Evolution and Present Role of the Histocompatibility Laboratory in Solid Organ Transplantation

by Daniel Ramon, Ph.D., D[ABHI], HCLD[ABB], Assistant Professor of Pathology & Director of Histocompatibility Laboratory

The purpose of this article is to review the origins and evolution of the Histocompatibility Laboratory, and describe how our current toolbox of assays allows us to support the transplant team by creating new options for donor selection, monitoring antibody removal treatments, as well as contributing to the surveillance of humoral rejection.

THE EVOLUTION

The foundational event that introduced the Histocompatibility Laboratory into Clinical Pathology was a report by Patel and Terasaki in 1969. Although previous studies described the presence of anti-HLA antibodies in sensitized patients by transfusion or pregnancies, this work was the first to highlight the strong association between a positive complement dependent cytotoxicity (CDC) crossmatch and the risk of hyperacute allograft rejection. (Table 1). The CDC crossmatch is performed by incubating donor lymphocytes cells with recipient sera; the existence of anti-HLA antibodies is demonstrated by the lysis of cells by the addition of a complement source.

Table 1. Significance of the positive crossmatch test in kidney transplantation.

<table>
<thead>
<tr>
<th>T-Cell</th>
<th>Accelerated Rejection</th>
<th>Functioning TX</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC Positive</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>CDC Negative</td>
<td>8</td>
<td>187</td>
</tr>
</tbody>
</table>

After the publication of this article a positive CDC crossmatch became a contraindication to transplantation and the best tool to predict short term graft survival. The CDC technique and its modifications were the main tool available to the Histocompatibility Laboratory for more than 30 years, but as was shown by the same article, 25% of the hyperacute rejection cases were missed by this technique, mostly explained by the low sensitivity and specificity of the assay.

It was not until the early eighties with the arrival of flow cytometers to the clinical laboratories when we learned that a big proportion of the anti-HLA
antibodies were missed by the CDC reaction. In 1983 the flow cytometric crossmatch was described by Garaboy. During the implementation years of this assay the clear advantage of a higher sensitivity method was tarnished by the lack of specificity due to a high rate of false positives. The main reason for these false positive results was due to non-specific binding of the Fc receptors on the surface of B cells to immunoglobulin molecules present in the serum of the recipient. The solution to this issue was described in 2001 with the removal of Fc receptors by treatment with the enzyme pronase. After the implementation of these modifications to the flow cytometric crossmatch, the hyperacute rejection cases caused by anti-HLA antibodies have been virtually eradicated.

Coincident to the discovery and implementation of the flow cytometric crossmatch, more sensitive and specific methods to detect anti-HLA antibodies were described. With the progress of methods to isolate and purify HLA molecules from cells, different solid phases were tested to immobilize these proteins. The result was the development of “cell independent” assays. The clear advantages of these new assays are the capability to detect antibodies without the need for viable cells, the increase in specificity without the need for a large panel of cells, and an increase in sensitivity to detect lower concentrations of the antigen-antibody complex.

Different surfaces and instrumentation were tested, but the combination of a 100-plex liquid bead array and a flow cytometric reading has produced the best results and performance for this purpose. A completely new level of specificity and sensitivity is attained when each of these 100 beads is conjugated with a different HLA antigen protein generated on recombinant cell lines. The flow cytometric crossmatch, reliable HLA typing by molecular techniques and this method known as Single Antigen Beads, expanded our service to areas not previously available for the Histocompatibility Laboratory. These are the currently available tools for the Histocompatibility Laboratory. From here we will review how our laboratory supports the solid organ transplant team at the University of Michigan Health System.

**HISTOCOMPATIBILITY SUPPORT FOR PRE-TRANSPLANT RISK ASSESSMENT**

For many years PRA% (Panel Reactivity Antibody) was used as the parameter to measure the risk of rejection and to select pre-transplant immunosuppression therapy. With the application of the new Single Antigen Beads assay, the PRA% has been replaced by the detection and characterization of anti-HLA Donor Specific Antibodies (DSA) in the serum of the recipient candidate. Retrospective studies have demonstrated that the absence of DSA, even in highly sensitized patients (PRA>80%), show a similar long term graft survival to those with 0% or low (1-30%) PRA values (Figure 2).

