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Meeting Report

National Conference to Assess Antibody-Mediated Rejection in Solid Organ Transplantation

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The process of humoral rejection is multifaceted and has different manifestations in the various types of organ transplants. Because this process is emerging as a leading cause of graft loss, a conference was held in April 2003 to comprehensively address issues regarding humoral rejection.

Though humoral rejection may result from different factors, discussion focused on a paradigm caused by antibodies, typically against donor HLA antigens, leading to the term 'antibody-mediated rejection' (AMR). Conference deliberations were separated into four workgroups: The Profiling Workgroup evaluated strengths and limitations of different methods for detecting HLA reactive antibody, and created risk assessment guidelines for AMR; The Diagnosis Workgroup reviewed clinical, pathologic, and serologic criteria for assessing AMR in renal, heart and lung transplant recipients; The Treatment Workgroup discussed advantages, limitations and possible mechanisms of action for desensitization protocols that may reverse AMR; and The Basic Science Workgroup presented animal and human immunologic models for humoral rejection and proposed potential targets for future intervention. This work represents a comprehensive review with contributions from experts in the fields of Transplantation Surgery, Medicine, Pathology, Histocompatibility, Immunology, and clinical trial design. Immunologic barriers once considered insurmountable are now consistently overcome to enable more patients to undergo organ transplantation.

Key words: Graft rejection, HLA antigens

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Introduction

The detection and treatment of allograft rejection has historically focused upon T-cell-mediated processes. The existence of vascular or humoral rejection was suspected, as antibodies reactive to donor HLA antigens were regularly found in the sera of recipients undergoing rejection (1). However, until recently, diagnosis of antibody-mediated rejection (AMR) was hampered by lack of a reliable histologic marker providing evidence of antibody deposition in biopsy specimens of rejecting grafts (2,3). Antibody-mediated rejection is typically unresponsive to conventional antirejection therapy (3), and therefore has recently been recognized as a major cause of allograft loss.

On April 23–24, 2003, a national conference was held at the National Institutes of Health to assess current knowledge regarding humoral rejection in solid organ transplantation. The objectives were to: develop a risk profile for recipient susceptibility to AMR; examine new criteria to diagnose AMR; assess the effectiveness of innovative treatment protocols; and develop immunologic strategies of basic science research. Workgroups addressed each of these topics, with participants selected for their expertise in these areas. Background papers and questions to focus interaction were distributed before the conference. The organizers summarized the findings of the various workgroups in this compendium and distributed them to participants who provided recommendations for the final report.

The Profiling Work Group Report

The assignment for the Profiling Work Group was to evaluate current techniques for establishing pre- and post-transplant risk of AMR. Strengths/limitations of commonly used detection methods including basic and enhanced complement dependent cytotoxicity (CDC), antiglobulin-enhanced CDC (AHG-CDC), and flow were discussed [reviewed in (4)]. In addition, the nature of antibodies (Ab) to HLA (both Class I and II), non-HLA and ABO, isotypes and autoAb were considered.

Testing before transplantation

The group concluded that: (1) A complete patient sensitization history which includes PRA, crossmatch (CXM) results, and transfusions, pregnancies and previous transplants are required to assess the risk of AMR for

renal, cardiac and lung transplant; (2) An individual cannot be reliably defined as unsensitized without a complete sensitization history; (3) Techniques at least as sensitive as AHG-CDC be used to determine the presence or absence of donor-specific Class I and Class II HLA antibodies (DSA); (4) The specificity of HLA Ab should be determined before transplantation and considered in algorithms for proceeding with transplantation and in the choice of immunosuppression; (5) The presence or absence of autoAb be established to facilitate interpretation of pretransplant CXM results; and (6) T- and B -cell CXMs should be performed before transplantation (unless the clinically indicated, i.e. a long cold ischemia time, and only then, if a complete and reliable sensitization history is negative).

Evidence supported the relevance to renal, heart and lung graft survival of Ab directed at Class II (5), as well as a history of Ab production (5–7). Lastly, the sensitivity of the final CXM should be equivalent to the sensitivity of the HLA Ab screening technique. Historic (peak PRA in a timeframe determined by the transplant center) and current sera should be included in the final CXM.

