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Antibody Mediated Rejection of the Cardiac Allograft

Christopher R. Ensor and Christina T. Doligalski
The Johns Hopkins Hospital & Tampa General Hospital, USA

1. Introduction

Antibody mediated rejection (AMR), also known as B-cell mediated rejection or humoral rejection, of the cardiac allograft was first clinically described in the late 1980's (Herskowitz et al., 1987) followed shortly thereafter by pathologic evidence to support a unique rejection process apart from cellular mechanisms (Hammond et al., 1989). This is in contrast to the progression of knowledge regarding cellular rejection, or T-cell mediated rejection, which was readily described in the early 1960's and is the target of most current maintenance immunosuppression agents. Unfortunately, AMR remains poorly understood due, in large measure, to its complicated presentation, pathophysiology, diagnosis, and treatment. The lack of clarity regarding AMR has been compounded by multiple small studies in varying populations with a multitude of treatment modalities and combinations. Additionally, several new agents have been recently utilized or hypothesized to be of utility, with varying success.

Given the complexity of this process, lack of standardization in diagnosis, and multiple proposed treatment options, several professional organizations have endeavored to come to a consensus on the subject of AMR in heart transplant recipients. Most recently in 2011, the International Society for Heart and Lung Transplantation (ISHLT) published their outcomes from a consensus conference regarding AMR in heart transplantation (Kobashigawa et al., 2011) as well as a breakout group working formulation regarding pathologic diagnosis of AMR in heart transplantation (Berry et al., 2011). While these two documents provide some direction for practitioners and transplant providers, many questions remained unanswered and the rapid evolution of novel therapies and strategies for treatment will likely change the field of AMR in the heart transplant population dramatically.

This chapter will look to lay a foundational knowledge of the pathophysiology, epidemiology, and diagnosis of AMR. Additionally, traditional therapies are described and evaluated with a highlight on the controversies surrounding their use; finally, novel and experimental therapies along with their potential impact on prevention and treatment of AMR are described.

2. Definitions

Antibody mediated rejection can be characterized in several different ways. First, it can be qualified based upon the temporal relationship it has to transplantation. Hyperacute AMR is

a well known, well described process by which a patient has previously been exposed to some antigen that a donor expresses, and upon transplantation a rapid, immediate antibody response occurs leading to graft dysfunction and most often graft loss within 24 hours. Treatment of hyperacute AMR rarely reverses the process to salvage the graft. Acute AMR occurs sometime after the 24 hour postoperative period, and is generally rapid in onset; treatment strategies may be moderately effective. Chronic or late AMR is a newly recognized, poorly understood process that usually occurs greater than one year following transplantation and is thought to be very slow in progression with poor response to therapy.

Additionally, AMR can be described as either occurring due to pre-sensitization or is the result of *de novo* antibody production. *De novo* AMR occurs when a recipient lacks donor specific antibodies (DSA) and has a negative cross-match at the time of transplant, but subsequently develops AMR at some point after transplantation. Alternatively, if a patient has been previously exposed to antigens that a donor expresses, they are said to be pre-sensitized and typically receive prophylactic or empiric treatment in the peri-operative period. If, however, antibodies reappear at some point in the post-transplant period a renewed AMR may occur.

3. Pathophysiology

The immune system can generally be divided in to two main arms: the T cell, "cellular", arm, and the B cell, "humoral", arm. While these systems are complex and largely integrated, they do originate independently. B cells begin in the bone marrow as progenitor B cells and through activation by encounters with antigens mature through pro B cell, pre B cell, immature, and finally mature B cells. Activated mature B cells are also known as plasma cells and are essentially antibody factories. Antibodies are specific to a single antigen, such as proteins expressed on the surface of a transplanted organ, that are created to attach and signal other parts of the immune system to attack the foreign substance. This immune activation by antibody signaling ultimately damages the allograft. Damage is thought to occur via complement cascade-mediated fixation and activation, which actively damages the foreign material and also acts as a biochemical "amplifier", signaling other parts of the innate and adaptive immune systems such as neutophils, pro-inflammatory molecules and cytokines for example, to relocate to the site of antibody adhesion and attack. One of the more unique aspects of the B cell arm of the immune system is that it retains memory. Once a person has been exposed to an antigen presenting cell (usually from a foreign physiologic source such as an organ or transfusion) and mounts an immune response, a memory B cell is created that, without active intervention, will always exist and will mount a more-rapid response to subsequent antigen presentation from the same source.

These antigens can be portions of viruses, bacteria, or fungus. Human cells also express antigens; the most commonly identified of which are human leukocyte antigens (HLA). While a person does not usually attack itself and therefore tolerates their own HLAs, this is not true for other human tissues that express various antigens and are introduced in to a patient such as in solid organ transplantation.