**VIRTUAL CROSSMATCH (VXM)**

Soon after the implementation of single antigen methods, we observed a strong correlation between these results and the flow cytometric crossmatch. In other words, with the current available tools we are now able to virtually...
predict the result of the crossmatch: the old concept of Virtual Crossmatch (VXM) has become a reality. Our experience has shown that the VXM prediction relies heavily on complete donor typing information as well as complete antibody profile information utilizing an extensive single antigen panel for Class I and Class II antigens. Utilizing this new technology, we have observed a higher frequency of antibody of difficult detection by the cellular techniques previously available to us. Clear examples are the antibodies directed to C, DQ, and DP loci. We have also learned that the previously known polymorphism in the alpha subunits of the DQ and DP molecules are capable of generating alloantibodies.

The detection of allele specific antibodies, which previously was an exception due to the limited cell panel available for CDC, are now frequently detected due to the possibility of simultaneously testing a large number of antigens and allele variants at the different loci. Our current knowledge of the alloantibodies repertoire leads us to rethink not only the need to include typing for loci HLAA, B, C, DR, DQA1, DQB1, DPA1, and DPB1, but also the need to reconsider the level of resolution required for better organ distribution.¹⁴ (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Virtual DSA Positive</th>
<th>Virtual DSA Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCXM Positive</td>
<td>515</td>
<td>28</td>
</tr>
<tr>
<td>FCXM Negative</td>
<td>83</td>
<td>854</td>
</tr>
</tbody>
</table>

Sensitivity = 86.1%; specificity = 96.8%; positive predictive value = 94.8%; negative predictive value = 91.1%.

**KIDNEY PAIRED DONATION (KPD)**

One of the areas where the Histocompatibility Laboratory plays a new and important role is in the Donor Exchange Program, which in our institution is known as the Kidney Paired Donation Program (KPD).¹⁵

Many factors are involved in the compatibility between a recipient candidate and a given donor. Among these factors, blood group incompatibility and the presence of DSA are the major barriers for transplantation. The KPD database stores the donor’s HLA typing information and the recipient’s anti-HLA profile from incompatible pairs and by a sophisticated algorithm identifies compatible donor/recipient exchange combinations. When a pair is identified by the software, all the virtual calculations are confirmed by an actual

**Spotlight on Daniel Ramon, Ph.D.**

Daniel Ramon, Ph.D., D[ABHI], HCLD[ABB], is a native of Argentina where he received his Bachelor of Sciences and Master of Sciences degrees in Biochemistry from the National University of San Luis, Argentina. He went on to receive his Ph.D. in Biochemistry from the same University, describing new methods to type HLA Class II alleles. His graduate studies were complimented by post-graduate residency training in clinical pathology with specialization in histocompatibility and immunogenetics at the Center for Medical Education and Clinical Research in Buenos Aires Argentina.

In the course of his residency training Dr. Ramon was invited to twice visit the Anthony Nolan Research Institute at The Royal Free Hospital in London as a Visiting Research Scientist.

After finishing his graduate and post-graduate education Dr. Ramon worked for a short time in industry exploring new technologies for HLA typing and is a co-author of two patents. He relocated to the United States to complete a Histocompatibility Director in Training Fellowship at Northwestern University in Chicago. After his fellowship Dr. Ramon was certified as a Diplomat of the American Board of Histocompatibility and Immunogenetics (ABHI) and as a High-complexity Clinical Laboratory Director by the American Board of Bioanalysis (ABB). In 2009 he was appointed Assistant Professor and Assistant Director of the Transplant Immunology Laboratory in the Division of Organ Transplantation in the Department of Surgery at Northwestern University Medical School.

Dr. Ramon traded royal purple for maize and blue in September 2010 when he joined the University of Michigan Medical School faculty as Assistant Professor in the clinical track and Director of the Histocompatibility Laboratory in the Department of Pathology. In his first three years he has made multiple important improvements in our world class HLA laboratory, emerging as a regionally and nationally recognized authority in his field. Over the course of his career he has made multiple important contributions to the peer reviewed literature. He currently serves as Chair of the Histocompatibility Committee for Gift of Life Michigan and is a member of the Scientific and Clinical Affairs, Director Training and Review, Abstracts, and Program Planning Committees of the American Society for Histocompatibility and Immunogenetics (ASHI).
flow cytometric crossmatch before proceeding with the transplant. When the compatible pairs are originated by the exchange of donors and recipients from two or more originally incompatible pairs, the approach is known as “closed chain”. The “open chain” approach is originated when an altruistic donor is included in the database and the algorithm identifies a compatible recipient, releasing the original incompatible donor. This donor is then evaluated to find a compatible recipient to continue the open chain.

