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AST Cutting Edge of Transplantation 2013 Meeting Report: A Comprehensive Look at B Cells and Antibodies in Transplantation

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Abstract

Antibody-mediated rejection (ABMR) represents a significant clinical challenge for solid-organ transplantation. Mechanistic understanding of ABMR is incomplete and diagnostic accuracy for ABMR is limited, and as a result, targeted treatment remains elusive and new treatment modalities are difficult to validate. Three-hundred-twenty-six participants from 15 countries met for the first Cutting Edge of Transplantation (CEOT) symposium organized by the American Society of Transplantation in Chandler, Arizona, February 14–16, 2013. During the three-day interactive symposium, presentations, moderated poster sessions, and round table discussions addressed cutting edge knowledge of B and plasma cell biology, mechanisms of antibody-mediated tissue injury, advances and limitations in ABMR diagnostics, as well as current and potential new treatment options for ABMR. The outcome of the meeting identified the following unmet needs for: a) Improved understanding of the regulation of B cell maturation and antibody response to enable targeted therapies; b) More precise diagnostics of ABMR, including molecular pathology, risk stratification by sensitive antibody testing, and monitoring of treatment effects; and c) Innovative multicentre trial designs that enhance observational power, in particular, in assessing synergistic multimodality therapies with reduced toxicities.

Keywords

B cells; antibody-mediated rejection

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Pathomechanisms of antibody-mediated tissue injury

B cell responses to alloantigen result in T cell-dependent germinal center (GC) reactions and the production of short-lived plasmablasts producing an early burst of high affinity class switched antibodies, long-lived plasma cells that provide long-term serologic memory, and memory B cells that play a dominant role in recall responses. Upon alloantigen re-encounter, memory B cells rapidly differentiate into new plasmablasts that produce a new burst of antibody production.

It was evident from the first CEOT presentation that a greater understanding of the basic biology of B cells will be required to develop targeted therapeutic anti B cells strategies in antibody-mediated rejection (ABMR). Michael Cancro provided an overview of B lymphocyte selection, homeostasis, and differentiation into germinal B cells, which is controlled by survival factors, mainly of the BLyS family cytokines and receptors. This family includes two ligands, BLyS and APRIL; as well as three receptors, BR3, TACI, and BCMA. B cells in the transitional and mature, pre-immune B lineage subsets rely on BLyS signals via the BR3 receptor for survival. When BLyS levels are elevated, B cells that would normally be negatively selected, instead survive to join mature naïve pools. Mounting evidence suggests analogous competitive checkpoints for germinal center B cells and plasma cells. Short-lived antibody-forming plasma cells adopt a TACI dominated BLyS receptor signature, whereas GC B cells profoundly down-regulate TACI but retain BR3, leading to a paucity of retained BLyS on GC B cells and the sole local source of BLyS in the GC from T follicular helper cells. Thus, if T cells are BLyS deficient, GC-dependent affinity maturation fails. Based on these recent observations the relevant question to answer is whether deliberately altering BLyS levels might be a means for manipulating the B cell repertoire to accommodate neo-self antigens introduced through transplantation rather than causing rejection.

Where B cells become activated and reside may directly alter the pathogenic response. Marcus Clark reported on new insights gained by examining cell-cell interactions between B and T cells in kidney biopsies with the use of a novel computational approach, termed cell distance mapping coupled to confocal microscopy, to quantitatively assess the spatial relationships between different cell populations *in situ*. Cell distance mapping revealed that T follicular helper-like cells, or T inflammatory helper cells, frequently infiltrated the renal tubulointerstitium in patients with lupus nephritis. T inflammatory helper cells were also observed in T cell-mediated rejection (TCMR) and mixed lymphocyte rejection cases. However, only in mixed lymphocyte rejection, which is additionally characterized by local antibody deposition, did T inflammatory helper cells form cognate pairs with B cells. These results suggest that in areas of diffuse inflammation, cell distance mapping reveals cellular organization and cell-cell interactions associated with *in situ* adaptive immunity, whether this plays a role in antibody-mediated rejection (ABMR) remains to be determined.

