NEW ADVANCES IN IMMUNOSUPPRESSIVE THERAPY FOR RENAL TRANSPLANTATION

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Interests: My academic interests are centered in the area of renal transplantation. I am interested in prevention and treatment of transplant rejection and the use of immunosuppressive agents in renal disease. My research interests also involve the area of transplantation-related complications and their prevention and treatment.

INTRODUCTION

I would like to initiate this presentation by reviewing the clinical course of two patients who received renal transplants at Parkland Memorial Hospital.

Case 1: A 10-year-old girl with chronic renal insufficiency secondary to congenital bilateral renal hypoplasia was referred to Parkland Hospital for living related donor kidney transplantation. The patient had growth retardation and progressive azotemia with a creatinine clearance of 3.5 cc/min. The prospective donor was her twin sister. Both twins were identical, as they shared one placenta. No HLA typing or cross-matching was available (more than 30 years ago). A full thickness skin graft (to the left upper arm) was performed and was not rejected during the 10-week period of observation. Donor-specific transfusions were given (145).

Dr. Paul Peters performed the kidney transplantation procedure. There was good urine output immediately in the operating room, and the BUN decreased from 83 mg/dl pre-operatively to 6 mg/dl by post-operative day #3. No immunosuppression was used. Patient had good renal function on follow-up.

Case 2: A 22-year-old man with end stage renal disease secondary to reflux nephropathy was referred to Parkland Hospital for living related donor kidney transplantation. He had been on hemodialysis for two years. The prospective donor was his sister, who was a two-haplotype match (HLA identical). Pre-transplant evaluation included negative cytotoxicity cross-matching, negative flow cytometry cross-matching, and negative skin cross-matching.

Transplantation was performed successfully. Patient had excellent urine output, and serum creatinine was 1.5 mg/dl by post-operative day #6. He was discharged on steroids and azathioprine as immunosuppression. Four weeks posttransplant, patient presented with fever and an acute rise in serum creatinine to 3.1 mg/dl. A renal biopsy confirmed the presence of severe acute cellular rejection. Patient was treated with intravenous steroids, and his immunosuppression regimen changed to include microemulsion cyclosporine A, mycophenolate mofetil, and steroids. His serum creatinine returned to his previous baseline of 1.5 mg/dl.

There are several issues illustrated by these two cases. First, successful renal transplants have been performed for many years. Second, the immune system is so efficient in discriminating self versus non-self that, except for genetically identical twins, most recipients can reject grafts even from an HLA identical sibling. Third, the advances in immunosuppression over the last several decades have made it possible to perform successfully an increasing number of transplants, not only from genetically related individuals but also from cadaveric donors.

The evolution of renal transplantation into a clinical discipline reflects the dedication and work of individuals from many countries aiming for the ultimate goal of successful organ transplantation. The first successful human kidney transplant was performed by Murray and colleagues in 1954 between two identical twins (117). Figure 1 outlines some of the most important landmarks in the history of renal transplantation. Several excellent reviews detail the progress in the field of renal

ADVANCES IN RENAL TRANSPLANTATION

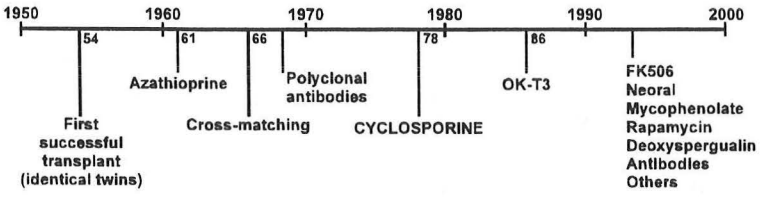


Figure 1

transplantation (118,119). Azathioprine was introduced in 1961 and made it possible to perform renal transplants with some predictable success. In the decade of the 1960s, tissue typing and cross-matching were introduced. Polyclonal antilymphocyte preparations were also introduced in the 1960s and employed more in the subsequent decades.

The major turning point in transplantation was the introduction of cyclosporine A (CsA). It was first used by Calne in 1978 and widely introduced in transplantation by the mid-1980s. The monoclonal antibody OKT3 was approved in 1986. The current decade has seen an unparalleled arrival of new immunosuppressive agents, including FK506, neoral, mycophenolate mofetil, rapamycin, deoxyspergualin, and numerous polyclonal and monoclonal antibodies, among others. Advances in basic understanding of the immune system and clinical care of transplant recipients will clarify the role of these and other agents in the future.

END STAGE RENAL DISEASE AND RENAL REPLACEMENT THERAPY

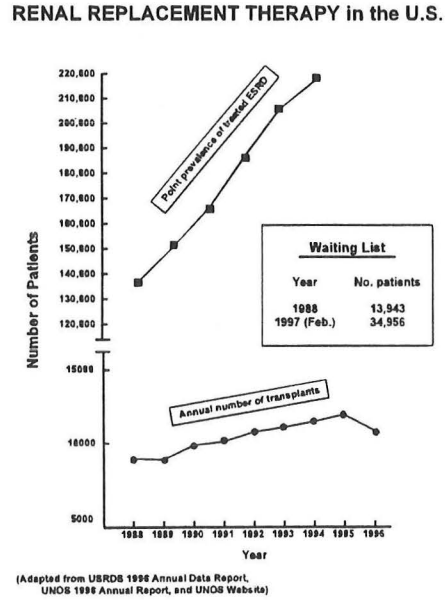


Figure 2

The magnitude of end stage renal disease as a public health problem in the United States is illustrated in Figure 2. The data on point prevalence of treated ESRD is derived from the USRDS Registry and includes all patients receiving renal replacement therapy (hemodialysis, peritoneal dialysis, or transplantation) and covered by Medicare (over 92% of dialysis patients and over 90% of kidney transplant recipients in 1993) (120,121). The data shows an increase in patients receiving renal replacement therapy of about 10% per year. The percentage increase for 1993 (last year reported) is less and

probably reflects an undercount of patients due to late submission of Medicare forms. Several explanations have been formulated for the increase in treated ESRD patients, including referral and acceptance of older and sicker patients, decreased mortality from other causes and subsequent progression to ESRD, decreasing death rates for ESRD patients, or an increase in causes of ESRD (123).

The annual number of renal transplants has slowly increased from about 9,000 in 1988 to close to 11,000 in 1996 (122). The cumulative number of patients with a functioning transplant continues increasing. As of 1993, about 60,000 of the 220,000 patients in the Medicare ESRD program were transplant recipients (121).

The limited supply of donors for kidney transplantation has brought an increase in the number of patients on the waiting list for a kidney transplant from 13,943 in 1988 to 34,956 as of Feb. 28, 1997 (122,124). The median waiting times for a cadaveric renal transplant have increased nationwide from 400 days in 1988 to 842 days in 1994, the last year from which waiting time data is available (122).

The large increase in the waiting time for renal transplantation is detrimental to most patients, as there is a long-term survival benefit for most patients following transplantation as compared to dialysis. Death rates based on adjusted Kaplan-Meier estimates by modality and year after transplantation consistently show lower mortality for cadaveric renal transplant recipients than for dialysis patients (122). Some of the differences in mortality for these two groups are likely related to a selection bias, as patients receiving transplants are generally healthier as a group than those remaining on dialysis. Nevertheless, some of the differences in mortality are probably related to an advantage of renal transplantation as a form of renal replacement therapy. In a report of all Michigan ESRD patients who started therapy between 1984 and 1989 and who were listed for transplantation, Port and colleagues found a slight increase in mortality early after transplant but a benefit in survival long-term (125). Recently, Port et al. have also noted a lower mortality for transplant recipients as compared to dialysis patients waiting for transplantation when reviewing national data (Port, personal unpublished observations).

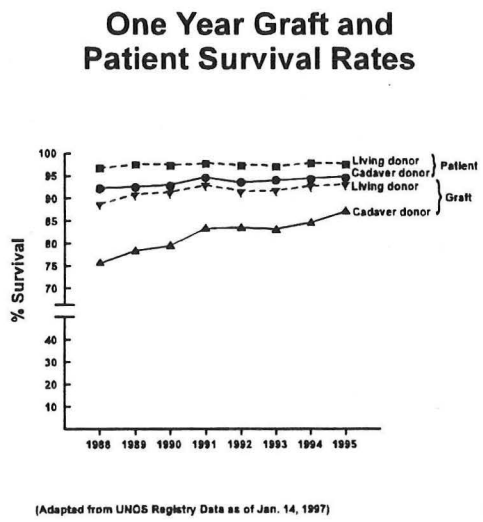


Figure 3

Renal transplantation offers excellent rates of patient and allograft survival at one year. Figure 3 illustrates the progressive improvement in success rates for living donor and cadaveric renal transplants in the United States. The UNOS/OPTN Scientific Registry reports for 1995 a one-year graft survival rate (Kaplan-Meier) of 87% and 93% for recipients of cadaveric donor renal transplants and living donor renal transplants respectively. The one-year patient survival rates are

94.6% for cadaveric renal transplant recipients and 97.5% for living related donor kidney transplant recipients in 1995 ((122) and UNOS Website). The availability of dialysis as a back-up therapy in case of transplant failure likely plays a major role in the very low mortality rates observed with renal transplantation.

At this point, it is important to consider the implications of the excellent one-year results in renal transplantation in terms of the evaluation of new immunosuppressive therapies. Hunsicker has termed this phenomenon "the price of success." A study designed to have a 90% power to detect a 30% reduction in the rate of renal graft loss at one year (for example, from 84% to 89%) would require a minimum of 1600-2000 patients (126). The logistics of such a study would be extremely difficult.

RENAL ALLOGRAFT SURVIVAL
First Cadaver Donor Kidney Recipients

Cohort (years)	1-year survival (%)	Half-life (years)
1975-79	49	7.2
1980-84	64	6.9
1985-89	78	7.9
1990-93	83	9.4
	p < 0.0001	p < 0.0001

Adapted from Gjertson et al., *Transplantation* 60: 1384, 1995

Table 1

Now that short-term renal transplant results are satisfactory, most of the attention has turned to long-term results, where the situation is quite different. Table 1 summarizes the survival rates for first cadaver donor kidney recipients as reported by Gjertson and colleagues based on data collected by the UCLA and UNOS kidney transplant registries (67). There has been a progressive improvement in one-year graft survival from less than 50% in the 1970s to more than 80% in the 1990s. The introduction of cyclosporine produced the single largest increase in one-year graft survival. Nevertheless, despite an almost 100% gain in one-year graft survival rates, the half life (or time to failure for half of the kidney's functioning after one year) has only increased by about two years in the last three decades (67).

The design of a trial to examine the effect of immunosuppressive therapies on long-term graft outcomes is very challenging. A study to detect with 90% power a 30% reduction in the annual rate of late graft loss from 6.9% to 4.9% would need to enroll over 4500 patients over more than four years with subsequent long-term follow-up (126). Again, the logistics of such a study on primary prevention of long-term graft loss would be formidable.

The difficulties with primary prevention trials have stimulated great interest in alternative endpoints (or surrogate markers). Acute graft rejection has become the most common endpoint in transplantation studies, and some investigators believe it is the main obstacle to long-term transplant success (20). Acute rejection can lead to graft loss and is an important risk factor for the development of chronic rejection. Treatment of acute rejection can increase morbidity and mortality and is expensive. Despite the attractiveness of acute rejection as a surrogate marker for long-term transplant outcome, analysis of the UNOS renal transplant database has not shown that reducing the number of episodes of acute rejection leads to sustained improvements in long-term transplant survival (126)}. Other alternative outcomes considered in the evaluation of new clinical

interventions include graft function, immunologic function, graft fibrosis (intermediate endpoint), adverse effects, costs, and utilization of resources and quality of life (126,127). Additional experience will be required to determine if the use of these endpoints will become a useful and valid tool in the long-term follow-up of transplant recipients.

