices have been reported to decrease sensitization.[63]

There have been two reports on outcome in patients with LVADs who subsequently underwent heart transplantation. John et al reported on 105 patients on LVAD support, with 66% (69 of 105) of patients developing HLA antibodies compared with only 6% (24 of 399) of non-bridged patients awaiting heart transplantation.[55] Among sensitized LVAD patients, 26 were treated with a pre-transplant immunomodulatory regimen consisting of IVIg and cyclophosphamide. After heart transplantation, 5-year survival, freedom from rejection and CAV were similar between the LVAD and non-bridged recipients. In another study, Gonzalez-Stawinski et al studied 238 patients (125 LVAD and 113 non-bridged patients).[56] The LVAD patients were more likely to be sensitized than non-bridged patients (20% vs 5%, $p < 0.01$). Eighteen LVAD patients (14%) received pre-transplant plasmapheresis compared with only 3 non-bridged patients (2.6%, $p < 0.01$). Immediately after transplantation, 27 LVAD patients received OKT3 and 6 LVAD patients received anti-thymocyte globulin, daclizumab or immunoglobulin therapy, compared with only 2 non-bridged patients who received OKT3. Similar to the previous study, post-transplant 5-year survival and freedom from rejection were comparable between groups. These studies suggest that LVAD patients have survival outcomes similar to those of non-bridged patients after heart transplantation, despite the significantly higher immunologic risk due to sensitization. It is possible that pre- and post-transplant immunomodulatory therapy counter this higher immunologic risk.

VIII. Desensitization Experience in Six Cardiac Transplant Programs (Columbia University, University of Berlin, Loyola Medical Center, University of California Los Angeles, University of Toronto and University of Wisconsin)

Six cardiac transplant programs presented their pre-transplant desensitization protocols. Most centers treated pre-transplant PRAs >50% and used a combination of plasmapheresis, IVIg and rituximab. Interestingly, no program had been using oral medications, such as

cyclophosphamide or azathioprine, which were mainstays of therapy in the past. The Loyola Cardiac Transplant Program reviewed their protocol of desensitization at the time of transplant. However, in all programs, including Loyola, quantitation of antibodies was not performed. Therefore, it is not clear whether the detected circulating antibodies actually required desensitization therapy. Examples of desensitization therapies are given in Table 2.

IX. Treatment of the Treated Pre-transplant Sensitized Patient After Cardiac Transplantation (University of California at Los Angeles Experience): Jignesh Patel, MD, PhD

Sensitized patients prior to heart transplantation are reportedly at risk for hyperacute rejection and for poor outcome after heart transplantation. It is not known whether reduction of circulating antibodies pre-transplant alters post-transplant outcome.

Methods and results—Between July 1993 and July 2003, we reviewed 523 heart transplant patients, of whom 95 had pre-transplant PRAs >10%. Twenty-one of 95 were treated pre-transplant for circulating antibodies. These 21 patients had PRAs >10% (majority at 50% to 100%) and were treated with combination therapy, including plasmapheresis, IVIg and rituximab, to reduce antibody counts. The 74 untreated patients with PRAs >10% (untreated sensitized group) and those patients with PRAs <10% (control group) were used for comparison. Routine post-transplant immunosuppression included triple-drug therapy (tacrolimus or cyclosporine, azathioprine or mycophenolate mofetil and corticosteroids). Circulating antibody levels pre-transplant decreased from a mean of 70.5% to 30.2%, which resulted in a negative prospective donor-specific crossmatch and successful heart transplantation. Compared with the untreated sensitized group and the control group, the treated sensitized group had similar 5-year survival (81.1% and 75.7% vs 71.4%, respectively, $p = 0.523$) and freedom from CAV (74.3% and 72.7% vs 76.2%, respectively, $p = 0.850$).

Conclusion—Treatment of sensitized patients pre-transplant appears to result in acceptable long-term outcome after heart transplantation.

Summary of the Break-Out Sessions from the Consensus Conference on Sensitization

Many clinically relevant issues arose during the consensus conference. These issues included the *identification*, *specificity*, *quantitation* and *clinical correlation* of circulating antibodies both pre- and post-transplant. The 71 participants of the consensus conference participated in smaller break-out sessions to address these topics and attempt to achieve consensus on the approach to the sensitized patient. A summary of these discussions is provided in what follows.

