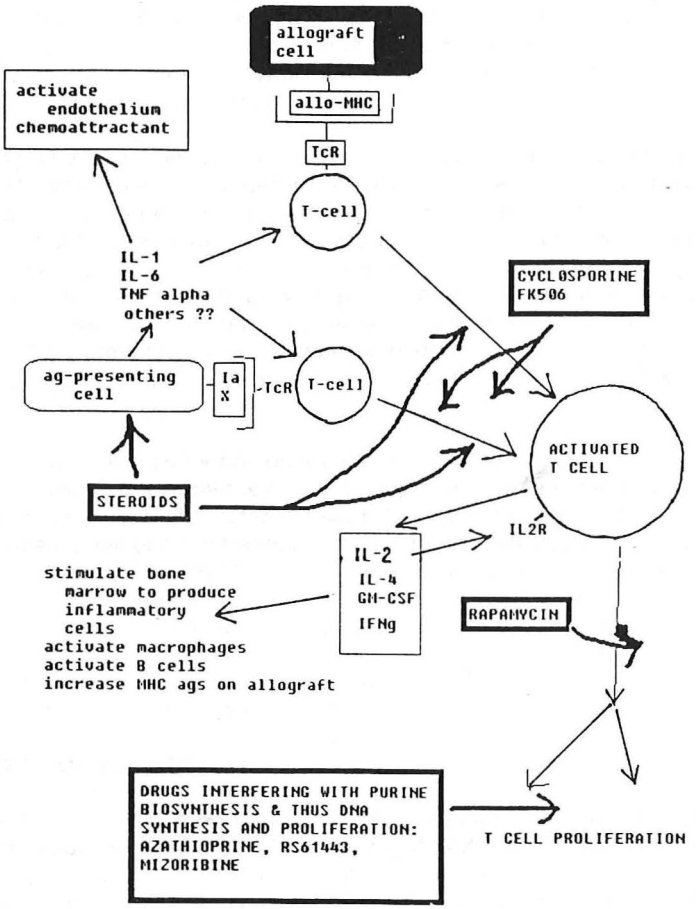
NEW IMMUNOSUPPRESSIVE THERAPIES IN RENAL TRANSPLANTATION



Medical Grand Rounds
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Associate Professor of Internal Medicine
April 16, 1992

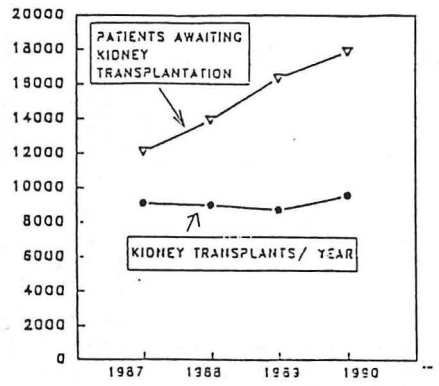
There is a dynamic interaction between clinical transplantation and basic immunology. On the one hand, new insights by basic scientists has resulted in new therapeutic agents which may totally revolutionize therapy in the next decade. On the other hand, in solving clinical problems, new insights into basic immunologic problems have emerged. My overall goal this morning is not only to tell you about new agents that may be part of the coming revolution in clinical transplantation, but also to share with you some of the excitment of what the search for these agents has taught us about basic immunologic processes.

I will begin by briefly reviewing the present state of renal transplantation. What do we do well? What could we do better? I will then move into the main part of this presentation and discuss new therapy and new insights into old therapy. First I will discuss drugs. I will discuss the major family of drugs that bind to immunophilins. This includes cyclosporine, one of the present mainstays of immunosuppressive therapy, and FK506 which has been well publicized in the lay press. Then, I will discuss new insights into the mode of action of steroids which continue to be important drugs for preventing and for treating rejections. I will also discuss drugs which inhibit lymphocyte proliferation. This includes the new agents RS61443 and mizoribine.

Second, I will discuss monoclonal antibodies. I will discuss the monoclonal antibody OKT3. This is presently the only FDA approved monoclonal antibody for use in humans. Furthermore, it illustrates many of the advantages and disadvantages of the use of murine monoclonals in treating human patients. Next, I will discuss the use of monoclonal antibodies specific for T-cell activation antigens, and the possibility of inducing antigen-specific tolerance with these non-specific agents. Then, I will discuss monoclonal antibodies against CD4 and theoretical advantages. Many of these monoclonal antibodies are currently in clinical trials. Finally, I will discuss exciting new work concerning the use of monoclonal antibodies against LFA and ICAM-1 which can induce tolerance in animal models.

PRESENT STATE OF THE ART.

Figure 1 makes two important points about renal transplantation in the U.S. First, transplantation is the preferred treatment of end-stage renal failure in those patients who are good surgical risks and able to comply with a chronic regimen of immunosuppressive medication. Many transplant recipients lead essentially normal lives. Women can have successful pregnancies. Men can be fathers. Over 10,000 patients receive renal transplants in the United States every year. The second point of Figure 1 is that the limiting factor in the number of patients who could benefit from transplantation is the availability of organs. No lecture to a general medical audience about transplantation is complete without a plea for your support in referring appropriate donors to your local Organ Procurement Organization or transplant team.



data for Fig 1 from Cate, F.H. & Laudicina, S.S. "Transplantation: White Paper - current statistical information about transplantation in America." compiled by The Annenberg Washington Program (Communications Policy Studies, Northwestern Univ.) and The United Network for Organ Sharing (The National Organ Procurement and Transplantation Network). 1991.

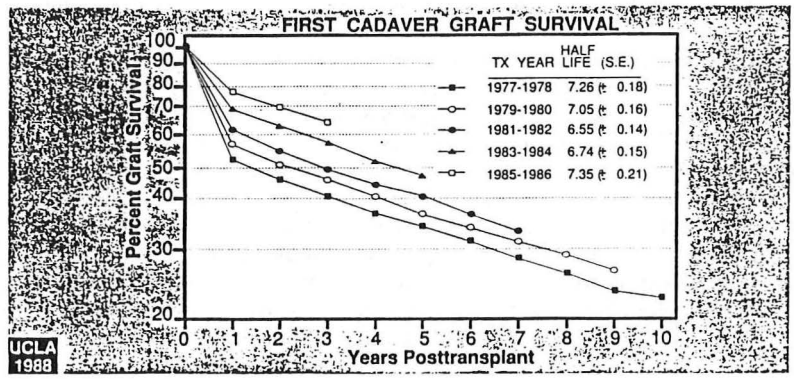
Figure 1

Part of the answer to the organ shortage problem is education of health care personnel.

Figure 2 shows the time after transplantation on the abscissa and the allograft survival on the ordinate (1). We can see that at the first year there have been marked improvements in renal allograft survival since 1977. Indeed survival has increased from 50% to approximately 80% in most centers. We are still losing approximately 20% of renal allografts in most centers during the first year. Furthermore, after the first year there continues to be a slow loss of allografts. This is linear and has not changed since 1977. Clearly, improved management of these patients would be extremely beneficial.

In addition, presently used immunosuppressive drugs, steroids, cyclosporine and various monoclonal antibodies have major serious side effects. Infection is a major complication. In particular, cytomegalovirus, pneumocystis, listeria, and tuberculosis are major, occasionally life-threatening, problems for these patients. In addition, certain malignancies are major problems for transplant patients. These include cancers of the skin--in particular basal cell and squamose cell cancers. Other major malignancies are B-cell lymphomas, perhaps caused by EBV virus. Such lymphomas occur in unusual places, such as the brain, lung, and GI tract. In addition, transplant patients have major problems with chronic active hepatitis, hyperlipedemia and accelerated atherosclerosis. These may be due to the present immunosuppressive regimens.

Although acute rejection can be reversed approximately 80% of the time,



Cadaver donor kidney graft survival of 5 cohorts since 1977. Transplants performed in every 2-year period showed a striking improvement in 1-year survival rates. It can be noted, however, that the subsequent loss rate of transplants has not been affected. When plotted on a natural logarithmic scale, the survival curves after 1-year posttransplantation were essentially linear. The half-life of 7.26 in 1977-78 does not differ from the 7.35 half-life of transplants performed in 1985-86. Cyclosporine (CsA), used in about 90% of the transplants during the latter period, did not influence long-term survival.

Figure 2

current therapy does fail in a significant number of cases. A core biopsy of the allograft is helpful in predicting which rejections will respond to currently available therapy. In general, a mononuclear interstitial infiltrate involving the tubules will usually respond to therapy. Inflammation involving blood vessels, including the glomeruli, is a bad prognostic sign. Endothelial disease is also a poor prognostic sign. Cyclosporine nephrotoxicity, at currently employed doses, has no pathognomonic feature, and will be discussed later. Kodachrome slides of representative core biopsies will be shown.