MECHANISMS OF ALLOGRAFT RECOGNITION AND DAMAGE

The immunosuppressive agents available today interfere with the immune response at different levels. Let us review the mechanisms of allograft recognition and damage. The introduction of a foreign vascularized organ elicits an antigen-specific immune response (1). T and B lymphocytes initiate anti-allograft antigen-specific immune responses. Non-specific injury to the allograft from donor trauma, surgical manipulation and ischemia, leads to recruitment of inflammatory cells and expression of cell surface molecules and soluble mediators that further amplify the antigen-specific immune response (2).

Alloantigen Recognition

The initial steps in allograft recognition require the coordinated activity of T cells and antigen presenting cells (3). The immune system of the recipient can recognize the donor allograft via the direct pathway or indirect pathway (4-6). Please see Figure 4.

In the direct pathway, recipient T cells recognize donor MHC or peptide (which may be derived from MHC) with MHC in the surface of an APC of donor origin. Donor dendritic cells can be "passenger leukocytes" and act as potent APCs in the direct pathway. Antigen presentation can occur in the allograft itself (peripheral sensitization) or in the recipient's lymphoid tissue (central sensitization) (7). Direct recognition of Class I MHC and peptide by recipient CD8+ T cells may play an important role in the early events of allograft recognition.

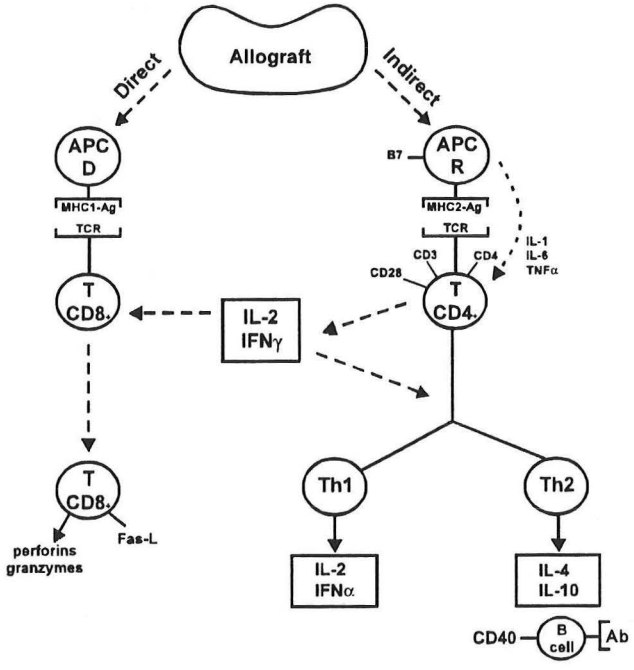


Figure 4

In the indirect pathway, recipient CD4+ T cells recognize donor peptides (which may be derived from MHC peptides) presented by recipient APCs. The peptides shed from the donor are taken by the recipient APCs and processed for presentation in the context of self MHC Class II for recognition by recipient CD4+ T cells.

T Cell Activation

Recognition of antigen in the context of MHC by the T cell via its T cell receptor/CD3 complex is the first step in T cell activation. A second signal which is antigen independent is necessary at this point, or the T cell may develop anergy or inability to respond to all antigenic stimulation (8). The second signal can be provided by interaction of the cell surface molecule B7 on APCs with its ligand CD28 on T cells (9,10). Cytokines derived from APCs such as TNF and IL-1 may also provide important second signals (1,11).

Stimulation of the T cell via its TCR/CD3 complex by peptide-MHC in the presence of costimulation (second signal) leads to T cell activation (3). There is redistribution of cell surface molecules, and CD4 molecules stabilize the interaction with Class II MHC while CD8 molecules stabilize the interaction with Class I MHC molecules. The CD3 complex associated with the TCR delivers signals to the interior of the T cell that result in activation of protein kinases, calcium fluxes, cytokine gene transcription, and DNA synthesis.

Two cytokines produced by CD4+ T cells play a pivotal role in the events following T cell activation. Interferon γ activates macrophages to release inflammatory mediators such as TNF and IL-1 and also upregulates expression of Class II MHC molecules, rendering the allograft more immunogenic. IL-2 provides the main stimulus for division of T cells (CD4 and CD8) resulting in clonal expansion, and for T cell differentiation.

Effector Functions

CD8+ T cells are cytotoxic and can destroy the allograft by two mechanisms (29):

(1) Release of perforins and granzymes (12): Perforins create perforations in the cell membranes and osmotic defects. Granzymes are proteases that enter the cytosol and trigger apoptosis.

(2) Expression of Fas-ligand (13,14): This membrane-bound effector binds to Fas in target cells, initiating a signaling process that leads to apoptosis.

CD4+ T cells can differentiate into two different phenotypes (15,16). Th1 cells produce IL-2 and interferon γ and are associated with cell-mediated immune responses. Th2 cells produce IL-4, IL-5, IL-10 and are associated with antibody-mediated responses.

CD4+ T cells can mediate cytotoxicity via Fas-ligand (14) or via a mechanism of DTH (17).

B cells are activated by stimulation by antigen via their immunoglobulin cell surface receptor. Costimulation is provided by cytokines (IL-2, IL-4, IL-10) and by cell surface molecules (CD40 ligand on T cells - CD40 on B cells). B cells proliferate and differentiate into plasma cells that produce antibodies. Allograft damage may occur by antibody deposition and fixation of complement or by ADCC (5,18). NK cells have also been shown to be important effectors in transplantation settings such as bone marrow transplantation (5).

In summary, recognition of the allograft by T cells on the recipient can lead to the generation of diverse mechanisms of allograft damage, including cytotoxic and helper T cells, donor-specific antibodies, and inflammatory cytokines.

IMMUNOSUPPRESSIVE AGENTS

The immunosuppressive agents available today can be classified according to their origin into two broad categories, xenobiotics and biologics (69). The xenobiotics are microbial products with immunosuppressive activity or chemically synthesized molecules that are structurally different from mammalian molecules. The biologics or biological agents are naturally occurring mammalian proteins or peptides derived from mammalian proteins.

Several classifications of the available xenobiotic agents have been proposed according to their site of action in blocking the immune response (3,19). In this presentation I will classify the immunosuppressive drugs available for renal transplant recipients as follows:

(a) Xenobiotics

1. Inhibitors of cytokine transcription (cyclosporine, tacrolimus)
2. Inhibitors of growth factor signal transduction (sirolimus, leflunomide)
3. Inhibitors of nucleotide synthesis (mycophenolate mofetil, azathioprine, brequinar, mizoribine, leflunomide)
4. Inhibitors of cell differentiation/maturation (deoxyspergualin)

(b) Biological agents

1. Polyclonal antilymphocyte preparations
2. Monoclonal antibodies

XENOBIOTIC IMMUNOSUPPRESSIVE AGENTS

I. INHIBITORS OF CYTOKINE TRANSCRIPTION

Mechanism of Action of Cyclosporine and Tacrolimus

Cyclosporine, tacrolimus and sirolimus bind a group of cytosolic proteins called immunophilins. Cyclosporine binds to the immunophilin cyclophilin, and tacrolimus and sirolimus bind to the immunophilin FKB12. The active intracellular inhibitor is the drug-immunophilin complex (1).

Cyclosporine and tacrolimus are inhibitors of T cell cytokine transcription, while sirolimus inhibits the transduction of signals derived by cytokines (3,19).

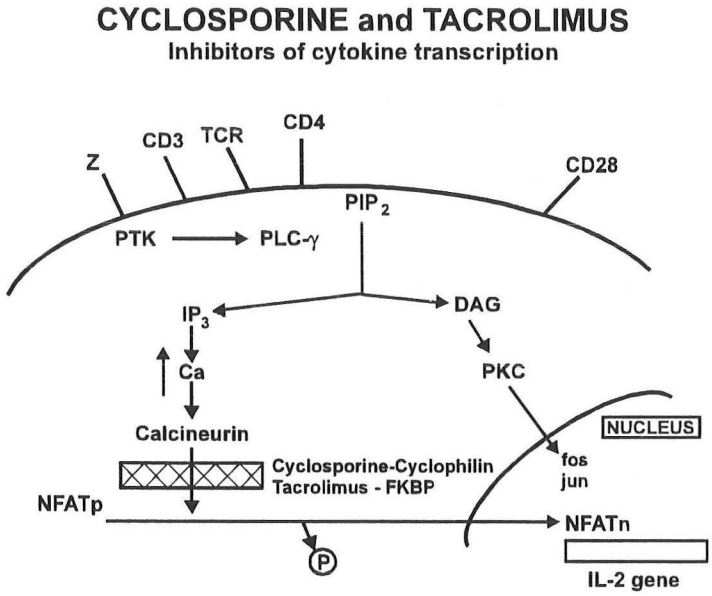


Figure 5

As previously outlined, T cell activation is initiated when the TCR binds alloantigen presented in the context of MHC (first signal) by an APC, and the T cell also receives an accessory signal by a cell surface molecule on an APC (second signal). The CD4 or CD8 molecule also binds to the MHC molecule. Conformation changes occur in the CD3 complex and zeta chain complex that transduce signals to the interior of the cell and that result in phosphorylation and activation of a series of protein tyrosine kinases (20-22,35) (please see Figure 5). Two principal effector pathways are activated: PLC and Ras-map kinase.

PLC γ (phospholipase C) lyses the membrane lipid phosphatidyl inositol (PIP2) to release inositol triphosphate (IP3) and diacylglycerol (DAG). DAG activates PKC (protein kinase C), which stimulates synthesis of the transcription factors jun and fos, which form AP-1. IP3 raises cytoplasmic calcium, which activates the calcium calmodulin-dependent phosphatase termed calcineurin. The removal of phosphate groups from NFATp (nuclear factor of activated T cells) allows NFATp to translocate into the nucleus and bind to promoter regions in the gene for IL-2, ultimately leading to IL-2 secretion (23,28). NFATp binds to promoter sites in the genes of several other cytokines, including IFN γ and IL-4. NFAT binding to IL-2 cytokine gene promoter sites occurs as part of a larger complex called NFATn, which also requires binding of additional transcription factors such as AP-1 (activator protein-1) formed by jun and fos protein dimers (22).

The complex of cyclosporine-cyclophilin or tacrolimus-FKBP binds to calcineurin and inhibits its phosphatase action (24,25). As a consequence, activation of IL-2 gene transcription does not occur and there is no T cell division or differentiation. NFAT is only found in T cells, which accounts for the specificity of cyclosporine and tacrolimus to inhibit T cell activation.

CsA may also exert part of its immunosuppressive action by stimulating production of transforming growth factor-ß (TGF-ß) (26). Among the properties of TGF-ß are inhibition of IL-2 stimulated T cell proliferation, inhibition of generation of cytotoxic T cells, and stimulation of tissue fibrosis (27). TGF-ß may be the mediator of immunosuppression and of fibrosis associated with the use of cyclosporine (26,27).

Cyclosporine

The introduction of cyclosporine has been one of the most important landmarks in the field of transplantation. Cyclosporine has made it possible to perform transplants of kidneys, livers, hearts, pancreas, and small bowel with predictable survival of the allograft for most patients (30). Cyclosporine has also been a successful agent in the treatment of numerous autoimmune disorders. The advances in understanding the mechanisms of action of cyclosporine is a prime example of the dynamic interaction between clinical transplantation medicine and basic immunology (1).