Pre-transplant assessment of rejection risk

Defining a risk for AMR and proclaiming a contraindication to transplant were distinguished. A consensus recommendation for *risk assessment* based on either a positive T- or B-cell CXM for AMR was achieved (Table 1):

- A current positive CDC or CDC-AHG CXM poses a high risk of AMR or early graft loss.
- A current positive CDC or CDC-AHG CXM is a contraindication to transplantation unless DSA can be reduced with desensitization protocols.
- A positive flow CXM or a remote (historic) positive CDC or CDC-AHG CXM poses intermediate risk for early acute rejection and may require augmented immunosuppression.
- Recipients with a negative flow or CDC-AHG CXM have low risk of AMR with conventional immunosuppression.
- Owing to the highly variable sensitivity of the CDC technique, current and remote negative CXMs, if obtained only by CDC, do not necessarily confer a low risk for AMR.

ABO and CXM incompatibility

The Profiling Work Group discouraged the performance of ABO or CXM incompatible transplants without an investigative protocol outlining plans for monitoring long-term effects on graft survival. Evaluation of the Ab following treatment and transplantation should be performed in every protocol that modifies HLA/ABO Ab before transplantation. More studies are needed to determine the safety and long-term efficacy of ABO- and CXM-incompatible transplants.

Post-transplantation monitoring

Emphasis was placed on monitoring DSA post-transplantation as a diagnostic/prognostic tool for AMR and how monitoring might differ for high- and low-risk patients. Distinguishing between preformed and *de novo* Ab and between DSA and third-party antibody were considered essential (8,9). Data correlating DSA with heart and lung AMR diagnosis and outcome were presented and it was noted that HLA Ab may presage chronic rejection as well (6). The group recommended that sera for HLA Ab analysis (e.g. solid-phase screen for class I and II) should be collected at the time of post-transplant biopsies. If an Ab is detected, HLA specificity analysis should be correlated with a C4d determination in the biopsy. Multi-center studies were recommended to correlate serial evaluation of HLA Ab with both AMR diagnosis and short and long-term allograft outcome as well as the role for HLA Ab monitoring at the time of stable renal function.

Table 1: Proposed kidney risk assessment for humoral rejection and early graft loss

	Contraindicated	High[1]	Intermediate[2]	Low[3]
Current positive CXM				
Direct CDC non-reducible	•			
Direct CDC modifiable		•		
AHG CDC		•		
Flow crossmatch			•	
Remote positive CXM				
Direct CDC			•	
AHG CDC			•	
Flow crossmatch			•	
Current and remote negative CXM				
Direct CDC			•[4]	
AHG CDC				•
Flow crossmatch				•

[1] Minimally requires pretransplant intervention and post-treatment/transplant monitoring.
[2] May require augmented immunosuppression and/or post-transplant monitoring.
[3] Conventional therapy may be used.
[4] See text.

Other future considerations

Additional studies were proposed to refine risk assessment and prognosis:

- Develop uniform pre- and post-transplant T- and B-cell CXM and HLA Class I and II Ab detection assays;
- Determine the clinical relevance of donor-specific IgM HLA Ab
- Determine the utility of translating HLA Class I and II Ab specificity analysis into 'virtual PRA' for use in organ distribution;
- Develop a virtual cross-matching algorithm for broader distribution of organs (for highly sensitized patients);
- Determine the role of antiendothelial/MICA Ab and other Ab that may cause C4d positivity when HLA Ab is negative;
- Determine the role of antiphospholipid/ANCA Ab in the appropriate setting (patients with thrombotic history and/or autoimmune diseases); and
- Develop therapeutic strategies based on the pretransplant risk assessment for AMR to increase access to transplantation and to improve outcomes in at risk patients.

Diagnosis Workgroup Report

The diagnosis workgroup was charged with developing diagnostic criteria for AMR after kidney, heart or lung transplantation. A common framework of graft dysfunction, histologic evidence of capillary injury, and identification of antidonor antibody was used to define AMR for each organ.