**HISTOCOMPATIBILITY SUPPORT FOR DESENSITIZATION PROTOCOLS**

The kidney paired exchange programs have proven to be an effective and dynamic option to find donors for highly sensitized patients. Unfortunately, this approach also faces the same HLA antigens frequency as the deceased donor pool and some of these patients will require a desensitization treatment to create the appropriate immunological conditions to proceed with the transplant and avoid complication due to the presence of a DSA.

The development of new treatments for the removal of antibodies, the precise detection of DSA, and the new capabilities to quantify concentration of alloantibodies has allowed the development of desensitization therapies. The Histocompatibility Laboratory has become a very important component of this program.

Various approaches have been described to remove or decrease the concentration of DSA, but most of them include different combinations of IVIG, plasmapheresis, Thymoglobulin, or anti-CD52 and anti-CD20. Due to the interference of these drugs with cellular assays, solid phase assays are the method of choice to guide the treatment.

The measurement of anti-HLA antibodies strength is necessary not only to monitor the effectiveness of treatment, but to evaluate the chances of removing a given DSA with a specific treatment. Not every antibody will be removed by these therapies and the program must assess the possibilities based on their experience and a comfortable level of risk. The most commonly used unit to measure antibody strength is Median Fluorescent Intensity (MFI) by the Luminex method; this value could also be transformed into MESF or SFI values. The other option is to run serial dilutions that in addition to the concentration also provide an idea of the avidity of the antibody, but the downside to this approach is that it is costly and labor intensive.

Due to the presence of DSA before desensitization or at the time of transplant, the immunological risk of developing AMR post-transplant is significantly high. Therefore, a close monitoring should be established to detect a revamp on the antibody level to decide clinical intervention. At the University of Michigan Health System, not only have we increased DSA monitoring, but we have also increased biopsy surveillance by stabilized protocol surveillance.

Our laboratory provides support to the University of Michigan Health System transplant team in both the Living and the Deceased Donor Desensitization Program.

**POST-TRANSPANT MONITORING**

As mentioned in multiple reports, the recurrence of DSA detected before the transplant is associated with a higher incidence of AMR and graft lost. Recent publications have demonstrated that even transplanted patients with low immunological risk developed de novo DSA in 15-23% of cases (Figure 3). Development of de novo DSA has been associated with poor mismatch at class II antigens and with a decrease in immunosuppression drugs in circulation due to lack of adherence or drug minimization strategies. The appearance of these de novo DSA could occur in the acute or chronic phases, associated with a higher incidence of AMR and decrease in long term graft survival. The early detection and a reduction in the concentration of de novo DSA by antibody removal treatment is associated with improvement in allograft survival. The implementation of surveillance protocols to detect de novo DSA after transplantation is a valuable tool to decide an early intervention, and adjustment of the anti-humoral therapy to rescue the graft and improve outcomes.

Figure 3. The graft survival of patients with de novo DSA versus those without.
COMPLEMENT ACTIVATION: C1q BINDING ASSAY

With the implementation of the new solid phase assay we lost the ability to distinguish complement fixing from non-complement fixing DSA. A recent modification to the single antigen assays includes the addition of a purified initial component of the complement cascade C1q. When an alloantibody capable of activating complement (mostly IgG1 and IgG3) recognizes the HLA molecule attached to the beads, the Fc receptors activate the C1q molecule. The detection of the deposited C1q molecule on the Luminex single antigen beads shows a higher sensitivity than the CDC reaction. A correlation with acute rejection and graft survival has been observed in kidney transplants. In our hands C1q proves to be an excellent tool to monitor antibody removal therapies in patients treated for AMR and a good predictor of graft lost after the treatment (Table 3).

Table 3. C1q Binding assay can predict outcomes after AMR treatment.

<table>
<thead>
<tr>
<th>Graft Loss</th>
<th>Non Responders</th>
<th>Complete &amp; Partial Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre post</td>
<td>pre post</td>
</tr>
<tr>
<td>IgG DSA (+)</td>
<td>34% (11)</td>
<td>34% (11)</td>
</tr>
<tr>
<td>C1q DSA (+)</td>
<td>34% (11)</td>
<td>34% (11)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Resolution of cAMR</th>
<th>Non &amp; Partial Responders</th>
<th>Complete Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre post</td>
<td>pre post</td>
</tr>
<tr>
<td>IgG DSA (+)</td>
<td>53% (17)</td>
<td>47% (15)</td>
</tr>
<tr>
<td>C1q DSA (+)</td>
<td>53% (17)</td>
<td>47% (15)</td>
</tr>
</tbody>
</table>

ANTIBODIES TO NON-HLA ANTIGENS

Thanks to the single antigen assays we have been able to clearly detect cases of AMR in the absence of DSA directed against HLA molecules. Cases with a clear histology of AMR are likely produced by alloantibodies to non-HLA antigens or autoantibodies against tissue specific autoantigens mostly expressed in the endothelial cells. However, the big limitations for the detection of these antibodies are the lack of knowledge about the targets and the need for a standardized technique.