Subsequently, the risk factors for development of AMR include anything that exposes patients to other human products and therefore creates more potential memory cells to respond to a transplanted organ. These include pregnancies, blood and blood product

transfusions, repeat transplantation, and, specific to heart transplantation, the widespread and growing use of extracorporeal and intracorporeal mechanical circulatory support devices such as left ventricular assist systems (LVAS), bi-ventricular assist devices, total artificial hearts, extracorporeal membranous oxygenators (ECMO), or intra-aortic counterpulsators (Reed et al., 2006).

AMR has recently been described as occurring across a spectrum, from completely asymptomatic circulating antibody to clinically overt organ rejection with hemodynamic compromise, graft loss, and decreased survival (Takemoto et al., 2004). Additionally, AMR has been described to contribute significantly to cardiac allograft vasculopathy (CAV), and often occurs in conjunction with acute cellular rejection as so-called mixed rejection (Montgomery et al., 2004).

4. Epidemiology

The true incidence of AMR has been difficult to define given the lack of standardization in diagnosis; however, it is generally accepted that AMR plays a much larger role in overall graft and patient survival than previously appreciated. The reported incidence of *de novo* AMR varies widely based upon the definitions used and at which point on the spectrum a study defines AMR. Epidemiologic studies in centers that perform protocolized endomyocardial biopsies have shown a wide variability in incidence of 3 – 51% (Michaels et al., 2003; Shahzad et al., 2011). Not surprisingly, those institutions that include circulating antibodies without evidence of graft dysfunction had a higher reported incidence of AMR.

Additionally, as the boundaries of transplantation have been expanded in recent years, the number of patients who present for transplantation highly pre-sensitized to other human antigens is on the rise. Based on a survey of the patients who experienced AMR at some point after transplant from 46 heart transplant centers, 35% (114/324) of patients were pre-sensitized prior to transplant, and of those 32% (37/114) were treated to attempt to reduce the amount of circulating antibodies prior to transplantation (Kobashigawa et al., 2011).

5. Diagnosis

Significant effort has been placed on standardizing the diagnosis of AMR of the cardiac allograft within the past 3 – 5 years. These efforts highlight that clinical factors, immunologic criteria, and pathologic criteria all play important roles. In 2004, a general staging of AMR was developed (Table 1), as were criteria for diagnosis of AMR in heart transplant recipients (Table 2). More recently, the ISHLT proposed a preliminary pathologic grading scheme similar to the 2004 guidelines (Table 3) with one major difference: the ISHLT workgroup recognized AMR as a diagnosis that can be made without evidence of circulating antibodies or clinical dysfunction.

5.1 Immunologic screening

Antibody screening tests have been clinically available for many years. These tests determine circulating antibody, but do not address very low level antibodies or antibodies that may be active but not in circulation. Prior to solid-phase antibody (SPA) testing, the presence of antibodies was determined utilizing cell-based assays. The mainstay of testing

	Circulating Antibody	C4d Deposition	Tissue Pathology	Graft Dysfunction
Stage I: Latent Humoral Response	Present			
Stage II: Silent Humoral Rejection	Present	Present		
Stage III: Subclinical Humoral Rejeciton	Present	Present	Present	
Stage IV. Humoral Rejection	Present	Present	Present	Present

Table 1. General AMR staging

Evidence of graft dysfunction	Present
Histologic evidence of tissue injury	*Endothelial swelling or denudation
	*Macrophages in capillaries
	Neutrophils in capillaries
	Interstitial edema, congestion and/or hemorrhage
Immunopathologic evidence for antibody action	Ig G, M, and/or A
	C3d and/or C4d and/or C1q in capillaries
	Fibrin in vessels
Serologic evidence of anti-HLA or other anti-donor antibody at time of biopsy	Present

* required histologic findings

Table 2. 2004 diagnostic criteria of acute AMR in heart transplant recipients

Category	Description	Definition
pAMR 0	Negative for pathologic AMR	Both histologic and immunopathologic studies are negative
pAMR 1 (H+)	Histopathologic AMR alone	Histologic findings present and immunopathologic studies negative
pAMR 1 (I+)	Immunopathologic AMR alone	Histologic findings negative and immunopathologic findings positive
pAMR 2	Pathologic AMR	Both histologic and immunopathologic findings present
pAMR 3	Severe pathologic AMR	Histologic findings of interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis, and/or karyorrhexis and marked edema

Table 3. 2011 ISHLT criteria for pathologic AMR

was complement-dependent cytotoxicity (CDC) assays which involve incubating patient serum with cells of known HLA types, rabbit sera as a source of complement, and finally cell dyes to determine the amount of cell death that has occurred. The HLAs tested cover a very wide spectrum of known HLAs, however not all HLAs are tested. Limitations of this test included its lack of sensitivity and specificity (Berry et al., 2011). Unfortunately, the differences in clinical impact of circulating donor specific antibodies (DSAs), anti-HLA antibodies, or non-HLA antibodies, comparatively, have not been fully elucidated.

