Immunosuppression for pediatric cardiac transplantation in the modern era

Bill A. Pietra*, Mark M. Boucek

Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO, USA

Abstract

With the advent of the T cell activation inhibitors such as cyclosporine, heart transplant success rates for pediatric patients have improved to the point that the initially restricted ages and indications have expanded considerably. Currently the half-life (50% still alive) for children transplanted in the early 1980s is approximately 12–14 years. Decades-long survival seems likely. Components and functions of the immune system are naïve and change during postnatal development. Maturation occurs not only in the first years of life, but well through adolescence and even into adult life. These age-dependent changes within the immune system greatly complicate any attempt to assess immune implications for the use of immunosuppression in children. Since the introduction of cyclosporine, immunosuppression regimens have been virtually unchanged, through the 1990s. Recently, there have been significant new immune pharmacological agents which are now commercially available, or still in investigational stages of development. The new maintenance immunosuppressive drugs are either inhibitors of de novo synthesis of nucleotides (purines or pyrimidines), or are immunophilin-binding drugs that inhibit signal transduction in lymphocytes. The newer inhibitors of de novo nucleotide synthesis include mycophenolate mofetil, mizoribine, brequinar and leflunomide. The immunophilin-binding drugs are cyclosporine, tacrolimus and rapamycin. Antibody preparations such as ATG, ATGAM and OKT3, as well as the newer biological agents, which specifically bind to the IL-2 receptor, basiliximab and daclizumab, are discussed. The potential for biologicals which inhibit co-stimulation are also discussed. There may be dramatic changes in protocols used clinically as a result of these new agents over the next decade. The increasing understanding of the alloimmune response as well as the clinical use of these newer drugs promise even better long-term results. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Immunosuppression; Immunosuppressive agents; Pediatric; Orthotopic heart transplantation; Cardiac allograft

1. Introduction

Heart transplantation for infants, children and adolescents with complex cardiac anatomic lesions [1] have now been successfully performed for up to 15 years [2,3]. With the advent of T cell activation inhibitors such as cyclosporine, heart transplant success rates for pediatric patients have improved to the point that the previously restricted ages and indications have expanded considerably. Currently the half-life (50% still alive) for children transplanted in the early 1990s is approximately 12–14 years [4]. Decades-long survival seems likely. Although there have been significant advances in survival of pediatric and particularly neonatal patients undergoing heart transplantation, most of the improvement in outcome is related to improved surgical as well as pre- and post-operative care of pediatric transplant patients [5]. Immunosuppression since the introduction of cyclosporine has been virtually unchanged. There are new immune pharmacological agents discovered since cy-
closporine, and novel agents are in investigational stages of development. We are now entering an era when there is both a significant improvement in our understanding of the immunobiology of allotransplantation, and the concomitant development of immunosuppressive agents which will allow us to take advantage of this knowledge. The increasing experience and newer drugs promise even better long-term results. Strategies, which are free of steroids and promote the induction of graft-specific tolerance, are reality. This report is an attempt to familiarize the transplant physician with immunosuppressive strategies of today and to provide an orientation to future directions.

2. Immune response to cardiac allografts

Antigens from the cardiac allograft are presented to the recipient immune system by two major pathways. Antigens shed from the graft are endocytosed and processed by host antigen-presenting cells (APC), for example host macrophages (Fig. 1). The antigens are processed to short peptides which are presented in the context of major histocompatibility complex (MHC) receptors on the host APC to the T cell receptor (TCR), on the T cell. This method of presentation is termed indirect. This is the usual form of antigen presentation during an infection. Solid organ transplantation is a unique experiment in nature, and as a result there is a second pathway by which the host immune system is presented with antigens from the graft. It involves the donor APC, which are passenger leukocytes and dendritic cells within the graft, and after transplantation they present directly to the host T cells. Both pathways are operational in human cardiac transplantation, which is more clinically important is unknown.

APC are able to present antigens to lymphocytes via MHC class I or II molecules on their surface. CD8 T cells are restricted to seeing antigens in the context of MHC class I, which is found on the surface of all cells in the body. CD4 T cells on the other hand are generally restricted to presentation by MHC class II molecules. Cells which express MHC class II are considered to be professional or semi-professional APC.

Once the TCR/CD3 complex is engaged with the proper MHC and peptide antigen an intracellular signal is delivered, termed signal 1 (Fig. 1). Co-stimulation, or signal 2, is required for T cell activation. Signal 2 consists of a cascade of receptor ligand interactions. Delivery of signal 1 through the TCR causes induction of CD40L (CD154) on the T cell. CD154 interacts with CD40 which is constitutively

Fig. 1. Overview of the site of action of immunosuppressive agents. AZA, azathioprine; CsA, cyclosporine; RAFT, rapamycin and FK 506 target; MMF, mycophenolate mofetil; RAPA, rapamycin; FK 506, tacrolimus; TCR, T cell receptor.
expressed on APC (macrophage, dendritic cells, B cells, etc.). Ligation of CD40 leads to the up-regulation of B7.1 (CD80) and B7.2 (CD86) co-stimulatory molecules on the APC. CD80/86 also interacts with CD28, constitutively expressed on most naïve T cells. In addition to numerous co-stimulatory signals, CD28 ligation leads to the induction of a second ligand for CD28, CTLA-4. This second ligand for CD28 appears to function as a negative signal to the T cell. Thus, co-stimulation appears to provide positive as well as negative signals.

Although many points still require clarification, it appears the general theme is that the T cell integrates the TCR/CD3 signal, signal 1 and CD28 signal 2 mediated events to lead to T cell activation and function. Signal 1 is considered to be an early event in T cell activation. Neither alone is sufficient for optimal T cell activation. Conditions for optimal induction of tolerance are the subject of intense research. Tolerance is generally thought to be an active process, which also requires both signal 1 and signal 2. There are experiments in animals which establish transfer of tolerance under conditions of antigen Ag presentation signal 1, and weak or blocked signal 2 Fig. 2. These two signals lead to the translocation and/or induction of nuclear binding proteins involved in cytokine gene expression and other T cell activation molecules. TCR-mediated signaling via the PKC pathway is responsible for mobilization of intracellular Ca^{2+} involving the calcium phosphatase, calcineurin. Calcineurin-dependent signaling is the target of cyclosporine (CsA) and tacrolimus. CD28 signaling does not appear to contribute to calcineurin-mediated signals and does not appear to be CsA sensitive. TCR/CD3 and CD28 signaling appears to intersect at the level of the Jun kinases (JNK). Also, CD28 signaling appears to lead to the translocation of nuclear factor kappa B (NFκB) into the nucleus, while TCR-mediated signals do not (Fig. 1). Other drugs such as mycophenolate mofetil (MMF) inhibit the proliferation of lymphocytes, which is considered to be late in the response [6].