Antibodies injury to allografts occurs through several mechanisms including localized fixation of complement. Jeffery Platt provided an overview of how complement-fixing antibodies activate the complement system upon binding to the graft. He discussed evidence

that the initial activation and subsequent responses of B cells can be regulated by complement. From his lecture it is obvious that improving our understanding of how complement directly, or through intermediaries such as heparan sulfate fragments and IL-1 α , injures allografts or confers accommodation constitutes an opportunity for novel therapeutics.

Robert Anthony addressed immune functions triggered by the IgG Fc region, which has a single N-linked glycosylation that is required for all interactions with Fc gamma receptors and C1q. The Fc glycan has tremendous heterogeneity, and over 30 distant glycoforms have been identified on IgG. Importantly, the composition of the Fc glycan dictates IgG effector functions. The addition of terminal sialic acid to this glycan reduces Fc γ R affinity and pro-inflammatory effector function while promoting binding to dendritic cell-specific ICAM-3 grabbing non-integrin (DC-SIGN) and anti-inflammatory activity. Sialylation on IVIG is critical for its potent anti-inflammatory activity, and deglycosylated or desialylated IVIG exhibit no anti-inflammatory activity. However, it remains to be investigated whether such modifications of IVIG have the potential to increase therapeutic efficacy in humans with ABMR.

Antibodies are considered specific for unique antigens, but emerging evidence of cross-reactivity may explain several aspects of alloimmunity. Emmanuel Zorn's studies suggest that polyreactive antibodies produced by B1 B cells contribute to ABMR and pre-sensitization. Somatically mutated memory B cells secreting polyreactive antibodies can be found at high frequency in the blood of patients with ABMR (1). Furthermore, high serum levels of polyreactive IgG pre-transplant correlate with reduced kidney allograft survival. Some of these polyreactive antibodies cross-react with HLA, suggesting that they may contribute to the overall serum reactivity. In addition, polyreactive antibodies have the capacity to bind to apoptotic cells and activate complement, leading to the deposition of C3d and C4d (2). How and why polyreactive IgG develops in patients awaiting transplant or in patients with ABMR remains to be clarified, as does their capacity to enhance ABMR pathology.

Can alloantibodies promote damage in complement-independent mechanisms, i.e. cause C4d negative ABMR? Joren Madsen promoted the idea that natural killer (NK) cells are necessary in chronic rejection of solid-organ allografts. There is emerging evidence that NK cells can facilitate antibody-mediated pathology. NK cells alone are probably not sufficient but require additional factors such as the presence of alloantibody or a viral infection to promote the NK cell pathway towards rejection and induce vascular lesions. The mechanisms whereby NK cells and alloantibody interact to induce chronic rejection are a major focus of ongoing research.

B cells can contribute more to immune responses than antibody generation and Frances Lund discussed how B cells could also play an important role in the control of CD8 $^{+}$ T cell responses. In mice infected with influenza, B cell depletion led to an accelerated contraction of CD8 $^{+}$ T cells specific to some but not all MHC class I restricted flu peptides. This effect was mediated by class switched or secreted IgG. Remarkably, B cell class switch recombination in this model did not appear to require T cell help. While the mechanisms

linking CD8+ T cell and humoral immunity are still unclear, these studies suggest that B cells contribute to shaping the CD8+ T cell response to viral antigens and thus suggests studying the relevance of this observation in the transplant setting.

Not only do B cells generate antibody and help shape the T cell response, but emerging evidence suggests that a subset can have immunosuppressive effects. David Rothstein discussed regulatory B cells (Bregs) that inhibit immune responses through IL-10 dependent mechanisms (3). While Bregs lack a specific marker, ~75% of all IL-10⁺ B cells express TIM-1, making this the most inclusive marker identified (4). TIM-1⁺ B cells from immunized mice can transfer Ag-specific tolerance to otherwise naïve allograft recipients. Moreover, Bregs could be induced by targeting TIM-1 or the related molecule TIM-4, suggesting that Breg based treatment strategies may be possible. On the other hand, B cell depletion prevents allograft tolerance by various agents, suggesting that Bregs may help establish the tolerogenic threshold. In humans, memory and transitional B cells can simultaneously express IL-10 and proinflammatory cytokines [Cherukuri A, under review]. The ratio of IL-10 to TNF α is highest in transitional B cells and correlates with Breg activity. The IL-10:TNF α ratio decreases with transplant rejection. Thus, measurement of both pro and anti-inflammatory cytokines is necessary to define human Bregs. Key future directions include finding more specific markers for Bregs and to find ways to inhibit antibody producing B cells without depleting Bregs.