5.1.1 Solid Phase Antibody detection

The recent advent of SPA detection has revolutionized immunologic screening. The so-called Luminex® (LABScreen, One Lambda Inc., Canoga Park, CA) single antigen bead (SAB) assay panel provides a comprehensive assessment of individualized IgG and IgM HLA antibodies present in the recipient using a multiplex platform (El-Awar et al., 2005). These beads are coated with fluorescein-tagged antigens, which fluoresce in the presence of the known HLA antibody. The degree of fluorescence, defined in units of mean equivalents of soluble fluorochrome or mean fluorescent intensity, is directly proportional to the circulating amount of the HLA antibody in question. This quantitation is critical when determining which antibodies to exclude from the potential donor pool, and during the depletion process of DSA in the post transplant period.

Despite this improved specificity, the positive predictive value (PPV) of the Luminex assay for AMR remains poor (45%); however, the negative predictive value for AMR is quite good (100%) in a recent analysis (Chin et al., 2011). In an effort to improve the PPV of the Luminex-SAB assay, the Immunogenetics Laboratory at Stanford University spiked an otherwise ordinary Luminex assay sample with purified human Complement-1q (C1q) and ran the sample. The results of the assay revealed a significant decrease in background antibodies, and focused the assay only on those HLA antibodies able to fix C1q. This addition improved the assay's PPV, dramatically, to 100% (Chin et al., 2011). This technique is currently in its infancy, but may result in enhanced utility of the Luminex assay over the decade to come.

5.2 Pathophysiology

Endomyocardial biopsies (EMB) at many centers are routinely performed in addition to those performed for any patient who exhibits signs and symptoms of graft dysfunction. It has been recognized that findings seen on histology are unique from those seen with acute cellular rejection or CAV. Some consensus regarding the findings for AMR was established recently. Pathologic findings are almost exclusively found in the capillary beds; common findings in AMR include endothelial swelling or denudation, deposition of macrophages or neutrophils in capillaries, and interstitial edema, congestion, and potentially hemorrhage in severe cases. Immunopathologic findings include deposition of IgG, M, or A, and positive staining for byproducts of the complement cascade including Complement-3d (C3d), Complement-4d (C4d), or C1q in the capillaries. Sometimes fibrin may also be found in the vessel beds. Table 3 outlines the grading criteria for pathologic AMR staging.

6. Treatment

When discussing treatment options, there are two major divisions for which these therapies have been studied. The first is for the removal of circulating antibodies prior to

transplantation, a process known as desensitization; the second is for treatment of AMR, whether it be a reactivation of a previously sensitized patient or *de novo* AMR. Desensitization may be performed to either remove circulating antibody in the weeks to months prior to a transplant in an effort to allow for a larger donor pool in highly sensitized patients, or to mitigate the risk of AMR in the early postoperative period when a patient is known to have mismatched antigens such as is the case with ABO incompatible transplantation or positive cross-matches at the time of transplantation. Treatment may be performed at any point in the spectrum of AMR, from treatment of asymptomatic circulating antibodies to the treatment of clinically significant graft dysfunction caused by antibody-mediated activation of the immune system, with the goals of halting current damage, reverse signs and symptoms of AMR, and long-term to prevent the development of CAV and improve allograft and patient survival. Figure 1 contains a proposed treatment algorithm.