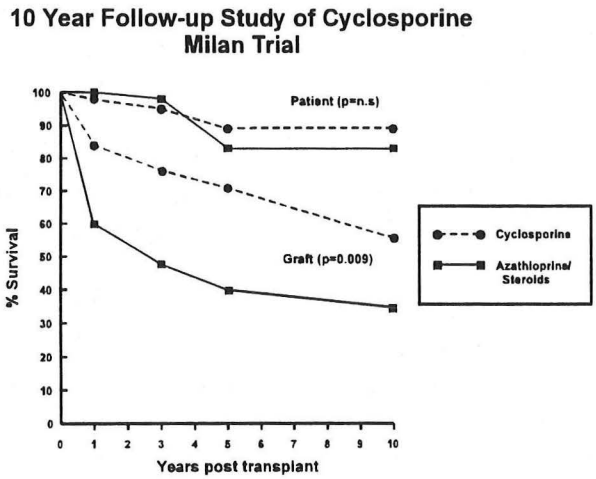
ONE-YEAR RENAL ALLOGRAFT SURVIVAL

Immunosuppression	Cyclosporine A	Azathioprine and Steroids	p
Canadian Trial	80%	64%	0.003
European Study	72%	52%	0.001

Adapted from: *N. Eng. J. Med.* 309: 809, 1983.
Lancet 2: 986, 1983.

Table 2

Cyclosporine is a cyclic endecapeptide derived from *fungus imperfectus*, and its immunosuppressive properties were discovered by Borel in 1972 (68). Several studies in the early 1980s reported better short-term renal graft survival for patients receiving cyclosporine. Table 2 is a summary of the results of the Canadian Multicentre Trial (31) and the European Multicentre Trial Group (32). In the Canadian Multicentre Trial, 232 cadaveric renal transplant recipients were randomized to receive cyclosporine and steroids vs the standard therapy at that time, which was azathioprine and steroids. Graft survival at one year was superior (80.4%) for the cyclosporine group than for the standard therapy group (64%). In the European Multicentre study, patients were randomized to cyclosporine monotherapy or to azathioprine and steroids. The one-year graft survival was better for the cyclosporine group (72%) than for the azathioprine/steroids group (52%).



Adapted from
Ponticelli et al., *Transplantation* 45: 908, 1988
J Am. Soc. Nephrol 7: 792, 1996

Figure 6

Recently, Ponticelli et al. have published data comparing 10-year renal allograft survival for patients treated with cyclosporine and patients treated with azathioprine and steroids (33). Figure 6 combines the short-term, intermediate and long-term follow-up results of a cohort of 108 patients who received

cadaveric renal transplants in Milan in 1983 (33,34). The allograft survival was significantly superior for cyclosporine treated patients at 1, 3 and 10 years. The number of rejection episodes was much higher for patients who were not receiving cyclosporine. There was a tendency for a longer half-life of grafts functioning after one year for those patients on cyclosporine, although not statistically significant. Patient survival and the incidence of serious side effects was similar for both groups.

Although cyclosporine confers a benefit in allograft survival compared to standard immunosuppression with steroids and azathioprine, there is a progressive loss of allografts over time that has not been significantly altered by the use of cyclosporine (67). As noted in Figure 6, patients on cyclosporine therapy continue experiencing a progressive loss of grafts over time.

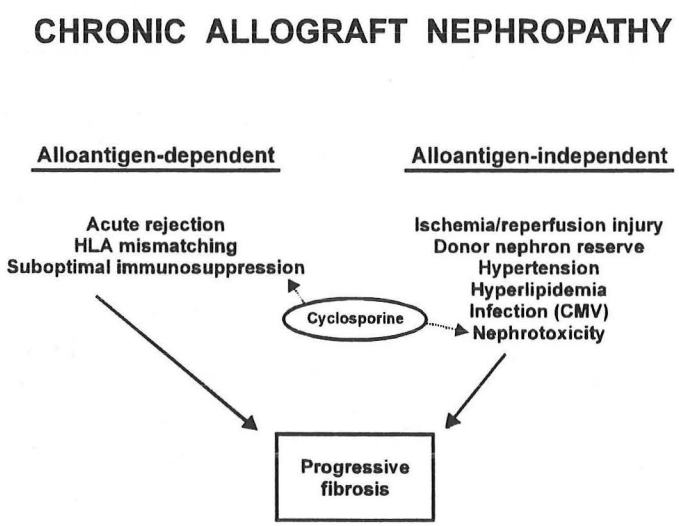


Figure 7

Most long-term losses of renal graft function are due to patient death or to chronic rejection. The term "chronic transplant rejection" or "chronic allograft nephropathy" is used to designate this entity, characterized by progressive narrowing of the arteries and arterioles, interstitial fibrosis, tubular atrophy, and glomerular sclerosis. Several excellent reviews have been written recently on this topic (36-41). A complete discussion of chronic allograft nephropathy is beyond the scope of this presentation. Figure 7 summarizes factors that have been associated with chronic allograft nephropathy or chronic rejection.

Alloantigen-dependent factors are those related to antigenic differences between donor and recipient and include previous episodes of acute rejection, HLA mismatching, and suboptimal immunosuppression. Alloantigen-independent factors include allograft injury from prolonged ischemia and/or subsequent reperfusion, suboptimal nephron mass (nephron dosing) from donor to recipient, hypertension, hyperlipidemia, infection (especially CMV),and nephrotoxicity.

The role of cyclosporine in chronic allograft nephropathy is not clear. Early after its introduction into clinical practice, it became apparent that cyclosporine carried a risk of acute and chronic nephrotoxicity (42,43). Withdrawal of cyclosporine is tolerated with stable renal function by some transplant recipients but may carry a significant risk of rejection and even allograft loss for some patients (44-48). Administration of cyclosporine has been shown to result in nephrotoxicity in recipients of non-renal solid organ transplants and in patients with autoimmune disorders (43). Cyclosporine can stimulate secretion of TGF-ß, which can lead to progressive fibrosis such as seen in chronic allograft nephropathy (26,54). Nevertheless, several studies have shown that many renal

transplant recipients tolerate long-term cyclosporine administration without progressive nephropathy (49-53).

Many investigators believe that many more kidney transplants are lost to rejection than to cyclosporine toxicity. Inadequate immunosuppression due to suboptimal exposure to cyclosporine has been associated with the development of acute and chronic rejection and with renal allograft loss (55-58).

Cyclosporine A Microemulsion

A serious limitation to the clinical use of the conventional preparation of cyclosporine has been its poor absorption and large variation in bioavailability, ranging from around 20-50% (mean of about 34%) (59). Cyclosporine is a cyclic polypeptide, neutral and highly insoluble in water. The conventional preparation is available as an olive oil based liquid and a corn oil based gel cap (please see Figure 8). Absorbtion of the conventional preparation of CsA through the GI mucosa occurs in the upper intestine and requires digestion by pancreatic enzymes and emulsification by bile into hydrophilic particles (60). Many factors affect the absorbtion of cyclosporine, including the administration of food, volume and content of bile, bowel motility, and GI tract intramural cytochrome P450 activity (58). Variations in these factors explain the high degree of intraindividual and interindividual variability in cyclosporine absorbtion.

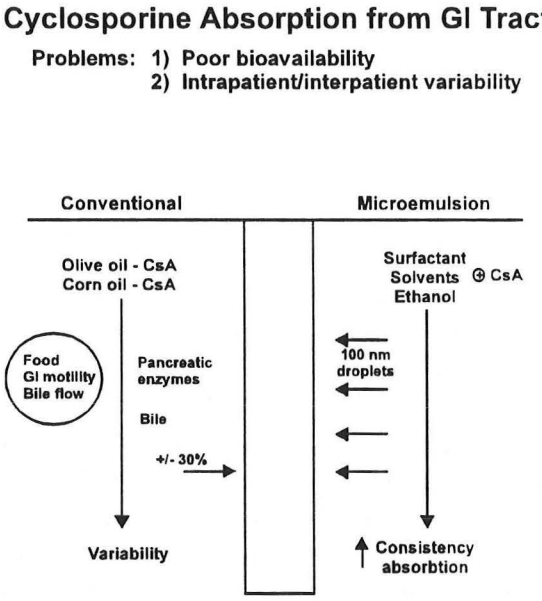


Figure 8

A new oral formulation of cyclosporine as a microemulsion was approved by the FDA in 1995. The microemulsion preparation of cyclosporine incorporates the drug in a preconcentrate that contains surfactant, lipophilic and hydrophilic solvents, and ethanol (60). When the microemulsion comes in contact with aqueous fluid in the intestinal tract, it immediately forms a transparent emulsified solution of droplets smaller than 100 nm in diameter and ready for absorbtion without the need for bile or pancreatic enzymes.

Several studies have compared the pharmacokinetic profiles of the stable renal transplant patients receiving the conventional CsA and microemulsion CsA preparations. The Canadian Neoral Renal Transplantation Study Group recently reported the findings of a prospective,

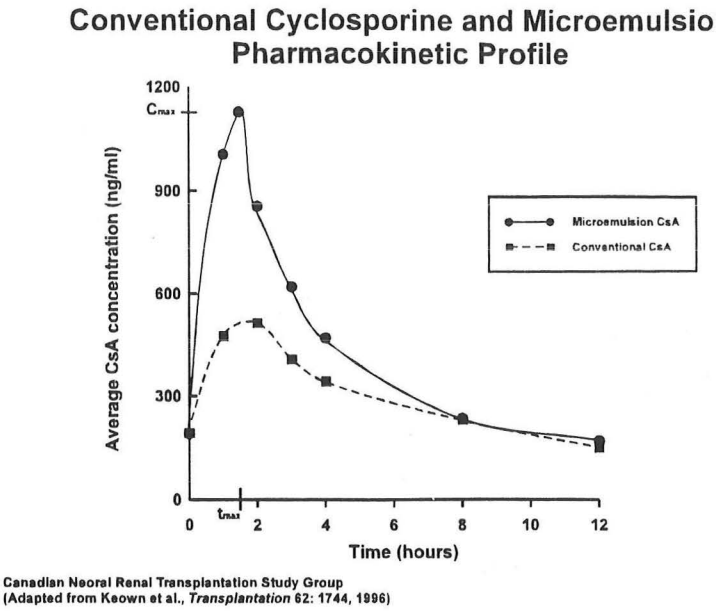


Figure 9

concentration-controlled pharmacoepidemiologic study comparing the conventional preparation of CsA with the new microemulsion preparation (61). In this study, 1097 stable renal transplant patients were randomized (2:1) to treatment with microemulsion or conventional CsA and followed for six months. Figure 9 shows a whole blood 12-hour concentration profile performed during the third month of the study. Doses of conventional and microemulsion CsA were adjusted to maintain similar trough levels (concentrations at time=0 hours or time=12 hours) at all times and were comparable for both groups. Although there was no significant difference in trough levels, there was a significant increase in exposure to cyclosporine as represented by the area under the curve of the concentration-time curve (AUC) for patients receiving the microemulsion preparation of CsA. The AUC remained higher for patients treated with microemulsion CsA after adjustments were made for the dose of CsA used. The adjusted values for AUC (0-12 hours) were 3525±961 ng.hour/ml for microemulsion CsA and 2556±788 ng.hour/ml for conventional CsA. The maximum concentration of CsA (Cmax) was higher for microemulsion CsA (1126 ng/ml) than for conventional CsA (627 ng/ml). The time to reach the maximum concentration of CsA (Tmax) was shorter for patients receiving microemulsion CsA (1.5 hours) than for those receiving conventional CsA (2.8 hours). Intrapatient and interpatient variability was reduced for patients receiving microemulsion CsA compared with conventional CsA.

Two recently published studies have compared the pharmacokinetics of microemulsion CsA and conventional CsA in *de novo* renal transplant recipients. The microemulsion CsA preparation showed higher bioavailability as evidenced by a higher dose-normalized AUC. Intrapatient variability of pharmacokinetic parameters was lower with microemulsion CsA, and there was a stronger correlation between the pre-dose trough concentration and the AUC (62,63). No significant differences in the incidents of adverse events have been observed between microemulsion CsA and conventional CsA in these studies despite the increased bioavailability of microemulsion CsA (61). Although the rates of acute rejection have been similar for both groups, fewer patients in the

microemulsion CsA groups have required monoclonal antibody therapy due to severe and resistant rejection (64).

Studies currently in progress may help elucidate if the use of microemulsion CsA with its improved bioavailability and less variability can result not only in less severe episodes of acute rejection but also in lower risks of chronic rejection and consequently bring improved long-term renal allograft outcomes. The possibility of worsening of chronic cyclosporine nephrotoxicity due to increased exposure to CsA will also have to be studied with long-term follow-up with patients treated with the microemulsion preparation of CsA.