Kidney: acute antibody-mediated rejection

The workgroup concluded that sufficient clinical data exist to substantiate the concept of acute AMR. Antibody-mediated rejection, identified in approximately 5–7% patients and 12–37% of biopsies taken for acute rejection, is typically resistant to standard therapies and is associated with a poorer prognosis than pure acute cellular rejection (ACR) (10). Antibody-mediated rejection has no distinguishing clinical features but typically occurs early after transplantation and causes rapid functional deterioration. However, AMR can also occur much later, particularly in the setting of reduced immunosuppression or noncompliance.

Participants generally agreed that the diagnosis of acute AMR should require graft dysfunction to distinguish clinical from subclinical rejection, a criteria not specified in the recent Banff criteria for AMR (11). Antibody-mediated rejection may occur with or without the features of ACR. The primary biopsy features of AMR are detection of the complement component C4d in peritubular capillaries combined with some evidence of acute tissue injury (Table 2). The work group did not consider endarteritis – a feature of cell-mediated rejection – as indicating AMR. Fibrinoid necrosis, and potentially, transmural arteritis are the two arterial lesions compatible with AMR (although not necessary or sufficient for the diagnosis) (10,12). Participants agreed that

Table 2: Diagnostic criteria of antibody-mediated rejection after kidney or heart transplantation

	Kidney		Heart
	Acute	Chronic	Acute
Clinical evidence of graft dysfunction	+	+	+
Histologic evidence of tissue injury	PMNs/macrophages/ thrombi in capillaries and/or fibrinoid necrosis and/or acute tubular injury	*Arterial intimal fibrosis/ *Duplication of glomerular basement membrane *Interstitial fibrosis/tubular atrophy *Laminated peritubular capillary basement membrane *Requires 3 of 4 criteria for diagnosis	^Endothelial changes: swelling or denudation ^Macrophages in capillaries Neutrophils in capillaries #Interstitial edema, congestion, and/or hemorrhage ^Required criterion for diagnosis #Finding in absence of OKT3 induction
Immunopathologic evidence for antibody action	C4d in PTC or Ig/C3 in arteries	C4d in PTC	Ig (G, M, and/or A) + C3d and/or C4d and/or C1q (equivalent staining in capillaries) Fibrin in vessels (optional)
Serologic evidence of anti-HLA or other antidonor antibody at time of biopsy	+	+	+

positive C4d staining of frozen tissue requires widespread, strong, linear, and circumferential peritubular capillary staining in either cortex or medulla, excluding scarred or necrotic areas. Similar criteria with the exception of strong staining were adopted for staining of paraffin-embedded, formalin-fixed material. It was acknowledged that C4d can be identified in allograft biopsies lacking morphologic evidence of rejection (13,14). Furthermore, non-HLA and/or non-ABO antibodies along with antibody-independent mechanisms may theoretically result in C4d deposition (15), although ischemia does not seem to be a cause (16). The final requisite diagnostic element is serologic evidence of antidonor (either anti-HLA or anti-ABO) antibody.

Kidney: chronic antibody-mediated rejection

Previous studies (17,18) have correlated circulating anti-HLA antibodies with the development of chronic rejection and graft loss. Two recent reports (19,20) have described the presence of histologic and ultrastructural changes of chronic rejection in association with C4d staining. Concomitant circulating antidonor antibody was identified with an approximate frequency of 90% (20). Subclinical C4d deposition has been reported in preliminary studies (19) and this may precede chronic glomerulopathy.

Literature pertaining to the diagnosis of chronic AMR is sparse, and thus chronic AMR has not yet gained wide acceptance. The workgroup, nevertheless, felt that a set of proposed diagnostic criteria for chronic AMR parallel to that for acute AMR would be useful to stimulate future studies (Table 2). The term 'chronic' was meant to connote a process extending over some time, rather than a designation of inactivity. Indeed, the presence of C4d itself argues for an active, immunologic process, as C4d positivity is transient, lasting days to weeks.