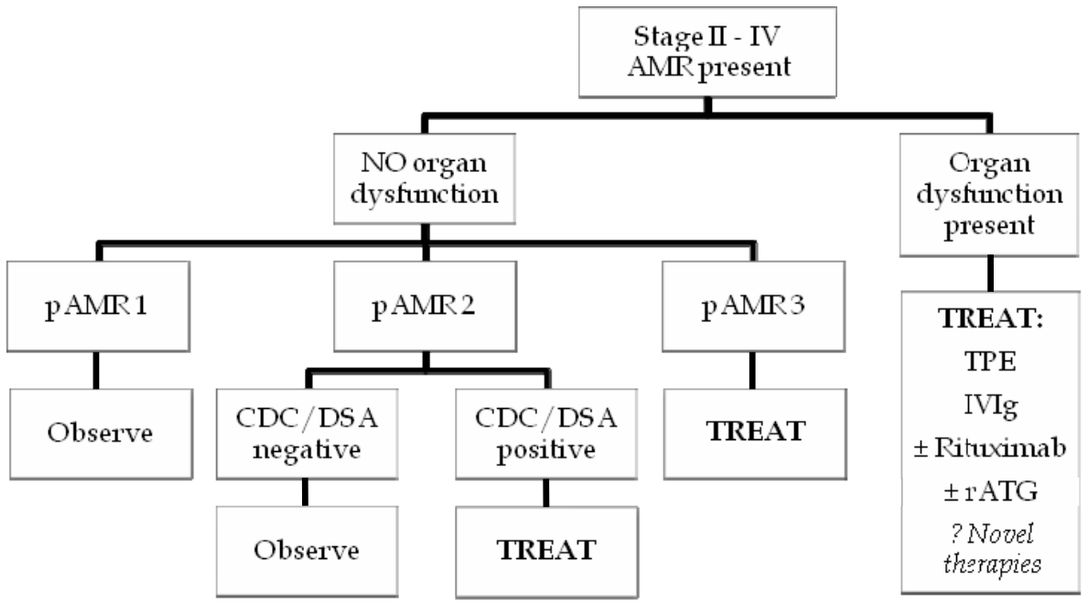

Fig. 1. AMR treatment algorithm. CDC, complement dependent cytotoxicity. DSA, donor specific antibody. IVIg, intravenous immunoglobulins. rATG, rabbit anti-thymocyte globulin. TPE, plasmapheresis.

6.1 Plasmapheresis

Plasmapheresis, or plasma exchange, has been used clinically for a variety of autoimmune conditions since the early 1970's and is generally considered a cornerstone for treatment of AMR. It is a process which physically removes circulating antibodies along with many other circulating proteins; generally 7 – 14 plasmapheresis sessions at varying intervals (from daily to every 3 – 4 days) are required for substantial removal of antibodies. Each session generally lasts 2 – 4 hours. It is an invasive procedure in which a large-bore central venous catheter must be placed and extracorporeal separation of blood occurs via either centrifuge or filtration, antibodies are removed and discarded, and finally blood is returned to the patient.

6.1.1 Plasmapheresis techniques

Three main techniques can be utilized: therapeutic plasma exchange (TPE), double-filtration plasmapheresis (DFPP), and immunoadsorption plasmapheresis (IAPP). Therapeutic plasma exchange involves separation of red blood cells from plasma, complete removal of all plasma, and finally administration of exogenous fresh-frozen plasma or albumin to replace the plasma removed. In many centers, protocols require repletion with both albumin and fresh-frozen plasma on alternating days to replete coagulation factors removed that are not present in exogenous albumin preparations. Double-filtration plasmapheresis separates plasma from red blood cells in the first step, followed by a second filtration of the plasma that separates large molecules from small molecules and sera, and the small molecules and sera are then infused with the endogenous red blood cells. The final technique, immunoadsorption, is theoretically similar to DFPP, but utilizes an immunochemical reaction in the second step to remove only immunoglobulins. TPE and DFPP are older, more established techniques and relatively inexpensive; the immunoadsorbent membrane utilized with IAPP is quite expensive and removes only circulating immunoglobulins, potentially leaving signaling molecules for AMR such as cytokines in circulation. However, an advantage of IAPP is the avoidance of replacement colloids like albumin and fresh-frozen plasma and the adverse effects that are associated with these products; plasma exchange is the predominant method utilized at most US transplant centers.

6.1.2 Data for plasmapheresis in heart transplantation

Plasmapheresis has been utilized significantly in both desensitization protocols as well as in the treatment of AMR in all solid organ transplants as well as heart transplant recipients. Among sensitized patients awaiting transplantation, the preoperative use of plasmapheresis with intravenous immunoglobulins (IVIg) produced similar intermediate term outcomes of rejection and allograft survival compared to non-sensitized patients (Larson et al., 1999; Leech et al., 2006; Pisani et al., 1999). For the treatment of AMR, plasmapheresis has been utilized as part of a multi-treatment modality with success. In 2006, Wang and colleges reported moderate success with 5 days of daily plasma exchange for 12 symptomatic AMR cases in conjunction with methylprednisolone 1gm/day (Wang et al., 2006). In surveying 6 major cardiac transplant centers, all report plasmapheresis as part of their initial management strategy for AMR. Unfortunately, no studies have compared therapy of AMR with or without plasmapheresis, so the actual contribution to good outcomes is impossible to determine at this point, however it is recommended as one of the first line strategies for treatment of AMR (Kobashigawa et al., 2011).

6.1.3 Considerations with plasmapheresis use

One major consideration with use of plasmapheresis is medication removal; medications that are highly protein-bound with low volumes of distribution will be readily removed by TPE or DFPP, and should be administered after the session is complete. In studies evaluating removal of medications in the setting of overdose, those medications with a Vd less than 0.2 L/kg and greater than 80% protein binding were most likely to be substantially removed (Sketris et al., 1984). Case reports have found minimal removal of calcineurin inhibitors, prednisone, or azathioprine (Balogun et al., 2001; Hale et al., 2000; Stigelman et al., 1984); however one case report of plasma exchange following administration of

basiliximab found significant removal (Okechukwu et al., 2001). Other agents with likely removal by plasma exchange of concern to transplant recipients include rituximab, (Darabi et al., 2006), vancomycin (Foral & Heineman 2001; Osman & Lew 1997; Sirvent & Borras-Blasco 2006), levothyroxine (Binemelis et al., 1987; Liel et al., 2003), and aminoglycoside antibiotics (Kale-Pradhan et al., 1995; Ouellete et al., 1983; Appelgate et al., 1981). Regardless of likelihood of removal by plasma exchange, every effort should be made to administer critical medications following a session to ensure adequate exposure.