Cyclosporine G (OG37-325)

Cyclosporine G, or norvaline-cyclosporine, is a naturally occurring cyclosporine that differs from CsA in that the α-amino butyric acid residue in position II of the cyclic endecapeptide has been replaced by L-norvaline (19).

Some early studies had shown similar outcomes in patient and graft survival with CsA and cyclosporine G but less nephrotoxicity with cyclosporine G (65). Other studies noted similar nephrotoxicity for both preparations but more hepatotoxicity associated with cyclosporine G (66). Due to its lack of immunological advantage and concern about hepatotoxicity, cyclosporine G has been withdrawn from clinical use.

Tacrolimus (FK506)

Tacrolimus (formerly known as FK506) is a cyclic macrolide that was originally isolated from a soil actinomycete, and its immunosuppressive properties were discovered by Ochai in 1985 (68). Tacrolimus exerts its immunosuppression by binding intracellularly to immunophilins termed FKBPs (FK506 binding proteins) (69). The complex of tacrolimus-FKB12 binds and inhibits the activity of calcineurin in a manner analogous to cyclosporine-cyclophilin blocking dephosphorylation of NFAT and T cell activation.

Tacrolimus has been approved for use in liver transplantation and is associated with fewer episodes of acute rejection and similar patient and graft survival than cyclosporine (70). Studies from single institutions have reported good results with tacrolimus as primary immunosuppression for renal transplantation (71). Two recent prospective multicenter trials have compared tacrolimus and cyclosporine in recipients of cadaveric renal transplants (72-75).

The one-year results of the U.S. Kidney Transplant Multicenter Study Group are summarized in Table 3. A total of 412 cadaveric renal transplant recipients were prospectively randomized at 19 centers to receive tacrolimus (n=205) or CsA (n=207). The one-year patient and graft survival were similar for both groups. Biopsy-proven acute rejection occurred in significantly fewer patients receiving tacrolimus than CsA. The rejection episodes were less severe in patients receiving tacrolimus, and significantly fewer patients on tacrolimus required antilymphocyte therapy for acute rejection. Nephrotoxicity, malignancies, and infectious complications were similar in both groups. More patients developed posttransplant diabetes mellitus in the tacrolimus group. Tumors were also more frequent in the tacrolimus group.

FK506 U.S. MULTICENTER STUDY
Kidney Transplant Results at One Year

Immunosuppression	Tacrolimus (FK506) N=205	Cyclosporine A N=207	p
Patient Survival	96%	97%	ns
Graft Survival	91%	88%	ns
Acute Rejection	31%	46%	0.001
Antilymphocyte Therapy (for acute rejection)	11%	26%	<0.001

Adapted from Pirsch et al., *ASTP* Abst. 345, 1996

Table 3

Patients with acute rejection that is refractory to standard therapy with steroids and antilymphocyte preparations have poor graft survival and increased morbidity and mortality with repeat therapy. Tacrolimus has been used in patients with refractory rejection as "rescue" therapy. Table 4 summarizes the results of two recent studies that report good outcomes with tacrolimus rescue therapy for renal allograft rejection (76-78).

The University of Pittsburgh Transplant Group had initial access to tacrolimus and has had success with graft salvage in 74% of patients and 94% patient survival in patients with refractory rejection at most recent follow-up (76,77). Many of these patients (85%) had failed not only high doses of steroids but also antilymphocyte preparations. Some recipients that were dialysis-dependent due to severe rejection and patients who had vascular rejection had favorable responses to rescue therapy with tacrolimus. Average serum creatinine for responders with a mean follow-up of 30 months was reported as 2.3 mg/dl. Some patients were able to discontinue their steroids without adverse consequences.

TACROLIMUS AS RESCUE THERAPY
Results in Refractory Acute Rejection

Study	Pittsburgh Study N=169	Multicenter Rescue Group N=73
Median Follow-up	30 months	12 months
Graft Survival	74%	75%
Patient Survival	94%	93%
Mean Serum Creatinine	2.3 mg/dl	2.2 mg/dl

Adapted from Jordan et al., *Transplantation* 63: 223, 1997
Woodle et al., *Transplantation* 62: 594, 1996.

Table 4

The Tacrolimus Kidney Transplantation Rescue Study Group has similarly reported excellent outcomes with graft salvage in 75% of patients and 93% patient survival (78). Good long-term renal function was reported with a mean serum creatinine at one year of 2.2 mg/dl.

The results of these two trials have generated much interest in the use of tacrolimus as therapy for acute rejection. Due to the limited therapeutic alternatives for patients with refractory rejection, neither of these two trials involved randomization with alternate treatments.

A recent study has reported successful resolution of refractory rejection with low doses of tacrolimus and no adverse effects (79). Tacrolimus has not been effective in the treatment of established chronic renal allograft rejection (115).

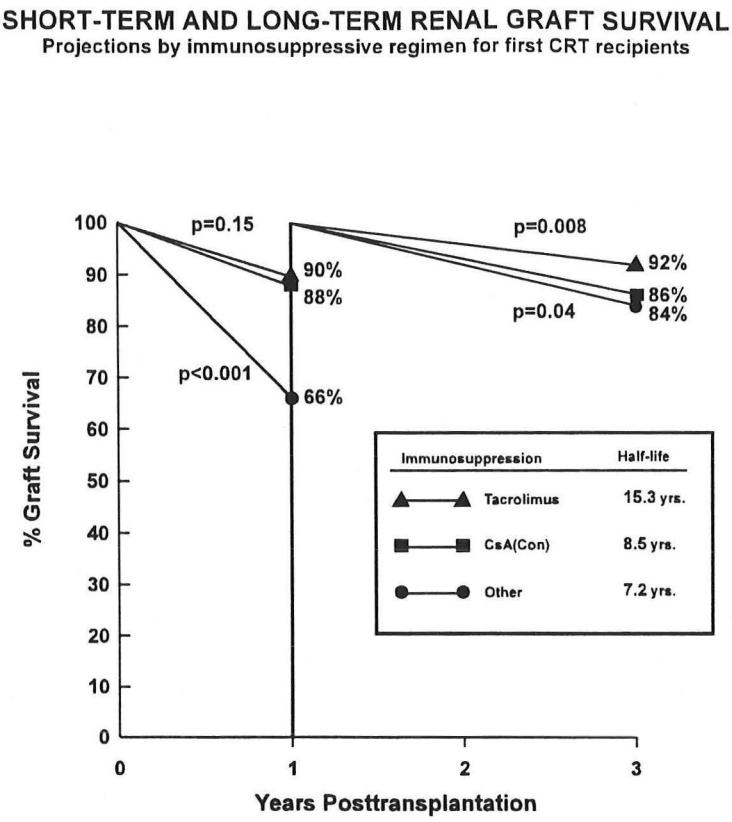


Figure 10

As mentioned earlier in this presentation, the significant advances in one year renal allograft survival rates observed after the introduction of CsA diminish over time, and the rates of graft loss after the first year are similar for CsA and previous immunosuppressive regimens. Tacrolimus may have a beneficial effect on long-term renal graft survival. Figure 10 summarizes the report from Gjertson et al. comparing the effects of CsA and tacrolimus in short-term and long-term graft survival (67). The report is based on the outcomes of 38,057 first cadaveric renal transplants performed from 1988 through 1994 at 224 centers that performed at least 10 renal transplants per year. The immunosuppressive therapies at discharge from the hospital (for grafts surviving more than 15 days) were based on CsA, tacrolimus or other (no cyclosporine, no tacrolimus). Graft survival at one year, three years, and long-term (as defined by half-life beyond one year for those kidneys functioning at one year) was determined. Figure 10 is adjusted for demographic variables previously reported to influence graft survival (recipient variables, donor variables, and transplant variables). The adjusted projections for one year kidney graft survival are better for tacrolimus (90%) and for CsA (88%) than for other therapies (66%). Among the kidneys that survived the first year, the adjusted three year graft survival rate under tacrolimus therapy (92%) is significantly better than for CsA (86%) or other immunosuppression (84%). The adjusted half-lives are projected as 15.3 years for tacrolimus, 8.5 years for CsA, and 7.2 years for other therapies. Possible factors contributing to the better long-term graft survival with tacrolimus may include lower rates of acute rejection and less requirement for corticosteroids (110). It needs to be emphasized that there were relatively few patients on tacrolimus in this cohort (544 out of 38,057 patients) and that patients on CsA were receiving the conventional formulation and not the microemulsion. Nevertheless, this report suggests that the use of tacrolimus may result in significant improvements in long-term renal allograft survival. Follow-up studies will be necessary to determine the long-term efficacy and safety of tacrolimus as compared to CsA microemulsion and other therapies.

Side Effect Profile of Tacrolimus and Cyclosporine

	Tacrolimus	Cyclosporine
Infections	↑	↑
Malignancies	↑▲	↑
Nephrotoxicity	↑	↑
Neurotoxicity	↑↑	↑
Hyperglycemia	↑↑	↑
GI disturbances	↑↑	↑
Hypertension	↑	↑↑
Hyperlipidemia	↑	↑↑
Cosmetic changes	?	↑↑

Table 5

The toxicities of tacrolimus and cyclosporine are very similar. Table 5 outlines some of the principal side effects associated with tacrolimus and how they compare with cyclosporine-based regimens. The occurrence of infections and malignancies is more related to the overall intensity of immunosuppression than to the use of tacrolimus or cyclosporine itself. An increased incidence of lymphoproliferative disease was initially noted in pediatric recipients of renal transplants treated with tacrolimus but has been less common as lower doses of tacrolimus have been used (79). Nephrotoxicity is seen with either tacrolimus or CsA. Neurotoxicity is more common with tacrolimus. The incidence of new onset diabetes mellitus posttransplant is higher with tacrolimus and has been described in up 19% of patients in some series (75). Gastrointestinal disturbances are more common with tacrolimus. Hypertension and hyperlipidemia are more commonly seen with CsA. Cosmetic changes such as hirsutism and gingival hyperplasia are uncommon with tacrolimus.

II. INHIBITORS OF GROWTH FACTOR SIGNAL TRANSDUCTION: SIROLIMUS (RAPAMYCIN)

Sirolimus (formerly known as rapamycin) is a cyclic macrolide antibiotic produced by the actinomycete *Streptomyces hygroscopicus* and with immunosuppressive properties discovered in 1977 (68). Sirolimus also binds to FKBPs, but differently from tacrolimus and cyclosporine, sirolimus does not inhibit calcineurin. The sirolimus-FKBP complex exerts its immunosuppressive action by interaction with an effector protein termed RAFT (rapamycin and FKBP target) (80,81). Sirolimus prevents cell cycle progression from G_1 to S, even after T cell stimulation by cytokines.

The precise mechanism of action of sirolimus has not been fully elucidated. It blocks calcium-dependent and calcium-independent (CD28/B7 pathway) events during G_1 and the second signals derived by IL-2 and other cytokines (3,81).