MECHANISMS OF RENAL ALLOGRAFT REJECTION

New immunosuppressive drugs are being sought to overcome the above problems with our current therapy. Before we can consider the mode of action of the newer immunosuppressive drugs, as well as new insights into the action of old immunosuppressive drugs, we need to consider the mechanisms of allograft rejection. Two different classes of T-lymphocytes are necessary for allograft rejection (2). One type of lymphocyte is the CTL or cytotoxic lymphocyte. An interaction of a CTL with its target cell is by itself not sufficient to result in lysis of the target cell. The CTL must receive accessory signals from a helper T-cell. These accessory signals include

interleukin 2, interferon gamma, and interleukin 6. After receiving these accessory signals, the CTL differentiates into a fully competent cell capable of killing the allograft kidney cell. The accessory signals are secreted by helper T-cells.

In Figure 3, we review the activation of the helper T-cell in greater detail. Shown at the top of the Figure, we see that the helper T-cell can interact directly with the allograft T-cell, receive accessory signals from antigen presenting cells, and become an activated helper T-cell. Alternatively, antigens from the allograft may be internalized by macrophages or dendritic cells. These antigens are then presented on the cell surface of the host macrophage in the context of IA or Class 2 major histocompatibility antigens. These then stimulate the helper T-cell. The relative importance of these two alternative pathways in the activation of helper T-cells is not well established at this time. Note, that in addition to presenting antigens to helper T-cells macrophages also secrete important accessory molecules, including interleukin 1, interleukin 6, and tumor necrosis factor alpha.

Note that two signals are necessary to stimulate helper T-cells (see review (3)). There is a cognate or antigen-specific interaction between the T-cell receptor and its specific antigen. There must also be stimulation of the T cell by accessory signals. The activated T-cell then secrete additional lymphokines, including interleukin 2, interleukin 4, GM-CSF, and interferon gamma. These lymphokines activate macrophages, activate B-cells, change the biology of the allograft, to make it more susceptible to rejection--i.e., increase MHC antigens on the allograft. In addition, the lymphokines stimulate bone marrow to produce more inflammatory cells. Thus, the activated T-cell performs many activities which coordinate the complex process of allograft rejection. In addition, the entire process is amplified when the activated T-cell proliferates.

DRUGS BINDING IMMUNOPHILINS: THE CYCLOSPORINES, FK506 AND ANALOGUES, RAPAMYCIN AND ANALOGUES.

A powerful class of immunosuppressive drugs bind to molecules called immunophilins. This class of drugs includes cyclosporine and FK506 which inhibits T-cell activation in response to stimuli delivered by the T-cell receptor. This class also includes rapamycin which inhibits further activation of the T-cells which are stimulated via their interleukin 2 receptor. The story of these drugs and how they work is an elegant example of the dialogue between clinical transplantation and basic immunology.

The first member of this family cyclosporine was discovered entirely by accident in 1970. Cyclosporine originally aroused interest because of its antibiotic activity. However, it was a big disappointment as an antibiotic. Instead of curing infected laboratory animals, it killed them. It turned out to be immunosuppressive. Rather than throwing cyclosporine away, Dr. Borell at Sandoz - Basel - astutely

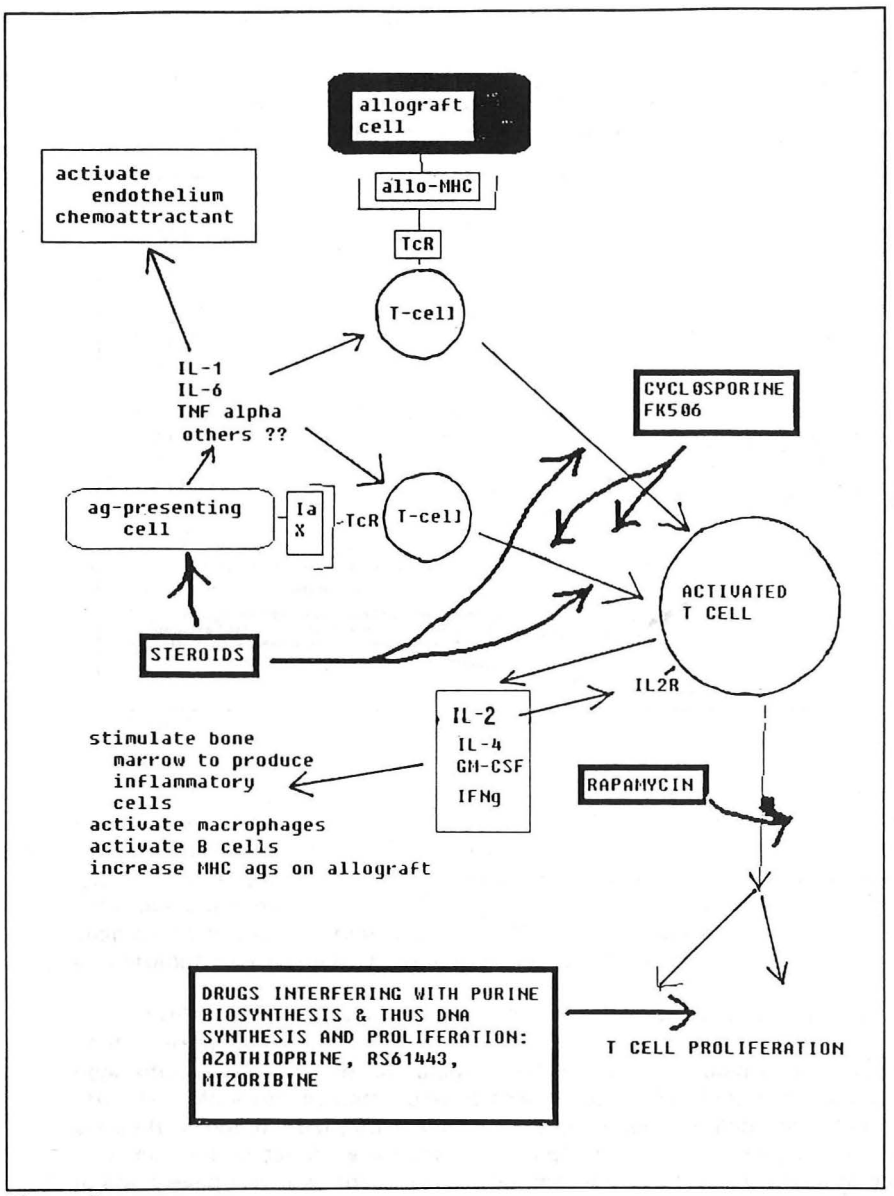
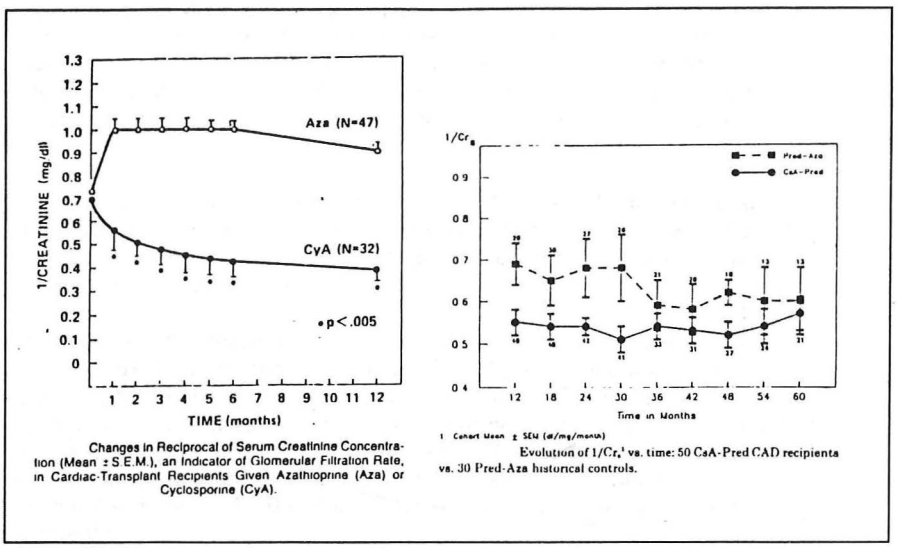

allograft cell
allo-MHC
TcR
T-cell
activate endothelium chemoattractant
IL-1
IL-6
TNF alpha
others ??
CYCLOSPORINE FK506
ag-presenting cell
Ia X
TcR
T-cell
ACTIVATED T CELL
STEROIDS
IL2R
IL-2
IL-4
GM-CSF
IFNg
stimulate bone marrow to produce inflammatory cells
activate macrophages
activate B cells
increase MHC ags on allograft
RAPAMYCIN
DRUGS INTERFERING WITH PURINE BIOSYNTHESIS & THUS DNA SYNTHESIS AND PROLIFERATION: AZATHIOPRINE, RS61443, MIZORIBINE
T CELL PROLIFERATION

Figure 3

realized its potential as an immunosuppressive agent (see review (4)).

Many clinical trials have compared cyclosporine and prednisone protocols versus the previously standard protocol of azathioprine and prednisone. These have all shown advantages to using cyclosporine (see review (5-7).