Pre-transplant Sensitization

The presence of circulating anti-HLA antibodies in the patient awaiting heart transplantation is associated with a high risk of hyperacute rejection and poor outcome after transplant, including an increased risk for first-year rejection, hemodynamic compromise rejection (symptoms of heart failure requiring inotropic support), decreased survival, and increased risk for the development of CAV.

Identification of circulating antibodies—Identification of circulating antibodies is achieved reliably through the use of solid-phase assays such as flow cytometry, Luminex and enzyme-linked immunoassay techniques. Current problems include standardization of the assays and a common language of reporting. These problems are being addressed by the

entire solid-organ transplant community, as this applies to all patients waiting for organ transplants. Retrospective studies are planned to: (1) compare different assays to detect circulating antibodies from different laboratories; and (2) assess the clinical relevance of circulating antibodies after transplantation. Multicenter, prospective studies using standard reference laboratory reagents prepared from human monoclonal antibodies are also being planned.

Another important issue is that some OPOs perform a donor-specific crossmatch for antibodies against only T cells (Class I anti-HLA antibodies) and not B cells (Class II anti-HLA antibodies). It is believed that Class I HLA antigens are constitutively expressed on donor endothelial cells and, therefore, if donor-specific antibodies are present in the recipient, hyperacute rejection could occur at the time of transplant. Class II HLA antigens are usually not constitutively expressed on donor endothelial cells and thus are involved mainly in delayed (several days) hyperacute rejection after transplant. Because both pre-transplant Class I and Class II anti-HLA antibodies significantly impact post-transplant outcomes, avoidance/reduction in both classes should result in improved long-term outcomes. Therefore, one suggestion of the consensus conference is that significant donor-specific Class II antibodies, in addition to Class I antibodies, should be avoided in the potential donor.

Specificity—If circulating antibodies are detected, then determination of antibody specificity is paramount. Solid-phase assays, such as the single-antigen bead assay, can identify the specific antibodies present in the pre-transplant patient. The corresponding HLA antigens can then be listed in the United Network for Organ Sharing (UNOS) online database as unacceptable antigens for any potential donor. It has been noted that different vendors coat the single-antigen beads with different concentrations of antigen, which may result in different results if compared with another vendor's beads. Standardization of antigen concentration on all beads is being pursued.

Quantitation—The amount of circulating antibody present in the patient waiting for a heart transplant plays an important role in patient outcomes. In the past, quantitation of antibodies has been performed by a dilution technique. The newer solid-phase assays make use of fluorescence technology and are more precise in quantitating antibody levels. These assays are described as mean fluorescent intensity (MFI), molecular equivalents of fluorescence (MESF) and standard fluorescent intensity (SFI). Such quantitative methods also need to be standardized across laboratories.

Virtual crossmatch—The virtual crossmatch is a relatively new method that increases the chances of finding an acceptable donor for the sensitized patient. With a virtual crossmatch, recipient blood samples are not required; donors with HLA antigens matching the recipient's HLA antibodies are avoided. Because it is difficult and costly to send recipient blood samples to all surrounding OPOs (to perform a prospective donor-specific crossmatch), the virtual crossmatch allows all donors (without a prospective donor-specific crossmatch) to be paired with the recipient, thus increasing the donor pool for that recipient.

Clinical correlation—If circulating antibodies are detected in the patient waiting for heart transplantation, what are the clinically relevant issues? The first issue is the quantity of these circulating antibodies (antibody strength/level) that would place that patient at risk for hyperacute rejection at the time of transplant as well as poor outcome later after transplant (this is discussed further in the section on post-transplant considerations). If circulating antibody levels in the pre-transplant patient are found to be significant, when should one

intervene and what then can be done to lower these antibody levels? And, does lowering of antibody levels make a difference in clinical outcome?

