Contemporary review of heart transplant immunology and immunosuppressive therapy

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Abstract

Background: Survival after heart transplantation (HT) has improved considerably since the first HT was performed in 1967 in Cape Town, South Africa. Understanding immunology behind organ rejection has paved the way for advances in the assessment of pre-transplant compatibility, development of newer and more specific immunosuppressive drugs, and management of rejection.

Objectives: Unlike medical therapy for heart failure, transplant protocols vary considerably between different centers. These variations in protocols generally reflect unique population characteristics and the availability of resources. This review article aims to provide a consolidated update on contemporary cardiac transplant medicine. We also aim to highlight local practice and its difference from our international counterparts.

Methods: A literature search was performed on Pubmed and Cochrane Central Register of Controlled Trials to identify trials and review articles that discussed heart transplant immunology and protocols. The International Society for Heart and Lung Transplant (ISHLT) guidelines were also reviewed. We focused on risk factors, prevention strategies, and treatment of cardiac rejection.

Results: A total of 48 articles were selected to provide a comprehensive overview of the contemporary practice of cardiac transplant immunosuppressive therapy. Comparisons were made with local data and practice protocols to highlight key differences.

Conclusion: Heart transplant covers a small subset of cardiac patients and much of the evidence is derived from empirical observations and retrospective analysis. This accounts for the heterogeneity in care and treatment protocols. More studies are needed to select best practices from around the world to further improve outcomes.

Keywords
Immunosuppression, cardiac transplant, graft rejection

Survival after heart transplantation (HT) has improved considerably since the first HT was performed in 1967 in Cape Town, South Africa.1 The median survival for HT recipients between 1982 and June 2016 was 11 years for adult recipients, while those performed before the 1980s were less than 2 years.2 Besides better surgical techniques and perioperative care, advances in immunosuppressive treatment is a key element in the improvement in survival.

Our understanding of the immunology behind organ rejection has allowed the development of specific inhibitors to prevent injury to the cardiac allograft. The immune system plays a vital role in protecting a person from microbial organisms that cause infection and aberrant cells that develop into cancer.3 Thus, the major challenge for transplant

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immunosuppression to prevent rejection and at the same time reduce complications of an attenuated immune response.

There are limited prospective randomized controlled trials that back the guidance on pre and post-transplant immunosuppression. Most transplant centers have different protocols for workup for transplant compatibility and subsequent immunosuppressive regimens.

The purpose of this review is to highlight key developments in heart transplant immunology, pre-transplant immune compatibility workup, immunosuppressive therapy, and rejection management. We also aim to highlight local practices in our center in Singapore.

**Immunology concepts (the two major subsystems of the normal immune system)**

Understanding the normal immune response towards foreign antigens allows us to comprehend the immunological basis behind rejection, the concepts of rejection prevention, and the mechanisms of action of immunosuppressive drugs.

The immune system’s response to a foreign antigen has two arms. The innate and adaptive immune systems. Both arms play an equally important response in protecting the host with some key fundamental differences.

The innate immune system comprises physical barriers such as skin and endothelial linings, chemical barriers such as gastric acid, and cellular barriers such as macrophages, dendritic cells, and monocytes. For transplant rejection, we will focus primarily on the cellular barriers. These cellular elements provide a rapid response to the inciting antigen within minutes to hours without prior exposure. This response happens each time without any immunological memory. Hence this aspect of the immune system is referred to as a rapid non-antigen-specific response.

This response is made possible through the recognition of damage-associated molecular patterns (DAMPs). These patterns are encoded in our genetics and are expressed by infected or damaged cells. These DAMPs serve as ligands for pattern recognition receptors (PRRs) that are found on cells comprising the innate immune system. This brings about the recruitment of immune cells to the site of infection or damage through the release of cytokines. Key cytokines that play this role include tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6). The final result is the destruction of the antigen.