Changes in Reciprocal of Serum Creatinine Concentration (Mean ± S.E.M.), an Indicator of Glomerular Filtration Rate, in Cardiac-Transplant Recipients Given Azathioprine (Aza) or Cyclosporine (CyA).

Evolution of $1/Cr_s$ vs. time: 50 CsA-Pred CAD recipients vs. 30 Pred-Aza historical controls.

Figure 4

Despite its clinical utility, there are major problems associated with cyclosporine. For one it is nephrotoxic. It is generally agreed that cyclosporine may cause acute nephrotoxicity by causing vasospasm of the afferent arteriole to the glomerulus. The may result from release of endothelin or thromboxane by endothelial cells damaged by cyclosporine. Why these particular endothelial cells are so susceptible to cyclosporine is unclear. Decreasing the cyclosporine dose may result in a prompt improvement in renal function (see review (8,9)).

Whether cyclosporine causes chronic nephrotoxicity remains an important controversial question. See Figure 4. Brian Myers at Stanford found chronic nephrotoxicity leading to end-stage renal failure in recipients of heart transplants (10,11). More recent data suggest that cyclosporine does not cause progressive allograft failure in most patients who have good serum creatinines at approximately one year and whose dose is kept at 3-4 mg/ kg (12). In those patients who cannot tolerate even this low dose of cyclosporine, the addition of azathioprine has been helpful in some cases (13). It is of note that cyclosporine does increase collagen

deposition in vitro (8), but the importance of this in vivo remains to be established.

Cyclosporine also causes hyperkalemia, hypertension, hyperuricemia and in rare patients causes a hemolytic uremic syndrome. In addition, cyclosporine at high doses may be toxic to the liver, causes neurologic abnormalities, gingival hyperplasia and gastrointestinal symptoms (14). There are, in addition, profound interactions with drugs (15). Of particular note is rifampin (16). It is common for renal transplant patients to require rifampin for treatment of acute tuberculosis. Such patients may require a five-fold increase in their cyclosporine dose to compensate for hypermetabolism induced by rifampin. The addition of azathioprine may be necessary. Unless the clinician compensates for the effects of rifampin on cyclosporine metabolism, rejection will occur.

Another example of a drug interaction is that of fluconazole and cyclosporine. It is common for transplant patients to receive fluconazole for fungal infections. This may increase cyclosporine levels such that acute renal failure occurs with its associated life-threatening complications.

A point worth emphasizing is that cyclosporine can cause a Type IV hyperchloremic, hyperkalemic metabolic acidosis (17). This can occur in patients with normal creatinines after renal transplant. A common story is that the patient receives a successful transplant, feels great, makes excellent urine, and mistakenly thinks that he can eat all the potassium rich foods that he likes. The serum potassium goes to 7, and the patient can have a life threatening or fatal arrythmia. This complication is more common in patients receiving Beta blockers, ACE-inhibitors, or non-steroidal anti-inflammatory agents.

The problems with cyclosporine have led to a search to understand how it works so that new, more effective agents with less side effects can be discovered. I would like to illustrate some of our understanding of how the immunophilins work at the present time.

Table I

The Immunophilins:
CYCLOPHILIN(s) (18 kd others 19, 20, 31, 43, 80 dk) bind to cyclosporine
FK506 BINDING PROTEIN(s) (12 kd others 60, 27, 13 kd) bind to FK506 and rapamycin
Ubiquitous, but especially high levels in brain, thymus and kidney.
Ubiquitous - fungi to man
peptidyl prolyl cis-trans isomerase (rotamase)

Table I summarizes what we know about the immunophilins (18). These proteins are peptidyl, prolyl, cis-trans isomerase. Also called rotamase. This means they catalyze cis-trans conformations of peptides, perhaps allowing them to fold into their active

configurations. Proteins highly homologous to the immunophilins are present throughout nature from E. coli to man. These proteins are ubiquitously distributed, but are present at especially high levels in the brain, thymus, and kidney. A number of immunophilins are now known to exist. These can be divided into two families. One family of immunophilins bind to cyclosporine A and G. The major member of this family has a molecular weight of 18 kDa and has been cloned and sequenced. Other members are less well studied. These include proteins of 19, 20, 31, 43, and 80 kDa. The other family of immunophilins are the so-called FK506 binding protein (FK506BP). These bind to the immunosuppressive drugs FK506 and rapamycin. The major protein here has a molecular weight of 15 kD; other proteins are of molecular weights 13, 27 and 60 kDa.

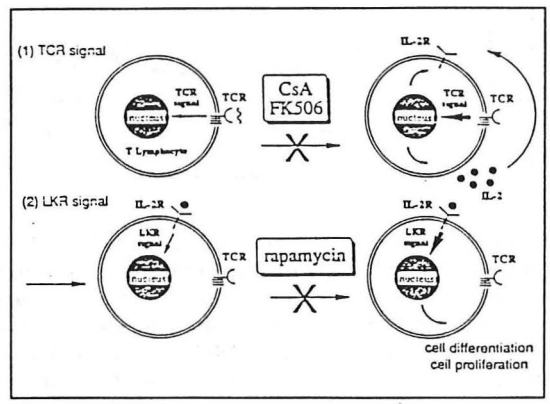


Figure 5

The discovery of the immunophilins led to the hypothesis that cyclosporine and FK506 and rapamycin inhibited T-cell activation by inhibiting the rotomase activity of these proteins. However, this hypothesis has major problems (18). One, the immunophilins are broadly distributed. Why does cyclosporine and FK506 act predominantly only on T-cells? Two, why will either cyclosporine or FK506 inhibit T-cell activation, when only one of two immunophilins is inhibited? Three, why are the concentrations of cyclosporine or FK506 required to inhibit T-cells far below the concentrations needed to saturate binding to the corresponding immunophilin? Four, why is there an imperfect correlation between an analogues' ability to inhibit immunophilin enzymatic activity and to inhibit T-cell activation? Five, rapamycin and FK506 both inhibit rotamase activity, but inhibit completely different steps in T cell activation (see Figure 5) (19).

A hypothesis which explains all the currently available data follows (18,20). As shown in Figure 6, drugs such as FK506 and cyclosporine actually have two domains. One domain binds to the immunophilin; the other domain is important in the inhibition of T-cell activation. The other part of this hypothesis states that the active immunosuppressive agent is not the drug but the drug immunophilin complex. See Figure 7. If FK506 binds to FK506 binding protein, the isomerase is inhibited, but the important fact is that the FK506-immunophilin complex binds to a, as yet unknown, component X. This inhibits the T-cell response after stimulation of the T-cell receptor.

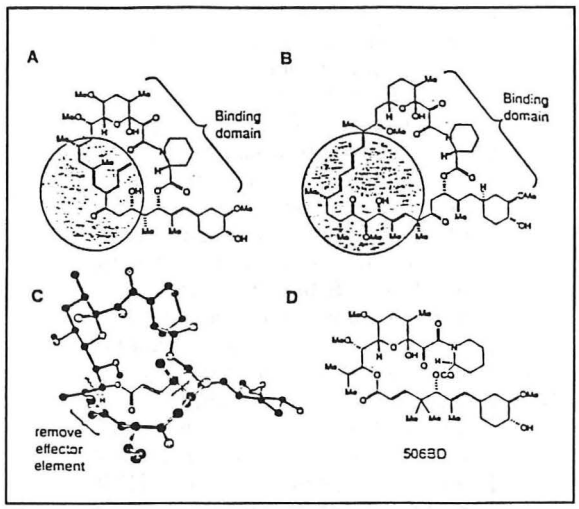


Figure 6

On the other hand when rapamycin binds to FK506 binding protein, it binds to the drug immunophilin complex binds to a different component. This inhibits the T-cell response to interleukin 2 but not to the T-cell receptor. Key to this hypothesis is the availability of an analogue called 506BD. This analogue binds to the isomerase, but does not have the domain necessary to allow the drug immunophilin complex to bind to either component X or Y and thus having immunosuppressive activity. This hypothesis makes several predictions. First, it predicts that 506BD should inhibit the effects of FK506 and rapamycin. It does this by binding the immunophilin and making it unavailable for formation of the FK506-immunophilin complexes which are inhibitory. Rapamycin, similarly, will bind up the immunophilin and make it unavailable for the formation of rapamycin-immunophilin complexes. In other words, FK506 should inhibit the suppressive effects of rapamycin on T-cell activation and rapamycin should, similarly, inhibit the immunosuppressive effects of FK506.

Component X is now known to be group of molecules made up of calcium, calmodulin, and calcineurin (21,22).

Figure 8A (23) shows an experiment supporting this hypothesis. Here we find that T-cell stimulation via a CD3 receptor is inhibited by FK506 but not by 506BD. However, when one adds increasing amounts of 506BD, the inhibitory effect of FK506 is reversed. Figure 8B (24) shows that FK506 reversese the inhibition of rapamycin, and that rapamycin and cyclosporine synergize. This is predicted by the hypothesis and has therapeutic implications.