The cellular response to a cardiac allograft is still a subject of intense debate and research. The CD4 T cell is required for allograft rejection. A sizable amount of evidence is emerging that the CD4 T cell can function both as an ‘effector’ and a ‘helper’ in the context of allograft rejection. Other significant effector cells are the cytotoxic CD8 T cells, and B cells, which are required for antibodies. Other antigen independent cells such as macrophages, neutrophils and natural killer cells also play a role once the inflammatory process has started. Although how CD4 T cells mediate ‘help’ is not completely understood, there are significant data that at least one way CD4 T cells mediate ‘help’ is through co-stimulatory molecules. CD40L (CD154) is predominately expressed by CD4 T cells, not by CD8 T cells. The CD154/CD40 interaction between CD4 T cells and APC leads to the induction/enhancement of CD80/86 on the APC. Activated APC can interact with CD8 T cells leading to their activation. Conditions that directly activate CD80/86 on APCs, can bypass the requirement for T cell ‘help’ (agonistic antibodies to CD40, infection with viruses, and inflammation) [7]. This late point may be important clinically (CMV infection and its association with acute rejection, for example).

3. Immune response in pediatric patients

Components and functions of the immune system change during postnatal development, not only in the first years of life, but well through adolescence and even into adult life. These age-dependent changes within the immune system greatly complicate any attempt to assess immunologic implications for the use of immunosuppression in children. For example, cord blood T cells of infants appear to be extremely sensitive to inhibition of proliferation by dexamethasone. This high sensitivity of cells to dexamethasone...
can still be observed in the first 2 weeks after birth, but it subsequently decreases. By 1 year of age the adult response pattern has been acquired [8].

Maturation of the adaptive immune system occurs early in development and by 14 weeks gestation the developing fetus has circulating differentiated T cells and B cells capable of responding to antigens. Many components of the immune system function less well in neonates compared with adults, giving rise to the concept of an ‘immunodeficiency of immaturity’. The adaptive significance of these alterations for neonatal and infant solid organ transplantation remains obscure. The most important differences appear to be: (1) reduction in bone marrow reserve of granulocyte precursors; (2) reduction in complement activity; (3) decreased ability to produce antibodies against non-protein antigens [9]; (4) increased percentage of T lymphocytes bearing an antigenically ‘naive’ cell surface phenotype and a correspondingly naïve functional program [10]; and (5) the percentage of natural killer cells is low, and the number and percentage of B cells increase after birth [11].

T cell-dependent responses in neonates have been shown to be naïve in comparison with adults. Although the CD4/CD8 T cell ratio remains stable, there are differences in the TCR Vβ usage in the neonatal immune system [12]. Phenotypically, neonatal CD4+ T cells and CD8+ T cells are more immature than in the adult. The expression of surface molecules such as integrins and adhesion on both CD4+ and CD8+ cells is low and increases post-natally [13]. There is a corresponding rise in serum sICAM-1 during the first month of life in healthy neonates suggesting activation and maturation of the neonatal immune system [14]. Neonates and infants have (10–20%) of CD4+ T cells as ‘naive’ cells (CD45RO+/RA+ CD31−), which are very low in adults. CD4+ ‘memory’ T cells have the phenotype CD45RO+/RA−. The activation and maturation of neonatal CD4+ T cells is particularly dependent upon the strength of CD28-mediated co-signal which dictates not only the cytokine profile released upon primary activation but also the response to IL-12. Lymphocytes have been described by Mosmann as Th1-like if they produce cytokines which are pro-inflammatory, and Th2-like if the cytokine profile promotes antibody production and tolerance [15]. Neonatal T cells are thought to have a Th2 bias.

Neonatal T cells can be driven towards a Th1 phenotype under strong CD28 co-stimulation conditions [IL-2, interferon-gamma (IFN-γ), and tumor necrosis factor-beta (TNF-β)], whereas in the context of low CD28 co-stimulation yields a Th2 response (IL-4 and IL-13) [16].

Healthy children differ markedly compared with adults in their ability to produce cytokines as suggested above (IL-2, IFN-γ, IL-4 and IL-6) [11]. Analysis of cytokine production demonstrates that all cytokines increase gradually and steadily after birth. IFN-γ and IL-10 production is low at birth, whereas IL-2 and IL-4 production are not. Maximal stimulation with mitogen demonstrates impaired cytokine production with markedly lower levels of all four cytokines produced compared with adult levels. When stimulated with antigens, median levels of IL-2 and IL-4 remained lower than adult values; IL-6 production was increased as was IFN-γ, albeit not significantly [17].

The effect of thymectomy, a common practice among neonatal cardiac surgeons at the time of congenital cardiac surgery or transplantation, is unknown. At 12 months post-thymectomy the percent of CD3+ and CD4+ T cells, but not CD8+ T cells, were found to be significantly less than in controls. Lymphocyte proliferation to a mitogen, phytohemagglutinin, and antibody response to tetanus toxoid were all normal at 12 months. No increased incidence of infection has been reported following thymectomy. Neonatal thymectomy results in only a modest decrease in T-lymphocyte levels, but no detectable decrease in immune function [18]. Its effect in the face of solid organ transplantation is unknown.

4. Corticosteroids: non-specific immunosuppression

Corticosteroids in combination with azathioprine represent the first clinically successful immunosuppression strategy, which saw widespread use in renal transplantation. Corticosteroids are potent immunosuppressive agents and are the first line of rejection therapy. Many centers continue to use corticosteroids as part of routine immunosuppression. Corticosteroids are non-selective agents and are the first line of rejection therapy. Many centers continue to use corticosteroids as part of routine immunosuppression. Corticosteroids are non-selective form of immune suppression that effects the immune system at many levels. Steroids block T cell activation at the nuclear level, preventing up-regulation of cytokine genes required for the alloimmune responses, including IL-1, IL-2, IL-3, IL-6, FasL, TNF-α, and INF-γ. Neonatal and infant lymphocytes may be more sensitive to the effect of steroids [8]. In pediatric patients, the side effects of corticosteroids, including growth retardation, have encouraged many programs to attempt to discontinue routine oral steroids [19].