The adaptive immune system provides an antigen-specific response through two major cell types; the T and B lymphocytes. The T-lymphocytes produce the cell-mediated immune response and B-lymphocytes through transformation into antibody generating factories called plasma cells produce the humoral immune response. The hallmark of the adaptive immune system is the ‘immunological memory’ which refers to its ability to retain specific memory of a target antigen. This allows it to launch a more intense and rapid immune reaction when encountering the same antigen.

During the first encounter with a pathogenic antigen, specialized cells from the innate immune system called the antigen-presenting cells (APCs) process these antigens and couple them with human leukocyte antigens. Human leukocyte antigens (HLA) are proteins that are expressed through transcription of genetic sequences called major histocompatibility complex (MHC). The two main sequences of MHC that are of importance are referred to as class I (A, B, C) and class II (DR, DQ, DP) MHC sequences. Class I HLA antigens are expressed in most nucleated cells. When these are presented on their cell surface with pathogenic antigens, CD-8 cytotoxic T lymphocytes will proceed to destroy the cell. Class II antigens, on the other hand, are expressed only on APCs, and these when embedded with pathogenic antigens serve as ligands to CD-4 helper T cells which activate the B lymphocytes and CD-8 T cells. During the first encounter, this response is slow, taking days to have full effect. But during a second exposure due to the presence of memory lymphocytes, the response is much quicker.

The innate and adaptive immune systems work synergistically in the clearance of the foreign antigen.

**Transplant rejection**

With the above understanding of immunology, we can discuss the risk factors and pathophysiology behind the rejection. Transplant rejection is sub-divided into hyperacute and acute rejection.

**Risk factors**

Certain patient populations are at a higher risk for acute rejection than others. This includes younger patients, black recipients, a higher number of HLA mismatches, those bridged with left ventricular assist device (LVAD), non-compliance to immunosuppression, and female donors or recipients. The female gender is associated with an increased risk of rejection due to pregnancy. Pregnancy offers a unique immunological environment, where the fetus harbors both paternal and maternal HLA whilst in the uterus. Maternal exposure to fetal elements and blood results in the production of anti-HLA antibodies against the paternal HLA antigens. The pre-existing anti-HLA antibodies naturally will result in an elevated rejection risk. Another group of patients that have increased sensitization is those with LVADs. Due to the long waiting time, many stage D heart failure patients are bridged to transplant with mechanical assist devices. It is postulated that because of increased transfusion of blood products and immunogenic components of the device, there is an increase in anti-HLA antibodies produced. Despite this increase in sensitization, one large retrospective analysis of 7686 patients showed that it does not result in a significantly increased risk of rejection or mortality. Our center’s data of 87 patients on the transplant list with LVAD therapy suggest that only a minority of patients (12/87) had an increased PRA post-implantation. Interestingly with time, the PRA levels reduced to baseline levels.

**Hyperacute rejection**

Hyperacute rejection is the immediate graft rejection that occurs due to pre-existing anti-graft antibodies.
Before transplantation, donor-recipient compatibility is vital in predicting the risk of rejection. Any two individuals who are not identical twins will express different HLA antigens. These differing HLA molecules themselves can act as non-self antigens that can activate an immune response. If the recipient had exposure to HLA antigens of the donor during his or her lifetime, this will result in pre-formed antibodies against the donor’s heart. This is referred to as sensitization. Preformed, potentially cytotoxic, antibodies play a major role in the catastrophic hyper-acute transplant rejection.\(^{21}\)

International Society of Heart and Lung Transplant (ISHLT) recommends that screening panel reactive antibody (PRA) be performed in all heart transplant candidates.\(^{22}\) The panel aims to detect the presence of circulating antibodies in a random panel of donor lymphocytes. A reaction of $>10\%$ reflects an increased risk for rejection.\(^{23}\)

In patients with elevated PRA, some centers will proceed to identify the anti-HLA antibody. This is performed using a solid-phase assay where beads coated with HLA agents are incubated with the recipient’s serum. If HLA-specific antibodies are present, they will bind to the specific bead that harbors the HLA antigen. Subsequently, fluorochrome-labeled antibody is added to the bound recipient antibody. A laser detector is then used to detect the bound antibodies.\(^{24}\) Identifying an antibody is not sufficient as some of these bound antibodies have weak ineffective interactions that do not result in a significant immune response. To identify the strength of interaction, the mean fluorescent intensity (MFI) can be calculated.\(^{25,26}\) An MFI of $>10,000$ is strongly predictive of cytotoxic response.

