Renal

CYCLOSPORINE NEPHROTOXICITY

MEDICAL GRAND ROUNDS

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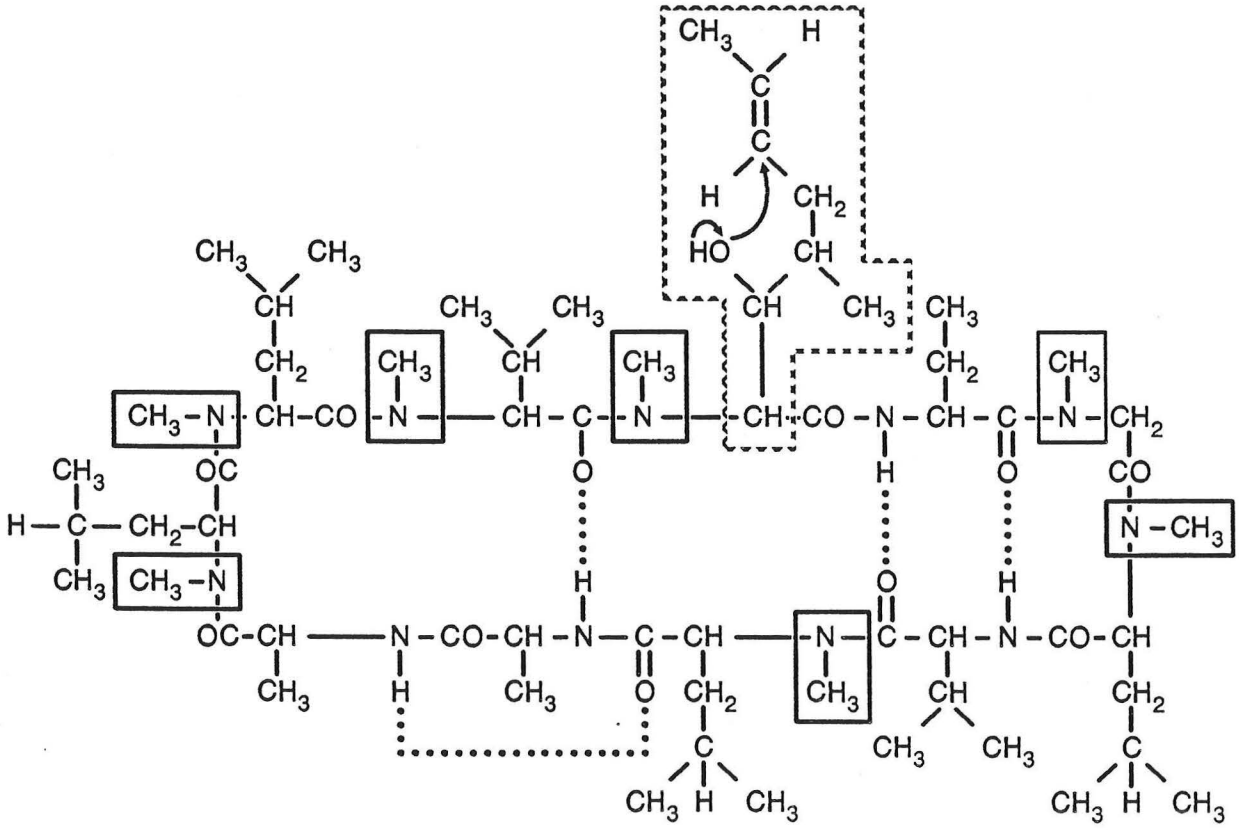

