A report on the first joint Transplantation Histocompatibility Conference – 2008: Summary and Follow-up

The first Transplantation Histocompatibility Conference, jointly sponsored by ASHI, ASTS, AST, UNOS, ISHLT, and NIH, was held in Chicago on March 3 - 4, 2008 and focused on HLA antibody testing using solid phase methodologies. The meeting was successful in directing attention to the role of HLA antibody in transplant success and the use of the newer solid phase methods to predict risk factors associated with HLA antibody. This was evidenced by the outpouring of papers on this subject and an unprecedented number of abstracts and presentations on HLA antibody at all of the transplant meetings during the last two years. Many feel that the developments in HLA antibody testing over the past decade have made this one of the most exciting times to be involved in HLA testing. The purpose of this article is to give a summary of what was discussed at the 2008 meeting, review some of the developments since the meeting and discuss new ideas and projects that should be pursued and discussed at the next meeting. A second Transplantation Histocompatibility Conference is scheduled for November 2010.

Summary report from Transplantation Histocompatibility Conference held March 3-4, 2008.

The introduction of solid-phase tests to identify antibodies directed against human histocompatibiltiy antigens (HLA) over the past decade represents an important addition to the tools available for measuring sensitization of organ transplant candidates. The UNOS Histocompatibility Committee estimates that at least 98% of laboratories are currently using solid-phase technologies. The use of purified HLA antigens and HLA antigens produced by recombinant DNA technologies permits a more accurate and precise identification of antibodies, particularly for patients who have been broadly immunized by prior transplants, pregnancies, and other exposures to allogeneic HLA antigens. These tests are far more sensitive than cell-based assays and the clinical importance of allo-antibodies at these low levels has not been well-established. A multi-disciplinary approach to clarify the clinical importance of HLA antibodies and how best to use the solid-phase tests in different clinical settings is clearly required. To that end, a Transplantation Histocompatibility Conference was held in 2008 that was jointly sponsored by the American Society of Histocompatibiltiy and Immunogenetics (ASHI), the American Society of Transplantation (AST), the American Society of Transplant Surgeons (ASTS), the United Network for Organ Sharing (UNOS), the International Society for Heart and Lung transplantation (ISHLT),and the National Institutes of Health (NIH). Fifty-one transplant professionals from various transplant programs and representatives from UNOS and NIH attended the meeting.

Issues discussed were: a) the correlation between pre-transplant donor-specific antibody levels identified with solid-phase HLA antibody tests and crossmatch results using cytotoxicity and flow cytometry assays, b) the ability of laboratories to identify donor-specific antibodies accurately in order to predict crossmatch results and facilitate transplantation for highly sensitized patients, and c) the benefits of identifying donor-specific antibodies that develop after transplantation. Presentations primarily focused on microparticle bead assays which allow independent analysis of antibodies to HLA Class I

or Class II antigens (phenotypic beads) or to individual HLA antigens (single antigen beads). Compared to cell-based assays, appreciably greater sensitivity is obtained with these beads using either Flow cytometry or Luminex instrumentation.

Dr. Peter Nickerson presented a Canadian study which showed excellent concordance between laboratories using solid-phase tests to detect strong and moderately strong antibodies. Less consistency was seen with weaker antibodies. Much discussion ensued regarding the difficulty in establishing a clinically relevant cutoff. It was noted that different cutoff values currently exist among labs and transplant programs. There was general agreement that a prospective, multicenter trial would be beneficial in establishing the clinical importance of weak antibodies.

Dr. Sue Leffell explained how the use of a calculated PRA allows each transplant program to establish their own level of sensitivity when entering unacceptable antigens. Patients that are candidates for desensitization protocols will enter only the strongest antibodies that are likely to be refractive to desensitization efforts.

Dr. Nancy Reinsmoen and Dr. Adriana Zeevi showed data that indicated an important role of donor-specific antibody in heart and lung transplants both before and after transplantation, suggesting that the more sensitive assays for antibody measurement would be helpful in evaluating candidates and recipients of most solid organ transplants. Dr. Andrea Zachary further discussed the utility of various solid phase immunoassays in post-transplant monitoring and in evaluating responses to desensitization treatments. She recommended that the schedule for post-transplant monitoring be customized according to risk factors for humoral sensitization (prior transplant, high responsiveness, pro-inflammatory events, etc.) All three presentations suggested that antibody levels assessed by solid phase assays correlated with clinical outcome.

Dr. Elaine Reed had data showing that signaling pathways within endothelial and smooth muscle cells were affected by the amount of HLA antibody bound to the cell surface. High levels of antibody favored a pathway that led to proliferation. This would be expected to produce thickening of blood vessels and luminal narrowing as seen in chronic graft rejection. Low levels of antibody, however, resulted in the stimulation of a pathway that led to up-regulation of genes associated with anti-apoptotic activity. This could result in lower susceptibility of cells to antibody-mediated damage.

Dr. Ron Kerman presented retrospective analysis which stressed the importance of the flow crossmatch as the final determinant of compatibility. This is especially true when antibodies against HLA-Cw, -DQ, and DP were not fully characterized. Drs. Stanley Jordan, Robert Montgomery, James Gloor, and Lloyd Ratner presented desensitization protocols and most recent results. All agreed that IVIG alone does not substantially deplete HLA antibodies, and plasmapheresis is needed to substantially lower antibody titer. Failure to reduce donor-specific antibody carried significant risk of antibody-mediated rejection. Quantitation of antibody level and correlation with outcome is of great interest to programs involved with desensitization protocols.