The drugs binding immunophilin have also elucidated important aspects of the regulation of T-cell activation (18,25,26). Shown in Figure 9 is a cartoon of a T-cell. Activation of the IL-2 gene requires that a nuclear factor NF-AT bind to the five prime flanking region of the gene. Work with FK506 has demonstrated that NF-AT has two components: A and B. After the T-cell interacts with the T-cell receptor complex, tyrosine kinase is activated. This activates phospholipase-C-gamma. This, in turn,

activates protein kinase C. This causes the new synthesis of component A and its movement into the nucleus. Activation of phospholipase-C also causes the release of inositol polyphosphates. These cause an increase in intracellular calcium. This, in turn, causes component B to move into the nucleus. Components A and B then bind, and these then activate the interleukin 2 gene. Cyclosporine and FK506 prevent the movement of component B into the nucleus. This hypothesis also explains the specificity of cyclosporine and FK506 for T-cell activation. The nuclear binding factor NF-AT is found only in T-cells. In particular, the component which is unique to T-cells is component B.

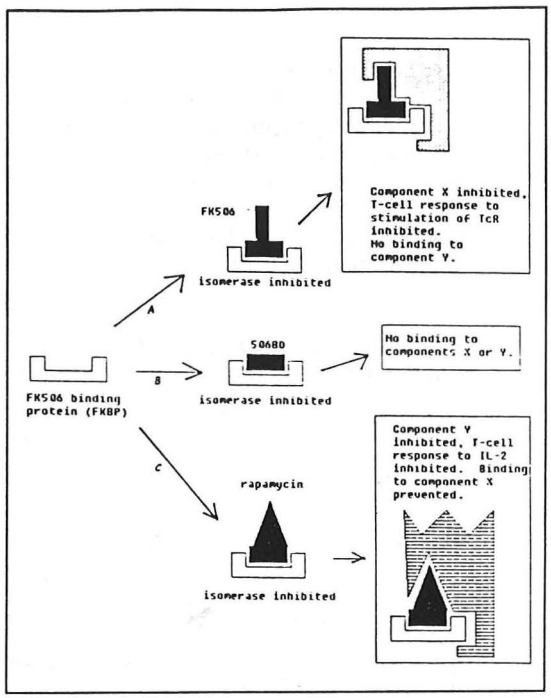


Figure 7

FK506 has undergone clinical trials particularly at the University of Pittsburgh in patients with liver and kidney transplants (27). It appears to be an effective immunosuppressive agent in these selected patients. Unfortunately, it also has marked nephrotoxicity, causes hyperkalemia. However, it seems to have far fewer hypertensive, and hyperlipidemia complications. Unfortunately, there are no randomized clinical trials comparing FK506 and cyclosporine, which is the standard clinical therapy nowadays.

Rapamycin has been used in experimental animals. Human trials using this agent will begin in the near future.

The University Transplant Program at Parkland is currently involved in a multi-center trial using cyclosporine G. This drug, which is closely related to cyclosporine A, has similar immunosuppressive activity but may be much less nephrotoxic. Obviously, this would represent a major step forward in the immunosuppressive therapy of these patients.

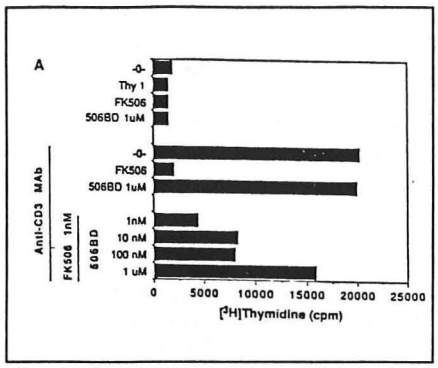


Figure 8a

STEROIDS.

Steroids remain important immunosuppressive drugs. They have significant inhibitory actions at a number of stages of allograft rejection. See Table 2. Steroids inhibit T-cell activation directly, and they act on the antigen presenting cell. They inhibit the ability of the antigen presenting cell to express IA and secrete lymphokines.

Although steroids and drugs binding immunophilins (cyclosporine, and FK506), both inhibit interleukin 2 gene transcription by T-cells, these drugs act by different mechanisms (28). Cyclosporine and FK506, as we have previously discussed, decrease the amounts of the nuclear factor NF-AT and AP-1. These nuclear factors NF-AT consisting of components A and B, and AP-1, consisting of the fos and jun proteins bind to the five prime flanking region of the IL-2 gene, and cause it to be activated. Cyclosporine and FK506 decrease the actual amounts of these nuclear binding proteins. Steroids, on the other hand, also inhibit the activation of the IL-2 gene. However, steroids do not decrease the amounts of either NF-AT nor the amounts of AP-1 in T-cells. Instead, steroids seem to prevent NF-AT and AP-1 from cooperating in a positive way with each other.

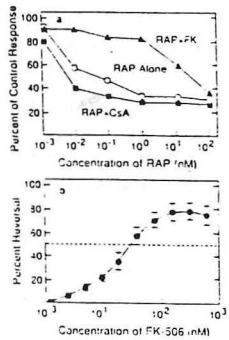


Figure 4 (a). Effects of FK-506 (311 nM) and CsA (208 nM) on the inhibition by RAP of the proliferative response of splenic T cells stimulated with IL-2 (100 U/ml) + PMA (10 ng/ml). Cultures were treated with various concentrations of RAP either alone (○) or in presence of FK-506 (▲) or CsA (■). Proliferation was measured by ^{3}H-TdR uptake at 48 h of culture. The control level of incorporation in cultures that received no immunosuppressant was 547.7 × 10^3 cpm. (b). Dose-dependency of the reversal by FK 506 of the inhibition of proliferation of IL-2 + PMA stimulated T cells induced by 1.1 nM RAP. By itself, this concentration of RAP caused 40 to 60% reduction of proliferation. The percent reversal of RAP inhibition was calculated as follows:

$$\% \text{ reversal} = 100 - \frac{\% \text{ inhibition RAP + FK-506}}{\% \text{ inhibition RAP alone}} \times 100.$$

Each point is the mean ± SEM of 5 experiments.

Figure 8b

Steroids also inhibit T-cell activation indirectly by their inhibitory effect on macrophages. See Figure 10 and 3. Steroids inhibit macrophage IA expression by inhibiting activation of the IA gene (29-31). Thus, after macrophages have ingested bits and pieces shed by allograft cells, these antigens cannot be presented to the T-cell. Recall that T-cell activation

requires that the T-cell receptor interact with the antigen in the context of IA. If steroids inhibit the expression of IA, they will also inhibit the ability of macrophages to present antigen 2 and activate T-cells.

T-cell activation also requires accessory signals in addition to the stimulus to the T-cell receptor (see Figure 3). Macrophages secrete many of these accessory signals which include interleukin 1, interleukin 6, TNF, and eicosanoids. Production of these accessory signals is also inhibited by steroids (32,33).

Table II

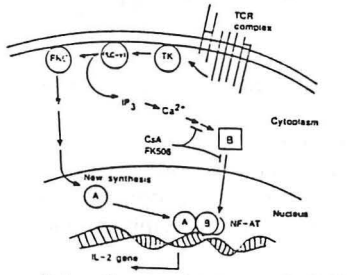
STEROIDS

- inhibit T-cell activation (inhibition of transcription of lymphokines)
- inhibit macrophage activities
  - expression of Ia (inhibition of transcription)
  - release of IL-1, TNFa (inhibition of translation)
  - inhibit eicosanoid release (? inhibition of phospholipase A2)

The ability of steroids to inhibit the macrophage production of TNF IL-1 and eicosanoids has further implications (34,35). A major event which occurs during allograft rejection is the infiltration of allograft tissue by inflammatory cells. See Figure 11. In other words, the inflammatory cells which are in the host's bloodstream must interact with the endothelium, such that the inflammatory T-cells, neutrophils, and monocytes translocate across the endothelium into the allograft tissue where rejection then occurs. The macrophage products TNF, interleukin 1, and eicosanoids act on allograft endothelial cells, such that they express signs, or molecules such as ICAM-1, and addressins. These molecules act as signs saying "Help needed here, exit," that cause the inflammatory cells to adhere to the endothelium and then to move across it.

Steroids do not inhibit activation of the genes for TNF and interleukin 1. Instead inhibition occurs at a post-transcriptional level. For TNF, translation is inhibited by steroids as demonstrated by Dr. Beutler at this institution (32). On the otherhand, steroids decrease the half-life of mRNA for interleukin 1 beta (33).

Steroids are widely used in transplantation (36,37). They are used as chronic maintenance therapy in North America. Many transplant centers in Europe use cyclosporine mono therapy, but over 50% of these patients are started on steroids after their first rejection. Steroids are also used to treat acute rejection. Perhaps their importance in preventing rejection is illustrated by the poor success, in many cases, of protocols that withdraw steroids early after transplantation. There are ongoing trials, which ask if steroids may be withdrawn late after transplantation .