It is unclear how current maintenance immunosuppression inhibits memory B cell and T cell responses in individual transplant recipients, and this is an issue that has relevance both to protective immunity to infection and to allograft rejection. Anita Chong presented a novel approach to quantifying the cellular immune response in the setting of maintenance immunosuppression. The effectiveness of maintenance immunosuppression (corticosteroids, calcineurin-inhibitor, purine-synthesis-inhibitor) in controlling memory B and T cell responses in stable transplant recipients was assessed by quantifying responses to influenza vaccine. A significant suppression of influenza-specific memory B cells differentiating into short-lived plasma cells and the influenza-specific IFN-gamma memory T cell response was observed in the majority of transplant recipients. Future studies are necessary to understand how immunosuppression compromises protective memory T and B cell responses, and to develop new assays to quantify the efficacy of immunosuppression on vaccination and alloimmunity in human transplant recipients.

Diagnosis and risk stratification of ABMR

When donor-specific antibody (DSA) is detected pre-transplant and avoided, the risk for ABMR is minimal (<5% develop ABMR). However, unrecognized it portends an increased risk for ABMR (55% develop ABMR) and graft loss (20% increase compared to no DSA) (5). Similarly, de novo DSA arising post-transplant (described in 10–40% of not pre-sensitized patients) portends an increased risk for late graft loss (graft survival without de novo DSA: 90% versus 50% with de novo DSA + ABMR) (6). Peter Nickerson described risk factors for the development of de novo DSA as the degree of class II mismatching, early TCMR preceding de novo DSA, and non-adherent behavior (60% of non-adherent patients develop DSA compared to 20% of adherent patients). Generally the onset of de novo DSA

precedes the development of proteinuria or graft dysfunction due to antibody-mediated chronic allograft pathology, e.g. transplant glomerulopathy.

With better DSA detection comes the question: what defines a positive DSA and when is it important? Howard Gebel led off a series of talks on current methodologies for detection of DSA. Based on sequence similarities and differences between HLA alleles, pre-transplant virtual crossmatching is a simple way to predetermine whether a recipient has the potential to express anti-HLA antibodies against any given donors by computer algorithms rather than in a test-tube (7). This is a highly efficient way to pre-determine the risk for ABMR that has positively impacted the allocation of deceased donor organs to patients awaiting transplantation. As a major result of virtual crossmatching, highly sensitized patients are being transplanted at a higher rate (80–90%) than ever before. Unfortunately, among patients who undergo desensitization and transplantation, there currently are no routine tests that distinguish recipients with a smooth post-transplant course from those who develop ABMR. One of the most contentious issues relates to how donor HLA antigens are defined as unacceptable: Are cutoff values used to identify the presence of antibodies appropriate, too high, or too low when DSA are identified? Do the antibodies that are reported result in a positive crossmatch? Do they fix complement? Are the antibodies to native or denatured HLA molecules? Thus a major challenge is to discriminate HLA-DSA that are clinically relevant from those that are not. Additional, more recently discovered factors such as the number and specificity of memory B cells, antibody affinity, titer, isotype, degree of sialylation need to be studied in more detail in regard to their potential role in ABMR. Major progress has been made recently in regard to the clinical value of assessing the complement fixing attributes of DSA by using a C1q assay. Loupy et al. demonstrated that patients with complement-binding DSA had the lowest graft survival (54% at 5-years), compared with patients with non-complement-binding DSA (93%) and patients without DSA (94%). Complement-binding DSA were also associated with an increased rate of ABMR, a more severe antibody-mediated graft pathology including increased deposition of C4d (8). Furthermore, there is also growing recognition that non-HLA alloantibodies (e.g., anti-endothelial cell or anti-AT1R antibodies) are potential additional risk factors to graft survival (9–11).