Heart: acute antibody-mediated rejection

As outlined in a recent review, several studies document the occurrence of AMR after heart transplantation, which increases susceptibility to allograft vasculopathy and compromises allograft survival (21). The ISHLT has not yet established criteria for cardiac AMR. However, the suspected prevalence of pure AMR is 7–18%, and AMR accompanying ACR is 23% (21). Of patients with AMR, 68% of those presenting early but only 13% of those presenting late exhibit graft dysfunction. Although 15–82% of patients have detectable circulating antibody (1) by 6 months after transplantation, the majority are asymptomatic. The presence of non-HLA antibodies such as antivimentin and/or antiendothelial cell antibodies may also have clinical relevance with similar clinical consequences (22).

The workgroup proposed criteria for cardiac AMR, requiring pathologic, immunopathologic, and serologic evidence in association with graft dysfunction (Table 2). Myocardial capillaries commonly show macrophages or neutrophils associated with endothelial activation or denudation. Interstitial hemorrhage and edema may also be seen but are nonspecific, as these features may also be seen in patients induced with OKT3 (23). Immunopathologically, capillaries show accumulation of immunoglobulins (IgG, IgM, and/or IgA) and complement components (C3d, C4d, C1q) and may show accumulation of fibrin when the process is ongoing or severe (23). If frozen section tissue is unavailable, immunopathologic features can be demonstrated in paraffin sections using a macrophage marker (CD68) in combination with a vascular marker (CD31 or CD34; 24,25), although published experience with C4d staining of paraffin-embedded heart tissue is lacking.

Heart: chronic antibody-mediated rejection

As noted above, circulating antibodies and a history of AMR correlate with increased cardiac allograft vasculopathy, although chronic AMR per se has not been defined.

Lung: acute antibody-mediated rejection

Historically, AMR of the lung has been associated with 'hyperacute rejection' clinically manifest by primary graft failure within min/h/days of transplantation in the setting of preformed antibodies to either donor HLA antigens or endothelial cells (26). However, it has been difficult to identify features distinguishing the clinical syndrome of primary graft failure from AMR/hyperacute rejection vs. endotoxemia and severe ischemia/reperfusion injury.

Acute rejection with a component of AMR is manifest by perivascular and peribronchiolar mononuclear infiltrates. As ISHLT rejection grades increase (A3 or A4), neutrophils are increasingly prominent (27). Prominence of B cells in the infiltrate may also indicate organ-based antibody generation and resistance to conventional therapy such as steroids (28). The histopathology of mixed ACR and AMR has been associated with CD20+ B cells and plasma cells in the inflammatory infiltrate, endothelialitis and small airway inflammation. Experience with C4d staining in lung biopsies is, however, limited (29). The presence of preformed HLA-specific antibodies and/or the development of *de-novo* DSA post transplantation have been documented in patients with high-grade and steroid refractory rejections (30).

Lung: chronic antibody-mediated rejection

Studies have examined post-transplant development of *de novo* anti-Class I/II antibodies and its association with chronic allograft dysfunction, known as bronchiolitis obliterans syndrome (BOS) (1,31). Although no histologic studies have elucidated the humoral contribution to BOS, B cells appear to be present in the inflammatory infiltrate.

General classification of humoral responses

The workgroup developed a general classification of humoral responses applicable to all organs that may facilitate future research (Table 3). Circulating antidonor antibodies, without histologic or immunopathologic sequelae, may be

Table 3: Putative stages of humoral response to an organ graft

I: Latent humoral response
Circulating antibody[1] alone (but without biopsy findings or graft dysfunction)
II: Silent humoral reaction (accommodation vs. prerejection state)
Circulating antibody[1] + C4d deposition (but without histologic changes or graft dysfunction)
III: Subclinical humoral rejection[2]
Circulating antibody[1] + C4d deposition + tissue pathology (but without graft dysfunction)
IV: Humoral rejection
Circulating antibody[1] + C4d deposition + tissue pathology + graft dysfunction

[1]Circulating antibody to HLA or other antigens expressed on donor endothelial cells.
[2]May differ among organs, as the ability to detect particularly mild degrees of graft dysfunction varies among organs.

considered a latent response. The significance of C4d in the absence of histologic evidence of tissue injury is unknown, potentially reflecting either a beneficial response such as accommodation and/or an adverse warning of future rejection. Finally, subclinical AMR – AMR without graft dysfunction – is just emerging as an entity with uncertain outcome.