6.2 Total Intravenous Immunoglobulins (IVIg)

Total intravenous immunoglobulins are likely the most often utilized product for desensitization and treatment of AMR across solid organ transplantation including cardiac transplant recipients. IVIg was originally developed in the 1980's as a replacement product for those patients with immune deficiencies, but anti-inflammatory and immunomodulatory effects were quickly understood. The actual mechanisms of immunomodulatory effects of IVIg have been widely postulated, however consensus has not been reached on exactly how IVIg may prevent or halt AMR; rather a "multi-hit" model has been proposed.

6.2.1 Mechanisms of action

Proposed mechanisms specific to the humoral immune system include increased apoptosis of B cells, neutralization of B cell survival signaling molecules, regulation of antibody production, and decreased B cell proliferation (Nimmerjahn & Ravetch 2008; Brandt & Gershwin 2006; Jordan & Toyoda 2009; Durandy et al., 2009). A multitude of mechanisms that affect other aspects of the immune system including T cells, neutrophils, NK cells, and so forth have been proposed as well. Dose-dependent effects have also been proposed, with low-doses (500 mg/kg) IVIg reported to have more pro-inflammatory effects whereas high-doses (1 – 2 gm/kg) exhibit anti-inflammatory and more immunoregulatory effects.

6.2.2 Data for IVIg in heart transplantation

While IVIg is one of the most common agents used in AMR of the cardiac allograft, data are surprisingly limited for its use. Four main studies have evaluated the utility of IVIg for desensitization prior to cardiac transplantation. Similar to the data seen with other therapy modalities, IVIg has been used in combination with either plasmapheresis, rituximab, or high dose corticosteroids. No studies have shown that IVIg alone can reduce antibody burden pre-transplant, and outcomes following successful transplant are conflicting (Shehata et al., 2010; Nussinovitch & Shoenfeld 2008; Pisani et al., 1999; John et al., 1999). In the treatment of AMR, data are more robust; although again no studies have evaluated the utility of IVIg alone.

6.2.3 Considerations with IVIg Use

One unique consideration for the use of IVIg is product selection. Currently, seven FDA approved products are available for use. The major considerations for product selection are stabilizing agents/sugar contents, the IgA content, anti-A and anti-B isohemagglutinin concentrations, and availability of the product. One of the known adverse effects of high-dose IVIg therapy is acute kidney injury (AKI). The likely contributors to AKI are sheer

protein load, osmolarity, and the excipient content of the product. Higher doses and therefore higher protein loads are associated with increased rates of adverse effects, as are products with sucrose as an excipient and those with high osmolarities. Efforts to decrease these effects include preparations that are liquid products with iso-osmolarity; unfortunately, the techniques utilized to achieve a more tolerable product have increased the titers of anti-isohemagluttinins. These products have therefore been associated with increased rates of clinically significant hemolysis. Clinical monitoring of patients with A, B, or AB blood types with prolonged duration or high doses of IVIg therapy is recommended (Jordan et al., 2011). Additionally, the IgA concentration varies across the available products. IgA depleted products must be used for patients with IgA deficiencies or antibodies to IgA, as IgA rich products may increase the risk of serious adverse reactions such as anaphylaxis. Finally, since IVIg is a pooled human product, it is limited by availability of donors and product demand. Subsequently, intermittent product shortage has been commonplace.

Cytomegalovirus hyperimmune globulin (CMVIg, Cytogam), has a unique historical perspective in solid organ transplantation. While CMVIg has been studied as specific prophlyaxis for cytomegalovirus (CMV) disease, at one point in time, supply of IVIg was greatly limited. During this IVIg shortage, centers utilized the one IVIg product that was available: CMVIg. Subsequently, many centers established efficacy for CMVIg in desensitization and the treatment of AMR, and continue to use this specific product, despite no known or theoretical advantages over total IVIg, purely for immunoregulatory effects.

6.3 Total Lymphoid Irradiation

Total lymphoid irradiation (TLI) is low dose, targeted radiotherapy directed at major concentrations of lymph nodes across the body as well as the spleen. This traditionally has involved radiation exposure to 3 major areas across the body: the first being the chest above the diaphragm and below the base of the skull, a peri-aortic and splenic field, and finally a pelvic field to encompass all pelvic and inguinal lymph nodes. By irradiating these areas, theoretically a long-term decrease in antibody production would occur and mitigate the contribution of the B-cell immune system to rejection and any subsequent rejection episodes (Salter et al., 1995).