Many regimens continue to include corticosteroids as part of a three-drug regimen (steroids, azathioprine and a calcineurin inhibitor) [20,21]. The dose for maintenance oral prednisone is in the range of 0.1–1 mg/kg per day. For rejection, methylprednisolone is given in a dose of 10–30 mg/kg every 12 h intravenously for six to eight doses. A tapering dose may then be used to return to maintenance oral doses of
prednisone or discontinue the steroids depending on the policy of each individual program.

It is now established that successful cardiac transplantation can be achieved in children without the use of steroids [22,23]. Ferrazzi reported on 40 pediatric cardiac transplant patients with a 5-year survival of 88% [24]. In pediatric renal transplantation steroid-free regimens are also being used successfully [25]. However, steroid withdrawal, even from low doses, is often associated with significant morbidity, including an increase in acute rejection in temporal association in the weeks following withdrawal. Acute rejection is reported to occur in the first 6 months post-withdrawal in as many as 24% of children [19]. The reasons for the increase in morbidity remain unclear, especially in light of the fact that steroid-free immunosuppressive regimens exist and are at least as successful as regimens which use steroids. Potentially, it may be more difficult to withdraw than to never use chronic steroids in an immunosuppressive regimen.

Adverse effects of steroids include sodium and fluid retention, hypertension, hyperglycemia and altered glucose metabolism, altered lipid metabolism resulting in elevated cholesterol and triglycerides, negative calcium balance and abnormal bone growth, muscle wasting, behavioral changes, increased appetite and impaired wound healing. Chronic high-dose steroid use results in the classic Cushingoid appearance of truncal obesity, buffalo hump, moon faces, acne, striae, hirsutism, osteoporosis and avascular necrosis, peripheral muscle wasting, cataracts and glaucoma, impaired physical hormonal maturation and impaired growth, and is now routinely avoided.

5. Immunophilin-binding drugs

Maintenance immunosuppressive drugs act by partially blocking rate-limiting steps in the immune response. The new maintenance immunosuppressive drugs are either: (1) inhibitors of de novo synthesis of nucleotides (purines or pyrimidines); or (2) are immunophilin-binding drugs that inhibit signal transduction in lymphocytes. Of the immunophilin-binding drugs, cyclosporine was the first drug of this class to reach clinical utility in the early 1980s and in effect began the modern era of solid organ transplantation. Significant improvements in survival occurred with the use of CsA in the early 1980s. This class of immunophilin-binding drugs inhibits T cell activation. They are the mainstay of immunosuppressive therapy (Fig. 2). They inhibit either the calcium-dependent phosphatase calcineurin (CN), tacrolimus and CsA, or signaling from growth factor receptors, rapamycin (RAPA). CsA inhibits early calcium-dependent events required for signal transduction and T cell activation mediated through the T cell receptor (TCR). CsA binds to cyclophilin to create a complex that inhibits CN. Inhibition of CN prevents activation of cytokine genes in T cells. Tacrolimus and CsA inhibit by similar mechanisms. Both subsequently inhibit T cell synthesis of cytokines such as IL-2, IL-3, INF-γ, IL-6 and IL-7 [26].

CsA can be given initially intravenously in a dose of 0.03–0.1 mg/kg per h. When oral medications can be tolerated the usual dose is approximately three times the intravenous dose or 2–6 mg/kg per day divided every 8 h in infants and every 12 h in older children. Blood levels of CsA must be monitored to insure efficacy and avoid toxicity. The trough therapeutic range for blood levels for the active compound, cyclosporine A, ranges from 100 to 300 ng/ml. Most protocols recognize the need for higher maintenance level immunosuppression early in the course of the transplant, decreasing to long-term maintenance immunosuppression at 1 year post-transplant. Patients with episodes of acute rejection, or late severe rejection are typically maintained at slightly higher maintenance levels. CsA levels should be minimized to reduce the side effects, occurrence of infection, and malignancy, yet achieve effective immunomodulation. One consideration in the interpretation of CsA levels is the unbound plasma fraction which varies from 0.5 to 4.0% in individual patients. The unbound fraction was higher in younger patients and was significantly lower in hypercholesterolemic transplant recipients than in normocholesterolemic patients [27]. Even with a therapeutic trough concentration, activity of the target enzyme is only reduced by 50%; however, there is considerable inter-patient variability. Throughout the dosing interval, enzyme activity parallels that of drug concentrations [28].

With CsA the most common side effects are nephrotoxicity (acute and chronic), hypertension, hyperkalemia, hirsutism, hyperlipidemia, glucose intolerance, gingival hyperplasia and neurotoxicity (tremor and seizures). End-stage kidney disease may develop in 1–3% of cyclosporine-treated adult heart transplant recipients, and older patients show a decreased glomerular filtration rate. However, immunosuppression of the pediatric patient with cyclosporine at therapeutic doses protects the cardiac graft against acute rejection, yet is compatible with normal glomerular function and leads to only minor tubular disturbances [29]. The same types of side effects are seen with both Sandimmune™ and Neoral™. Due to its improved formulation and consistent absorption, side effects with Neoral may be less frequent, particularly in children [30]. The toxicity of CsA can be broad but is largely dose-dependent. The pharmacokinetics of CsA is different in children compared with adults. The bioavailability of CsA is particularly variable in
children. The newer microemulsion preparation (Neoral) improves bioavailability [31]. Oral absorption of CsA is incomplete and erratic in both children and adults. Due to small bowel absorption of this drug, infants and neonates have relatively decreased absorption. Absorption and bioavailability in the pediatric heart transplant patient is only 25%, similar to renal transplant patients [32]. Neoral, the newer microemulsion formulation of CsA, provides a higher degree of dispersion, making CsA immediately available for absorption. The bioavailability of Neoral is greater with a shorter $T_{\text{max}}$ in heart transplant patients compared with Sandimmune [33]. Because of its improved absorption many protocols have switched pediatric patients to Neoral. Conversion typically requires a 5–25% reduction in milligram dose. Increased CsA metabolism in children compared with adults has also been noted. Many toddlers and infants require t.i.d. dosing, while older children and adolescents appear to be maintained on b.i.d. regimens similar to adults. Cost issues may also be a consideration when choosing a formulation, since a generic form of CsA is now available.