Another novel method of identifying cytotoxic anti-HLA antibodies is through the use of the C1q assay.\(^{27}\) C1q is part of the complement cascade which is responsible for the formation of the membrane attack complex which results in cell lysis. Hence, identification of C1q positive anti-HLA antibody can predict the importance of the antibody present.

Kittleson and Kobashigawa\(^ {28}\) suggested that, in patients with identified strong binding anti-HLA antibodies, to proceed to assess the calculated PRA (cPRA). To do this the transplant center has to identify the frequency of the identified anti-HLA antibodies in a reference population. If the frequency of these identified antibodies is high then the cPRA value will be elevated. This would mean the chances of finding a compatible match is lower. The author suggests that patients with cPRA of more than $50\%$ undergo desensitization.

The above pre-transplant workup is performed for patients waiting on the transplant list. It allows transplant centers to predict the success of transplant for a given patient and allows for desensitization in high-risk patients. However, during acute transplant activation where a procured donor heart is available. The selection of a compatible recipient has to occur more quickly. In previously identified low-risk patients a virtual crossmatch can be performed by reviewing the stored recipient anti-HLA antibody profile with remote HLA typing of a deceased donor.\(^ {29,30}\) For all other patients, a prospective crossmatch using complement-dependent cytotoxicity (CDC) crossmatch must be performed. A CDC crossmatch is performed using a fresh sample of donor lymphocytes and mixing it with recipient serum in the presence of complement.\(^ {31}\) This allows one to detect preformed cytotoxic antibodies in a test tube. The presence of such a reaction will be considered a contraindication for transplant.

Locally, we perform PRA on our potential transplant candidates every 6 months. Unlike our western counterparts, the prevalence of clinically significant anti-HLA antibodies is very low. For these reasons, we have not had to perform desensitization in Singapore. With careful PRA screening and donor-recipient crossmatching, hyperacute rejection has never occurred in the 90 transplant cases in our center.\(^ {30}\)

**Acute rejection**

Acute transplant rejection is subdivided into the more common acute cellular rejection (ACR) and the less common antibody-mediated rejection (AMR). It most commonly occurs in the first 3–6 months after transplantation.

There are two mechanisms underpinning ACR; the direct and indirect pathways. The direct pathway starts with the donor’s APCs migrating from the graft to the lymph node. While in the indirect pathway the recipient’s APCs process donor antigens and present donor HLA fragments to the T-cells.\(^ {32}\) Once the T-cell gets activated it produces cytokines such as interleukin-2 (IL-2) via a calcineurin-dependent pathway. At the same time, IL-2 receptors are released to the surface of T-cells. IL-2 binds to IL-2 receptors which result in the clonal proliferation of T-cells via two primary pathways, the proliferation via the mammalian target of rapamycin and cyclin/cyclin-dependent kinase pathway.\(^ {33}\) This will bring about cytolysis of the graft.

Antibody-mediated rejection is a result of antibodies produced against the vascular elements of the donor’s heart.\(^ {34,35}\) Once these antibodies bind to the HLA receptors on the endothelial lining of the vessels they activate the complement cascade that brings about damage to the vessels. AMR that occurs early within the first month is either due to the development of donor-specific antigens or pre-existing antibodies.\(^ {31}\) It is associated with greater graft dysfunction and hemodynamic compromise. The ISHLT published in 2013 the grading of AMR based on histology and immunopathological findings from biopsy specimens.\(^ {36}\)

While it is easy to consider ACR and AMR in isolation, it has been reported that up $24\%$ of rejection cases have both forms of rejection occurring simultaneously.\(^ {37}\)