TLI has been utilized in the treatment of rejection among solid organ transplant recipients for more than 20 years. The majority of data supporting TLI in the treatment of cardiac AMR comes from a single center, which reported TLI therapy for recurrent rejection or rejection with hemodynamic compromise in 73 patients between 1990 and 1996. TLI was delivered as 80 cGy twice weekly for a total of 5 weeks, and was associated with a significant reduction in risk of rejection. This benefit was seen for approximately 4 years. No changes in long-term outcomes such as CAV or survival were seen. Unfortunately, myelodysplasia (MDS) or acute myelogenous leukemia (AML) did develop in 7 patients. This reported risk of leukemias has significantly limited the utility of TLI, and has lead to consensus recommendations to avoid its use in treatment of AMR (Kobashigawa et al., 2011).

6.4 Photopheresis

Extracoroporeal photopheresis (ECP) is a procedure similar to plasmapheresis where approximately 700mL of blood is removed and separated. The plasma is then incubated

with a photosensitizing agent, 8-methoxypsoralen, under UV-A radiation. This process covalently binds the photosensitizing agent to DNA and cell surface. These irradiated lymphocytes, monocytes, and dendritic cells decrease down-stream signaling for immune activation. Although the exact mechanisms are unknown, it is not believed that photopheresis has a majority of its benefit on B cells, but rather T cells; specifically T regulatory cells are affected. Each session is performed for approximately 1 – 3 hours, and sessions vary from daily to weekly.

6.4.1 Data for photopheresis in heart transplantation

ECP gained popularity in the heart transplant population for prevention of rejection when results from a multi-center randomized control trial evaluating standard immunosuppression with or without ECP found a 2 fold decrease in overall incidence of acute rejection. In this study, ECP was performed 2 days in a row every week for 4 weeks, then every 2 weeks for 2 months, and finally every month for 3 months (Barr et al., 1998). Unfortunately, there was no decrease in time to rejection or incidence of rejection with hemodynamic compromise. No studies to date have been performed to specifically evaluate the role of ECP in prophylaxis or treatment of AMR, although the Barr study did demonstrate a significant reduction in HLA antibody levels.

6.4.2 Considerations with photopheresis use

One consideration to be taken regarding photopheresis is the requirement for a central venous catheter; however, it is reported to be better tolerated than plasmapheresis, has minimal side effects, and has been shown to be safe in case reports of heart transplant recipients up to 3 years (Marques & Schwartz 2011). Reported adverse effects include malaise, low-grade fever, and gastrointestinal upset. Risks associated with invasive central lines, such as infection and thrombosis, are present as well.

6.5 Cyclophosphamide

Cyclophosphamide is an alkylating nitrogen mustard chemotherapeutic agent that was approved by the FDA in 1959 and has been used to treat a variety of neoplastic and autoimmune conditions. Its cytotoxic effects arise from intra- and interstrand DNA cross-linking, leading to DNA inactivation and cell death; immunosuppression arises from selective suppression of B-lymphocyte activity as well as a general lymphopenia of both B cells and T cells. Theoretically, cyclophosphamide provides the advantage of avoidance of rebound B cell proliferation with more short-term strategies like plasmapheresis and IVIg that do not alter B cell production.

6.5.1 Data for cyclophosphamide use in heart transplantation

Several small case series have reported success with the use of cyclophosphamide and IVIg for desensitization prior to cardiac transplantation. The first reported a PRA decrease from 64% to 14% with the combination of IVIg and cyclophosphamide, leading to successful transplantation (De Marco et al., 1997). More recently, Itescu and colleagues reported their experience with 16 sensitized LVAS patients awaiting cardiac transplantation, who received monthly treatment with cyclophosphamide 0.5 – 1 g/m2; all 23 patients were successfully

transplanted. No differences in adverse events compared to other sensitized patients occurred in this single center experience (Itescu et al., 2002).

6.5.2 Considerations with cyclophosphamide use

The clinical utility of cyclophosphamide, however, has been limited by the potential adverse effect profile and the increased utilization of B cell specific monoclonal antibodies. Primary toxicities associated with cyclophosphamide use include hematologic toxicities, hemorrhagic cystitis, and infertility/teratogenicity. Additionally, there is a risk of secondary malignancies such as leukemia, lymphoma, and skin cancers.

6.6 Anti-Thymocyte Globulin (ATG and rATG)

Anti-thymocyte globulins are polyclonal antibody products derived from either equine (ATG or Atgam®) or leporine (rATG or Thymoglobulin®) sources. Horses or rabbits are inoculated with human lymphocytes; serum is then removed from the animals and the antibodies against human CD3-bearing T cells are collected and purified. These products have been used generously throughout solid organ transplantation as both induction agents at the time of transplant as well as agents for the treatment of rejection, with a shift in clinical practice to almost exclusive use of the rabbit preparation based on studies showing better tolerability and potentially improved outcomes.