Drug and food interactions are common with immunosuppressive agents and should be carefully evaluated when starting new drugs, even antibiotics or foods such as grapefruit. These interactions include medications that increase blood or plasma concentrations of CsA (e.g. calcium-channel blockers, corticosteroids, doxycycline, ketoconazole, erythromycin, oral contraceptives), decrease blood or plasma concentrations of CsA (anticonvulsants, nafcillin, rifampin), or increase nephrotoxicity of cyclosporine (aminoglycosides, amphotericin B, acyclovir, diuretics, non-steroidal anti-inflammatory drugs) [34].

Tacrolimus (FK 506) has a similar mechanism of action as cyclosporine, but tacrolimus also acts at a different site in the IL-2 activation pathway of lymphocytes (Fig. 1). Tacrolimus binds to FK binding protein-12 (FKBP-12) to create a complex that inhibits CN. It is approximately 100 times more potent than CsA in the inhibition of secretion of activating cytokines in vitro [6]. Tacrolimus has been extensively used in the pediatric heart and heart–lung transplant patient population [35,36]. However, the relative clinical effectiveness of tacrolimus vs. microemulsion CsA appears similar in terms of outcomes (number of rejection episodes and mortality). In a European adult study, 82 heart transplant recipients were randomized to treatment (2:1 ratio) with either tacrolimus (n = 54) or CsA-based therapy (n = 28). No significant differences were evident between the two treatment groups in either rejection or survival rates at 1 year. Kaplan–Meier estimates of the freedom from rejection were 26.3% and 18.5%, respectively, for the tacrolimus and cyclosporine treatment groups ($P = 0.444$). The overall rates of infection, impaired renal function (31.5% vs. 21.4%), and glucose intolerance (7.0% vs. 4.3%) did not differ significantly between the tacrolimus and cyclosporine treatment groups [37]. In this study no significant differences in short-term outcome was seen between the two drugs, although patients with induction therapy in either group had fewer episodes of acute rejection.

In the Pittsburgh experience, use of tacrolimus has allowed success at weaning steroids [36]. Anemia, renal toxicity, hyperkalemia, chronic diarrhea and allergic were the most common adverse effects of tacrolimus in the Pittsburgh experience. Renal dysfunction was seen in 20 patients (41%) with elevated creatinine levels between 1 and 2 mg/dl. Five patients (11%) had levels greater than 2 mg/dl. Severe anemia developed in eight patients (16%), two of whom had parvovirus. Moderate anemia developed in 21 patients (43%). Eosinophilia occurred in 19 patients; 11 of 19 patients (58%) had allergic symptoms. There was one case of diabetes mellitus, and this has also been reported by others. Hypertension, gingival hyperplasia, coarsening of facial features and hirsutism were not seen [38].

Tacrolimus can be given intravenously in a dose of 0.03–0.05 mg/kg per h. When given p.o. the dose is usually 0.05–0.1 mg/kg per day divided b.i.d. Trough blood levels must be monitored. The therapeutic range is 5–15 ng/ml. The toxicity of tacrolimus is also broad. Side effects of tacrolimus are both similar to cyclosporine and unique. Like CsA, FK 506 has renal toxicity and neurologic side effects. Unlike CsA, hirsutism and gingival hyperplasia are not seen with tacrolimus, but anemia and diabetes can occur [32,39]. Most patients are still treated with CsA as the primary T cell activation inhibitor. Multi-institutional studies comparing CsA with tacrolimus have not shown a clear clinical advantage for tacrolimus in heart transplant recipients [37].

Rapamycin (RAPA, Sirolimus) is the newest agent in this class. RAPA acts at a more distal site in the lymphocyte activation cascade by blocking transcription of activation genes. RAPA inhibits Ca$^{2+}$-dependent proliferation of T cells. RAPA also inhibits IL-2-dependent and independent proliferation of T cells. Rapamycin inhibits signaling from growth factor receptors, such as IL-2R. Rapamycin binds to FKBP to create a complex that engages a protein(s) called TOR (target of rapamycin), or RAFT (rapamycin and FKBP target), which may be kinases. The result is a block in the ability of cytokine receptors to activate cell cycling, interfering with clonal expansion. RAPA is readily absorbed in adults and has a half-life of approximately 30 h [6]. There are no reported interactions of RAPA and CsA. The side effect profiles appear not to overlap. It may be more efficacious...
than either CsA or tacrolimus, and combinations with CsA have been promising in early clinical trials [40]. The early experience with this drug is in conjunction with cyclosporine [41]. Target of Rapamycin (TOR), the as of yet unidentified intracellular binding protein of RAPA, also binds tacrolimus, making concomitant use of RAPA and tacrolimus theoretically less attractive [42].

RAPA was recently approved for use in clinical transplantation in the United States. There are no reports of its use in the pediatric heart transplant population. In Europe, results with this drug have been promising in adults [43]. Renal graft survival (98% Sirolimus vs. 90% CsA), patient survival (100% vs. 98%), and incidence of biopsy-confirmed acute rejection (41% vs. 38%) were similar. Serum creatinine was lower with RAPA, significantly ($P \leq 0.05$) so at 3 and 4 months, and serum uric acid and magnesium were normal [44]. Reported side effects associated with RAPA included hypertriglyceridemia (51% vs. 12%), hypercholesterolemia (44% vs. 14%), thrombocytopenia (37% vs. 0%), leukopenia (39% vs. 14%), and, of lesser importance, increased liver enzymes and hypokalemia. These abnormalities improved 2 months after transplantation when the RAPA target was reduced to 2 mg/d. No Renal graft survival (98% Sirolimus vs. 90% CsA), patient survival (100% vs. 98%), and incidence of biopsy-confirmed acute rejection (41% vs. 38%) were similar. Serum creatinine was lower with RAPA, significantly ($P \leq 0.05$) so at 3 and 4 months, and serum uric acid and magnesium were normal [44]. Reported side effects associated with RAPA included hypertriglyceridemia (51% vs. 12%), hypercholesterolemia (44% vs. 14%), thrombocytopenia (37% vs. 0%), leukopenia (39% vs. 14%), and, of lesser importance, increased liver enzymes and hypokalemia. These abnormalities improved 2 months after transplantation when the RAPA target was reduced to 2 mg/d. No