Figure 11 expands on the previous description of T cell activation and illustrates possible sites of action of sirolimus (22,80). After stimulation of the T cell receptor (by peptide presented in the context of MHC) plus a second signal (such as CD28 stimulation by B7 in antigen-presenting cells), the T cell advances from G_0 (resting phase) to G_1 (activated). IL-2 and other cytokines present engage their own receptors and lead to activation of protein tyrosine kinases. PI3-kinase is activated and phosphorylates a membrane lipid, PI, leading eventually to activation of protein kinase B (PKB) (22). Although the steps are not clearly understood yet, interaction between the sirolimus-FKBP complex (SRL-FKBP) with a protein termed RAFT is associated with inhibition of p70S6 kinase

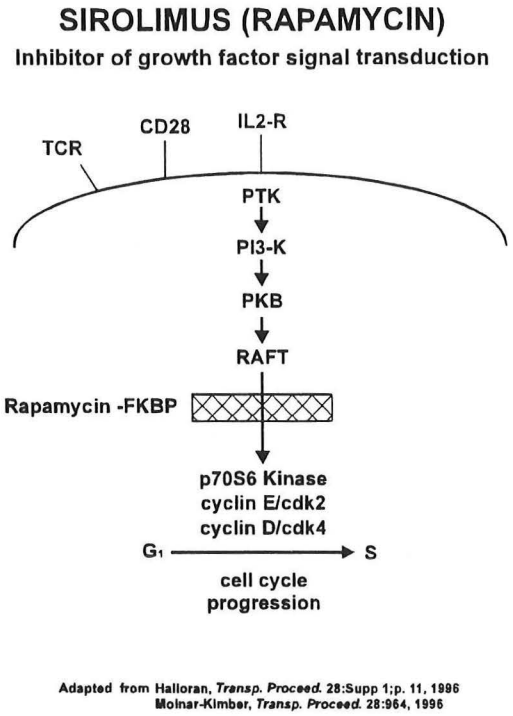


Figure 11

and prevention of the generation of the active enzyme complexes cyclin/CDK2 and cyclin D/CDK4. Inhibition of these factors and enzymatic complexes blocks the progression from G_1 to S in the cell cycle and the expansion of T cell clones.

Sirolimus may also interfere with T cell function at a different site. Stimulation via CD28 is calcium-independent and down-regulates IKB, an inhibitory protein that prevents the translocation of the transcription factor NF-kB from the cytoplasm. After CD28 signaling, NF-kB can move into the nucleus to initiate transcription of IL-2 and other cytokines. Sirolimus can prevent down-regulation of IkB, and there is persistent inhibition of NF-kB (116).

Sirolimus binds to a different immunophilin than CsA, and the combination of the two immunosuppressive agents offers the potential advantage of synergism without additive toxicity (83). Figure 12 summarizes the six-month results of a Phase II trial of sirolimus in renal transplant recipients performed by the Sirolimus Multicenter Study Group. A total of 149 patients were entered into the study and 98 completed the six months of the study. Patients were treated with two doses of CsA: full dose or half dose. A control group was treated with full dose CsA, steroids, and placebo, and had a rate of biopsy-proven rejection of 40%. Addition of sirolimus at a dose of 1 $mg/m^2/d$ or 3 $mg/m^2/d$ to full dose CsA resulted in a significant reduction in the rate of acute rejection to 10% and 11% respectively (82).

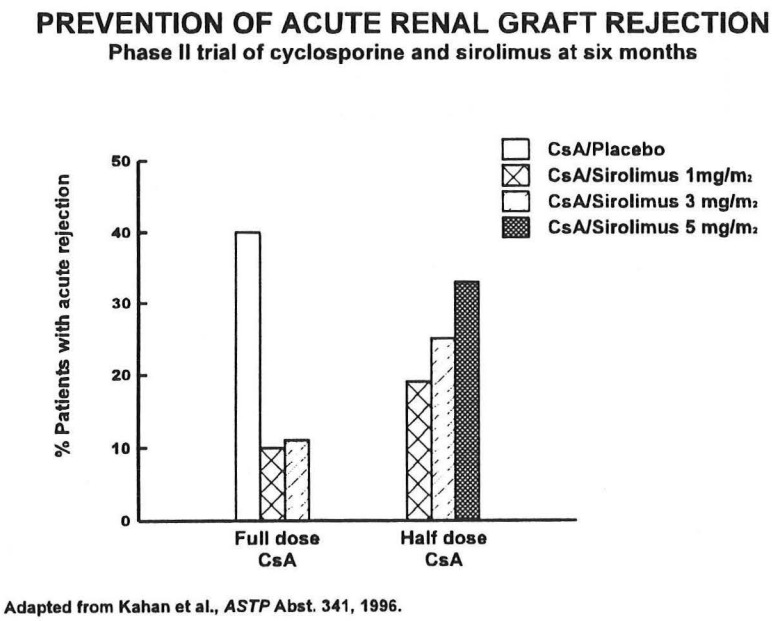


Figure 12

Among the group of patients receiving half dose CsA, sirolimus did not reduce the acute rejection rate significantly when added at 1 mg/m^2/d (19% rejection), 3 mg/m^2/d (25% rejection), or 5 mg/m^2/d (33% rejection).

The combination of sirolimus with CsA appears to provide synergistic immunosuppression with lower rates of acute rejection. Use of lower doses of CsA and/or reduction/discontinuation of steroids has been possible in some renal transplant recipients treated with sirolimus (81). There are case reports of sirolimus effectiveness in refractory acute renal allograft rejection (84).

Sirolimus has a different side effect profile than CsA (85). Nephrotoxicity, hypertension, and neurotoxicity have not been significant with sirolimus in early reports. Thrombocytopenia, leukopenia, and hyperlipidemia are more common in sirolimus-treated patients.

Multicenter studies now in progress should help define the role of sirolimus in renal transplantation.

III. INHIBITORS OF NUCLEOTIDE SYNTHESIS

Proliferation of lymphocytes requires purine and pyrimidine nucleotides for DNA synthesis. Several immunosuppressive agents exert their action by inhibition of nucleotide synthesis and preventing lymphocytes from entering the S phase (see Figure 13).

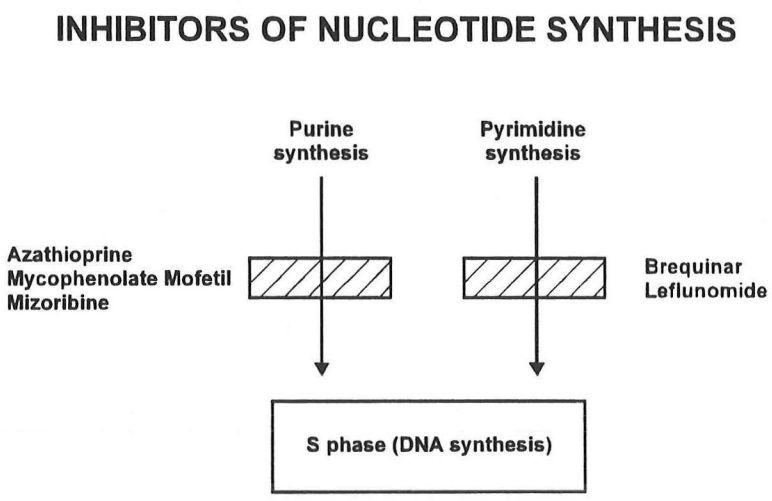


Figure 13

Azathioprine was the first immunosuppressive agent widely used in transplantation, and it interferes with purine synthesis. The new agents, mycophenolate mofetil and myzoribine, also interfere with purine synthesis. Brequinar sodium and leflunomide are inhibitors of pyrimidine biosynthesis.

Mycophenolate Mofetil (RS61443)

Mycophenolate mofetil (formerly known as RS61443) is a morpholinoethyl ester prodrug of the fungal antibiotic mycophenolic acid (MPA) (68). Figure 14 illustrates the two pathways of purine biosynthesis. Selection of MPA as an immunosuppressive drug originated from observations of the effects of hereditary abnormalities in the purine biosynthetic pathways (86,87). In the *de novo* pathway, purine bases are assembled from ATP and ribose 5-phosphate. Children with inherited deficiencies in adenosine deaminase (ADA), a key enzyme in the *de novo* pathway of purine synthesis, suffer from a severe combined immunodeficiency. They have an accumulation of adenosine and deoxyadenosine nucleotides compared to guanosine and deoxyguanosine nucleotides At the other extreme, children with another hereditary enzymatic deficiency, Lesch-Nyhan syndrome (HGRPTase deficiency), which

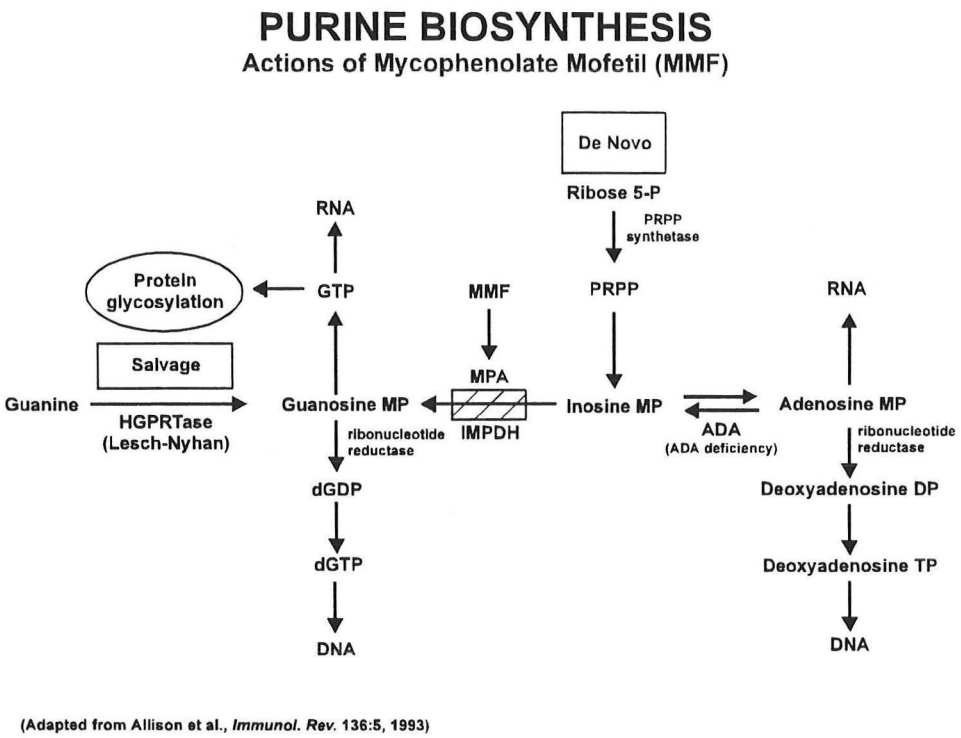


Figure 14

affects the salvage pathway of purine synthesis but not the *de novo* pathway, have a severe neurological disease but no immunodeficiency. In children with Lesch-Nyhan, the *de novo* pathway is functional and lymphocyte function is normal.

The observation that lymphocytes are markedly dependent on the *de novo* pathway and limited in their ability to use the salvage pathway led to the selection of MPA. Mycophenolate mofetil (MMF) is well absorbed in the GI tract and is rapidly metabolized to MPA, which is a potent, selective, non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), which is the enzyme catalyzing the first committed step toward the synthesis of guanosine nucleotides (87). Inhibition of IMPDH leads to depletion of guanosine nucleotides and dGTP required for DNA synthesis. The accumulation of adenosine nucleotides and depletion of guanosine nucleotides leads to a decrease in function of phosphoribosyl-1-pyrophosphate (PRPP) synthetase, which decreases the rate of the *de novo* purine synthesis. Furthermore, the accumulation of deoxyadenosine nucleotides inhibits ribonucleotide reductase, which generates dGTP for DNA synthesis. The net result of these actions of MPA is inhibition of DNA synthesis and therefore inhibition of proliferation of T and B lymphocyte clones responding to antigen stimulation (87).

Another possible effect of MPA is inhibition of protein glycosylation. GTP is necessary for the transfer of fucose and mannose residues to cell surface glycoproteins, which are ligands for selectins. MPA, via depletion of GTP, may interfere with the glycosylation of cell surface glycoproteins and the recruitment of lymphocytes and monocytes into sites of inflammation. This action would explain the efficacy of MMF in ongoing rejection (21,86,87).

MMF is more selective and potent against lymphocytes than other cell types due to the increased dependence of lymphocytes on the *de novo* pathway of purine biosynthesis as compared to other cell types. In addition, activated lymphocytes express a particular IMPDH isoform (Type II) which is more sensitive to MPA than the Type I isoform expressed in resting lymphocytes (87).