Accordingly Anat Tambur discussed the critical need to use virtual crossmatching to obtain accurate and complete identification of donor antigens, at a resolution obtained by the single antigen tests. The immediate unmet needs are for a UNOS database available to capture high resolution typing information for the donor and recipient. Most specifically this relates to HLA-DQ antigens, which should be assigned as DQA/DQB heterodimer combinations, as is present in the Luminex single antigen assays and expressed on the cell surface. This level of information will address future needs in assigning antibody recognition of HLA epitopes, understanding the immune-biology of this phenomenon, identifying relevant DSA pre-transplant, and assessing the risk of developing de-novo HLA-DSA post transplantation.

Lee Ann Baxter-Lowe presented data demonstrating that use of single antigen microarrays to characterize HLA antibodies underlies substantial improvements in predicting the results of cell-based crossmatches and transplant outcomes. These microarrays revealed that antibodies against HLA -C and -DQ antigens are common, but their clinical significance

remains controversial. For HLA-C, genetic factors that cause substantial differences in the level of expression and its role as a ligand for inhibitory receptors on NK and certain T cells can contribute to inconsistent observations. For HLA-DQ, antibody epitopes involving both HLA-DQ alpha and beta chains, differences in levels of expression, and technical artifacts have impeded investigation. Nevertheless, emerging data suggest that HLA-DQ antibodies are clinically important, particularly when the antibodies appear after transplant. Basic and clinical investigation is needed to clarify the role of HLA-C and -DQ antibodies. However, it is only with standardization of antibody detection assays that current and subsequent studies can be interpreted properly. Recent NIH funded studies among seven laboratories in North America demonstrated that, indeed, standardization of antibody detection assays is feasible (12).

Exactly where and how do DSA cause damage? Elaine Reed described mechanisms through which de novo DSA cause endothelial tissue damage. The major targets of the humoral immune response are HLA class I and class II as well as non-HLA molecules expressed on the endothelium of the allograft (13). Crosslinking of HLA class I and class II molecules by antibodies stimulates a series of complement-independent intracellular signaling events leading to endothelial cell activation, actin cytoskeleton remodeling, proliferation and migration (14;15). Investigation of the proximal signaling events demonstrated a mutual dependency between HLA class-I and integrin $\beta 4$ to stimulate signal transduction, increased intracellular calcium, mobilization of Weibel-Palade bodies resulting in increased P-selectin expression and monocyte adherence to the endothelium (16). Administration of recombinant P-selectin ligand-1 (PSLG1) dramatically reduces macrophage recruitment to MHC class I stimulated endothelium *in vivo*, suggesting to explore this a potentially clinically useful approach for diagnostics and therapeutics of ABMR (16).

Robert Colvin and Michael Mengel discussed current and novel diagnostic approaches to better define clinically relevant ABMR. The morphological correlates of DSA mediated tissue injury are microcirculation inflammation and remodeling in the allograft and deposition of C4d in the microcirculation (17). However, neither of these features is absolutely specific or sensitive for diagnosing ABMR and cases of C4d-negative ABMR are now well recognized while microcirculation inflammation and remodeling can be seen in other disease entities (18;19). On the other hand intimal arteritis (i.e. Banff v-lesions) have per consensus been attributed to TCMR, but recent data indicate that at least a subset of these cases is cause by DSA (20). Accordingly the diagnostic criteria for ABMR have been revised after the 2013 Banff meeting and now include C4d-negative ABMR and v-lesions as a potential feature of ABMR (21). But still, limited reproducibility of staining for C4d (22) and in assessing the respective Banff lesions exists, which together, result in a lack of precision for diagnosing, staging, and grading of ABMR and thus impaired ability to conduct and monitor respective clinical trials (23). Recent insights into the molecular mechanisms of antibody-mediated tissue injury offer to increase the diagnostic precision for ABMR. Increased expression of endothelial and NK cell associated transcripts have been identified as molecular correlates of DSA-mediated tissue injury (24–26). Although also not absolutely specific for ABMR, quantification of the molecular expression levels offers the potential to assess disease activity and thus to tailor and monitor treatment. Thus, concerted

interdisciplinary efforts to integrate clinical, serological, histological, and molecular correlates of antibody-mediated tissue injury are underway for achieving precision diagnostics in ABMR (27).