6. Anti-metabolites (antiproliferative agents)

6.1. Azathioprine

Azathioprine (Imuran) along with steroids was the original regimen used for immunosuppression in solid organ transplantation. Azathioprine remains a mainstay in most clinical heart transplant protocols and it is the most commonly used agent to block immune cell proliferation. It is a non-selective inhibitor causing generalized bone marrow suppression and subsequent immunosuppression as an anti-metabolite that affects all rapidly dividing cells. The usual dose is 1–2 mg/kg per day as a single dose, and drug affect is monitored by the white blood count. The white blood count is usually maintained at >4000/ml and <12000/ml. Toxicity of azathioprine is largely related to the therapeutic effect of bone marrow suppression. Hepatotoxicity has also been reported.

6.2. Cyclophosphamide

Cyclophosphamide is another antimetabolite that has been used for years in solid organ transplantation. It has been used primarily as an adjunct, for a short course in patients thought to have rejection mediated by a humoral or B cell response. Little data exists that this strategy is efficacious in any group of patients.

6.3. Methotrexate

Methotrexate has seen wider acceptance as an adjunctive maintenance therapy for patients with chronic or severe rejection which has failed other measures [49]. Methotrexate therapy for recurrent rejection in pediatric patients is given at a dose of 10 mg/m² per week over three doses. With treatment the rejection
rate (rejections per patient-month) fell in treated patients to a rate similar to patients who did not have recurrent rejection [50]. In contrast, in another study of adults with recurrent rejection, it was found that even after reversal of rejection by methotrexate, patients requiring methotrexate for the treatment of persistent/recurrent rejection continued to have higher rejection rates than patients not requiring methotrexate. However, patients treated with methotrexate had lower rates of cardiac allograft vasculopathy [51].

6.4. Newer anti-proliferative agents

The new inhibitors of de novo nucleotide synthesis include mycophenolate mofetil (MMF), mizoribine (MZ), brequinar (BQR) and leflunomide (LEF).

6.4.1. Mycophenolate Mofetil

Mycophenolate Mofetil (MMF, Cellcept) is a morpholinoethyl ester of mycophenolic acid (MPA). MPA is a potent non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH). MMF is rapidly converted to MPA, which is a specific inhibitor of purine synthesis via the de novo pathway, which is required in lymphocytes. MPA reduces the pools of guanine nucleotides, and increases some adenine nucleotides, inhibiting the cell cycle. Since lymphocytes lack the salvage pathway, MMF can selectively block lymphocyte proliferation without the side effects of non-specific bone marrow suppression. Thus, the number of specific effector T and B lymphocytes is reduced by limiting clonal expansion. Hence, MMF blocks the late events in lymphocyte activation. The use of MMF in heart transplant patients has been shown to be efficacious. Limited data exist on its use in the pediatric heart transplant population. The improved selectivity compared to azathioprine may provide more effective immunosuppression [52]. MMF is used in place of azathioprine in combination with CsA, or tacrolimus, coupling drugs that inhibit late and early events, respectively [53].

The usual dose of MMF in children is 25–50 mg/kg per day but absorption varies widely and higher doses may be required. In our experience, the pediatric heart transplant patient requires doses of 100–125 mg/kg per day early post-transplant (Table 1). In adults and adolescents the dose of MMF is 3 g/day divided b.i.d. Blood levels are not widely available but are useful due to inter-individual variability. The effective blood level for mycophenolic acid has been reported as 3–7 ng/ml [54]. Toxicity of MMF can include bone marrow suppression but most commonly is gastrointestinal in as many as 30% of patients. However, in pediatric patients it is uncommon to discontinue the medication because of GI side effects, which appear to be subject to tachyphylaxis.

6.4.2. Mizoribine

Mizoribine (MZ), like MMF, acts to inhibit de novo purine synthesis, by inhibition of inosine monophosphate dehydrogenase (IMPDH). MMF is a non-competitive, while MZ is a competitive inhibitor of IMPDH. The relative clinical effectiveness of MMF vs. MZ is not known. MMF has been approved in a number of countries. MZ has been approved in Japan.

6.4.3. Brequinar and leflunomide

The inhibitors of de novo pyrimidine synthesis, brequinar (BQR) and leflunomide (LEF), act on the enzyme dehydroorotate dehydrogenase. Neither is currently in clinical trials in transplantation. LEF may have a role in the treatment of rheumatoid arthritis. Brequinar is unlikely to have a role in clinical transplantation.

6.5. Other agents

Deoxyspergualin (DSG) a parenteral drug in development for induction or antirejection therapy. It may inhibit intracellular chaperoning by Hsc70, a member

---

Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>0–1 years</th>
<th>1–5 years</th>
<th>5–10 years</th>
<th>10–16 years</th>
<th>&gt; 16 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>No. of patients with therapeutic level</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>No. of therapeutic levels</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Dose to achieve therapeutic level (mg/kg)</td>
<td>125 ± 29</td>
<td>101 ± 51</td>
<td>67 ± 31</td>
<td>47 ± 24</td>
<td>49 ± 17</td>
</tr>
<tr>
<td>Dose to achieve therapeutic level (mg/m²)</td>
<td>2189 ± 696</td>
<td>2254 ± 887</td>
<td>1833 ± 931</td>
<td>1573 ± 833</td>
<td>1792 ± 579</td>
</tr>
</tbody>
</table>

a Therapeutic level: > 3 ng/ml.
b Number of patients in that age range on the date of MPA level.
of the heat shock protein family. It may have its principal effect by inhibiting the activation of transcription NFκB in APC and monocytes [6]. DSG is unlikely to have a role in clinical transplantation.