Participants were divided into small discussion groups, concentrating on the relevance of HLA antibody on abdominal or thoracic transplantation. A major area of concern was the clinical relevance of low levels of antibody that could only be detected using Flow or Luminex-based assays. Thoracic transplant physicians felt that heart transplant patients can tolerate low level antibody concentrations better than renal transplant patients. A

separate organ-specific cutoff for may be necessary when identifying unacceptable antigens. All physicians would like to see some sort of standardization or normalization of the results so that run-to-run and lot-to-lot variations are minimized. Channel shifts, MFI, MESF, and titrations have been used to approximate antibody strength. Collaborative studies are needed to establish the best way to assess antibody concentration using results obtained from solid phase testing. Best use of these assays can only be achieved when antibody results can be correlated with crossmatch results and clinical outcome. The proper interpretation of crossmatch results was also an issue of concern. Positive crossmatches should correlate with the presence of donor-specific HLA antibody. Likewise, positive crossmatches should correlate with graft rejection and graft survival. The concept of an immune risk profile for each patient was discussed. Risk could be assigned based on presence of HLA-specific antibody, quantity or strength of antibody, and crossmatch results. This could lead to customization of protocols for induction, desensitization, or monitoring based on the level of risk for the patient.

There was general agreement that retrospective studies of patients transplanted prior to the institution of solid-phase testing would be informative. Sera used for the final crossmatch could be re-tested using solid-phase assays for low level donor-specific antibody, including anti-HLA-Cw, -DP, and DQ-alpha. The clinical outcome for transplants unknowingly performed in the face of donor-specific antibodies would provide valuable information on clinical relevance. Education and discussion among both clinicians and laboratory professionals is clearly beneficial, and histocompatibility professionals should be able to provide information to the transplant community using a common language and a unified accepted approach based on clinical data.

Progress since 2008 meeting

The American Transplant Congress (ATC) meeting (May 29 – June 3, 2009) featured five different symposia that included topics on HLA antibody testing. There were also 47 abstracts accepted for oral or poster presentation. At the ASHI Annual meeting in November 2009, there were 83 abstracts submitted that involved HLA antibody and 11 on non-HLA antibody. The acronyms DSA (donor-specific antibody) and MFI (mean fluorescence intensity) have become commonplace expressions in transplant settings. Numerous abstracts examined the correlation between Luminex MFI values and crossmatch results or graft survival. While many labs have reported a correlation between MFI and antibody strength, there continues to be variability between runs, between technologists, and between laboratories. Several approaches have been suggested to try to normalize the results so that they can be used to monitor the effect of desensitization protocols or to identify windows of opportunity for transplantation after desensitization. The use of MFI, SFI, Ratio (MFI/Neg), MFI normalized to lowest bead MFI, Relative Ratio normalized to Pos/Neg ratio, and MFI normalized to affinity have all been used to establish antibody strength. These attempts to "normalize" the results show promise in reducing run-to-run variations. The possible effect of epitope-sharing beads on the MFI of a donor-specific antibody was also discussed.

A renewed interest in flow cytometry cutoff values has also been noted as laboratories are correlating antibody levels with crossmatch results. Improved prediction of crossmatch results using solid phase antibody testing (virtual crossmatch) will be possible as laboratories "fine tune" crossmatch cutoffs based on antibody levels. This will become increasingly important as transplant centers become involved in donor-exchange programs.

There were also several new studies on post-transplant monitoring using solid phase HLA antibody results. Several reports showed excellent correlation between the development of HLA antibody and graft rejection and ultimate survival.

Future Directions

While much progress has been made in identifying HLA antibodies, new questions have emerged. There is still disagreement among programs on what is "clinically relevant". Collaborative studies are needed to establish clinically relevance for each type of organ transplant. It is becoming evident that there may not be one answer that suits all transplant programs. The presence of low levels of HLA antibody should be viewed as a risk factor and not necessarily a contraindication to transplant. The level of acceptable risk will be dependent on the condition and sensitization status of the recipient, the immunosuppressive regimen, desensitization protocols, and rescue therapies available.

Quantitation of HLA antibody is vital if we are to provide usable information to the physicians. The focus of the next joint meeting should be to review different methods that have been used to determine antibody strength. It is important that we find a reliable, reproducible, and cost-effective way to provide this information. Antibody strength is essential when identifying unacceptable antigens, when selecting appropriate candidates for desensitization protocols, when identifying donor-specific antibodies post-transplant, and when monitoring the effect of rescue protocols. The interpretation of antibody strength may be affected when the epitope is shared by multiple beads in the panel. In addition, there have been reports of prozone-like effects causing MFI to increase with dilutions.

A collaborative study is needed to establish clinical relevance of low levels of HLA antibody for each organ system. Transplant programs should select transplants that have failed in the past five years and re-test archived serum using newer solid-phase methods for pre-formed donor-specific antibody. An equal number of transplant that were successful from the same time period should also be tested. The results should be analyzed using different methods available for quantitation (MFI, Ratio, Relative Ratio, MFI normalized to lowest bead, etc.). Instructions for determining antibody strength using different methods can be provided to ASHI laboratories. Preliminary data can hopefully be presented at the November meeting with recommendations for the best way to quantitate HLA antibody. Lastly, non-HLA antibodies need to be discussed and their clinical relevance evaluated.

The joint transplant conference has been successful in opening lines of communication between laboratories and transplant physicians. Progress is much faster when we work together and share experiences and information. This is truly an exciting time to be involved in the field of transplantation.