A majority of our understanding about ABMR has been in kidney transplant recipients, and the importance and effects in other solid organ transplant settings may be different. Jon Kobashigawa emphasized that antibody-mediated injury after heart transplantation is associated with increased development of cardiac allograft vasculopathy and mortality (28–31). But the importance of detected DSA after heart transplant has been much more contentious compared to other organ transplants. A 2010 ISHLT-sponsored ABMR Consensus Conference determined that ABMR would be defined by pathology only with cardiac dysfunction and the presence of donor-specific antibodies (DSA) used as descriptors to help guide therapy (32). However, asymptomatic ABMR is associated with increased vasculopathy development and mortality (33). In addition, DSA is not always detected in the presence of pathology confirmed ABMR which may be due to absorption of DSA to the graft and small studies have suggested that low binding DSA in the first year after transplant may not have greater rejection risk (low DSA may reflect a state of accommodation?). More comprehensive studies in the area of heart transplantation are still needed to better define the clinical significance of DSA and ABMR.

ABMR Treatment

A consistent message at the CEOT meeting was, that if possible, DSA should be avoided at transplantation and strategies to minimize the risk for de novo DSA should be a mandatory part of clinical management (e.g. allocation points for Class II matching, avoiding drug minimization in patients at risk for de novo DSA (i.e. Class II mismatch, or early TCMR), early recognition of non-adherence).

But if no alternative exists, what is the best combination to use for desensitization? Stanley Jordan discussed the >10 year experience of the Transplant Immunotherapy Program at Cedars-Sinai with desensitization using intravenous immunoglobulin (IVIG) alone or in combination with Rituximab. IVIG alone was shown to be effective in reducing anti-HLA antibodies and in improving transplant rates from 17% to 35% in the NIH-sponsored placebo-controlled trial (IG02 study) (34). However, the use of high-dose IVIG alone often was not sufficient to allow successful transplantation for all patients, and severe ABMR can occur after transplantation. Evaluation of the combination of IVIG + Rituximab (35) in a single center trial was associated with reduction in time required for desensitization to 5+/-6 months and improved rates of transplantation from 17% to 80% compared to IVIG alone. In a subsequent placebo controlled trial, (NCT01178216), IVIG + placebo to IVIG + Rituximab were compared for desensitization. This trial was halted due to 3 severe ABMR events, all in the placebo arm. Based on these observations, the combination of IVIG + Rituximab was considered superior to IVIG alone. Similarly the DSA burden during treatment of clinically manifest ABMR can be more effectively reduced by the combination of plasmapheresis + IVIG + Rituximab (delta mean intensity of fluorescence –80%) compared to IVIG alone (delta mean intensity of fluorescence –30%) (20;36).

Steve Woodle described results using an innovative prospective, iterative trial design of bortezomib-based desensitization. This approach using Bayesian statistical models provided enhanced observational power in a relatively small study of 52 treated patients. The results in this study indicate that bortezomib-based designs can significantly reduce anti-HLA antibody levels with response rates in over 80% of patients treated. This result is a higher response rate than that achieved at most centers using IVIG alone (25–50%).

Proteasome inhibitors like bortezomib have an array of effects on immune responses. The primary mechanism by which proteasome inhibitors delete plasma cells is thought to be via induction of apoptosis through endoplasmic reticulum stress. It is likely that normal plasma cells (much like myeloma cells) exhibit a spectrum of sensitivity to PIs, which may explain variations in clinical responses to proteasome inhibitor therapy. Clinical experience with proteasome inhibitors in transplant first involved patients with ABMR refractory to IVIG, rituximab and plasmapheresis. Proteasome inhibitor therapy has also been reported to reverse ABMR when applied as front line treatment of ABMR. Recent reports have demonstrated that proteasome inhibitors based ABMR regimens (like IVIG-based regimens) provide optimal results in early ABMR, with late ABMR demonstrating a greater degree of resistance.