7. Total lymphoid irradiation (TLI)

Total lymphoid irradiation (TLI) is used as an adjunct to the treatment of resistant or recurrent rejection in addition to a course of anti-T cell antibody and a change in chronic immunosuppression [55]. The use of TLI in pediatrics is limited [56]. The typical irradiation regimen is given in an inverted Y-shaped mantle. The protocol usually calls for 80cGy/treatment given bi-weekly for 10 treatments. In our limited experience this regimen was most effective if treatments were begun within 6 months of transplant. With treatment the rejection rate (rejections per patient-month) fell in treated patients to a rate similar to patients who did not have recurrent rejection (Pietra, unpublished data). The treatment was well tolerated. With development of newer more specific immunosuppressive agents it is unlikely that this therapy will ever see widespread use in pediatrics.

8. Cytolytic biological agents

8.1. Polyclonal antibodies

ATGAM (equine) is a polyclonal anti-T cell antibody preparation made in horses by immunizing with human T cells. The product is available for intravenous use containing 50 mg/ml of horse gamma globulin. The usual dose is 15 mg/kg per day and is continued for 7–14 days to treat acute rejection.

ATG (rabbit thymoglobulin) is a polyclonal anti-T cell antibody preparation made in rabbits by immunizing with human thyocytes. The product is available for intravenous use containing 25 mg of rabbit gamma globulin. The usual dose is 1.5 mg/kg per day and is continued for 7–14 days to treat acute renal rejection. It is not approved for use in heart transplantation. ATG (rabbit) is now commercially available and approved for use in renal transplantation. Trials for use in heart transplantation at designated centers are ongoing.

Antithymocyte serum (ATS), a polyclonal antibody preparation raised in rabbits, has been used as induction of immune prophylaxis in pediatric heart transplant patients [57,58]. ATS use is typically associated with a decreased incidence of early acute rejection [59]. Others have used drugs such as OKT3, and even CsA for induction by starting the drug pre-transplant [60]. In a retrospective comparison of ATS and OKT3 for induction therapy in pediatric heart transplant patients, induction therapy was found to decrease late deaths, particularly in recipients less than 6 months of age. Polyclonal antibody (ATS) appeared to be superior to OKT3 for this study. No increased risk of infection or malignancy was noted [58].

8.3. Monoclonal antibodies

OKT3 is an agent with very powerful immunosuppressive properties. The target for this drug is the CD3 molecule, which is universal on T cells. This agent appears to be very effective anti-rejection therapy. It is typically held in reserve for resistant rejection or hemodynamically significant rejection. Its use as induction therapy appears to be more controversial. It is a murine monoclonal antibody, made via hybridoma technology. It is available for intravenous use, in a 5 mg/5 ml preparation. Typical dose is 5 mg/day for 7–14 days. In children typical dose is 2.5 mg/day for 7–14 days.

The side effect profile for both polyclonal and monoclonal antibody preparations are similar. There is a larger risk with the use of monoclonal preparation such as OKT3. Side effects include fever, chills, rash, arthralgia and leukopenia. The risk of viral infection is increased. The risk of subsequent neoplasia is also increased. Sensitization to murine antibodies may preclude subsequent treatments or increase the risk of anaphylaxis. Patients receiving heterologous (made in animals) antibody treatments should have a skin test prior to the dose. Patients who have been treated should have serum sent for the appropriate antispecies, rabbit, or horse antibodies to determine sensitization.

Anaphylaxis has been reported in patients treated with heterologous antibody preparations and may not be predicted by the results of prior skin testing. Physicians should be prepared for this reaction prior to administration of the dose. Frequent monitoring is required for the first several doses. A specific syndrome of pulmonary distress, edema and hypertension has been reported after treatment with OKT3. This probably reflects the tremendous cytolytic effect of this drug and the subsequent release of inflammatory mediators and cytokines from lysed cells. Pretreatment with acetaminophen and benadryl, or corticosteroids is recommended [20,61].

Therapeutic monitoring with polyclonal and mono-
clonal antibody preparations are performed with direct staining of lymphocytes pre- and post-administration of the drug. A dramatic decrease in T cells and T cell subsets (CD4 and CD8) is typically seen. Care is required in the interpretation of flow cytometry results. Particularly with OKT3, T cells may recover but may not express normal levels of CD3 on their surface. Other T cell markers will identify these cells (CD4, CD8, CD45 for example). Early failure to deplete T cells may indicate an inadequate dose, or perhaps is an indication of the presence of a sensitized recipient. Since the biological effect of this drug is not strictly determined by the T cell numbers in the peripheral blood, efficacy should be determined by evidence of reversal of graft rejection.

8.4. Newer experimental biological agents

Biological agents directed at various targets on both antigen presenting cells and on lymphocytes represent the greatest promise in transplantation. They represent the beginning of an era when the paradigm of immunosuppression turns toward graft tolerance. Although biological agents such as OKT3 have been a mainstay in cardiac transplantation for years, there is now a shift in the design of these agents. Newer agents are intended to be less cytolytic, but rather block key signals required for immune activation (Fig. 1).

8.4.1. IL-2 inhibition

IL-2 is thought to be one of the critical cytokines required for the activation and proliferation of the immune response. At least part of the mechanism of action of CsA and tacrolimus is the inhibition of IL-2 production. Blockade of the IL-2/IL-2R pathway is an emerging strategy in solid organ transplantation. Preliminary studies with a murine IgG1 anti-IL-2R (BT563) antibody used for rejection prophylaxis in conjunction with CsA as induction therapy did not reduce the incidence of acute rejection in heart transplant recipients [62]. In orthotopic liver transplantation the same anti-IL-2R antibody was shown to decrease acute rejection [63]. Subsequently, two commercially available products (daclizumab and basiliximab) are either humanized or chimeric anti-IL-2R antibodies. These drugs are expected to find use in solid organ transplantation.

8.4.2. Daclizumab

Daclizumab (Zenapax) is a humanized IgG1 monoclonal antibody against the alpha-chain of the interleukin-2 receptor (IL-2R, CD25). The IL-2R is expressed by T cells participating in allograft rejection, but not by resting T cells. In a randomized double-blind, prospective phase III clinical trial, daclizumab reduced the incidence of acute rejection episodes and improved patient survival following renal transplantation. Typical administration of daclizumab was a regimen of five doses (1.0 mg/kg every other week, for a total of five doses) in addition to dual or triple immunosuppression [64–66].