Treatment Work Group Report

Two protocols have been established for reducing anti-HLA antibody to overcome a positive CXM or rescue organs undergoing AMR: high-dose intravenous immune globulin (IVIG) (32,33) and plasmapheresis (PP) combined with low-dose CMV hyperimmune globulin (CMVIg) or IVIG (34–36). There are several agents and interventions that can augment the potency of these protocols including splenectomy and anti-CD20 antibody, as well as traditional immunosuppressive and induction medications. These protocols, developed to desensitize patients in preparation for transplantation, are currently being used at some centers to treat AMR. They are described in Table 4 with important features highlighted below.

High-dose IVIG protocol

An *in vitro* IVIG CXM test has been developed which identifies patients most likely to benefit from IVIG therapy *in vivo* (37). Intravenous immune globulin is incubated *in vitro* with titrated patient's sera to determine the degree of inhibition. Through 2003, a total of 60 patients were enrolled in the Cedars Sinai desensitization program. Fifty-four received transplants and six are awaiting a deceased donor renal transplant. All transplanted patients had reductions in PRA values and negation of CDC CXM, although some still had a positive flow CXM at the time of transplantation. Outcomes at 2 years post transplant for 42 patients treated with this protocol were recently summarized (37).

Table 4: Strategies of antibody removal

High dose IVIG (1–2 g/kg) (32,33)
Protocol
In vitro PRA test to identify patients most likely to benefit from IVIG therapy (45)
Responders started on IVIG 2 g/kg on HD over 4 h
Monthly × 4 doses
Immunosuppression starts at time of transplant
Transplantation with deceased donor kidney
For live donors 1–4 doses – repeat crossmatch after each dose
Endpoint of therapy
Negative enhanced CDC crossmatch
Mechanism
Many putative immunomodulatory pathways identified
Antiidiotypic networks probably important (40)
Advantages
Can be used to desensitize patients on the waiting list
Less rebound in absence of donor antigen
Less expensive than plasmapheresis
Ease of administration
Disadvantages
Nonresponders
Need different techniques to follow DSA titers
Less rapid Ab removal, unproven for high-titer DSA
Toxicity and batch-to-batch variability
Plasmapheresis/low-dose CMVIg (100 mg/kg) (34–36)
Protocol
QOD plasmapheresis (PP): one volume exchange replaced with albumin or FFP
CMVIG: 100 mg/kg following each PP
PreTx: Tacrolimus, MMF started with 1st PP/ICMVIg
Steroids and Daclizumab added at transplant
For ABO-incompatible recipient or high-risk CXM positive recipient – laparoscopic splenectomy or anti-CD20
PP/CMVIg continued post-transplantation (3–5 QOD treatments)
Endpoint of therapy
For Anti-HLA antibody: Negative AHG CDC crossmatch
For ABO incompatibility: Isoagglutinin titer $\leq$1 : 16
Mechanism
Rapid reduction in anti-HLA or isoagglutinin Ab
Ab reduction allows immunomodulation at a lower Ig dose
Induces donor-specific unresponsiveness (HLA) or accommodation (ABOI)
Advantages
Predictable kinetics of plasmapheresis
No evidence of 'nonresponders', works for high titer DSA
Able to easily follow DSA levels during/after therapy
Disadvantages
Rebound occurs unless the transplant immediately follows preconditioning – not currently appropriate for patients waiting for a deceased donor transplant
Expensive and resource intensive
Probably more immunosuppressive
Anti-CD20 (35)
Mechanism
Rapid and durable ablation of the B-cell compartment
Advantages
Probably reduces precursor cells responsible for clonal expansion during AMR
May produce more effective antibody reduction when combined with plasmapheresis or IVIG

Table 4: Continued.

Well-tolerated, little apparent toxicity
Effect on the immune system is temporary
Disadvantages
Plasma cells persist in the spleen and bone marrow
Does not appear on its own to reduce DSA titers
Immunosuppressive
Splenectomy (35)
Mechanism
Reduces plasma cells, precursor cells, B-cell immune surveillance capabilities
Advantages
Proven efficacy in reducing graft loss in ABOI transplants
Can be performed using minimally invasive techniques
May produce more effective antibody reduction when combined with plasmapheresis or IVIG
Disadvantages
Life-long risk of sepsis from encapsulated bacteria
Does not appear on its own to reduce DSA titers
Effect on immune system is permanent

Approximately 30% of recipients had rejection episodes and the 2-year graft survival rate was 89%.