It was clear from the formal talks and from informal discussion during the CEOT that we are on the cusp of major advances in understanding and treating antibody-mediated allograft dysfunction. Yet we still need advances in understanding the basic biology and methods to translate that information to validated clinical approaches and outcomes. A major unmet clinical need in the area of ABMR was pointed out by Denis Glotz as the lack of systematic and scientifically rigorous multi-center investigations of therapeutic efficacy, with most data come from single center studies. The number of controlled, randomized trials for treating patients with ABMR remains very limited (<10 with a total of <50 patients enrolled). Thus new strategies for clinical trials and new multi-institutional groups are needed to test new compounds for the modification of B-cells, complement and antibody responses in transplantation.

In summary the first CEOT meeting identified clear unmet needs in the area of ABMR: We need to further improve the understanding of the regulation of B cell maturation and antibody response to enable the development of targeted therapies; More precise diagnostics of ABMR are needed for risk stratification and monitoring of treatment effects by molecular pathology and sensitive antibody testing; Innovative multicentre trials need to be designed with the aim to enhance observational power, in particular, in assessing synergistic multimodality therapies with reduced toxicities.

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Abbreviations

ABMR	antibody-mediated rejection
AST	American Society of Transplantation
Bregs	regulatory B cells
CEOT	Cutting Edge of Transplantation
DSA	donor-specific antibodies
GC	germinal center
IVIG	intravenous immunoglobulin
NK	natural killer
TCMR	T Cell Mediated Rejection

Reference List

1. Porcheray F, Devito J, Helou Y, Dargon I, Fraser JW, Nobecourt P, et al. Expansion of polyreactive B cells cross-reactive to HLA and self in the blood of a patient with kidney graft rejection. *Am J Transplant.* 2012; 12(8):2088–97. [PubMed: 22510337]
2. Porcheray F, Fraser JW, Gao B, McColl A, Devito J, Dargon I, et al. Polyreactive Antibodies Developing Amidst Humoral Rejection of Human Kidney Grafts Bind Apoptotic Cells and Activate Complement. *Am J Transplant.* 2013
3. DiLillo DJ, Matsushita T, Tedder TF. B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer. *Ann N Y Acad Sci.* 2010; 1183:38–57. [PubMed: 20146707]
4. Ding Q, Yeung M, Camirand G, Zeng Q, Akiba H, Yagita H, et al. Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J Clin Invest.* 2011; 121(9):3645–56. [PubMed: 21821911]
5. Amico P, Honger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. *Transplant.* 2009; 87(11): 1681–8.