8.4.3. Basiliximab

Basiliximab (Simulect) is a chimeric monoclonal antibody, approved for use in renal transplantation. Basiliximab is infused over 30 min as a single 20 mg dose on day 0 and 4 post-operatively. Serum concentrations in excess of 0.2 mg/ml were found sufficient to saturate IL-2R epitopes on circulating T lymphocytes. Concentrations were above this threshold for 26 ± 8 days (range 16–46) at the 40-mg total dose level and for 32 ± 11 days (range 22–51) at the 60-mg total dose level [67,68]. In two large randomized clinical renal transplant trials, patients were treated with either basiliximab, or placebo, and CsA with steroids. Basiliximab reduced the proportion of patients who experienced biopsy-confirmed acute rejection episodes by 28% and 32%, respectively, vs. placebo (P < 0.012). Prophylactic therapy is well tolerated, had an adverse event profile comparable to placebo, and significantly reduced the number of acute rejection episodes in renal allograft patients within the first year after transplantation [69,70]. The incidence of steroid-resistant first rejection episodes that required antibody therapy was also reduced in the basiliximab group (10% vs. 23.1%, P < 0.001) [70].

Both daclizumab and basiliximab specifically bind to the IL-2R and block signaling through this receptor. Both drugs have been used as rejection prophylaxis therapy and have shown some efficacy in reduction of other immunosuppression or decrease in acute rejection. Although the use of these drugs for prophylaxis of rejection is established in the kidney, the treatment of rejection with IL-2R antibody does not appear promising.

8.5. Co-stimulation inhibition

The rejection of the transplanted allograft is dependent on T cell activation, which requires T cell receptor engagement by antigen and co-stimulatory signals delivered by T cell surface molecules such as CD28. The CD28 receptor on T cells interacts with its ligand B7 on activated B cells/macrophages as the co-stimulus to support T cell activity. The natural ligand for CD28, B7.1 and B7.2 (CD80/86) are expressed in both constitutive and inducible forms. Expression of the inducible form, B7.1 is the most co-stimulatory and may peak at 48 h following antigen presentation. CTLA4-Ig is a fusion protein (a soluble CD28 receptor analog) that binds both B7s and inhibits CD28 activation. CTLA4-Ig has been shown to block the
CD28-mediated co-stimulatory signal and inhibit immune responses in vitro and in vivo (Fig. 1). CTLA4-Ig can induce transplantation tolerance in the adult murine cardiac allograft model [71]. CTLA4-Ig was found most effective 48 h following antigen presentation in a murine cardiac allograft model perhaps reflecting induction of tolerance at a time of maximal CTLA4-Ig/B7.1 blockade. CTLA4-Ig given later, at a time when the recognition/rejection process has already begun, was not effective [72]. CTLA4-Ig and anti-CD40 ligand (CD 154) prevent renal allograft rejection in primates, and anti CD154 alone prevents renal allograft rejection in primates [73]. Prolonged anti-CD40 ligand (CD154) therapy improves primate cardiac allograft survival [74].

In animal models many biological agents are showing promise in inducing long-term graft survival and tolerance in the absence of other immunosuppression. Agents known to induce tolerance in addition to those discussed above include anti-CD4, anti-CD45RB, anti-LFA, and donor specific transfusions (DST) under the cover of some antibody such as anti-CD4. These models may lead to a clinically useful protocol to induce tolerance directly, but more likely they will help to unravel the rules which mediate rejection and tolerance.

9. Management of immunosuppression

9.1. Pre-operative

Evaluation of the immune system is an important part of the pretransplant evaluation. Although it is common to check recipient and donor human leukocyte antigen (HLA) status, this information is not normally part of the decision-making process when determining donor suitability. Retrospective studies have suggested that HLA compatibility is rare but may lessen rejection and improve graft survival in heart transplantation [75]. In part due to the constraints of cold ischemic time (usually approx. 4 h) and the ongoing organ donor shortage, prospective HLA matching is not routinely utilized in heart transplantation. Panel reactive antibodies (PRA), another part of the immune system, are assayed to determine whether the recipient has any preformed, circulating anti-HLA alloantibodies. Because of the concern that circulating donor-specific HLA alloantibodies may decrease graft survival, patients with a significantly elevated PRA before transplantation often undergo a prospective cross-match between donor and recipient before acceptance of a donor organ [76]. However, this can severely limit the donor pool available to a recipient and there is evidence that patients with elevated PRAs may have a higher degree of allograft loss and a higher mortality after transplantation despite a compatible cross-match [77]. Because of the significant difficulties associated with finding a compatible cross-match in patients with elevated PRAs, multiple treatment modalities, including intravenous immunoglobulin, plasmapheresis, azathioprine and cyclophosphamide, have been utilized to decrease the PRA of highly sensitized patients [78,79].

9.2. Peri- and post-operative

Immunosuppression is begun in the perioperative period (Table 2). Many institutions begin T cell activation inhibitors (e.g. cyclosporine) and azathioprine just before the transplant operation. High-dose corticosteroids are given intraoperatively and continued for 48 h after which time they can be discontinued or decreased to a low-dose maintenance regimen. Additional immunosuppressive medications may be given post-operatively in the form of monoclonal anti T cell antibodies directed toward the CD3 epitope (OKT3), polyclonal antibodies directed toward multiple T cell epitopes (ATGAM, ATG) or chimeric or humanized monoclonal antibodies directed toward the IL-2 activation pathway (CD25) [80]. Rejection incidence reaches a peak at approximately 21 days, and approximately two-thirds of all rejection episodes occur in the first 3 months post-transplant.