Intravenous immune globulin also appears to be effective in treating AMR in heart and kidney allograft recipients (33). In 10 patients with severe rejection of heart or renal allografts and demonstrable antibody to donor-mismatched antigens, IVIG rapidly reduced this antibody and recurrent rejection was not observed in nine of these patients. Other investigators have suggested that IVIG is useful in the management of OKT3 or thymoglobulin-resistant forms of acute rejection (38,39).

The initial mode of action of IVIG may be neutralization/elimination of DSA by anti-idiotypic antibodies present in IVIG (40). Rapid reversal of inflammation and organ dysfunction suggests IVIG diminishes circulating levels of DSA and inhibits B-cell production of DSA (41). Intravenous immune globulin may also inhibit complement-mediated endothelial cell injury facilitated by Fc fragments with high avidity for the complement components C3b and C4b (42). In experimental models of AMR, C3b-stimulated alloantibody responses and initiated class switch from IgM to high-affinity IgG alloantibodies (31).

Plasmapheresis and low-dose CMVIg protocol

Plasmapheresis/CMVIg has been used at Johns Hopkins to transplant 62 patients with a pretransplant-positive AHG or flow CXM to their live donor. Among patients in whom desensitization was initiated, 95% were transplanted. After desensitization and transplantation, DSA titers are monitored and PP/CMVIg treatments are continued until DSA is eliminated. Donor-specific Class I and Class II HLA antibodies remained undetectable indefinitely in 90% of desensitized patients, while third-party anti-HLA antibody persisted (34,36). Three-year patient and graft survival was 94.3 and 86.8%, respectively. There are historical and humoral factors that are associated with an increased risk of AMR and graft loss (35). Patients classified as 'high risk' receive anti-CD20 and/or splenectomy in addition to PP/CMVIg before transplantation.

The same protocol has been applied to recipients of ABO-incompatible grafts. Plasmapheresis/CMVIg is continued pretransplant until ABO isogglutinin (IgG) titers are reduced to $\leq$16. Titers are maintained at or less than 16 for the first 2 weeks after transplantation with additional PP/CMVIg treatments. After engraftment, circulating isoagglutinin persists without apparent consequences to the graft (accommodation). Hopkins has achieved a graft survival rate of 94.2% for the 17 ABO-incompatible transplants performed to date.

Plasmapheresis (43) and PP/CMVIg therapy (36,37) have also been used as rescue therapy for AMR after renal transplantation with a goal of eliminating DSA. Plasmapheresis was performed every other day with each treatment followed by a low-dose (100 mg/kg) CMVIg infusion. Treatment was augmented with anti-CD20 or emergent splenectomy in cases of severe rejection. Cellular rejection, which frequently accompanies AMR, was treated with pulse steroids or antilymphocytic antibody.

The Treatment Workgroup concluded that effective therapy exists to desensitize in preparation for positive CXM and ABO-incompatible transplantation and reverse post-transplant AMR. While the two discussed treatment modalities have proven efficacy for antibody removal, they have yet to be tested in rigorous prospective, multicenter studies. Participants recommended that each protocol component be rigorously investigated. The major obstacle to wider implementation is the resource-intensive nature of these protocols. The long-term function of grafts transplanted after desensitization or rescued from AMR is unknown, as is any price paid for the enhanced immunosuppression (especially protocols utilizing splenectomy and/or anti-CD20 antibody) in terms of infectious or malignant complications.

Basic Science Work Group Report

The goal of the Basic Science Work Group was to define the boundary of basic knowledge and the unanswered questions of humoral rejection. The clinical condition is the result of a pathological state incited by antidonor antibodies (AMR), in conjunction with other factors such as complement, endotoxin, and by other agonists particularly directed against toll-like receptors. Alloreactive T cells, natural killer cells, platelets, and phagocytes acting directly on donor blood vessels may induce clinical and pathologic entities resembling AMR. The Basic Science Work Group considered the end lesion of humoral/vascular rejection to be brought about by ischemia and/or hypoxia. Whether ischemia or

hypoxia indeed constitute the final pathogenic step could be ascertained by manipulating independently the availability of oxygen (e.g. through the use of various types of cross-linked hemoglobin) and blood flow. The outcome could, in turn, suggest therapeutic strategies for rescuing grafts.