The quantity of circulating antibodies in the pre-transplant patient appears to be paramount in mediating the risk of hyperacute rejection at the time of transplant. It is generally accepted that a prospective negative cytotoxic crossmatch is associated with a low likelihood of hyperacute rejection. The question arises, at what antibody-level (strength) threshold in the pre-transplant patient would result in a positive prospective cytotoxic donor-specific crossmatch (and subsequent high risk for hyperacute rejection)? Recent data have shown that a correlation between the strength of anti-HLA antibody detected in solid-phase assays and association with crossmatch tests and transplant outcome can be determined.[64,65] This approach to determine specific measurements of antibody strength in solid-phase assays that predict a positive crossmatch has been adopted in many histocompatibility laboratories and their transplant programs. Currently, in the UCLA program, antibodies with MFI >5,000 on single-antigen Luminex beads correlate with flow-positive T- and B-cell crossmatches. Antibodies with MFI > 10,000 on single-antigen Luminex beads may have cytotoxic potential and, if donor-specific, could precipitate hyperacute rejection. The determination of an antibody level threshold is important for a virtual crossmatch as those antibodies that exceed this threshold would have their corresponding antigen listed as unacceptable for any potential donor. For example, if a patient has a 90% PRA screen with specificities revealing anti-HLA antibodies with high antibody levels (MFI >5,000 is selected in this case) for the HLA antigens A2, B27, B56 and DR3, then a donor with these HLA antigens would be considered unacceptable. The accurate prediction of the crossmatch results, however, depends not only on the antibody strength but also on the density of HLA molecules on the cell surface, which can vary among individuals.

If a circulating antibody-level in a pre-transplant patient should exceed a pre-determined antibody-level threshold toward any donor, this should correlate not only with a positive prospective donor-specific cytotoxic crossmatch but also to a positive donor-specific flow cytometry crossmatch. Currently, in the UCLA program, a donor-specific antibody level of 5,000 to 7,000 MFI on single-antigen Luminex beads correlates with donor-specific flow cytometry crossmatch-adjusted median channel shifts of 50 to 200 channels (using a cytometer with a 1,024-channel scale). It has been reported in kidney studies that a flow cytometry donor-specific crossmatch threshold of <200 median channels is acceptable to proceed with transplantation. This has not been established in the heart transplant field. Retrospective studies to determine this are in the planning stage.

Selecting an antibody-level threshold is also dependent on the program's degree of risk and the severity of illness of the sensitized patient. If a heart transplant program feels equipped to handle antibody-mediated rejection or has an extremely ill patient, then a higher antibody-level threshold can be selected to increase the donor pool for that specific patient. The higher the antibody-level threshold, the fewer unacceptable antigens listed, because the corresponding antibodies are lower than the designated threshold. Alternatively, a conservative program would employ a lower antibody-level threshold, which could limit the donor pool but may lower the risk for hyperacute rejection.

Complex cases exist where there are multiple circulating anti-HLA antibodies present with some antibody levels just below the threshold (e.g., if a MFI of 5,000 is the chosen threshold, MFIs between 3,000 to 5,000 are considered moderately elevated). In these cases one might face scenarios by which two moderately elevated antibodies to a potential donor might pose a risk for acute or hyperacute rejection. Therefore, this might result in refusal of that specific donor organ. This evaluation process might involve organizing a panel with

experienced members to jointly decide what antigen combinations would be unacceptable (and/or what antigen combinations would be acceptable).

The decision to proceed with desensitization therapy should be dependent on the percent chance that any donor will be available for the sensitized patient. This decision can be determined through the use of unacceptable antigens. Once these unacceptable antigens are designated (as noted earlier), they would be placed onto the UNOS website (http://www.unos.org/resources/frm_CPRA_Calculator.asp). The UNOS website would then provide a calculated PRA (cPRA), which will give the percentage chance that any donor will *not* be compatible given the designated unacceptable antigens. If the probability of finding a compatible donor is low (i.e., cPRA >50%, which means there is a greater than 50% chance of an unacceptable donor), then desensitization protocols might be considered for this patient. This probability cutoff (of 50% in this case) is also open to determination by each program.

If circulating antibodies that are above a certain level or strength (listed as corresponding unacceptable antigens) can be reduced by desensitization therapies to a level below a pre-determined threshold (and, therefore, not listed as unacceptable antigens), then more donors may be available for that patient. Various desensitization therapies have been reported, but the optimal protocol has yet to be established. High-dose IVIg has been reported by several heart transplant programs to be effective in lowering circulating antibody. Plasmapheresis has also been demonstrated to be effective, although the optimal frequency and duration is not known. Kidney transplant studies have suggested that plasmapheresis is helpful in the short term but after a short time the circulating antibodies return. Rituximab, a monoclonal antibody selective against CD20 on B cells, has also been used to reduce circulating antibodies, with a variable response.