MMF can inhibit arterial smooth muscle cell proliferation *in vitro* as well as recruitment of mononuclear cells into vascular lesions of chronic rejection in animal models (87). It has been suggested that these actions of MMF may provide unique properties in preventing the development or progression of chronic rejection. In addition to inhibition of T and B cell proliferation, MMF also blocks generation of cytotoxic T cells and antibody production, especially in primary antibody responses (87).

MPA does not produce chromosome breaks like other inhibitors of nucleotide synthesis. MPA addition to *in vitro* culture systems has resulted in inhibition of proliferation of EBV-transformed B cells (86). These two properties could theoretically lead to reductions in malignancies in patients treated with MMF compared to some other immunosuppressive agents.

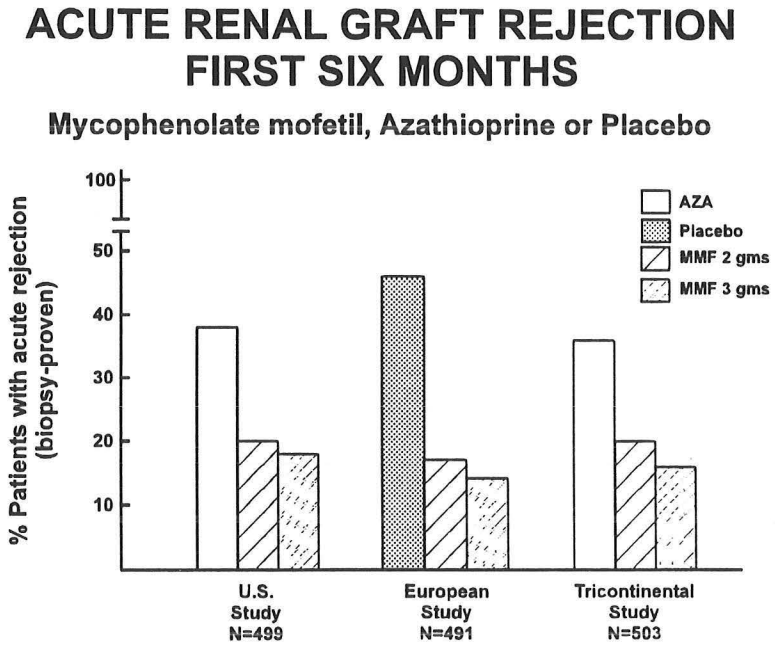


Figure 15

Three large multicenter clinical trials have examined the efficacy and safety of MMF for the prevention of acute cellular rejection in recipients of cadaveric renal transplants (see Figure 15). In the U.S. study (United States Renal Transplant MMF Study Group), 499 recipients of a first cadaveric renal transplant were randomized to receive azathioprine at 1-2 mgs/kgs/day, MMF 2 gms/day, or MMF 3 gms/day (90). All patients received steroids and CsA and had induction with antithymocyte globulin. Biopsy-proven rejection during the first six months posttransplantation occurred in 38% of patients receiving azathioprine, 19.8% of patients receiving MMF 2 gms, and 17.5% of patients receiving MMF 3 gms, for a

statistically significant difference between the azathioprine group and the MMF groups.

In the European study (European MMF Cooperative Study Group), 491 first or second cadaveric renal transplant recipients on an immunosuppressive regimen based on steroids and cyclosporine were randomized to receive placebo, MMF 2 gms/day, or MMF 3 gms/day (91). No antilymphocyte induction therapy was used in these patients. The percentage of patients with biopsy-proven rejection during the first six months was 46.4% for the placebo group, and 17% and 13.8% for the MMF 2 gms and MMF 3 gms respectively.

In the Tricontinental study (Tricontinental MMF Renal Transplant Study Group), 503 patients in Australia, Europe and Canada treated with steroids and cyclosporine were randomized to receive azathioprine, MMF 2 gms/day, or MMF 3 gms/day (92). No antilymphocyte induction treatment was used. Biopsy-proven acute rejection during the first six months occurred in 35.5% of patients in the azathioprine group, and in 19.7% in the MMF 2 gms group and 15.9% patients in the MMF 3 gms group.

The three multicenter studies with MMF also examined several other endpoints and the results were similar across the studies. Graft and patient survival at six months did not differ between patients receiving azathioprine (or placebo in the European study) and patients on MMF 2 gms or MMF 3 gms. Patients receiving azathioprine (or placebo) had shorter times for onset of a first episode of acute rejection, required more courses of antirejection treatment and antilymphocyte preparations, and had more severe rejections by histologic criteria (U.S. study and Tricontinental study).

MMF has been approved by the FDA for prevention of acute rejection and is part of the standard immunosuppression regimen in many centers now. An efficacy analysis of pooled data from the three multicenter studies after one year of follow-up has confirmed a reduction of acute rejection in patients receiving MMF 2 gms/day or MMF 3 gms/day (93). Most episodes of acute rejection occur early after transplant. The MMF trials have not shown any catch-up or increase in acute rejections after the early period. One year follow-up data has not shown any difference in graft or patient survival at one-year.

MMF has also been studied as therapy for acute refractory cellular rejection. The MMF Renal Refractory Rejection Study Group compared the efficacy and safety of MMF (3 gms/day) with high dose intravenous steroids (5 mg/kg/day x 5 days) in 150 patients with acute cellular rejection refractory to previous therapy, including administration of antilymphocyte preparations (94). Eligible patients were recipients of a first or second cadaveric renal transplant or living related donor kidney transplant. Patients were receiving steroids and CsA as part of their immunosuppression regimen. Patients randomized to intravenous steroids could remain on azathioprine. Patients could not be on dialysis or have serum creatinines more than 5 mg/dl for entry into the study. This was a six month, open label, randomized, multicenter trial with a 12 month postenrollment follow-up included. Table 6 summarizes the results of the trial. The primary endpoint was graft and patient survival at six months. Graft loss or death was 26% for the IV steroids group compared to 14% for the MMF group. This difference was not statistically significant. Subsequent rejection or treatment failure (premature termination for any reason, including death, graft loss or an adverse event) occurred in 64% of patients in the intravenous steroids group compared to 39% patients in the MMF for a significant difference. At 12 month follow-up, death or graft loss was more common for

TREATMENT OF REFRACTORY ACUTE CELLULAR REJECTION

Comparison of Steroids and Mycophenolate Mofetil (MMF)

Immunosuppression	IV Steroids N=73	MMF N=77	p
Graft loss or death (6 months)	26%	14%	0.062
Subsequent rejection/ treatment failure (6 months)	64%	39%	0.001
Graft loss or death (12 months)	32%	18%	0.042

(Adapted from MMF Refractory Rejection Study Group. *Transplantation* 61:722, 1996)

Table 6

patients treated with intravenous steroids (32%) than for patients treated with MMF (18%), a difference clinically and statistically important. MMF is now considered effective therapy for refractory acute cellular rejection and for prevention of subsequent rejections.

MMF has been well tolerated in the multicenter clinical studies (90-92,94). The most common side effects are gastrointestinal, including nausea, vomiting, and diarrhea. There has been a higher incidence of CMV tissue invasive disease (GI tract). Leukopenia, thrombocytopenia, and anemia do occur in some patients with administration of MMF. Posttransplantation lymphoproliferative disease has also been reported in patients receiving MMF, but its incidence is not significantly higher than with other immunosuppressive regimens. MMF 3 gms/day has slightly more toxicity than MMF 2 gms/day, and currently it is recommended to use 2 gms/day for most transplant recipients. Some high risk transplant patients may require a higher dose. It is not known at this time if prevention of acute rejection with MMF can be accomplished with its administration for a short period of time or if it should be administered indefinitely. A study to examine if steroids can be safely withdrawn in patients receiving MMF and CsA is currently in progress.

Azathioprine

The introduction of azathioprine in the early 1960s allowed for transplantation of kidneys from donors that were not genetically identical. Azathioprine is first converted to 6-mercaptopurine in the liver and then to thio-inosine monophosphate. There are several possible mechanisms of azathioprine induced inhibition of purine synthesis (1,68). Azathioprine inhibits the conversion of IMP to AMP and GMP. In lymphocytes, 6-mercaptopurine inhibits proliferation mainly by depletion of adenosine nucleotides (1). Several enzyme systems (IMPDH, PRPP-phosphorybosyl phosphatase and adenylosuccinate synthetase) are also inhibited by 6-mercaptopurine (68,86).

Most of the early immunosuppressive regimens in renal transplantation incorporated azathioprine (104). Before the introduction of cyclosporine, the addition of azathioprine to steroids resulted in higher rates of renal graft survival (111,128). Azathioprine is still used in some patients who received transplants many years ago. The addition of azathioprine to a CsA based immunosuppressive

regimen does not appear to result in significant improvements in long-term graft survival for the majority of patients (88,89).

Mizoribine

Mizoribine is an imidazole nucleoside which after phosphorylation acts as a competitive inhibitor of IMPDH and blocks the *de novo* synthesis of purines (68). It may inhibit DNA repair mechanisms. Mizoribine has been used in living related donor kidney transplantation in Japan with similar efficacy to azathioprine (112). Renal elimination requires dose adjustment for renal function (19). Mizoribine has not been actively studied in the United States.

Brequinar Sodium

Brequinar sodium is an inhibitor of dehydroorotate dehydrogenase (DHODH), a key enzyme in the *de novo* pyrimidine biosynthetic pathway (113). It may also have some inhibitory effect on cytidine deaminase in the salvage pathway (68). Brequinar has been reported to decrease the incidence of steroid resistant rejection (114). It has a narrow therapeutic index, causing thrombocytopenia and mucositis (113). It is not clear if brequinar will be further developed for use in transplantation.

Leflunomide

Leflunomide is a synthetic prodrug that is cleaved into its active metabolyte, A771726. It appears that at low concentrations leflunomide inhibits DHODH and the *de novo* pathway of pyrimidine synthesis, resulting in inhibition of lymphocyte proliferation (69). A771726 in high concentrations appears to inhibit tyrosine kinases associated with transduction of signals from growth factor receptors (3). Leflunomide appears to be relatively safe and is currently being studies in patients with rheumatoid arthritis (118).

IV. INHIBITORS OF CELL DIFFERENTIATION AND MATURATION

Deoxyspergualin

Deoxyspergualin (DSG) is a synthetic analog of spergualin, a product derived from a soil bacillus (95). It was initially derived as an antitumor agent but was found to have action as an immunosuppressive agent and to affect T cells, B cells, and antigen-presenting cells such as monocytes.