T-cell signalling induces the appearance and activity of nuclear factor of activated cells (NF-AT). Two components of NF-AT combine to create the DNA-binding activity. The simplest hypothesis is that NF-AT is a DNA-binding protein that has two subunits, both of which are required for DNA binding, with one subunit being the pre-existing cytosolic component and the other being the induced nuclear component. Cyclosporin A (CsA) and FK506 block the translocation of the cytosolic component to the nucleus. It is not yet known whether these drugs block the action of a calcium-signalling pathway on the NF-AT component, or whether they block the ability of the component to translocate following action of the calcium signalling pathway, so arrows are shown for both of the possible steps at which these agents may act. TCR, T-cell antigen receptor; TK, protein tyrosine kinase; PLC-γ1, phosphoinpase C-γ1; PKC, protein kinase C; A and B, the nuclear and cytosolic components of NF-AT, respectively.

Figure 9

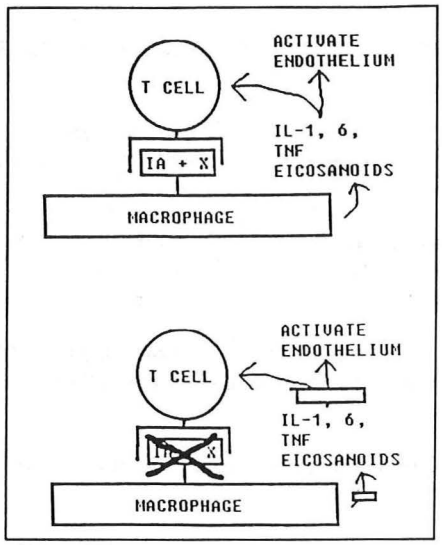


Figure 10

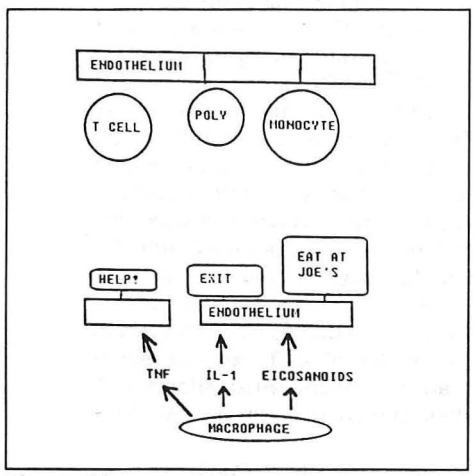


Figure 11

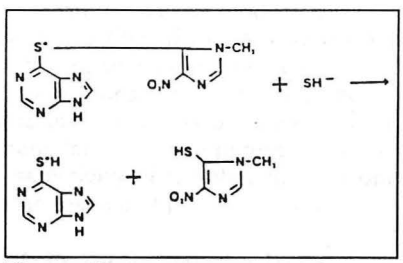


Figure 12

DRUGS INTERFERING WITH PURINE BIOSYNTHESIS AND THUS DNA SYNTHESIS AND LYMPHOCYTE PROLIFERATION.

Drugs interfering with purine biosynthesis have an important place in immunosuppression. Purines are important in the following cellular processes (38):

- as activated intermediates in biosynthetic pathways. For example: GDP-intermediates are important in glycoprotein production, including cell-surface receptors on lymphocytes which are important for their function - TcR, VLA4, etc. S-adenosylmethionine carries an activated methyl group.

- as metabolic regulators. GTP in regulation of G-proteins, cAMP as second messenger for many hormone activities. ATP alters protein activities by participating in their phosphorylation.

- adenine nucleotides are components of three major coenzymes: NAD +, FAD, CoA.

- ATP is the "universal currency of energy."

- adenine and guanine are precursors of RNA and DNA.

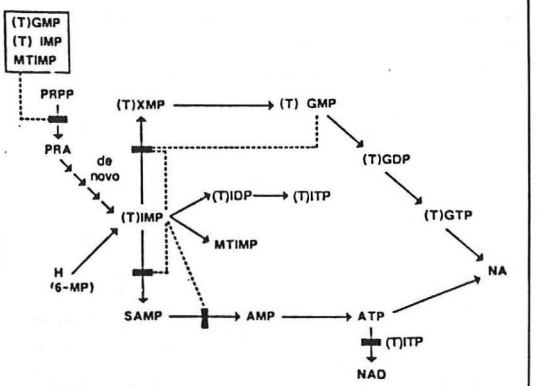
Interfering with one or more of these processes in lymphocytes would be expected to lead to immunosuppression.

Before discussing the new drugs RS61443 and mizoribine, I will discuss an old friend, azathioprine. The development of azathioprine resulted from work leading to understanding the purine and pyrimidine biosynthetic pathways. This work resulted in a Nobel prize in 1988 to George Hitchings, James Black, and Gertrude B. Elion. Two events must occur before azathioprine becomes an active immunosuppressive agent (39). First, as shown in Figure 12, azathioprine must be converted into its active metabolite 6-mercaptopurine. Indeed the advantage of administering the prodrug is that slow sustained release of active agent occurs as glutathione in red cells slowly convert azathioprine into 6-mercaptopurine. Second, as shown in Figure 13, the 6-mercaptopurine must be converted to thioinosinic acid by hypoxanthine-guanine phosphoribosyltransferase. The thioinosinic acid and its metabolites then inhibit multiple enzymatic steps leading to synthesis of adenosine and guanosine nucleotides. This should inhibit DNA synthesis as well as all other pathways involving these purines. Exactly which are important for immunosuppression is not known. The mechanisms underlying the greater sensitivity of lymphocytes, as opposed to other rapidly replicating cells such as gut epithelia and platelets is not known.

Successful pregnancies do occur in transplant patients taking azathioprine.

Although 6-mercaptopurine does cross the placenta, fetal tissues contain little hypoxanthine-guanine phosphoribosyltransferase and the drug cannot be be converted into its active agent thioinosine (37).

Two new drugs, which also inhibit purine synthesis, are now undergoing clinical trials in Japan and the U.S. These drugs both inhibit inosine monophosphate (IMP) dehydrogenase and thus prevent the synthesis of of guanosine nucleotides. Both drugs inhibit lymphocyte proliferation in vitro without inhibiting interleukin 2 production. The effects of the drugs are reversed by the addition of guanosine nucleotides (40,41). See Table 3. RS61443 is the morpholinoethyl ester of mycophenolic acid, the active agent (42). RS61443 is better absorbed by the GI tract than mycophenolic acid. Mizoribine is converted to its active monophosphate derivative intracellularly (43).



Pathways in the anabolism of 6-MP and loci of the nucleotides derived from 6-MP. In addition, (T)GDP is converted to d(T)GDP and d(T)GTP for incorporation into DNA. Abbreviations not in the text: PRA, phosphoribosylamine; (T)XMP, thio equivalent of xanthine monophosphate; (T)GMP, (T)GDP, (T)GTP, thio equivalents of guanosine monophosphate, diphosphate, and triphosphate, respectively; (T)IDP and (T)ITP, thio equivalents of inosine diphosphate and triphosphate, respectively; SAMP, adenylosuccinate; AMP and ATP, adenosine monophosphate and triphosphate; NAD, nicotinamide-adenine dinucleotide; NA, nucleic acid.

Figure 13

Table III

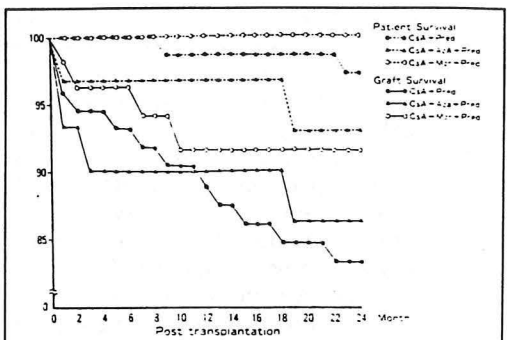
Table 3. Comparison of anti-proliferative agents sometimes used, or proposed for use, in transplantation

azathioprine
- inhibits purine metabolism. multiple effects including inhibition of both adenosine and guanosine production.
- relatively specific for lymphocytes and polys

inhibitors of inosine monophosphate dehydrogenase (RS61443, mizoribine)
- inhibits guanosine production.
- relatively specific for lymphocytes and polys
- no effects on IL-2 production, no effects on monocyte function.
- inhibitory effect on lymphocyte proliferation reversed in vitro by addition of GTP

Animal studies using these agents are extremely promising. There are no published human trials using RS61443, although data will probably be published in the upcoming meetings of the American Association of Transplant Surgeons and Physicians. Figure 14, and Table 4 (43) give data of a small trial comparing mizoribine against azathioprine. Overall the data suggest that the new agent is similar to azathioprine and has similar toxicity.

OKT3.