Antibody monitoring—A protocol for monitoring of antibodies has not been established. All patients waiting for heart transplantation have an initial blood test to detect circulating antibodies. For pre-transplant patients without detectable circulating antibodies, PRA screens should be obtained every 6 months. For patients with circulating antibodies, PRAs should be checked every 3 months. Patients with VAD support should have PRAs checked every month. After blood transfusions and infections, PRAs should be checked 1 to 2 weeks after the event. After desensitization therapy, PRAs should be checked 1 to 2 weeks after therapy.

Post-transplant Considerations

Monitoring—Post-transplant donor-specific antibody monitoring should be performed daily for the first week after heart transplantation for those patients who are considered high risk for antibody-mediated rejection to identify a possible amnestic antibody response that could lead to a delayed hyperacute rejection. This can be performed with use of donor cells in the form of a donor-specific cytotoxic cross-match. If significant donor-specific antibody is identified, then appropriate therapy can be initiated. This therapy can include thymoglobulin, plasmapheresis, IVIg and/or rituximab.

Immunosuppression—For patients who received desensitization therapy prior to transplant and for those patients who are considered high risk for antibody-mediated rejection, the empiric use of thymoglobulin has been recommended. This can be followed by the use of IVIg, plasmapheresis and/or rituximab. Maintenance immunosuppression with tacrolimus, mycophenolate mofetil and corticosteroids is recommended as a result of a multicenter, randomized trial suggesting that this regimen had the most favorable profile for

prevention of any treated rejection (which includes cellular and antibody-mediated rejections).[66]

Consensus Statements for Pre-Transplant Sensitization

The recommended frequency for antibody screening and identification is as follows:

- If no evidence of sensitization, a frequency of every 6 months is advised.
- In patients with detectable circulating antibodies, a frequency of every 3 months.
- In LVAD recipients, the optimal frequency is once per month.
- With “interceding events” (such as blood transfusions) we recommend a PRA screen at 1 to 2 weeks after the event.
- After desensitization therapy, PRAs should be checked 1 to 2 weeks after therapy.
- In all others (pediatric, retransplant, parous women), a frequency of every 3 months is advised.

Testing methodology:

- Identify circulating antibodies with a solid-phase assay such as flow cytometry.
- Delineation of complement fixation capability of detected antibodies should be reported.
- Anti-HLA Class I and II specificities must be defined (any HLA antibody directed against HLA-A, -B, -Cw, -DR and -DQ).
- Quantitate circulating antibodies to assess for unacceptable antigens and to obtain the calculated PRA.
- In the absence of international standards, each center must develop thresholds for definitions of unacceptable antigens.
- Consider the use of the virtual crossmatch (utilizing the unacceptable antigens) to increase the donor pool for any one sensitized individual.

Desensitization recommendations:

- If the calculated PRA is significant (the cutoff for significance is dependent on the transplant program; for example, greater than 50% chance that a donor is not acceptable), then desensitization therapy should be considered.
- Desensitization therapy may include IVIg and possibly rituximab.
- Plasmapheresis followed by IVIg may be considered for urgent transplants (Status 1A patients).
- A call for development of an international registry that proposes to use archived samples and follow patients prospectively was expressed.

Consensus Statements for Post-Transplant Considerations

Post-transplant considerations include:

- Measure post-transplant donor-specific antibodies at pre-determined time periods.
- Consider the use of thymoglobulin as induction therapy for post-transplant patients considered high risk (includes those treated with desensitization therapy prior to transplant) for antibody-mediated rejection.

- Consider the use of tacrolimus, mycophenolate mofetil and corticosteroids as maintenance immunosuppression therapy for those patients at high risk for antibody-mediated rejection.