The mechanism of action of DSG has not been completely elucidated, but it appears that its immunosuppressive action involves binding of DSG to a chaperone protein, HSC70 (a member of the HSP family), to inhibit cytokine synthesis and antigen presentation. Figure 16 illustrates a possible mode of action of DSG via inhibition of the translocation of the transcription factor NF-kB (22,95). NF-kB is a member of the rel family of transcription factors, and its predominant form is a heterodimer composed of p50 and p65 subunits. In unstimulated cells, NF-kB is under the inhibitory control of IKB (inhibitor kappa B) in the cytoplasm. After cellular stimulation, IKB dissociates and NF-kB is translocated into the nucleus with the help of the molecular chaperone

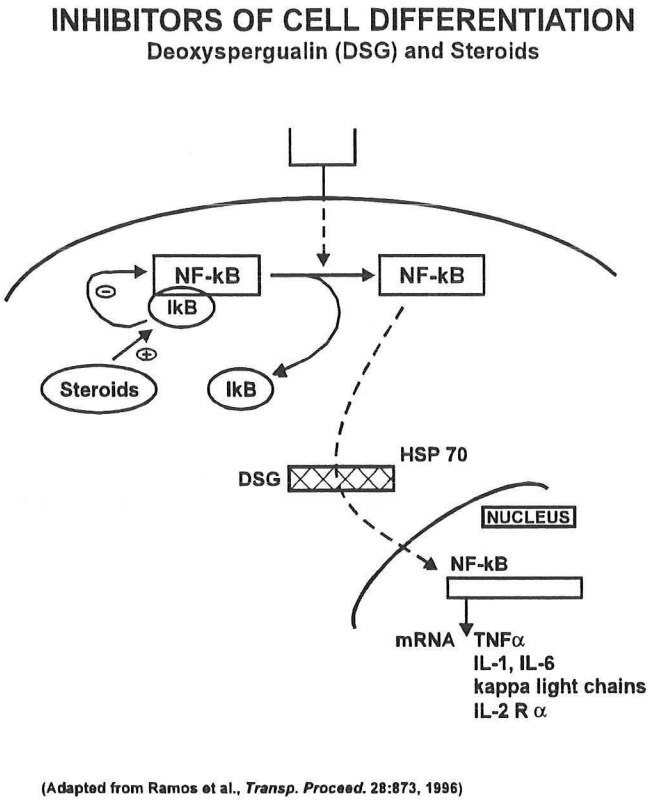


Figure 16

HSC70. Once inside the nucleus, NF-kB functions as a transcription factor. DSG appears to bind to HSP70 and prevents this chaperone molecule from facilitating the translocation of NF-kB into the nucleus. As a consequence, there is inhibition of transcription of TNFα, IL-1, and IL-6 in monocytes, immunoglobulin kappa light chains in B cells, and the alpha subunit of the IL-2 receptor in T cells (95). HSP70 may also play a role in antigen processing and presentation by APCs. Inhibition of the chaperone function of HSP70 by DSG could therefore interfere with antigen presentation by monocytes and other APCs (22,95).

The clinical efficacy and safety of DSG has been studied in several small trials. The Japan Collaborative Transplantation Study of DSG reported a 76% efficacy of DSG in the treatment of acute rejection within the first six months posttransplant (96). A randomized trial comparing DSG with a monoclonal antilymphocyte preparation (OKT3) for the treatment of steroid resistant rejection in 25 patients reported reversal of rejection (at least temporarily) in 58.3% of patients treated with DSG and 61.5% of patients treated with OKT3 (97). DSG is relatively well tolerated, and its main side effects are numbness of the face and limbs, gastrointestinal disturbances, and bone marrow suppression, including anemia, leukopenia, and thrombocytopenia. A large U.S. multicenter phase III trial of the safety and efficacy of DSG in the setting of acute renal transplant rejection was recently suspended. At this time it is not clear what will be the future of DSG as an immunosuppressive agent in renal transplantation.

Corticosteroids

Corticosteroids are synthetic derivatives of the adrenal hormone cortisol and not considered as xenobiotic agents. Nevertheless, given their capabilities to interfere with cell differentiation and maturation, they will be discussed in this section. Steroids have both immunosuppressive and anti-inflammatory properties.

There has been significant progress in understanding the mechanism of action of steroids. They bind a cytoplasmic glucocorticoid receptor and then translocate into the nucleus, where they can

block transcription of several genes. Steroids suppress IL-2 gene transcription, although the IL-2 gene does not have a glucocorticoid response element. The glucocorticoid receptor does interfere with AP-1 transcription factor and with the interaction between NFAT and AP-1 to initiate cytokine transcription (100,101). A recent finding explains the inhibitory actions of steroids upon several cytokines and cellular processes. Steroids induce transcription of the IKB gene (98,99). The inhibitory protein IKB prevents the interaction of the transcription factor NF-kB with its target genes and the cellular processes dependent on NF-kB are inhibited. Steroids exert inhibition of cytokine transcription, antigen presentation, the arachidonic acid cascade, and eicosanoid production, and affects expression of adhesion molecules (1). Recent studies have reported that corticosteroids can inhibit T cell mediated terminal maturation of dendritic cells (102) and induce apoptosis of peripheral blood T lymphocytes (103).

Steroids have been used in clinical transplantation for many years. Studies from the azathioprine era (before the availability of cyclosporine) reported improvements in one year graft survival when steroids were used as part of the maintenance immunosuppressive regimen in selected patients (104,105). Both oral and intravenous steroids can reverse more than 70% of the initial episodes of acute rejection (106).

Given the multiple adverse effects associated with long-term use of steroids, there have been multiple trials to determine if steroids can be withdrawn (or avoided) from a CsA based immunosuppressive regimen. A retrospective review of first cadaveric renal transplant recipients in the Collaborative Transplant Study revealed higher patient and graft survival for patients who had their steroids discontinued (110). A meta-analysis of seven randomized trials of steroid avoidance or withdrawal early after transplant (first three to six months) reported an increase in the risk of acute graft rejection but similar patient and graft survival at two years (107). In a recent trial of elective withdrawal of steroids more than one year posttransplant, 26% of the patients who had their steroids discontinued experienced rejection, although no grafts were lost to rejection during the 14 month follow-up period (108). The Canadian Multicentre Transplant Study Group has reported long-term follow-up of a placebo randomized trial involving discontinuation of steroids (109). Although during the first two years of the study there was no difference in graft survival, the five year actuarial graft survival was 73% for patients receiving placebo compared to 85% for patients receiving steroids, a difference statistically significant. A recent trial of late steroid withdrawal (one to six years posttransplantation) has noted that, although patients did not experience definite acute rejection episodes upon withdrawal of steroids, they had an insidious rise in plasma creatinine compared to patients receiving steroids (111,157).

It appears that although steroids can be discontinued in a large proportion of patients, there is a higher risk of acute rejection short-term and perhaps of graft loss long-term that may not be apparent in studies with short follow-up. Several trials are now in progress to determine the safety of steroid withdrawal in regimens based on some of the new immunosuppressive agents.

BIOLOGICAL AGENTS

The biological immunosuppressive agents include polyclonal and monoclonal antibodies, and they constitute a group of drugs that can be effective preventing the development of an immune response or suppressing an ongoing immune process (131). Experimental evidence suggests that some antibody preparations can successfully induce a state of antigen-specific immunological unresponsiveness (tolerance) in the absence of chronic immunosuppression (130).

Antibody preparations can be directed against diverse targets. They can exert their action by interacting with cell surface receptors and leading to: (1) destruction of cells involved in the immune response; and (2) inhibition of antigen recognition, costimulation, and interaction with adhesion molecules (131).

Figure 17 illustrates sites of action of several of the antibodies currently available.

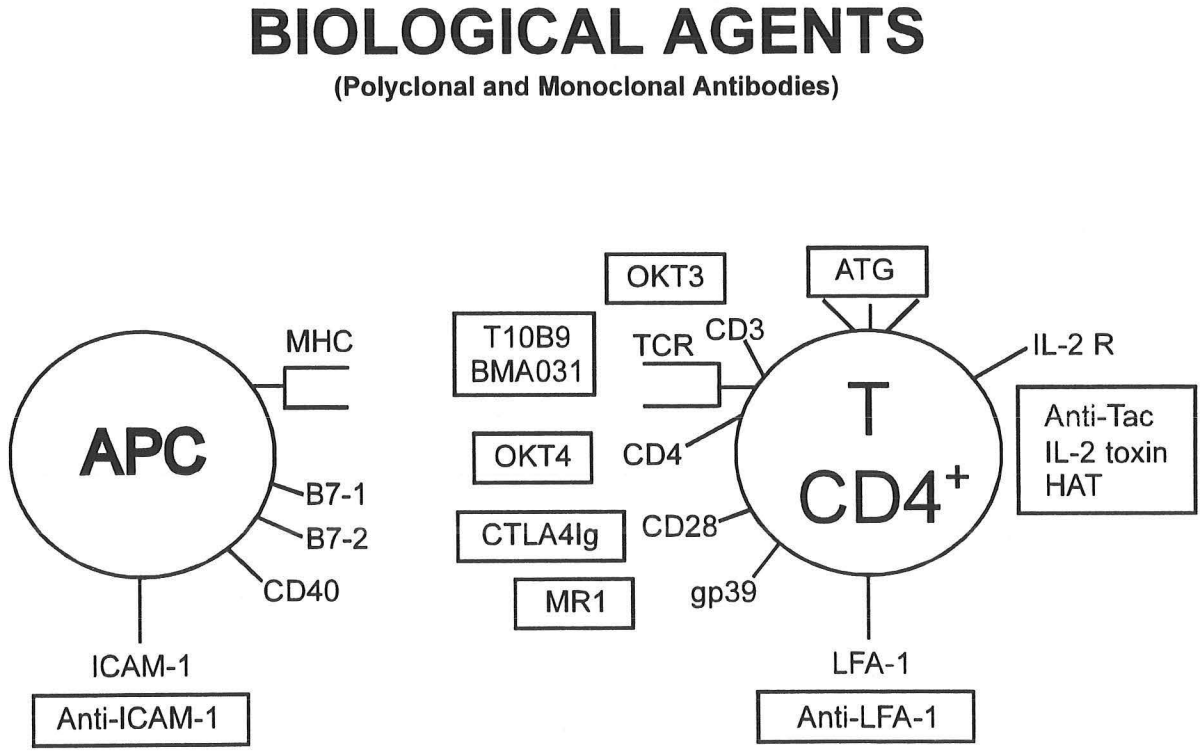


Figure 17

POLYCLONAL ANTIBODIES

Polyclonal antilymphocyte preparations interact with multiple cell surface proteins and were the first antibodies introduced into clinical transplantation (133). Their mechanism of action is not completely clear, but it likely involves destruction of T cells and neutralization (inactivation) of their functions (131). Polyclonal antibody preparations such as antilymphocyte globulin and antithymocyte globulin are effective in reversing first episodes of rejection and steroid-resistant rejections (132,156). Polyclonal antibody preparations are also effective as prophylactic agents against rejection and can delay the onset of rejection (154). Polyclonal antibody preparations have been associated with lower rates of delayed function as compared to other antibody preparations (153). The use of ALG has been associated with improvements in one year graft survival for African-American patients receiving steroids, cyclosporine and azathioprine in a single center report (152).

MONOCLONAL ANTIBODIES

Monoclonal antibodies are products of hybridoma technology and have the advantage of homogeneity in an antibody preparation of precise specificity.

Anti-CD3 Antibodies

The monoclonal antibody OKT3 is a murine IgG immunoglobulin that reacts with mature human peripheral T cells and was the first monoclonal antibody introduced into clinical practice (151). OKT3 recognizes the epsilon chain in the CD3 complex of the T cell receptor. The CD3 complex is essential for signal transduction after antigen recognition by the TCR (1). The mechanism of action of OKT3 initially involves cell marginalization and trapping by opsonization into the reticuloendothelial system. Subsequently, there is antigenic modulation of the CD3 complex, so that any T cells appearing in the peripheral circulation lack a functional TCR/CD3 complex (131).

In a large multicenter trial involving patients receiving baseline immunosuppression with steroids and azathioprine, OKT3 was found to be effective in reversing 94% of first episodes of acute renal transplant rejections compared to a rate of 75% rejection reversal with steroids (155). A retrospective review of patients receiving sequential quadruple immunosuppression with steroids, azathioprine, ALG and CsA noted that primary cadaveric renal transplant recipients had a 32% improvement in two year renal graft survival if they received OKT3 for primary treatment of a first rejection episode as compared to steroids (136). OKT3 has also been effective in reversing episodes of acute rejection resistant to therapy with steroids and reversing episodes of acute vascular rejection (134,135,146).

There has been considerable interest in the use of OKT3 for the prevention of a first episode of rejection. Opelz has reported a retrospective analysis from the Collaborative Transplant Study showing superior three year graft survival for first cadaveric recipients receiving OKT3 prophylaxis with sequential addition of cyclosporine (147). The benefits of OKT3 prophylaxis were especially apparent in patients who were presensitized (pannel reactive antibodies greater than 50%), black recipients, and pediatric recipients.