Our center has seen eight rejection cases in the 90 transplanted patients with seven cases of ACR and one case of AMR.\(^ {20}\)

**Prevention and treatment of rejection**

**Induction therapy**

Based on the immunological concepts discussed above, rejection risk is highest in the immediate and early post-transplant period. An ischemic injury that occurs before graft reperfusion will result in the upregulation of DAMPs and the innate immune system.\(^ {7,8}\) This activation will subsequently result in the activation of the adaptive immune system.\(^ {9}\) Induction therapy refers to a state of greater immunosuppression to reduce the risk and development of graft rejection. A secondary benefit is the delayed initiation of
traditional immunosuppressive drugs that have a nephrotoxic potential. Of note, only 40% – 50% of heart transplant centers around the world use the induction strategy. In a Cochrane review of 22 randomized controlled trials looking at induction strategies, five trials with a combined total of 606 subjects compared induction versus no induction. Major outcome measures such as mortality, infection, allograft vasculopathy, and post-transplant lymphoproliferative disorders (PTLD) showed no difference. The only positive finding was a reduction in acute rejection in those given IL-2 receptor antagonists.

There are three induction strategies employed around the world: IL-2 receptor antagonist, polyclonal anti-thymocyte antibody, and alemtuzumab. These agents are generally coupled with methylprednisolone.

IL-2 receptor antagonists are monoclonal antibodies that bind to IL-2 receptors (also known as CD25 antigen) found on T-cells which prevents the activation and proliferation of the lymphocyte. The most commonly used IL-2 receptor antagonist is recombinant monoclonal antibody basiliximab. Due to its monoclonal nature, it provides safer and more predictable therapeutic immunosuppressive response than that of the polyclonal antibodies. Its relative ease in administration makes it a popular agent. 20 mg of basiliximab is administered before graft reperfusion in the operative theatre and a further 20 mg is administered on day four post-transplant.

Polyclonal anti-thymocyte antibody is a polyclonal antibody derived from either rabbits or horses. They are produced by immunizing these animals with human thymocytes and harvesting the anti-thymocyte immunoglobulin G induced. It depletes T-cells through complement-mediated lysis. Rabbit-derived antibody is considered superior to horse-derived antibody. The main concern with these agents is the risk of serum sickness and cytopenia. The administration of the drug is also difficult, requiring weight-based dosing and pre-medication with steroids, antihistamines, and antipyretics. White cell and platelet counts need to be monitored closely and the dose adjusted or discontinued during the 2–5 days of therapy. A less common but upcoming new agent is Alemtuzumab. It is a humanized rat monoclonal antibody that targets the CD52 antigen expressed on both T and B cells. The main advantage of this agent is its ability to produced prolonged immunosuppression that prevents ACR in the first 12 months with lower doses of maintenance immunosuppressive agents. No difference in hematological or infectious complications has been observed in comparison with standard protocols despite prolonged lymphopenia. It is usually given as a single dose of 30 mg during surgery. In Singapore, our cardiac transplant protocol adopts routine induction therapy with methylprednisolone and IL-2 receptor monoclonal antibody basiliximab in all our cardiac transplant recipients.

Maintenance

Maintenance therapy refers to chronic immunosuppression therapy to reduce the risk of rejection over the recipient’s lifetime. The immune system plays a vital role in the prevention of infection and cancers, hence marked suppression increases these complications dramatically. Since the introduction of calcineurin inhibitors (CNI) in 1983, many different immunosuppressive agents have come to market. There are two key concepts in maintenance therapy to highlight. The use of a multi-drug approach with little overlapping in function and toxicity. And secondly, to have protocols to avoid over-suppression. This will enhance the benefit of rejection avoidance while minimizing toxicity. The commonly used strategy is the combination of calcineurin inhibitors, cell cycle inhibitors, and steroids. Some centers use proliferation signal inhibitors over calcineurin inhibitors or in combination with them.