Recommendations for Clinical Trials

- Mechanistic trials that center around the principles of antibody removal (plasmapheresis), antibody binding (IVIg) and antibody suppression (rituximab/cyclophosphamide) should be conducted.
- Desensitization trial. Similar to the kidney experience, a randomized trial of high-dose IVIg vs high-dose IVIg plus rituximab. Sensitized patients with a calculated PRA >25% to 50% (meaning more than 25% to 50% of donors would be unacceptable) would be eligible for this study. The end-points would include time to transplant, effective lowering of circulating antibodies and effective lowering of the calculated PRA.
- A randomized, controlled trial of thymoglobulin in patients immediately post-operatively, with a CDC-negative, but flow-positive, crossmatch.
- A randomized, controlled trial of triple-therapy approach (post-operative IVIg, plasmapheresis and rituximab) vs traditional immunosuppression alone in patients at high risk for antibody-mediated rejection.
- A randomized trial on the treatment of patients with post-transplant asymptomatic donor-specific antibody with IVIg/rituximab vs no therapy.

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Appendix: Consensus Conference Attendees

Keith Aaronson, MD, University of Michigan Medical Center; Juan Alejos, MD, David Geffen School of Medicine at UCLA; Nicholas Banner, FRCP, Harefield Hospital (UK)

David Baron, MD, Newark Beth Israel Medical Center; Robert Bourge, MD, University of Alabama at Birmingham; Dragan Bucin, MD, PhD, University Hospital (Sweden)

Charles Canter, MD, St. Louis Children's Hospital; Bernard Cantin, MD, PhD, Hospital Laval (Canada); Anil Chandraker, MD, FRCP, Brigham and Women's Hospital; Patricia Chang, MD, University of North Carolina, Chapel Hill; Daniel Cook, PhD, One Lambda

Paul Corris, MD, Freeman Hospital (UK); Lawrence Czer, MD, FACC, FACP, Cedars-Sinai Medical Center; Teresa De Marco eF. MD, University of San Francisco Medical Center; David DeNofrio, MD, New England Medical Center; Brooks Edwards, MD, Mayo Clinic

Howard Eisen, MD, Drexel University College of Medicine; David Feldman, MD, PhD, Ohio State University; Daniel Fishbein, MD, University of Washington, Seattle; James George, PhD, University of Alabama at Birmingham; Michael Givertz, MD, Brigham and Women's Hospital; Lee Goldberg, MD, University of Pennsylvania; Alain Heroux, MD, Loyola University Medical Center; Manfred Hummel, MD, PhD, German Heart Institute (Germany); Sharon Hunt, MD, Stanford University Medical Center; Maryl Johnson, MD, FACC, University of Wisconsin School of Medicine; Ingo Kaczmarek, MD, LMU Munich (Germany); Andrew Kao, MD, Mid America Heart Institute; Anne Keogh, MBBS, MD,

University of New South Wales (Australia); Ronald Kerman, PhD, University of Texas Medical School; James Kirklin, MD, University of Alabama; Michelle Kittleson, MD, PhD, David Geffen School of Medicine at UCLA; Jon Kobashigawa, MD, David Geffen School of Medicine at UCLA; Robert Kormos, MD, University of Pittsburgh; Hans Lemkuhl, MD, Deutsches Herzzentrum Berlin (Germany)

JoAnn Lindenfeld, MD, University of Colorado; Joren Madsen, MD, Massachusetts General Hospital; Donna Mancini, MD, New York Presbyterian Hospital; David Markham, MD, University of Texas Southwestern; Mandeep Mehra, MD, University of Maryland School of Medicine; Leslie Miller, MD, Washington Hospital Center; Takeshi Nakatani, MD, PhD, National Cardiovascular Center (Japan); Gerard O'Driscoll, MD, PhD, Royal Perth Hospital (Australia); Jayan Parameshwar, FRCP, Papworth Hospital (UK); Jignesh K. Patel, MD, PhD, David Geffen School of Medicine at UCLA; Si Pham, MD, University of Miami/ Jackson Memorial; Richard Pierson, MD, University of Maryland, Baltimore; Sean Pinney, MD, Mount Sinai School of Medicine; Barbara Pisani, DO, St. Luke's Medical Center, Milwaukee; Jeffrey Platt, MD, Mayo Foundation; Elain Reed, PhD, David Geffen School of Medicine at UCLA; Nancy Reinsmoen, PhD, Cedars-Sinai Medical Center; E. Rene Rodriguez, MD, Cleveland Clinic; Joseph Rogers, MD, Duke University Medical School; Marlene Rose, PhD, Harefield Hospital (UK); Bruce Rosengard, MD, Massachusetts General Hospital; Heather Ross, MD, Toronto General Hospital (Canada); Stuart Russell, MD, Johns Hopkins Hospital; Marc Semigran, MD, Massachusetts General Hospital; John Smith, MD, Harefield Hospital (UK); Randall Starling, MD, MPH, Cleveland Clinic; Susan Stewart, FRCP, Papworth Hospital (UK); Nicole Suciu-Foca, PhD, Columbia University College of Physicians & Surgeons