6.6.1 Data for Anti-Thymocyte Globulin in heart transplantation

While ATG has been utilized extensively in heart transplant recipients, no studies have evaluated their use in AMR; rather decreased overall rejection rates have been reported (Renlund et al., 1989; Ladowski et al., 1993; Macdonald et al., 1993; Schnetzler et al., 2002; De Santo et al., 2004). In both induction and rejection protocols, daily infusions are given for as little as 3 to at most 14 days. Additionally, no desensitization protocols have reported to utilize ATG. Given the lack of data, however, there is a theoretical benefit for the use of these polyclonal antibodies in the treatment of AMR or mixed ACR/AMR. While the majority of antibodies derived are against T-cells, many other cells are potentially affected, including B-cells, HLA heavy chains, plasma cells, platelets, white blood cells, red blood cells, and so on. In fact, the dose-limiting side effects are typically thrombocytopenia and leucopenia.

6.6.2 Considerations with Anti-Thymocyte Globulin use

In addition to pancytopenia, both products are associated with significant infusion reactions, cytokine release syndrome, and rarely anaphylactic reactions. These infusion reactions are significantly more pronounced in patients that have repeated previous exposure to either source animal and depending on the patient's history may warrant avoidance of a particular product. Pre-medication prior to the infusion with steroids, acetaminophen, and diphenhydramine are recommended. More serious adverse effects include serum sickness, which presents as a delayed reaction with flu-like symptoms, lymphadenopathy, blurred vision, and rash. At the cellular level, increased levels of IgG, IgM, IgE, and acute phase reactants are seen with serum sickness reactions. The effects on humoral activation of serum sickness are as yet unknown.

6.7 Rituximab

Rituximab is a chimeric humanized high-affinity monoclonal antibody targeted against the CD-20 receptor, which is borne by both B-cell progenitors and mature B-lymphocytes. B-cell depletion with rituximab occurs via three mechanisms: complement-dependent cytotoxicity, antibody-dependent cytotoxicity, and induction of apoptosis (Smith, 2003). Problematically, rituximab is only efficacious against circulating B-cells and progenitors and does not penetrate the spleen; thus, should be most efficacious against *de novo* antibodies which have been quickly recognized and intervened upon. In contrast, long-standing antibodies, such as those present in pre-sensitized candidates, should be relatively resistant to rituximab as the plasma cell from which they are generated is no longer circulating in the central compartment.

6.7.1 Data for rituximab in heart transplantation

Rituximab has been used across the spectrum of solid organ transplant recipients both for desensitization and AMR, albeit with minimal quality evidence of efficacy. The data describing rituximab use in cardiac transplantation is case report and case series in nature. Problematically, such case reports describe the addition of rituximab to traditional therapies such as TPE, IVIg, cyclophosphamide, and ATG complicating the evaluation of the efficacy of rituximab alone. Regimens used are also heterogeneous, ranging from 375 mg/m^2 to 1 gram fixed dose for 1 to 4 doses once weekly to twice monthly (Kcazmarek et al., 2007). Given this, it is nearly impossible to quantitate the potential benefit or role of rituximab for AMR in the cardiac allograft. Rigorous evaluation in a systematic fashion of rituximab therapy is desperately needed.

6.7.2 Considerations with rituximab use

Rituximab is generally well tolerated; however, some severe adverse effects have been reported. Leucopenia is relatively common after administration owing in large measure to suppression of B-lymphocyte differentiation. Hemodynamic effects, particularly transient hypotension, have been reported. Anaphylaxis is possible, but incredibly rare. Initially, small rituximab test-doses were required to evaluate anaphylaxis risk; however, these have been relegated due to poor positive predictive value. Since rituximab depletes CD-20 bearing B-cell progenitors, it may be associated with an increased risk of recurrent infections; particularly those of a viral nature that rely on innate memory B-cells for protection such as CMV. The data regarding infectious risk in transplant recipients is qualitatively poor. In a case series of 8 cardiac transplant recipients, 3 patients developed infections (Garrett et al., 2005).

7. Novel and experimental therapies

Recently, alternatives to the previously discussed and more traditional therapies have emerged. These agents target plasma cells both in circulation and in the spleen, B-cell activating factors (BAFF), and the complement cascade. It should be noted that, at present, data supporting the use of these agents in cardiac transplantation is minimal. However, as is the case with many of the traditional therapies, these agents have both been used or are being actively studied in highly sensitized renal transplant recipients (RTR).