There are two types of CNIs in production, cyclosporin (older) and tacrolimus. Calcineurin is responsible for the transcription of IL-2, TNF alpha, GCSF, and interferon-gamma. By inhibiting calcineurin, IL-2 mediated T-cell activation and proliferation is reduced. Tacrolimus has overtaken cyclosporin as the CNI of choice due to its significantly attenuated metabolic derangements such as hypertension and hyperlipidemia. It is also shown to be less nephrotoxic. New-onset diabetes on the other hand is more common with tacrolimus. In terms of efficacy, the incidence of biopsy-proven moderate to severe ACR is less with tacrolimus.

Several major CNI related toxicities need to be considered. Patients will need regular monitoring of their electrolytes and renal function. Hypokalaemia and hypomagnesemia are common and can result in life-threatening arrhythmias. Skin cancer risk is also increased by up to 200 times and early detection is vital in preventing metastatic disease. Cyclosporin has been shown to cause gingival hyperplasia and hirsutism which can affect body image and compliance.

Drug-drug interactions are common with CNIs due to their metabolism through the cytochrome P450 system. Drugs with lesser known but potentially important interactions include laxatives, narcotics, and antacids. Laxatives through increased intestinal transit can reduce the absorption time of the drugs resulting in lower blood levels. In contrast, narcotics slow intestinal transit and increase drug levels. Antacids have divalent cations that increase absorption. These drugs are commonly prescribed drugs, particularly in the early postoperative period.

Mycophenolate mofetil (MMF) and azathioprine are the two main anti-metabolite therapies available. Their mechanism of action is through the depletion of guanosine nucleotides required by T and B lymphocytes for proliferation. Allowing suppression of both ACR and AMR. MMF has largely replaced azathioprine as the drug of choice as its shown to have significantly less mortality and rejection.

Gastrointestinal side-effects can be a major limiting factor in prescribing MMF. Mycophenolate sodium, a newer formulation that is enteric-coated delays the release of the drug to improve tolerability. Anti-metabolite therapies are not nephrotoxic which make them good agents to combine with CNIs. The main side-effect that requires close monitoring is dose-related leukopenia. Patients admitted with infections will require dose attenuations or cessation of the drug to reduce worsening sepsis.
Proliferation signal inhibitors bind to FK binding protein similar to tacrolimus but they inhibit the mammalian target of rapamycin (mTOR). mTor carries out the downstream actions of IL-2 in the immune response. The two drugs in this class are sirolimus and everolimus. The benefit of mTOR inhibitors compared to CNIs is the lower rates of nephrotoxicity and allograft vasculopathy. A large multicenter randomized trial showed that everolimus at doses greater than 3 mg/day is associated with greater mortality than MMF. Compared to MMF, there is also higher reported discontinuation due to adverse reactions. The United States Food and Drug Administration (FDA) has issued a black box warning for everolimus use. Surgical wound healing is also reduced with these agents. For these reasons, CNI is still preferentially used in most centers unless patients develop complications of CNI therapy.

Pneumonitis and lymphedema in patients taking mTOR inhibitors are two unique complications that transplant physicians must be aware of. Patients with pneumonitis generally present with dyspnea and pleural effusion. Discontinuation of the drug results in resolution in 2–4 weeks and rechallenge is not recommended. In severe cases, a patient might require a period of steroid therapy. The exact pathophysiology of mTOR inhibitor-related lymphedema is not understood but it is thought to result from interference of lymph drainage. Both these conditions are commonly mistaken for fluid overload and rejection and treated inappropriately.

Steroids are the cornerstone of therapy in the early stage of transplant 6–12 months. Unlike the agents mentioned above, a steroid is a nonspecific agent that blocks multiple inflammatory pathways. It can block the innate immune system post graft reperfusion and by doing so prevents activation of the adaptive immune system via APCs. Steroids are weaned actively and stopped within the immediate post-induction period due to increased mortality from infections. Surgical wound healing is also reduced with these agents. For these reasons, CNI is still preferentially used in most centers unless patients develop complications of CNI therapy.