B cells in antibody-mediated rejection

The properties of the B-lineage cells that produce those antibodies are seminal to the understanding of AMR. Anti-blood group antibodies independent of T-cell help should be distinguished from anti-MHC antibodies generated with T-cell help. Why are T-cell-dependent anti-MHC antibodies produced in recipients who have adequate suppression of cell-mediated immune responses? Some small measure of T-cell responsiveness may persist despite treatment. Alternatively, T-cell help might be bypassed through suitable cross-linking of B-cell receptors if donor cells or fragments of donor cells bearing MHC molecules are presented to alloreactive B cells.

Which B cells actually produce these antibodies, are they memory B cells or plasma cells, and what regulates the function of alloreactive B cells? A vital regulator may be complement, which can promote B-cell responses, and under other circumstances contribute to B-cell tolerance. The main difficulty in studying B-cell responses in patients is poor access to the B cells that make alloreactive antibodies, residing in lymph nodes and spleen. Antibodies in circulation may not represent pathogenic (highest affinity) antibodies, as these may be bound to the graft.

Most investigators believe that immunoglobulin initiates rejection by activating the complement system, but which components of complement are needed and which events are triggered by complement activation is far from certain. Another important question is whether the complement that mediates tissue damage comes from the circulation exclusively, or whether it may be in some cases produced in the graft.

Endothelium and antibody-mediated rejection

Most investigators believe that endothelium is the predominant target of humoral injury in AMR. 'Activation' of endothelium leading to production of new procoagulant and proinflammatory substances or loss of anticoagulant and anti-inflammatory properties may be important in pathogenesis. If endothelium is needed for basic hemostasis and host defense, then therapeutic strategies should focus on the immune system rather than the endothelium. Furthermore, if the endothelium can protect against injury, as in accommodation, then inhibiting the functions of the endothelium may not be an optimal strategy.

Accommodation

Accommodation, acquired resistance to humoral injury, has been described in clinical and experimental systems (44). Accommodation clearly exists in ABO-incompatible allografts. There is general agreement that accommodation is an important phenomenon that may be necessary to the survival of grafts in individuals who generate humoral immunity against the donor. To the extent that accommodation is needed, understanding its basis and learning how to induce it deliberately should be central goals in this field.

T cells

In addition to promoting T-cell-dependent B-cell responses, alloreactive T cells may contribute to 'humoral' or vascular rejection directly. Clearly, T cells can interact directly with blood vessels to incite damage. The frequency of T-cell-mediated vascular injury may be diminished as immunosuppression has improved.

Therapy for antibody-mediated rejection

The best therapy for AMR has emerged from the empiric studies noted above. However, improvements in therapeutic regimens and revolutionary changes may derive from a rational consideration of the pathogenesis of the disease. How do therapeutic modalities directed against AMR work? What are the mechanisms of action of IVIG and plasmapheresis? Understanding the basic means by which these and other treatments control rejection may allow the development of less toxic and less expensive therapies. For example, if IVIG provides a source of soluble MHC, then the soluble molecules might be generated by other means without taxing the short supply of gamma globulin. Similarly, if plasmapheresis functions by modifying the function of B cells, then those functions might be modified directly without using this expensive form of therapy. It may be possible to generate protective antibodies or to drive B-cell responses away from production of pathogenic antibodies. There was general agreement that understanding the mechanisms of therapeutic modalities will require the use of laboratory animals, rather than the use of clinical subjects so that the variables can be eliminated or reduced in number.

Conclusion

Immunologic barriers once considered insurmountable are now consistently overcome to enable more patients to undergo organ transplantation. The current treatment of humoral rejection has effectively rescued patients previously destined to rapid graft failure. These treatments have been extended to conditioning regimens targeted to the elimination of donor-specific antibody. The horizon of these interventions is promising, as basic science models of the future address the accommodation of an allograft in a patient tolerant of donor antigens.