6. Wiebe C, Nickerson P. Posttransplant monitoring of de novo human leukocyte antigen donor-specific antibodies in kidney transplantation. *Curr Opin Organ Transplant*. 2013; 18(4):470–7. [PubMed: 23695596]
7. Gebel HM, Bray RA. The evolution and clinical impact of human leukocyte antigen technology. *Curr Opin Nephrol Hypertens*. 2010; 19(6):598–602. [PubMed: 20720491]
8. Loupy A, Lefaucheur C, Vernerey D, Prugger C, van Huyen JP, Mooney N, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med*. 2013; 369(13):1215–26. [PubMed: 24066742]
9. Taniguchi M, Rebellato LM, Cai J, Hopfield J, Briley KP, Haisch CE, et al. Higher Risk of Kidney Graft Failure in the Presence of Anti-Angiotensin II Type-1 Receptor Antibodies. *Am J Transplant*. 2013; 13(10):2577–89. [PubMed: 23941128]
10. Tinckam K, Campbell P. Angiotensin II Type 1 Receptor Antibodies: Great Expectations? *Am J Transplant*. 2013; 13(10):2515–6. [PubMed: 23924147]
11. Zitzner JR, Shah S, Jie C, Wegner W, Tambur AR, Friedewald JJ. A prospective study evaluating the role of donor-specific anti-endothelial crossmatch (XM-ONE assay) in predicting living donor kidney transplant outcome. *Hum Immunol*. 2013; 74(11):1431–6. [PubMed: 23777928]
12. Reed EF, Rao P, Zhang Z, Gebel H, Bray RA, Guleria I, et al. Comprehensive Assessment and Standardization of Solid Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA. *Am J Transplant*. 2013; 13(7):1859–70. [PubMed: 23763485]
13. Zhang Q, Reed EF. Non-MHC antigenic targets of the humoral immune response in transplantation. *Curr Opin Immunol*. 2010; 22(5):682–8. [PubMed: 20833523]
14. Zhang X, Rozengurt E, Reed EF. HLA class I molecules partner with integrin beta4 to stimulate endothelial cell proliferation and migration. *Sci Signal*. 2010; 3(149):ra85. [PubMed: 21098729]
15. Ziegler ME, Souda P, Jin YP, Whitelegge JP, Reed EF. Characterization of the endothelial cell cytoskeleton following HLA class I ligation. *PLoS ONE*. 2012; 7(1):e29472. [PubMed: 22247778]
16. Valenzuela NM, Hong L, Shen XD, Gao F, Young SH, Rozengurt E, et al. Blockade of p-selectin is sufficient to reduce MHC I antibody-elicited monocyte recruitment in vitro and in vivo. *Am J Transplant*. 2013; 13(2):299–311. [PubMed: 23279566]
17. Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, et al. Antibody-mediated rejection criteria - an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant*. 2003; 3(6):708–14. [PubMed: 12780562]
18. Mengel M, Husain S, Hidalgo L, Sis B. Phenotypes of antibody-mediated rejection in organ transplants. *Transpl Int*. 2012; 25(6):611–22. [PubMed: 22533649]
19. Sis B, Mengel M, Haas M, Colvin RB, Halloran PF, Racusen L, et al. Banff '09 Meeting Report: Antibody Mediated Graft Deterioration and Implementation of Banff Working Groups. *Am J Transplant*. 2010; 10(3):464–71. [PubMed: 20121738]
20. Lefaucheur C, Loupy A, Vernerey D, Duong-Van-Huyen JP, Suberbielle C, Anglicheau D, et al. Antibody-mediated vascular rejection of kidney allografts: a population-based study. *Lancet*. 2013; 381(9863):313–9. [PubMed: 23182298]
21. Haas H, Sis B, Racusen L, Solez K, Glotz D, Colvin R, et al. Banff 2013 Meeting Report: Inclusion of C4d-Negative Antibody-Mediated Rejection and Antibody-Associated Arterial Lesions. *Am J Transplant*. 2013 In Press.
22. Mengel M, Chan S, Climenhaga J, Kushner YB, Regele H, Colvin RB, et al. Banff initiative for quality assurance in transplantation (BIFQUIT): reproducibility of C4d immunohistochemistry in kidney allografts. *Am J Transplant*. 2013; 13(5):1235–45. [PubMed: 23464533]
23. Mengel M, Sis B, Haas M, Colvin RB, Halloran PF, Racusen LC, et al. Banff 2011 Meeting Report: New Concepts in Antibody-Mediated Rejection. *Am J Transplant*. 2012; 12(3):563–70. [PubMed: 22300494]
24. Dean PG, Park WD, Cornell LD, Gloor JM, Stegall MD. Intragraft gene expression in positive crossmatch kidney allografts: ongoing inflammation mediates chronic antibody-mediated injury. *Am J Transplant*. 2012; 12(6):1551–63. [PubMed: 22335458]
25. Hidalgo LG, Sis B, Sellares J, Campbell PM, Mengel M, Einecke G, et al. NK cell transcripts and NK cells in kidney biopsies from patients with donor-specific antibodies: evidence for NK cell

