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Ph.D. School of University of Medicine and Pharmacy CRAIOVA

Research Topic: Anti-HLA Antibody in Solid Organ Transplantation

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Keywords: Ab, antibody; AMR, antibody mediated rejection; BMNR LLPC, bone marrow niche resident long lived plasma cell; CDC-PRA, complement-dependent cytotoxic panel reactive antibody; cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; ER, endoplasmic reticulum; FDA, Food and Drug Administration; iAb, immunodominant antibody; iDSA, immunodominant donor-specific antibody; IRB, Institutional Review Board; IVIg, intravenous immune globulin; MCS, mean channel shifts; MFI, mean fluorescence intensity; PI, proteasome inhibitor; PRA, panel reactive antibody; SAB, single antigen bead; ACR, acute cellular rejection; ASHI, American Society for Histocompatibility and Immunogenetics; BKV, BK virus; Bortez, bortezomib; CMV, cytomegalovirus; HLA, human leukocyte antigen; MAR, mixed acute rejection; MMF, mycophenolate mofetil; POD, post-op day; rATG, rabbit antithymocyte globulin; Ritux, rituximab; SCr, serum creatinine;

General Section

Multiple end-organ diseases benefit today from transplantation of vascular allografts: kidney, pancreas, heart, lung, intestine, liver, limbs, facial grafts etc. Furthermore, non-vascular tissue grafts, such as hematopoietic and other stem cell transplantation are under substantial expansion.

After transplantation, antibody-mediated rejection (AMR) represents a major risk factor for allograft dysfunction and/or graft loss**.**

Detection of circulating anti-HLA antibody in solid-organ transplantation has continuously improved over the past decade. Both cellular and solid-phase assays are in use for detection of anti-HLA antibodies. Multiplex and flow-based techniques are reported as most sensitive for antibody detection, followed by ELISA and CDC methods. The results of solid-phase methods seem to be less influenced by IgM, auto- and non-HLA antibodies, as well as by cytolytic protocols. Considering the

advantages and limitations of each assay, a combination, rather than a single method, may provide the best approach to determine the level of sensitization and the specificity of anti-HLA antibody in transplant recipients.

Specific Section. Personal Contributions

I had the chance to study this field for the last fifteen years. The major interest of the present research work is represented by the dynamics of anti-HLA antibody both before, and after solid organ transplantation. Knowledge about humoral allo-response dynamics is of fundamental importance in the clinical follow-up and therapeutic protocols, and for patient prognosis, as well.

The objectives of the present thesis are to describe the dynamics of the humoral immune response towards HLA antigens both in transplant candidates, as well as in transplanted patients, in the context of various therapeutic strategies.

The main hypotheses will be that: a) Sensitized candidates do not have a natural tendency for a decrease in antibody response; b) Antibody patterns (antibody strength, specificity class, subclass) and therapeutic response in early AMR are different from late AMR; c) therapeutic response is also different in early versus late AMR; d) therapeutic response is influenced by the antibody pattern.

Immediate clinical applications are represented on the one hand by the increase in transplantability of candidates with high levels of antibodies and, on the other hand, by strategies to control the pathology mediated by antibody response against allografts.

HLA Antibody before Transplantation

Antibody Strength Can Predict Crossmatches. The DSA strength correlates well with crossmatch results. An MFI of 6540 predicted a positive antihuman globulin T-cell crossmatch. This was the first study to assess DSA strength based on proficiency test consensus.

Antibody Monitoring in Sensitized Renal Transplant Candidates
The first prospective iterative trial of proteasome inhibitor-based therapy for reducing HLA antibody levels. Five phase trial with complex antibody strength dynamics, dilutional studies, class and epitope specific analysis. This report also provides evidence that bortezomib-based regimens can provide desensitization for HLA sensitized kidney transplant recipients without concomitant IVIg therapy.

Antibody Monitoring in Sensitized Heart Transplant Candidates. We reported that sensitization prior to transplantation was associated with increased wait list mortality, longer wait list times, increased all-cause mortality after listing (regardless of subsequent transplantation), and earlier development of post-transplant coronary artery disease. Furthermore, we report desensitization followed by successfully heart transplantation both in adult, and in pediatric patients. Antibody reactivity measured by Luminex MFI can be used to monitor the response to desensitization protocols in transplant candidates without prospective crossmatches

Antibody Monitoring in Sensitized Lung Transplant Candidates. In addition to a negative virtual crossmatch, a detailed sensitization history and sensitive antibody testing might be of interest in lung transplant candidates, irrespective of their age.

HLA Antibody after Transplantation

In summary, for the question "When are de novo anti-HLA antibody developed/detected?", we saw that 1) Early (< 6 months) de novo HLA antibody were predominantly anti-HLA Class I and correlated with early AMR, while 2) late de novo HLA antibody were in majority anti - Class II, and correlated with late rejection episodes.

Impact of Anti-HLA Antibody in Lung Transplantation

HLA antibodies are associated with perivascular high-grade acute rejection. Significance of the research: This is the first report in literature to demonstrate the association of HLA donor-specific alloantibody with perivascular acute rejection in

lung transplantation. Prospective clinical study. It opens the field of mixed, cellular and humoral acute rejection in lung transplantation with consequences on post-transplant monitoring and therapeutically strategies.

HLA-specific antibodies are risk factors for lymphocytic bronchiolitis and chronic lung allograft dysfunction. This is the first report in literature to demonstrate the association of HLA donor-specific alloantibody with airway acute rejection in lung transplantation, as an intermediate stage towards chronic allograft dysfunction. Prospective clinical study.

C4d Deposition in Lung Allografts Is Associated with Circulating Anti-HLA Alloantibody. Significance of the research: This is the first report in literature to demonstrate the association of HLA donor-specific alloantibody detected by sensitive solid-phase methods with complement activation via classical pathway and specific, linear, subendothelial C4d deposition in lung allografts (C4d staining on paraffin specimens). It shows that alloantibody is not only a risk factor, but also a pathogenic mechanism in lung allograft rejection. It opens the field of defining antibody-mediated rejection criteria in lung transplantation.

- HLA Antibodies Are Associated with Elevated Soluble C4d
- HLA Antibodies Are Associated with Bronchiolitis Obliterans Syndrome and Graft Loss
- HLA-Specific Antibody and Therapeutic Protocols in Lung Transplantation

Impact of Anti-HLA Antibody in Heart Transplantation

- HLA Antibody and Acute Rejection in Pediatric Heart Transplantation
- Donor-specific Anti-HLA Antibodies of IgG3 Subclass Correlate with High-Grade Rejection after Cardiac Transplantation

Impact of Anti-HLA Antibody in Renal Transplantation

- HLA Antibody and Structural Matching

- HLA Antibody and Focal C4d Deposition in Renal Transplantation
- Antibodies as Monitoring Sentinels for Immunosuppression Weaning
- Antibody in Randomized Controlled Studies
- Proteasome Inhibitor-based Therapy for Antibody Mediated Rejection
- IgG3 Subtype Is a Marker of Late AMR
- IgG Subtype and Pathology Scores
- Donor-Specific Antibody and HLA Loci

Impact of Anti-HLA Antibody in Liver and Intestine Transplantation

- Liver transplant recipients weaned off immunosuppression lack circulating donor-specific antibodies
- Antibody Identification in Biopsy Eluates

Overall, the author of the present thesis has published in the field of humoral allo-immune response of solid-organ transplantation:

- 38 ISI full length manuscripts;
- Over 150 research papers (PubMed indexed);
- H - index = 23 (Scopus);
- 1144 citations/847 citing articles (without self-citations, ISI Thomson Reuters web of science)