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Appendix: Participants by Workgroup

Conference organizers: Steven K. Takemoto, PhD, and Francis L. Delmonico, MD.

Workgroup 1: Profiling for Antibody-Mediated Rejection Pre- and Post-Transplant

Chairs: Peter Nickerson, MD, and J. Richard Thistlethwaite, MD, PhD.

Recorder: Dolly B. Tyan, PhD, Diplomate ABHI.

Participants: Robert A. Bray, PhD, Diplomate ABHI, David M. Briscoe, MD, Ilias I.N. Doxiadis, PhD, Thomas C. Fuller, PhD, Howard M. Gebel, PhD, F. Carl Grumet, MD, Mary S. Leffell, PhD, Diplomate ABHI, ABMLI, Alan B. Leichtman, MD, Marc I. Lorber, MD, Susan Martin, Joshua Miller, MD, Thalachallour Mohanakumar, PhD, Karen A. Nelson, PhD, Diplomate ABHI, James A. Schulak, MD, Alan Ting, PhD, Agathi Varnavidou, MA, BSc, ASCP, John P. Vella, MD, FRCP(I), FACP, James J. Wynn, MD

Workgroup 2: Diagnosis of Humoral Rejection

Chairs: Adriana Zeevi, PhD, Diplomate ABHI, Jon Kobashigawa, MD, Robert B. Colvin, MD.

Recorder: Sandy Feng, MD, PhD.

Participants: Denise Y. Alveranga, MD, William M. Baldwin, III, MD, PhD, Michael C. Fishbein, MD, Susan. V. Fuggle, DPhil, FRCPath, Lillian Gaber, MD, Robert S. Gaston, MD, Mark Haas, MD, PhD, M. Elizabeth H. Hammond, MD, Silviu Itescu, MD, Ronald H. Kerman, PhD, Shamila Mauiyyedi, MD, Robert M. Merion, MD, Robert A. Metzger, MD, Volker Nickeleit, MD, Lorraine C. Racusen, MD, Heinz Regele, MD, Nancy L. Reinsmoen, PhD, E. Rene Rodriguez, MD, Millie Samaniego, MD, Kim Solez, MD, Jeffrey S. Stoff, MD, Nicole Suciu-Foca, PhD, Paul Terasaki, PhD, Sam Yousem, MD.

Workgroup 3: Treatment of Humoral Rejection

Chairs: Robert A. Montgomery, MD, PhD, and Stanley Jordan, MD.

Recorder: Arthur J. Matas, MD.

Participants: Carl J. Cardella, MD, David J. Cohen, MD, Daniel J. Cook, PhD, Matthew Cooper, MD, Gabriel M. Danovitch, MD, Connie L. Davis, MD, Richard N. Fine, MD, James M. Gloor, MD, Stuart J. Knechtle, MD, Leslie W. Miller, MD, Mark David Pescovitz, MD, Lloyd E. Ratner, MD, Ron Shapiro, MD, Mark D. Stegall, MD, Nina Tolkoff-Rubin, MD, Francis H. Wright, MD, Andrea A. Zachary, PhD, Diplomate ABHI.

Workgroup 4: Basic Science of Humoral Rejection

Chairs: Jerzy Kupiec-Weglinski, MD, and Jeffrey L. Platt, MD.

Recorder: Hamid Rabb, MD.

Participants: Lawrence Agodoa, MD, Enver Akalin, MD, Hugh Auchincloss, MD, Marilia Cascalho, MD, PhD, Stephen Desiderio, MD, PhD, Rene J. Duquesnoy, PhD, Wayne W. Hancock, MD, Paul C. Kuo, MD, MBA, Joren C. Madsen, MD, DPhil, John C. Magee, MD, Nader Najafian, MD, Charles G. Orosz, PhD, Thomas C. Pearson, MD, PhD, Richard N. Pierson III, MD, David J. Pinsky, MD, Jordan S. Pober, MD, PhD, Elaine F. Reed, PhD, Judith M. Thomas, PhD, Flavio Vincenti, MD, Peter A. Ward, MD, Barbara Wasowska, PhD.