Antibody-mediated rejection is mediated by B-cell activation, which removes only immunoglobulins. The latter is considered to be ACR. Immunomodulation can also be performed with antibodies produced by B-lymphocytes. B-lymphocyte reduction can also be performed using Rituximab, bortezomib, or polyclonal antithymocyte antibodies. The duration of therapy is usually 5–14 days.

Cardiac allograft rejection surveillance

Prevention of rejection is key but when this fails it is important to identify rejection early. The symptoms in the early stages can be non-specific. These include fatigue, nausea, and mild shortness of breath. All of which are not unreasonable experiences in the weeks to months after major surgery. It is for this reason, transplant centers have strict surveillance cardiac biopsy protocols in the first 6–12 months. In our center, a patient gets weekly biopsies for the first month followed by fortnightly biopsies in the second month, and finally monthly biopsies for the remainder of the year.

There is evolving technology using noninvasive and highly sensitive biomarkers in the detection of cardiac allograft rejection, such as gene expression profiles (GEPs), Allomap and donor-derived cell-free DNAs (dd-cfDNA). Biomarkers-based rejection surveillance strategies have been shown to be noninferior to endomyocardial biopsy surveillance in preventing serious clinical events in numerous trials. However, GEPs and dd-cfDNA are not available in Singapore.

Treatment of acute rejection

There are many reasons for rejections and these can be divided into preventable and unpreventable. Preventable reasons include ensuring adherence, avoiding drug interactions, and addressing drug intolerances. Unpreventable causes include younger age group, female patients, higher HLA mismatches, prior MCS, and re-transplant.

Acute cellular rejection is more common than AMR. ACR is T-cell mediated and the focus of therapy is on T-cell depletion and interruption of its function. The mainstay of treatment is high-dose steroids which act by inhibiting cytokine synthesis. Patients are given high-dose pulse steroids in the form of methylprednisolone 250–1000 mg/day intravenously for 3 days. Response to therapy is determined from biomarkers such as troponin, hemodynamic data, and electrical stability. In patients who respond, steroids are tapered and weaned according to clinical response. Those who do not show signs of improvement will require the addition of polyclonal antithymocyte antibodies. The duration of therapy is usually 5–14 days.

Antibody-mediated rejection is mediated by B-cell lymphocytes which transform into antibody-producing factories. The treatment of AMR is more complex with many lines of therapy to select from. Like ACR, steroids are given to blunt the cytokine response. The dosing is similar to ACR. Immunomodulation can also be performed with intravenous immunoglobulins (IVIg). This consists of normal IgG obtained from the pools of plasma of several thousand healthy blood donors. They block the Fc-receptor to inhibit antibody function and aid in the clearance of the circulating antibody. High weight-based dosing is required at 1–2 g/kg body weight. Transplant physicians must monitor for anaphylactoid reactions, kidney injury, and thrombosis risk.

Circulating antibodies can also be reduced mechan-ically with plasmapheresis. There are two major techniques used. Plasma exchange where there is non-selective removal of proteins and immunoadsorption plasmapheresis which removes only immunoglobulins. The latter is less efficient in reducing the circulating antibodies and is also not widely available. There are no randomized control trials looking at plasmapheresis in AMR despite its wide use for this purpose.

Besides targeting the antibodies produced by B-lymphocytes. B-lymphocyte reduction can also be performed with rituximab, bortezomib, or polyclonal antithymocyte antibodies. Rituximab is a monoclonal antibody against CD 20 antigen that is found on B-cells. Bortezomib inhibits plasma cells via reversible blockade of 26S proteasome.
Future of cardiac transplant

A cardiac transplant offers the best quality of life for stage D heart failure and advances in immunosuppressive therapy can ensure longer graft survival and quality of life. Newer more selective and less toxic agents will allow us to prevent rejection without blunting vital functions of our immune system.

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K.A. researched the literature and conceived the study. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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