ENDOTHELIAL PRECURSORS CELLS CROSSMATCH

For the screening of antibodies directed to an unknown endothelial target our laboratory performs a flow cytometric crossmatch that uses Endothelial Precursor Cells: XM-ONE. These cells are found in circulation at a concentration of 2-3%. The advantage of this test versus other previously described endothelial crossmatches is that it uses donor cells when available and not surrogate cell lines.

The following targets have been recognized and we have the ability to detect antibodies against them in the laboratory:

DETECTION OF ANTIBODIES AGAINST MICA

Antibodies against major histocompatibility Class I related molecules (MICA) have been associated with AMR and graft lost. These antigens are not present on the resting endothelium; the expression is induced under stress conditions like endothelial cell activation or inflammations. Thanks to familiarity with the HLA class I molecules there are available molecular typing methods and a single antigen assay for the screening of these alloantibodies.

DETECTION OF ANTIBODIES AGAINST AT1R

Another target capable of generating antibodies associated with allograft rejection and vasculopathy is the Angiotensin II Type 1 receptor (AT1R). Our laboratory is in the validation process of a commercially available ELISA assay for the detection of these antibodies.

Other targets have been identified like vimentin, tubulin, myosin and collagens among others but no standardized assay are available yet for clinical use.

OTHER AREAS OF SERVICE

The Histocompatibility Laboratory provides high resolution HLA typing and antibody screening for the Bone Marrow and Hematopoietic Stem Cell transplant team. We also have the capability to provide low or high resolution HLA typing for Disease Association evaluations.

REFERENCES


23. Ramon DS. C1Q Binding assay can predict outcomes after AMR treatment Manuscript in preparation.


Test Updates

New Tests

**GDI1 GENE SEQUENCING**

The MLabs Molecular Genetics Laboratory began sequencing the GDI1 gene (OMIM: 300104) effective August 28, 2013. GDI1 belongs to a family of highly conserved proteins that play a critical role in the recycling of Rab GTPases for vesicular transport. GDI1 protein is ubiquitously expressed in the brain during early development (Bachner et al. Hum Mol Genet 4:701-708, 1995). Mutations in GDI1 are associated with X-linked non-syndromic mental retardation (OMIM:300849) that is moderate to severe in males and mild in females (Bienvenu et al., Hum Mol Genet, 1998, 7 (8): 1311-1315).

**SLC7A7 GENE SEQUENCING**

The MLabs Molecular Genetics Laboratory began sequencing the SLC7A7 gene (OMIM: 603593) effective August 28, 2013. Mutations in SLC7A7 are associated with Lysinuric Protein Intolerance (LPI) (OMIM:222700), an autosomal recessive disorder caused by defective cationic amino acid (arginine, lysine, ornithine) transport at the basolateral membrane of epithelial cells in the intestine and kidney. LPI is characterized by recurrent episodes of vomiting and diarrhea, mild hyperammonemia, stupor and coma after a protein-rich meal, poor feeding, aversion to protein-rich foods, failure to thrive, hepatomegaly, splenomegaly, and hypotonia. Long term characteristics include poor growth, osteoporosis, respiratory insufficiency, alveolar proteinosis, progressive glomerular disease, and proximal tubular disease. Treatment of LPI consists of protein-restricted diet and supplementation with oral citrulline. Under proper dietary control and supplementation, the majority of the LPI patients are able to have a nearly normal life. However, severe complications including pulmonary alveolar proteinosis and renal insufficiency may develop even with proper treatment (Tanner et al., J Pediatr 150(6): 631–634, 2007).

Test Methodology, Reference Range, and Specimen Handling Changes

**PT AND PTT REFERENCE RANGES**

Due to a change in lot number of thromboplastin, effective May 2, 2013, the reference range for the PT (Prothrombin Time) assay will change from 9.8 - 12.5 seconds to 9.5 - 12.0 seconds.