OKT3 PROPHYLAXIS

Rejection rates and long-term graft survival

Induction Therapy	OKT3 (N=159)	Cyclosporine (N=153)	p
Rejections per patient (1 year)			
All patients	0.83	1.21	0.002
Cold ischemia > 24 h	0.87	1.35	0.008
Graft survival (5 years)			
All patients	73%	66%	0.182
Cold ischemia > 24 h	71%	56%	0.045

(Adapted from Abramowicz et al. *Kidney Int.* 49:768, 1996)

Table 7

The results from the combined data of two prospective randomized trials of OKT3 prophylaxis vs early use of cyclosporine (in addition to steroids/azathioprine) are summarized in Table 7 (137). The number of rejections per patient was higher for patients in the CsA group. Graft survival at five years was similar when all patients in both groups were compared. Among the recipients with cold ischemia times greater than 24 hours, OKT3 prophylaxis resulted in fewer episodes of rejection per patient and significantly better graft survival at five years (71% for OKT3 vs 56% for CsA).

Several problems can be associated with the use of OKT3 and other murine antibodies. Patients may develop human antibodies against the mouse antibody (1). The use of recombinant DNA technology to manufacture "humanized" antibodies in which the hypervariable region of the antibody molecule (from rodent origin) is joined to human variable and constant regions may help circumvent this problem (129). A capillary leak syndrome, or "first dose reaction," can occur after the first administration of OKT3 due to cytokine release from T cells and monocytes crosslinked by OKT3 via the Fc receptors (1). Alterations in the Fc regions of the anti-CD3 antibody so that they cannot react with Fc receptors on monocytes may help eliminate this problem.

Infections and malignancies can be seen with the use of polyclonal and monoclonal antibodies due to their potent immunosuppressive properties. Several studies have compared OKT3 and polyclonal lymphocyte preparations, and their effectiveness appears to be similar (138,139).

Anti-T Cell Receptor Monoclonal Antibodies (Alpha/Beta Chains)

Monoclonal antibodies against monomorphic epitopes in the alpha/beta chains of the TCR have also attracted interest in transplantation. T10B9 is an anti-TCR monoclonal antibody that has shown similar effectiveness to OKT3 in treatment of acute rejection (149). BMA031 is another murine monoclonal antibody against the alpha/beta chains of the TCR effective in reducing early episodes of acute rejection posttransplantation (148). There has been a high degree of sensitization against these antibodies. At this time, it does not appear that anti-TCR antibodies directed against the alpha/beta chains will replace OKT3.

Anti-CD4 Antibodies

There have been several trials with antibodies against the CD4 molecule, which recognizes MHC Class II-antigen and also functions as a coreceptor for the TCR/CD3 complex (131). OKT4A is a murine anti-CD4 antibody that has been used in a few transplant recipients and has been well tolerated (129). Trials with anti-CD4 antibodies will be closely followed in the next few years.

Anti-IL-2 Receptor Antibodies

Activated T cells express a high affinity IL-2 receptor complex in their surface, which is composed of at least three protein subunits: IL-2Rα (CD25, Tac), IL-2Rß and IL-2Rγ (144). The fact that only activated T cells express high affinity IL-2 receptor has stimulating enormous interest in targeting this activation marker. 33B3.1 (anti-Tac chain antibody) showed similar effectiveness to OKT3 in prevention of acute rejection (150). The effectiveness of 33B3.1 in ongoing acute rejection has been more limited (129). The clinical limitations of murine monoclonal antibodies such as anti-Tac molecules are likely related to inefficiency of murine antibodies fixing human complement, recognition of different epitopes in the IL-2 receptor, and low affinity of the antibody for the IL-2 receptor (1,131).

Chimeric constructs of IL-2-toxins can be very effective in recognizing and destroying targets, but concerns about toxicity against other cells has prevented their use in transplantation (1).

Most of the recent interest has centered in the use of humanized anti-Tac antibodies (HAT). Early studies have found HATs effective as prophylaxis for rejection, well tolerated, and easy to use due to a prolonged half-life (144). There should be many reports on the use of HATs in the next few years.

Anti-Adhesion Molecule Antibodies

Adhesion molecules play a very important role in many steps of the immune response and inflammatory processes. Leukocyte function-associated antigen-1 (LFA-1) is expressed on T cells, and its main ligand is the intercellular adhesion molecule (ICAM-1) expressed on APCs and endothelial cells (135). Administration of BIRR1 (anti-ICAM-1 antibody) has been associated with a reduction in episodes of delayed graft function and rejection in patients at high risk due to high sensitization and prolonged preservation (140). Similarly, a multicenter trial comparing a monoclonal anti-LFA-1 antibody with a polyclonal antilymphocyte globulin as induction treatment noted a trend towards less need for dialysis (earlier recovery of renal function) in patients receiving anti-LFA-1. Certainly, the possibility of interfering with the immune response and the inflammatory response associated with peritransplantation injury/ischemia will stimulate more studies on the role of monoclonal antibodies against adhesion molecules.

Costimulatory Blockade (CD28:B7 and gp39:CD40)

As previously discussed, T cells require two signals for activation. Signal one is provided via the TCR with antigen recognition and provides for the specificity of the T cell response (141). Signal two is provided via a costimulatory molecule. Interaction of the cell surface molecules B7-1 or

B7-2 in APCs with their ligand CD28 on T cells appears to be the most important form of second signal (9,10).

CTLA4 is another cell surface protein in T cells which interacts with B7-1 and B7-2 but delivers a negative signal that leads to cell cycle arrest in T cells (141). CTLA4Ig is a protein made by the fusion of CTLA4 and IgG heavy chain and can block the interaction of CD28 with B7-1 and B7-2. In the absence of a second signal, the T cell develops anergy. Animal experiments have shown that administration of CTLA4Ig can prolong the survival of renal and cardiac allografts (141).

Activated T cells also express gp39, which serves as a ligand for CD40, present in APCs (142). Animal studies have shown increased expression of gp39 and CD40 during rejection. Administration of the monoclonal antibody MR1 (which blocks gp39) leads to prolonged survival of allografts. Furthermore, recent experiments have shown that simultaneous blockade of CD28-B27 (with CTLA4Ig) and gp39-CD40 (with MR1) can promote long-term survival of fully allogeneic skin grafts in mice and prevent the development of chronic vascular rejection of cardiac allografts (143). There have been no reports on the extension of CD28-B27 blockade or gp39-CD40 blockade experiments in rodents to human transplant recipients. Studies in this area may have enormous implications in transplantation immunology.

RENAL TRANSPLANT IMMUNOSUPPRESSION TODAY

The large number of immunosuppressive agents available for use in renal transplantation today offers the opportunity to employ diverse agents which act in different steps of the immune response. The selection of a particular immunosuppressive strategy for a given patient is complex and requires careful consideration of all the options available. All the immunosuppression agents available today, however, are relatively nonspecific, and their use carries the risk of immunodeficiency and other nonimmune toxicities (20). From a renal transplantation perspective, choices of immunosuppressive therapy have to be made in three different stages: induction, antirejection, and maintenance therapy. Induction therapy is applied early after transplantation and aims to reduce early episodes of rejection and optimize the opportunities for long-term graft survival. Antirejection therapy is intense and concentrated over a limited period of time with the emphasis on reversing a state of heightened immunological activity. Maintenance therapy is required for long periods, and its aim is to prevent rejection and damage of the allograft.

Figure 18 summarizes an approach to renal transplant immunosuppression at the present time. I realize that there may be many variations to this model and that constant revisions are necessary as the reports of clinical studies become available.

Most transplant recipients receive steroids and cyclosporine immediately before or after transplantation. Some centers substitute tacrolimus for cyclosporine. Induction therapy with polyclonal antibodies or monoclonal antibodies is effective but carries a higher risk of complications and is very expensive. It is usually reserved for patients at risk for delayed function and for high

IMMUNOSUPPRESSIVE STRATEGIES

Renal Transplantation

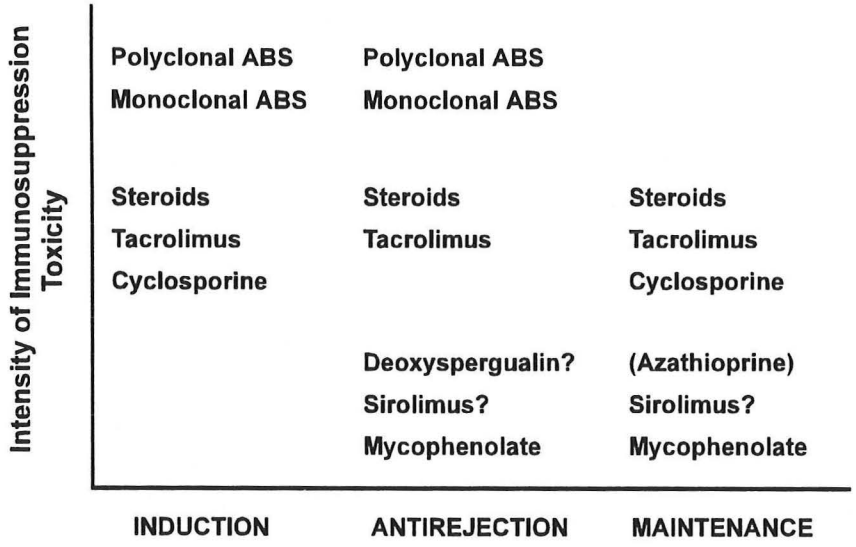


Figure 18

immunologic risk recipients, such as recipients of a repeat transplant or patients who are highly sensitized.

Steroids have been the first-line agent for the treatment of acute rejection for many years and are still considered the first choice in most centers. Polyclonal and monoclonal antibodies can be more effective for a first episode of acute rejection, but given the concern about their toxicity, they are usually reserved for steroid resistant episodes of rejection. Mycophenolate mofetil and tacrolimus have been shown to be effective in refractory episodes of rejection and are now considered as adequate alternatives in the setting of rejection. Deoxyspergualin may also be effective in the treatment of rejection, but it is not clear if it will be further developed for use in transplantation. Sirolimus offers promise as an anti-rejection drug, but the clinical data is still limited.

Cyclosporine or tacrolimus are the foundation of the maintenance regimen for most renal transplant recipients. Mycophenolate mofetil has rapidly gained acceptance in the transplant community and is now used as maintenance therapy for most new renal transplant recipients in the United States. Steroids are still used by most transplant centers in the United States, although many centers in Europe avoid steroids as part of their immunosuppression or discontinue them early after transplantation. There are several studies now in progress trying to determine how to best identify what patients can be safely treated without steroids. Azathioprine is part of the maintenance therapy of patients who received their renal transplants in the past, but mycophenolate mofetil has taken its place as maintenance therapy for new transplant recipients. Sirolimus has appeared to be very effective in early clinical trials, and the absence of nephrotoxicity makes it a very attractive agent for long-term use in renal transplantation.

The last four decades have witnessed the evolution of renal transplantation into one of the best examples of the progress that can be accomplished by the interaction of basic science and clinical medicine. Renal transplantation leads to successful outcomes for most patients. Patient and graft survival has progressively improved over the last several years. There are more choices in immunosuppressive therapy at the present time, and better drugs are available for the care of transplant recipients. Renal transplantation can increase not only the survival but also the quality of life for most patients. A successful renal transplant also results in cost savings to society compared to alternative forms of therapy.

Renal transplantation still faces many challenges. Transplant recipients still suffer from many short-term and long-term complications related to the use of immunosuppressive drugs and suboptimal allograft function. The number of patients on the waiting list for organ transplants continues to rise, and options to expand the donor pool and/or use alternative sources of organs such as xenotransplantation are actively being explored. Research into alternative forms of renal replacement therapy is active. The ultimate goal still remains the prevention of end stage renal disease.

I would like to return now to the first case I discussed earlier in this Grand Rounds presentation and provide some long-term follow-up. This transplant recipient, who received a living related donor kidney transplant from her identical twin sister, is now more than 30 years from her transplantation operation. She has normal renal function. She has never received any immunosuppressive agents and has never suffered from any transplant-related complications. This patient has achieved the ultimate goal of all transplantation immunosuppressive strategies: TOLERANCE.

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