- involvement in antibody-mediated rejection. *Am J Transplant*. 2010; 10(8):1812–22. [PubMed: 20659089]
26. Sis B, Jhangri G, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial gene expression in kidney transplants with alloantibody indicates antibody-mediated damage despite lack of C4d staining. *Am J Transplant*. 2009; 9(10):2312–23. [PubMed: 19681822]
 27. Mengel M, Campbell P, Gebel H, Randhawa P, Rodriguez ER, Colvin R, et al. Precision diagnostics in transplantation: from bench to bedside. *Am J Transplant*. 2013; 13(3):562–8. [PubMed: 23279692]
 28. Kaczmarek I, Deutsch MA, Kauke T, Beiras-Fernandez A, Schmoeckel M, Vicol C, et al. Donor-specific HLA alloantibodies: long-term impact on cardiac allograft vasculopathy and mortality after heart transplant. *Exp Clin Transplant*. 2008; 6(3):229–35. [PubMed: 18954302]
 29. Smith JD, Banner NR, Hamour IM, Ozawa M, Goh A, Robinson D, et al. De novo donor HLA-specific antibodies after heart transplantation are an independent predictor of poor patient survival. *Am J Transplant*. 2011; 11(2):312–9. [PubMed: 21219570]
 30. Tambur AR, Pamboukian SV, Costanzo MR, Herrera ND, Dunlap S, Montpetit M, et al. The presence of HLA-directed antibodies after heart transplantation is associated with poor allograft outcome. *Transplant*. 2005; 80(8):1019–25.
 31. Zhang Q, Cecka JM, Gjertson DW, Ge P, Rose ML, Patel JK, et al. HLA and MICA: targets of antibody-mediated rejection in heart transplantation. *Transplant*. 2011; 91(10):1153–8.
 32. Kobashigawa J, Crespo-Leiro MG, Ensminger SM, Reichenspurner H, Angelini A, Berry G, et al. Report from a consensus conference on antibody-mediated rejection in heart transplantation. *J Heart Lung Transplant*. 2011; 30(3):252–69. [PubMed: 21300295]
 33. Wu GW, Kobashigawa JA, Fishbein MC, Patel JK, Kittleson MM, Reed EF, et al. Asymptomatic antibody-mediated rejection after heart transplantation predicts poor outcomes. *J Heart Lung Transplant*. 2009; 28(5):417–22. [PubMed: 19416767]
 34. Jordan SC, Tyan D, Stablein D, McIntosh M, Rose S, Vo A, et al. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. *J Am Soc Nephrol*. 2004; 15(12):3256–62. [PubMed: 15579530]
 35. Vo AA, Lukovsky M, Toyoda M, Wang J, Reinsmoen NL, Lai CH, et al. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med*. 2008; 359(3):242–51. [PubMed: 18635429]
 36. Lefaucheur C, Nochy D, Andrade J, Verine J, Gautreau C, Charron D, et al. Comparison of combination Plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. *Am J Transplant*. 2009; 9(5):1099–107. [PubMed: 19422335]

Appendix – List of Speakers

Robert Anthony, PhD, Massachusetts General Hospital

Lee Ann Baxter-Lowe, PhD University of California, San Francisco

Michael Cancro, PhD University of Pennsylvania

Anita Chong, PhD The University of Chicago

Marcus Clark, MD The University of Chicago

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Anthony Jevnikar, MD London Health Science Center

Stanley Jordan, MD Cedars-Sinai Medical Center

Jon Kobashigawa, MD Cedars-Sinai Heart Institute

Frances Lund, PhD University of Alabama at Birmingham

Joren Madsen MD, DPhil Massachusetts General Hospital

Jonathan Maltzman, MD, PhD University of Pennsylvania

Eric Meffre, PhD Yale University School of Medicine

Michael Mengel, MD University of Alberta

Robert Montgomery, MD, DPhil Johns Hopkins Medicine

Peter Nickerson, MD University of Manitoba

Jeffrey Platt, MD University of Michigan

Elaine Reed, PhD University of California, Los Angeles

David Rothstein, MD University of Pittsburgh

Daniel Salomon, MD The Scripps Research Institute

Mark Schnitzler, PhD Saint Louis University

Dorry Segev, MD, PhD Johns Hopkins University

Mark Stegall, MD Mayo Clinic, Rochester

Anat Tambur, DMD, PhD Northwestern University

Paul Terasaki, PhD Terasaki Foundation Laboratory

Lori West, MD, DPhil University of Alberta

E. Steve Woodle, MD University of Cincinnati

Emmanuel Zorn, PhD Massachusetts General Hospital

Appendix – Planning Committee

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