7.1 Plasma cell targeted agents

Bortezomib, a 26S proteasomal inhibitor, results in the selective apoptosis of highly active plasma cells both in circulation and in the spleen. The proteasome is responsible for proteolysis of misfolded, damaged, or unneeded proteins within the cell; the inhibition of which leads to cell-cycle arrest and apoptosis (Everly et al., 2008). Only one case report has been published that describes the efficacy of bortezomib for refractory AMR in a cardiac transplant recipient (Eckman et al., 2009). Likewise, only one case series of 7 patients has been reported that describes the successful use of bortezomib for desensitization in candidates refractory to traditional therapies (Patel et al., 2010). The use of bortezomib is likely to expand in cardiac transplantation; particularly as longer-term safety data emerges.

Belimumab is targeted at the BAFF B-lymphocyte stimulator (BLyS) and proliferation-inducing ligand (APRIL). Targeting these tumor necrosis factor-family ligands ultimately results in the apoptosis of mature B-lymphocytes via suppression of BLyS and APRIL-mediated antiapopototic effects during B-cell differentiation and maturation (Bossen & Schneider, 2006). This agent has shown promise in the treatment of systemic lupus erythematosis and is being actively trialed for desensitization in renal transplant candidates.

7.2 Complement cascade targeted agents

Eculizumab, a humaninzed anti-C5 monoclonal antibody, depresses the formation of the membrane attack complex (C5b9) in response to circulating DSA. There are no such reports describing the use of this agent in cardiac transplant recipients; however, 5 anecdotal cases are known to us. In all cases, eculizumab was paired either with traditional means of antibody depletion (TPE and IVIg), bortezomib, or both. A larger experience is known in renal transplantation. Eculizumab was given as prophylaxis of AMR in 10 highly sensitized RTR who received pre-transplant desensitization with TPE and IVIg. Half of the patients developed high DSA titers post-transplant, but no incidences of AMR were recorded in 12 months of follow-up (Stegall, M., et al. 2009). Additionally, eculizumab was given as sole treatment to 16 highly sensitized RTR, and compared to a similarly sensitized cohort of patients who did not receive therapy. The incidence of AMR in the first post-transplant month was substantially less in the eculizumab patients (6.25% vs. 40%); however, 6 patients developed chronic AMR (Cornell et al., 2010). This suggests that eculizumab may be best-utilized when paired with antibody-depletion measures, such as TPE. It should be noted that combining eculizumab with rituximab will render rituximab ineffective as its predominant pro-apoptotic mechanism is complement-mediated.

Cinryze, human C1-esterase inhibitor, is collected from human donors in whole blood, purified via pasteurization and nanofiltration, and is currently approved for hereditary angioedema. The potential role of supraphysiologic concentrations of human C1-esterase inhibitor in the downregulation of the complement cascade notwithstanding, no reports currently exist that describe the role of Cinryze in transplant recipients. However, Cinryze is being actively studied in renal transplantation and may provide an alternative for C5b9 inhibition.

8. Conclusions

Antibody-mediated rejection is a poorly characterized, understood, and studied disease process in solid organ transplantation today. There are active efforts across all organ

systems to better-define the pathogenesis of DSA in the development of AMR, the long-term consequences of its development, and the best methodology to screen and manage patients who are pre-sensitized. Relative to AMR in the heart transplant community, until the newly derived definition of AMR is uniformly adopted and utilized, it is unlikely that quality retrospective research will be completed on a large scale. However, there are ongoing efforts at high-volume centers to study the novel and experimental therapies in an effort to enhance transplantability of highly-sensitized candidates and manage AMR when it subsequently develops. Traditional measures, such as TPE and IVIg, remain the cornerstone of AMR therapy. TLI, photophoresis, and cyclophosphamide have fallen out of favor due to adverse effects or lack of efficacy. The role of rituximab is unclear as it has never been subjected to rigorous clinical evaluation and has mechanistic disadvantages relative to its novel alternative, bortezomib. Finally, the horizon is bright for the use of complement-antagonists to reshape how we manage pre-sensitized patients in an effort to enhance their candidacy for transplantation.

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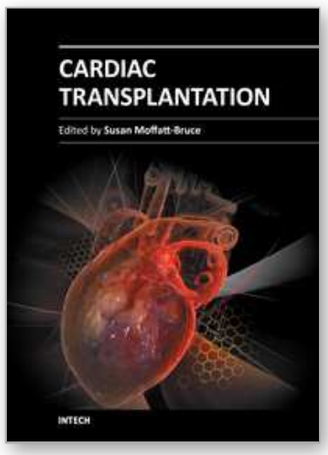

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We are truly in an era of change not only in terms of technology but in the type of patient we are caring for. That is why I feel this book is exciting in that it presents the team approach to the transplant patient. I am confident that the pioneers of cardiac transplantation would be pleased with our response to challenges in healthcare today and be pleased with the final product.

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51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Phone: +86-21-62489820
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