Actuarial graft survival rate of recipients under three different immunosuppressive treatments.

Figure 14

The T-cell receptor complex is actually a complex of the T-cell receptor and a five protein complex called the CD3 complex. The epsilon chain of the CD3 complex is the target for OKT3. This is is the only monoclonal antibody presently approved by the FDA for use in transplantations. OKT3 causes the disappearance of CD3 positive T-cells and later in its course, the disappearance of the entire CD3 T-cell receptor complex from the surface of T-cells. Obviously, if there are no T-cells able to see the graft because they don't have any T-cell receptors, rejection cannot occur. A series of injections of OKT3 causes the disappearance of CD3 positive cells, in fact all T-cells. T-cells which are CD2, CD4 or CD8 positive, but which do not have CD3 or T-cell receptors, then reappear (44).

The effectiveness of OKT3 was shown in a randomized multi-center study (45). The University Transplant Program at Parkland Memorial Hospital was a participant in this study. Patients either received OKT3 or three doses of methoprednisolone to treat acute rejection, as shown in Figure 15. Table 5 shows that 94% of the patients responded to OKT3 therapy, whereas only 75% responded to steroid therapy. The next Figure 16 shows that the OKT3 treated group continue to do better than the steroid treated group, even when followed for a period of over a year.

Despite its effectiveness OKT3 has major life threatening complications. It causes a capillary leak syndrome, probably because the T-cells are activated before they die. If the capillary leak is into the lungs, life threatening pulmonary edema can occur. All candidates for OKT3 treatment must be within 3% of their so-call best weight, and they must have a chest x-ray without failure, and absence of physical signs causing failure. The so-called first dose reaction is due to the release of lymphokines, particularly TNF-alpha (46). TNF-alpha is markedly increased in the

Table IV

Table 2. Incidence of complications within 3 months

Complication in living related recipients	CsA – Pred	CsA – Aza + Pred	CsA – Mzr + Pred
NO	58	15	42
Liver Dysfunction	8 (13.8%)	1 (6.7%)	9 (21.4%)
DM	7 (12.1%)	5 (33.3%)	2 (4.8%)
Leukopenia	3 (5.2%)	5 (33.3%)	3 (7.1%)
Hypertension			
2 weeks	30 (51.7%)	6 (40.0%)	25 (59.5%)
3 months	28 (48.3%)	7 (46.7%)	24 (57.1%)
Infection			
Bacterial	4 (6.9%)	1 (6.7%)	1 (2.4%)
Bacteruna	12 (20.7%)	1 (6.7%)	1 (2.4%)
Viral	11 (19.0%)	4 (26.7%)	15 (35.7%)
GI-complication	2 (3.4%)	0	1 (2.4%)
Nephrotoxicity	7 (10.0%)	0	0

Complication in cadaveric recipients	CsA – Pred	CsA – Aza + Pred	CsA – Mzr + Pred
NO	14	15	12
Liver Dysfunction	4 (28.6%)	2 (13.3%)	1 (8.3%)
DM	2 (14.3%)	0	2 (16.7%)
Leukopenia	2 (14.3%)	5 (33.3%)	5 (41.7%)
Hypertension			
2 weeks	6 (42.9%)	5 (33.3%)	2 (16.7%)
3 months	7 (50.0%)	5 (33.3%)	3 (25.0%)
Infection			
Bacterial	2 (14.3%)	1 (6.7%)	0
Bacteruna	2 (14.3%)	5 (33.3%)	0
Viral	2 (14.3%)	5 (33.3%)	5 (41.7%)
GI-complication	0	0	0
Nephrotoxicity	0	0	0

serum following the first injection of OKT3. However, the important point is that there is very little TNF-alpha made after the second or third injection of OKT3. If one injection of OKT3 causes severe side-effects, the clinican should either stop and don't give the patient anymore OKT3, or he or she should continue knowing that the response to the second dose is likely to be less dangerous. A serious error is to give the first dose, to skip a day or two, and then give another dose of OKT3, because a first dose reaction will occur all over again. Steroids are helpful in preventing the release of TNF-alpha (see discussion above) and are routinely used to decrease first dose reaction.

The first dose reaction is associated with the Fc portion of the OKT3 antibody molecule. The murine isotopes IgG-2A causes more first dose activity than IgG-1 which causes more than IgG-2B. The severity of the first dose reaction is related to T-cell debility of the anti-T-cell receptor CD3 antibody to activate T-cells in vitro. Shown in Table 6 (47,48) are a number of monoclonal antibodies against the CD3 or the T-cell receptor. Some of them are the murine IgM, or IgG-2B classes, and it is hoped that these, such as T10-B9.1A-31, or OKT3D will turn out to have much less first dose reaction but also be efficacious.

Another problem of treatment with OKT3 is the appearance of human anti-murine OKT3 antibody. These generally peak one to two weeks after the first course of therapy. They are likely to be clinically important in reducing the efficacy of a second course of OKT3 if the titre is greater than >1:1000. Indeed, if a patient needs more than one course of therapy with murine antibody, it is recommended that their T-cell levels in the blood by monitored by flow cytometry. A level of less than 50 CD3 positive cells per cubic millimeter is generally recommended (44).

Table V

Table 3. Efficacy of Treatment with OKT3 or Steroids for Acute Renal-Allograft Rejection.

	OKT3	Steroids	P Value
		incidence (%)	
Reversal of rejection (according to different criteria)			
Serum creatinine level plus clinical indexes	58/62 (94)	45/60 (75)	0.009
Serum creatinine level alone	55/62 (89)	37/60 (62)	<0.001
One-year follow-up			
Patient survival			
Actual	53/62 (85)	52/58* (90)	
Life table	— (85)	— (90)	0.47 (NS)†
Kidney survival			
Actual	36/53 (68)	25/52 (48)	
Life table	— (62)	— (45)	0.029

*Two patients were lost to follow-up
†NS denotes not significant

MONOCLONAL ANTIBODIES SPECIFIC FOR ACTIVATED T-CELLS.

Figure 17 shows that it may be possible to induce antigen specific immunosuppressive with monoclonal antibodies against antigens found only on activated T cells (49). Presumably, at the time of transplant, only those T-cells against the kidney will be activated. These T-cells will have cell-surface antigens unique to activated T-cells. These would be killed by monoclonal antibodies against these activation antigens. Thus, T-cells against desirable antigens, such as tuberculosis, might be spared. This has led to clinical trials testing monoclonal antibodies against the IL-2 receptor, which is such a T-cell activation antigen. These include 33B3.1 which has been used in Nantes, France, Anti-tack, Boston, BB10 in Germany, and YTH-906 in Cambridge. See Table 7 (47,48,50-52). In the future, toxin-conjugated anti IL-2 receptor antibodies or toxin-conjugated IL-2 antibodies may be used.

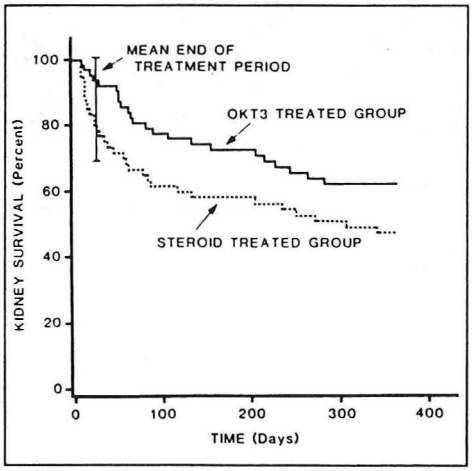


Figure 16

Figure 18 shows the effect of one of the anti P55 IL-2 receptor antibodies on acute rejection (50). Ten patients were tested, 6 patients responded, 4 failed to respond. This same antibody has also been used in maintenance and induction therapy. It has been compared with ATG which is standard therapy and no difference has been found (52).

ANTIBODIES TO CD4.

The CD4 molecule is an accessory molecule which stabilizes interactions between T-cells and their targets. It has long been known that antibodies against CD4 can induce tolerance if administered at the same time as antigen. Table 8 shows that there are a number of anti

CD4 antibodies presently undergoing, or soon to undergo, clinical trials. These include: OKT4, OKT4A, humanized OKT4, BL4, MT151, and MT412 (47,48,53,54). Table 9 (53) shows that infusions of OKT4A monoclonal antibodies into cynomolgus monkeys prevent rejection. Monkeys treated with low or high dose OKT4 had kidney transplants survived for approximately 40 to 50 days, whereas control animals lost their allografts within the first week or two. In addition, peripheral blood lymphocytes taken from treated monkeys was specifically tolerized against donor cells. See Figure 19.