9.3. Treatment of rejection

Institutional preference usually dictates whether endomyocardial biopsy or echocardiography is used as the primary rejection surveillance tool. The vast majority of initial rejection episodes can be successfully reversed by high-dose corticosteroids alone or in conjunction with anti T cell antibodies (Table 3). Intra-
Table 3

Treatment of rejection

Mild (no symptoms)
Increase or change maintenance immunosuppression
Oral prednisone (50–100 mg/day × 7 days)

Mild/moderate (minimal symptoms + myocyte destruction)
Solumedrol i.v. 25–75 mg/kg per day Q 12 h × 8 doses

Severe (hemodynamically significant, + myocyte destruction)
Solumedrol i.v. 25–75 mg/kg per day Q12 h × 8 doses
Anti-T cells Antibody (ATGAM, ATG, OKT3)
A change in chronic immunosuppression either
(a) Adjust CsA and/or tacrolimus dose
(b) Add short course MMF in place of azathioprine
(c) Consider new combination of rapamycin with CsA or rapamycin with MMF

Persistent or multiple and severe (chronic or + myocyte destruction)
Solumedrol i.v. 25–75 mg/kg per day Q 12 h × 8 doses
Anti-T cells Antibody (ATGAM, ATG, OKT3)
A change in chronic immunosuppression either
(a) Adjust CsA and tacrolimus dose
(b) Add short course MMF in place of azathioprine
(c) Short course of methotrexate (6 weeks)
(d) Total lymphoid irradiation (TLI) (particularly if > 6 months post-transplant) 80 cGy/dose twice weekly × 10 doses
(e) Consider new combination of Rapamycin with CsA or Rapamycin with MMF

Transplant coronary artery disease (TCAD, IVUS or angiographic)
Potential strategies for treatment
(a) Treat as severe acute rejection and add/change chronic immuno-suppression MMF and/or Rapamycin and/or
(b) Consider adding adjunctive drugs
Diltiazam and/or
HMG-CoA inhibitors

venous methylprednisolone (10–30 mg/kg i.v. Q 12 h)
is the first line in rejection therapy. Repeat episodes of rejection can be more difficult to treat and can result in graft loss and patient demise.

9.4. General complications of immunosuppression

Since two of the main functions of the immune system are fighting infection and tumor surveillance, all immunosuppressants put the patient at risk for infection and malignancy. In addition, individual immunosuppressant medications have significant adverse effects, even when properly utilized in appropriate doses. Hypertension is a common complication after transplant and may represent in part an adverse effect of CsA, but data from the ISHLT/UNOS database showed a stronger association between hypertension and chronic steroid use. Catastrophic neurologic complications have resulted from severe post-operative hypertension, this requires urgent treatment. Chronic hypertension appears to be less prevalent in pediatric heart transplant recipients than in adults, but is still a problem requiring treatment, even in centers who use a steroid-sparing maintenance immunosuppressive protocol [24]. CsA may also cause acute and chronic renal insufficiency, and monitoring for renal insufficiency in children should be lifelong. Treatment options are limited but CsA levels should be maintained long-term as low as can be tolerated in patients without increasing risks of rejection. In adults, verapamil has been shown to improve renal function in some patients on CsA after transplantation [81]. Neurotoxicity is another recognized complication of CsA, occurring in as many as 10–25% of patients, with a peak in the early post-operative period [82]. Symptoms include tremors, restlessness, dysesthesias of the palms and soles, seizures and altered mental status.

Both CsA and corticosteroids cause hypercholesterolemia. Hypercholesterolemia has been shown to be a common problem after pediatric heart transplantation [83], but little is known of the longitudinal lipid profiles of these patients. Pediatric heart transplant recipients who were switched from a regimen of cyclosporine, azathioprine and prednisone to a regimen of tacrolimus and azathioprine demonstrated a significant decrease in serum lipid levels [32]. It is hoped that the use of steroid-free or reduced steroid immunosuppressive regimens in children will lessen the incidence of lipid disorders.

9.4.1. Infection

Pediatric heart transplant recipients are at risk for serious and opportunistic infections, including bacterial, viral (especially cytomegalovirus), and protozoal (Pneumocystis) infections, particularly in the first 6 months after heart transplantation when immunosuppression is greatest [84]. Due to the significant post-operative risk of infection, many centers use some form of prophylaxis against fungal (nystatin), cytomegalovirus (acyclovir or gancyclovir in recipients who receive cytomegalovirus-positive donors), and/or protozoal (trimethoprim/sulfamethoxazole) infections, although exact indications and duration of prophylaxis are unknown.

9.4.2. Immunization

Transplant programs have recommended that in the non-immunized child, immunizations should follow the usual schedule for polio (using killed virus), DPT, hepatitis B and Haemophilus beginning between the third and fourth post-operative months [85]. At this time, live viral vaccines are contraindicated. Thus, it is recommended that MMR be deferred, although siblings can receive it. Some have recommended the trivalent split-virus influenza vaccine for pediatric solid organ transplant recipients, their household contacts, and health care workers [86]. There are significant
concerns that pediatric heart transplant recipients may have less of an antibody response to pneumococcal vaccine [87]. As with all children, pediatric heart transplant recipients have fevers and require prompt evaluation for these. Fortunately, the majority of acute febrile illnesses in these patients are non-serious and can be managed safely in an outpatient setting [88,89].

9.4.3. Malignancy

An increased risk of malignancy is a well-recognized complication after organ transplantation. In pediatric heart transplant recipients, the incidence of neoplasms appears to be equal to or greater than adult recipients, and the majority are lymphoproliferative disorders that often respond to reduction in immunosuppression [90,91]. When primary Epstein-Barr virus infection develops after transplant, preventative measures may be warranted [91]. The level of immunosuppressive therapy should be maintained as low as is compatible with good allograft function [92] and antiviral therapy with acyclovir should be considered. Because of the concern of skin cancers, children should be advised to avoid excessive sun exposure and use sunscreen.

10. Conclusions

We are now entering an era when there is both a significant improvement in our understanding of the immunobiology of allotransplantation, and the concomitant development of immunosuppressive agents which will allow us to take advantage of this knowledge. The establishment of allograft tolerance in animals has been achieved. Adapting this technology for clinical use is underway. Strategies, which are free of steroids and promote the induction of graft specific tolerance, are reality. There may be dramatic changes in protocols used clinically as a result of these new agents over the next decade.

References

plasma unbound fraction in heart and lung transplantation recipients. Ther Drug Monit 1999;21:8–16.


[63] van Gelder T, Baan CC, Balk AH et al. Blockade of the interleukin (IL2/IL-2 receptor pathway with a monoclonal anti-IL-2 receptor antibody (BT563) does not prevent the