Anat Tambur, PhD, Northwestern University; David Taylor, MD, Cleveland Clinic Foundation; Patricia Uber, PharmD, University of Maryland; Hannah Valantine, MD, Stanford University School of Medicine; Adrian Van Bakel, MD, PhD, Medical University of South Carolina; Lori West, MD, PhD, University of Alberta (Canada)

Adriana Zeevi, PhD, University of Pittsburgh; Andreas Zuckermann, MD, Medical University of Vienna (Austria).

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Table 1

Sensitization Consensus Conference Survey Results (April 8, 2008)[a]

Assays used by surveyed centers to detect circulating antibodies:

- 78% use Luminex
- 65% use flow cytometry
- 61% of centers use complement-dependent cytotoxicity
- 4% use enzyme-linked immunoassay
- 91% of centers perform antibody specificities

Treated sensitized patients (since January 2000–present):

- Total number of heart failure patients referred for transplant: 4,640
- Total number of treated sensitized patients referred for transplant: 362

  Percent of patients referred for transplant that were treated sensitized patients: 8%

Experience with sensitized patients on ventricular assist devices (VADs):

- Total number of sensitized patients on VADs: 141 This represents 39% of all treated sensitized patients
- 14% of programs have a special protocol to treat sensitized patients on VADs

Treatment of the sensitized patient:

- Average threshold PRA level for initiation of treatment: 35% (range 10–100%)
- 48% with elevated anti–B-cell circulating antibodies (without elevated anti–T-cell antibodies)
- 65% of centers use virtual crossmatch
- 48% of centers will transplant across a donor specific antibody
- On average, 45% (range 0–100%) of treated sensitized patients had a significant reduction (≥50%) in circulating antibodies
- On average, 73% (range 13–100%) of treated sensitized patients underwent successful heart transplantation
- 43% of centers use a special protocol for immunosuppression and/or post-operative therapies for transplanted treated sensitized patients

Current therapies used by surveyed centers to reduce circulating antibodies:

Therapy	Initial therapies	Secondary therapies
IVIg	65%	48%
Plasmapheresis	57%	52%
Rituximab	30%	39%
Mycophenolate	43%	26%
Cyclophosphamide	39%	30%
Methotrexate	4%	4%
Tacrolimus	4%	4%
Daclizumab	4%	4%
Azathioprine	4%	0%

[a] A total of 23 participating centers were represented.

Table 2
Examples of Desensitization Therapies

	Dose	Frequency
Plasmapheresis	(A) 1.5 volume exchanges	(A) 5 consecutive days
		(B) 5 times, every other day
		(C) 2–3 times/week until transplant
		(D) 5 times, every other day, every 2–4 weeks
	(F) 1.5 volume exchanges	
Intravenous immunoglobulin (IVIg)	(A, B) 2 g/kg IV divided over 2 days	(A) Every 2–4 weeks
	(C) 2–3 g/kg IV divided over 4 days	
	(D) 0.1 mg/kg IV	(D) Every 2–4 weeks
	(E) 100 mg/kg IV	(E) Every 4 weeks
	(F) 20 g (of 10% IVIg)	
	(G) 150 g (of 10% IVIg) divided over 3 rounds	(G) Every 2 weeks
Rituximab	(A) 1 g IV	(A) Weekly times 4
	(C) and (E) 375 mg/m^2	(C) Times 2 doses
		(E) Weekly times 4
	(G) 500 mg	(G) Every 2 weeks
Cyclophosphamide (used in the past)	(A) 1 mg/kg orally	(A) Daily
	(C) 0.5–1 g/m^2 IV	
	(D) 1 mg/kg orally	

(A) = UCLA; (B) = Stanford University; (C) = University of Maryland; (D) = University of Toronto; (E) = University of Wisconsin; (F) = Loyola Medical Center; (G) = University of Berlin. IV, intravenous.