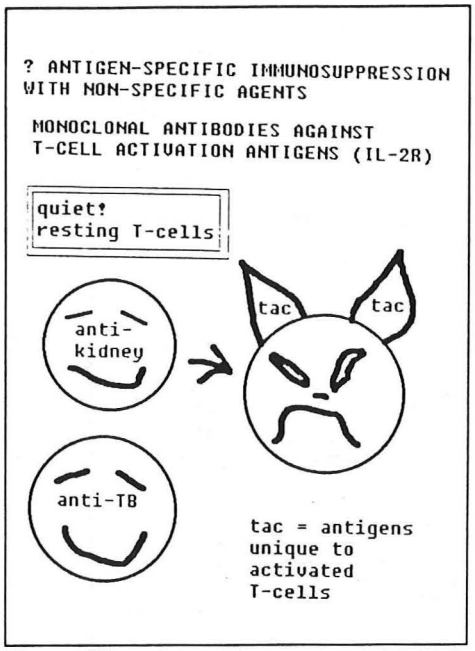


Figure 17

ANTIBODIES AGAINST ADHESION MOLECULES ICAM-1 AND LFA-1.

These molecules are important in two aspects (55). First, they stabilize interactions between CD4, T-lymphocytes, and antigen presenting cells allowing the T-cells to be activated. These molecules also stabilize interactions between CTL and their targets allowing their targets to be killed. Furthermore, as shown in Figure 11, LFA-1, ICAM-1 are important adhesion molecules which allow translocation of inflammatory cells into the allograft. It will therefore be expected that monoclonal antibodies against these molecules would have important immunosuppressive activities. Table 10 shows that cardiac allografts survive indefinitely in mice after treatment with monoclonal antibodies against LFA-1 and ICAM-1 (56).

Monoclonal antibodies against ICAM-1 are now undergoing clinical trials in humans.

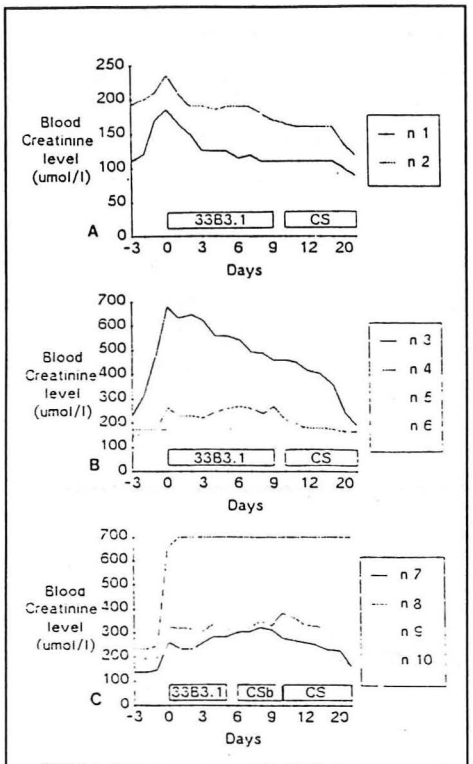


FIGURE 1. Patient response to anti-IL-2-R MoAb treatment in 10 ongoing rejection episodes. (A) Immediate response (N=2). (B) Delayed response (N=4). Blood creatinine returned to prerejection levels after the end of the MoAb course when Cs (1 mg/kg) was given. (C) no response (N=4). Rescue treatment (Cs boluses) was administered (day 6) because of MoAb ineffectiveness.

Figure 18

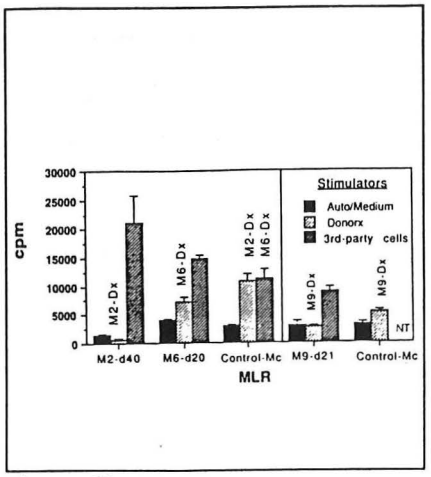


Figure 19

Table VI

Murine monoclonal antibodies against the TcR-CD3 complex. Side-effects IgG2a > IgG1 > IgG2b. Correlated with ability to activate T cells in vitro.

mAb/ class	target	T-cell activation	status
T10B9.1A-31 IgM	TcR monomorph	??	trials
BMA-031 IgG2b	above	low	suspended
OKT3D IgG2b	CD3	low	trials
WT 32 IgG2a	CD3	??	trials
OKT3 IgG2a	CD3	high	in use

Table VII

Monoclonal reagents specific for activated T cells

Monoclonal anti-IL-2R (CD25) antibodies - undergoing clinical testing. All anti-p55.

33 B3.1 (Nantes)

anti-TAC (Boston)

(BB10) BT 563 (Germany)

YTH-906 (Cambridge)

Toxin-conjugated anti-IL-2R antibodies - animal studies only, cancer patients.

Toxin-conjugated IL-2 - animal studies only, cancer patients.

Table VIII

Anti-CD4 antibodies
OKT4 and OKT4A - murine IgG2a - monkey studies - undergoing human trials
Humanized OKT4
BL4 - murine IgG2a - human trials
MT 151 - murine IgG2a - human trials
MT 412 - humanized ??

Table X

Treatment	n	Survival days	Mean survival (days ± SD)
None	6	7, 7, 8, 8, 8, 10	8.0 ± 1.1
M18/2	6	7, 8, 8, 9, 9, 10	8.8 ± 1.2
YN1/1.7	6	11, 12, 12, 13, 15, 23	14.3 ± 4.5
KBA	6	17, 20, 25, 30, 38, 47	29.5 ± 11.3
YN1/1.7 plus KBA	9	>70, >70, >70, >70, >70, >70, >70, >70, >70	>70

Table IX

TABLE 1. Allograft survival of immunosuppressed recipients

Treatment groups[a]	Animal	Allograft survival (postop. days)[b]	% Sup. of pretransplant MLR by OKT4A[c] (tested at 50/5/0.5/0.05 μg/ml)
CsA-sub	M190	15	NT
	M290	13	NT
OKT3	M1189	9	70/90/33/19
OKT4A			
Low-dose	M2888	49	76/66/24/33
	M2688	36	95/73/56/6
2-bolus	M1589	36	30/18/15/3
	M2089	30	55/72/26/40
	M2189	30	54/55/41/3
High-dose	M289	51	89/79/52/54
	M689	56	-/72/60/20
	M889	36	55/53/22/5
	M989	22	55/58/34/14
	M1089	30	34/30/48/19
	M2289	40	NT

[a] Details of treatment protocols are given in *Materials and Methods* and have been published elsewhere (*12*).
[b] Determined by recipient survival days as described elsewhere (*12*).
[c] Defined as described in *Materials and Methods*.

Literature cited:

1. Cho, Y.W. and Terasaki, P.I. Long-term survival. In: *Clincal Transplants 1988*, edited by Terasaki, P. Los Angeles: UCLA Tissue Typing Laboratory, 1988, p. 277-282.

2. Abbas, A.K., Lichtman, A.H. and Pober, J.S. *Cellular and molecular immunology*, Philadelphia:W.B. Saunders Co, 1991. pp. 1-417.

3. Jenkins, M.K. Models of lymphocyte activation: the role of cell division in the induction of clonal anergy. *Immun. Today* 13(2):69-73, 1992.

4. Shevach, E. The effects of cyclosporin A on the immune system. *Ann Rev Immun* 3:397-423, 1985.

5. Wood, R.P., Katz, S.M. and Kahan, B.D. New immunosuppressive agents. *Transpl. Sci.* 1:34-46, 1991.

6. Gruger, S.A., Chan, G.L.G., Canafax, D.M. and Matas, A.J. Immunosuppression in renal transplantation: I. Cyclosporine and azathioprine. *Clin. Trans.* 5:65-85, 1991.

7. Lorber, M.I. Cyclosporine: lessons learned - future strategies. *Clin. Trans.* 5S:505-516, 1991.

8. Kopp, J.B. and Klotman, P.E. Cellular and molecular mechanisms of cyclosporin nephrotoxicity. *J. Am. Soc. Neph.* 1:162-179, 1990.

9. Perico, N., Dadan, J. and Remuzzi, G. Endothelin mediates the renal vasoconstriction induced by cyclosporine in the rat. *J. Am. Soc. Neph.* 1:76-83, 1990.

10. Myers, B.D., Ross, J., Newton, L., Luetscher, J. and Perlroth, M. Cyclosporine-associated chronic nephropathy. *NEJM* 311:699-705, 1984.

11. Myers, B.D., Newton, L., Boshkos, C., et al. Chronic injury of human renal microvessels with low-dose cyclosporine therapy. *Transplantation.* 46:694-703, 1988.

12. Lewis, R.M., Janney, R.P., Golden, D.L., et al. Stability of renal allograft function associated with long-term cyclosporine immunosuppressive therapy - five year follow-up. *Transpl* 47:266-272, 1989.

13. Delmonico, F.L., Conti, D., Auchincloss, H.Jr., et al. Long-term, low-dose cyclosporine treatment of renal allograft recipients: a randomized trial. *Transplant.*

49:899-904, 1990.