Effective September 18, 2013, the reference range for the PTT (Activated Partial Thromboplastin Time) assay has changed from 22-32 seconds to 22-29 seconds. This change is due to a new lot of aPTT reagent.

**Discontinued Tests**

**ENTEROVIRUS CULTURE, STOOL**

Effective September 3, 2013, the MLabs Microbiology Laboratory will no longer offer Enterovirus culture for stool specimens due to the low number of requests for this test and specimen toxicity. The stool specimen is frequently toxic to cell lines used for culture and in the majority of cultures incubation time is discontinued early due to cell line toxicity. The utility of this testing is poor.

**METHADONE, URINE**

Effective April 9, 2013, the MLabs Chemical Pathology Laboratory has discontinued the Methadone, Urine screen. The Methadone Metabolite (EDDP) assay is available for qualitative detection of methadone metabolite (2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine or EDDP) in urine.

**STREPTOCOCCUS PNEUMONIAE IGG 7 SEROTYPES**

Effective July 2, 2013, Mayo Medical Laboratories has discontinued the Streptococcus pneumoniae IgG Antibody Panel, 7 Serotypes. The Prevnar-7 vaccine, on which this test was based, has largely been discontinued and replaced with Prevnar-13. The Streptococcus pneumoniae IgG Antibody Panel, 23 Serotypes is recommended for assessing the response to active immunization with nonconjugated, 23-valent vaccines or determining the ability of an individual to respond to polysaccharide antigen(s).
MLabs News

U-M OFFERS NEW EARLY DETECTION TEST FOR PROSTATE CANCER

Effective September 25, 2013, the MLabs Michigan Center for Translational Pathology (MCTP) Laboratory is now offering the Mi-Prostate Score, or MiPS test to provide a personalized prostate cancer risk assessment for patients with elevated Serum PSA for estimating prostate cancer risk in patients without a previous negative biopsy.

More than 1 million men will undergo a prostate biopsy this year, but only about one-fifth of those biopsies will result in a cancer diagnosis. The reason is that the traditional prostate cancer screening test – a blood test to measure prostate specific antigen, or PSA – does not give doctors a complete picture. Now, the University of Michigan Health System has begun offering a new urine test called Mi-Prostate Score to improve on PSA screening for prostate cancer. The test incorporates three specific markers that could indicate cancer and studies have shown that the combination is far more accurate than PSA alone.

“Many more men have elevated PSA than actually have cancer but it can be difficult to determine this without biopsy. We need new tools to help patients and doctors make better decisions about what to do if serum PSA is elevated. Mi-Prostate Score helps with this,” says Scott Tomlins, M.D., Ph.D., assistant professor of pathology and urology at the University of Michigan.

Mi-Prostate Score was developed from a discovery in the lab of a genetic anomaly that occurs in about half of all prostate cancers, an instance of two genes changing places and fusing together. This gene fusion, T2:ERG, is believed to cause prostate cancer. Studies in prostate tissues show that the gene fusion almost always indicates cancer.

The new urine test looks for the T2:ERG fusion as well as another marker, PCA3. This is combined with serum PSA measure to produce a risk assessment for prostate cancer. The test also predicts risk for having an aggressive tumor, helping doctors and patients make decisions about whether to wait and monitor test levels or pursue immediate biopsy.

The combined MiPS test is now available only from the University of Michigan MLabs to provide patient specific prostate cancer risk assessment.

Note that the MiPS test is performed using the same Gen-Probe Progensa urine transfer tubes as the PCA3 assay. For questions regarding How to Send a Specimen, please call MLabs at 800-862-7284 or visit us at www.mlabs.umich.edu.

U-M DEPARTMENT OF PATHOLOGY NEWS

Evan Farkash M.D., Ph.D., previously a Fellow in Renal Pathology at Massachusetts General Hospital/Harvard Medical School has joined the Department of Pathology September 1, 2013 as a Clinical Lecturer in Renal Pathology.

Madelyn Lew, M.D., previously a Cytopathology Fellow at Brigham and Women’s Hospital/Harvard Medical School, joined the Department of Pathology in July as an Assistant Professor in the Cytopathology Section.

Jeffrey Myers, M.D., A James French Professor of Pathology, Director of Anatomic Pathology and Director of MLabs, was installed as President of the United States and Canadian Academy of Pathology (USCAP) at the recent 2013 USCAP Annual Meeting in Baltimore.