14. Stiller, C.R. and Keown, P.A. Cyclosporine therapy in perspective. In: *Progress in Transplantation, vol 1*, edited by Morris, P.J. and Tilney, N.L. New York: Churchill Livingstone, 1984, p. 11-45.

15. Ptachicinski, R.J., Venkataramanan, R. and Burchart, G.J. Clinical pharmacokinetics of cyclosporine. *Clin. Pharmacokin.* 11:107-132, 1986.

16. Langhoff, E. and Madsen, S. Rapid metabolism of cyclosporine and prednisone in kidney transplant patients on tuberculostatic treatment. *Lancet* 2:1303, 1983.

17. Kamel, K.S., Ethier, J.H., quaggin, S., et al. Studies to determine the basis for hyperkalemia in recipients of a renal transplant who are treated with cyclosporine. *J. Am. Soc. Neph.* 2:1279-1284, 1992.

18. DeFranco, A.L. Signal transduction: immunosuppressants at work. *Nature* 352:754-755, 1991.

19. Bierer, B.E., Mattila, P.S., Standaert, R.F., et al. Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK506 or rapamycin. *PNAS* 87:9231-9235, 1990.

20. Schreiber, S.L. Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Sci* 251:283-287, 1991.

21. Friedman, J. and Weissman, I. Two cytoplasmic candidates for immunophilin action are revealed by affinity for a new cyclophilin: one in the presence and one in the absence of CsA. *Cell* 66:799-806, 1991.

22. Liu, J., Farmer, J.D., Lane, W.S., Friedman, J., Weissman, I. and Schreiber, S.L. Calcineurin is a common target of cyclophilin-cyclosporine A and FKBP-FK506 complexes. *Cell* 66:807-815, 1991.

23. Bierer, B.E., Somers, P.K., Wandless, T.J., Burakoff, S.J. and Schreiber, S.L. Probing immunosuppressant action with a nonnatural immunophilin ligand. *Sci* 250:556-559, 1990.

24. Dumont, F.J., Melino, M.R., Staruch, M.J., Koprak, S.L., Fischer, P.A. and Sigal, N.H. The immunosuppressive macrolides FK-506 and rapamycin act as reciprocal antagonists in murine T cells. *J.I.* 144:1418-1424, 1990.

25. Moody, H.R., Urmson, J., Ramassar, V., Vincent, D. and Halloran, P.F. Evidence

that MHC Class II expression in normal kidney is cytokine-induced, not constitutive. *J. Am. Soc. Neph.* abstract, 1991.

26. Emmel, E.A., Verweij, C.L., Durand, D.B., Higgins, K.M., Lacy, E. and Crabtree, G.R. Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation. *Sci* 246:1617-1620, 1989.

27. Fung, J.J., Abu-Elmagd, K., Todo, S., et al. FK506 in clinical organ transplantation. *Clin. Trans.* 5S:517-522, 1991.

28. Vacca, A., Felli, M.P., Farina, A.R., et al. Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. *J.E.M.* 637:646, 1752.

29. Fertsch, D., Schoenberg, D.R., Germain, R.N., Tou, J.Y.L. and Vogel, S.N. Induction of macrophage Ia antigen expression by rIFNg and down-regulation by IFN-a/b and dexamethasone are mediated by changes in steady-state levels of Ia mRNA. *J.I.* 139:244-249, 1987.

30. Zimmer, T. and Jones, P.P. Combined effects of tumor necrosis factor-alpha, prostaglandin E2, and corticosterone on induced Ia expression on murine macrophages. *J.I.* 145:1167-1175, 1991.

31. Politis, A.D., Sivo, J., Driggers, P.H., Ozato, K. and Vogel, S.N. Modulation of interferon consensus sequence binding protein mRNA in murine peritoneal macrophages: induction by IFNg and down-regulation by IFNa, dexamethasone, and protein kinase ilnhibitors. *J.I.* 148:801-807, 1992.

32. Han, J., Thompson, P. and Beutler, B. Dexamethasone and pentaoxyifylline inhibit endotoxin-induced cachectin/ tumor necrosis factor synthesis at separate points in the signaling pathway. *J.E.M.* 391:394, 1990.

33. Lee, S.W., Tsou, A.P., Chan, H., et al. Glucocorticoids selectively inhibit the transcription of the interleukin 1 beta gene and decrease the stability of interleukin 1 beta mRNA. *PNAS* 85:1204-1208, 1988.

34. Pober, J.S. and Cotran, R.S. The role of endothelial cells in inflammation. *Transplant.* 50:537-544, 1990.

35. Butcher, E.C. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 1033:1036, 1991.

36. Gruber, S.A., Chan, G.L.C., Canafax, D.M. and Matas, A.J. Immunocuppression

in renal transplantation: II. Corticosteroids, antilymphocyte globulin, and OKT3. *Clin. Trans.* 5:219-232, 1991.

37. Walker, R.G. and d'Apice, A.J.F. Azathioprine and steroids. In: *Kidney Transplantation: principles and practice*, edited by Morris, P.J. Philadelphia: W.B. Saunders Co., 1988, p. 319-342.

38. Stryer, L. *Biochemistry*, New York:W.H. Freeman & Co., 1988. Ed. 3

39. Elion, G.B. The purine path to chemotherapy. *Sci* 244:41-47, 1989.

40. Turka, L.A., Dayton, J., Sinclair, G., Thompson, C.B. and Mitchell, B.S. Guanine ribonucleotide depletion inhibits T cell activation: mechanism of action of the immunosuppressive drug mizoribine. *JCI* 87:940-948, 1991.

41. Eugi, E.M., Almquist, S.J., Muller, C.D. and Allison, A.C. Lymphocyte-selective cytostatic and immunosuppressive effects of mycophenolic acid in vitro: role of deoxyguanosine nucleotide depletion. *Scand. J. Immunol.* 33:161-173, 1991.

42. Sollinger, H.W., Eugui, E.M. and Allison, A.C. RS-61443: mechanism of action, experimental and early cllnical results. *Clin. Trans.* 5S:523-526, 1991.

43. Kokado, Y., Ishibahi, M., Hiang, H., Takahara, S. and Sonoda, T. Low-dose ciclosporin mizoribine and prednisolone in renal transplantation: a new triple-drug therapy. *Clin. Trans.* 4:191-197, 1990.

44. Colvin, R.B. and Preffer, F.I. Laboratory monitoring of therapy with OKT3 and other murine monoclonal antibodies. *Clin. Lab. Med.* 11(3):693-714, 1991.

45. Ortho multicenter transplant study group A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. *NEJM* 313:337-342, 1985.

46. Chatenoud, L., Legendre, C., Ferran, C., Bach, J.F. and Kreis, H. Corticosteroid inhibition of the OKT3-induced cytokine-related syndrome - dosage and kinetics prerequisites. *Transplant.* 51:334-338, 1991.

47. Heffron, T.G. and Thistlethwaite, J.R. New monoclonal antibodies. *Transpl. Sci.* 1:64-70, 1991.

48. Land, W. Monoclonal antibodies in 1991: new potential options in clinical immunosuppressive therapy. *Clin. Trans.* 5S:493-500, 1991.

49. Strom, T.B. and Kelly, V.E. Toward more selective therapies to block undesired

immune responses. *Kid. Int.* 35:1026-1033, 1989.

50. Cantarovich, D., Le Mauff, B., Hourmant, M., et al. Anti-interlukin 2 receptor monoclonal antibody in the treatment of ongoing acute rejection episodes of human kidney graft - a pilot study. *Transpl* 47:454, 1989.

51. Kirkman, R.L., Shapiro, M.E., Carpenter, C.B., et al. A randomized prospective trial of anti-TAC monoclonal antibody in human renal transplantation. *Transpl* 51:107-113, 1991.

52. Soulillou, J.P., Cantarovich, D., Le Mauff, B.L., et al. Randomized controlled trial of a monoclonal antibody against the interleukin 2 receptor (33B3.1) as compared with rabbit antithymocyte globulin for prophylaxis against rejection of renal allografts. *NEJM* 322:1175-1182, 1990.

53. Cosimi, A.B., Delmonico, F.L., Wright, J.K., et al. Prolonged survival of nonhuman primate renal allograft recipients treated only with anti-CD4 monoclonal antibody. *Surg.* 108:406-414, 1990.

54. Morel, P., Vincent, C., Cordier, G., Panaye, G., Carosella, E. and Revillard, J.P. Anti-CD4 monoclonal antibody administration in renal transplanted patients. *Clin. Immun. Immunopath.* 56:311-322, 1990.

55. Dustin, M.L. and Springer, T.A. Role of lymphocyte adhesion receptors in transient interactions and cell locomotion. *Ann. Rev. Imm.* 9:27-66, 1991.

56. Isobe, M., Yagita, H., Okumura, K. and Ihara, A. Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. *Sci* 255:1125-1127, 1992.