MEDICAL GRAND ROUNDS

APRIL 16, 1987

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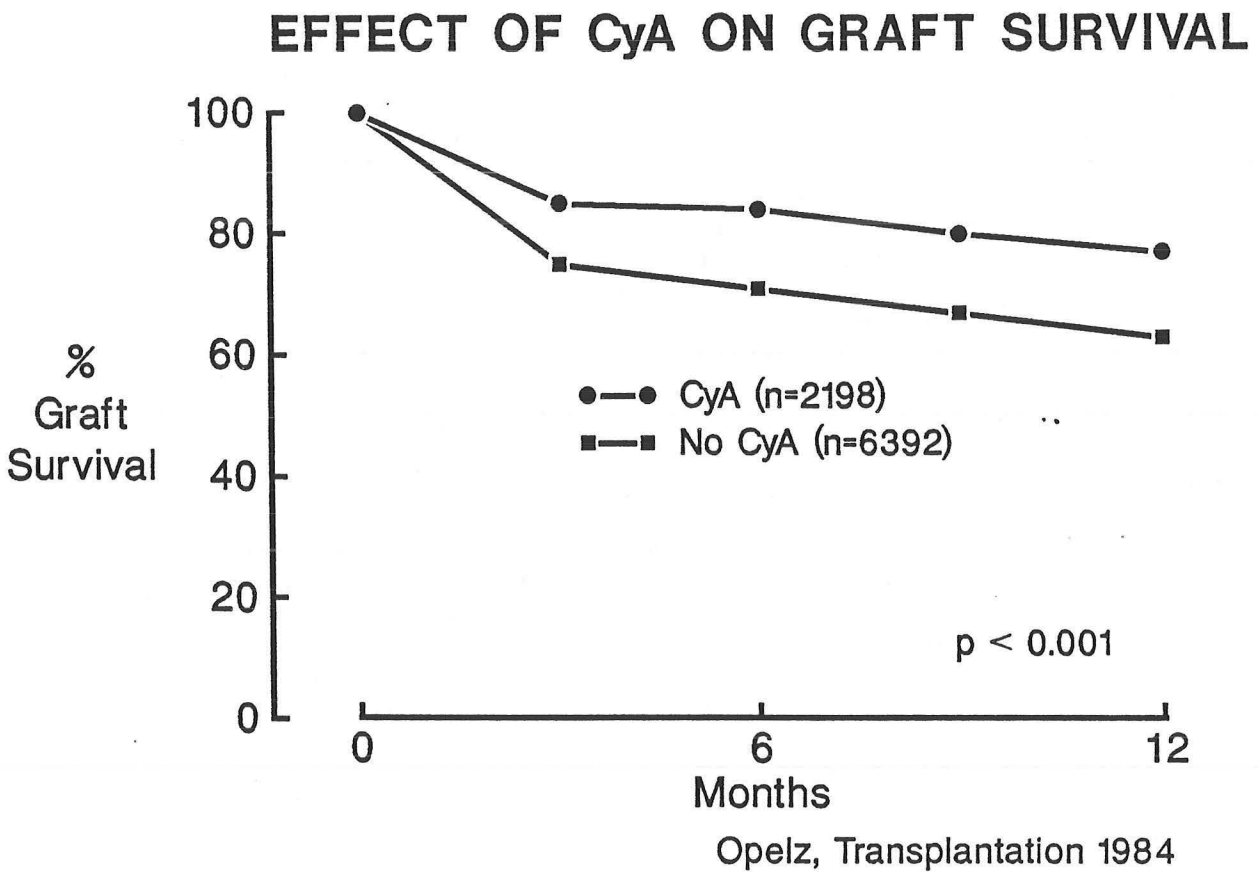
I. INTRODUCTION

Fewer new medicines have made as much an impact on clinical practice as has cyclosporine A, a peptide byproduct of a rare fungus which resides below the frozen tundra of Scandinavia. The drug has completely altered our approach to organ transplantation. It is possible to divide the history of renal transplantation into three specific eras. Era one was a period of experimentation in which various immunosuppressive protocols were examined and ultimately abandoned. During this first era, transplantation was performed in carefully selected patients whose other medical problems were small and success could be considered to be maximal. Although allograft success improved during this time, it remained meager with high mortality rates. The advent of azathioprine launched the transplant world into a second era of activity. This second era was characterized by graft survival results which permitted renal transplantation to be a clinically useful endeavor. As the patient pool capable of receiving a transplant expanded and selection criteria for recipients loosened, graft survival results actually declined in era two. Patient survival improved so that it may be possible to explain some of the graft survival results as the result of the practice of salvaging patients at the expense of grafts. Even with the use of azathioprine as part of the immunosuppressive regimen, transplantation remained an experimental activity for all other solid vascularized organs save for the early success for heart transplantation at a small group of institutions. Since the release for general use of cyclosporine A in 1983, the transplant world has been thrust into a third era characterized by an extraordinary improvement in graft survival with further reduction in patient mortality rates specifically for renal transplantation. At the University of Texas Health Science Center at Dallas, Parkland Memorial Hospital Transplant Program, cyclosporine has improved graft survival more than 30% over what was experienced throughout the azathioprine period.

TRANSPLANT RESULTS/PMH-UTHSCD

	CAD			LRD		
Year	N	1 Yr	Pt	N	1 Yr	Pt
1977-83	56±8	55±2	88±3	-	78	95
1985	83	73	93	13	96	100
1986	84	80	98	11	100	100

The actuarial graft in patient survival figures for 1986 reflect data as good as any transplant program in the entire United States. The point, however, is that these superb results are universal with the use of cyclosporine as part of the immunosuppressive regimen.



Moreover, transplantation of other solid, vascularized organs such as the heart and the liver are now routinely performed with good results. Extension of clinically beneficial transplantation to pancreas, lung, and heart-lung is now a possibility in man.

The importance of cyclosporine to the general internist has been growing as the drug has been tried in an ever expanding list of disorders whose common feature at this time is a supposed immunologically based pathogenesis. Table I is just a partial list of the disorders for which cyclosporine has already been tried. Clinical trials for the use of the immunosuppressive agent in a wider range of clinical diseases that would impinge on all specialties of Internal Medicine have been planned or on the way.

TABLE. DISEASES FOR WHICH CYA HAS BEEN USED

1. Uveitis
2. Myasthenia Gravis
3. Multiple Sclerosis
4. ITP
5. Diabetes Mellitus
6. Hemolytic Anemia
7. Hashimostos Thyroiditis

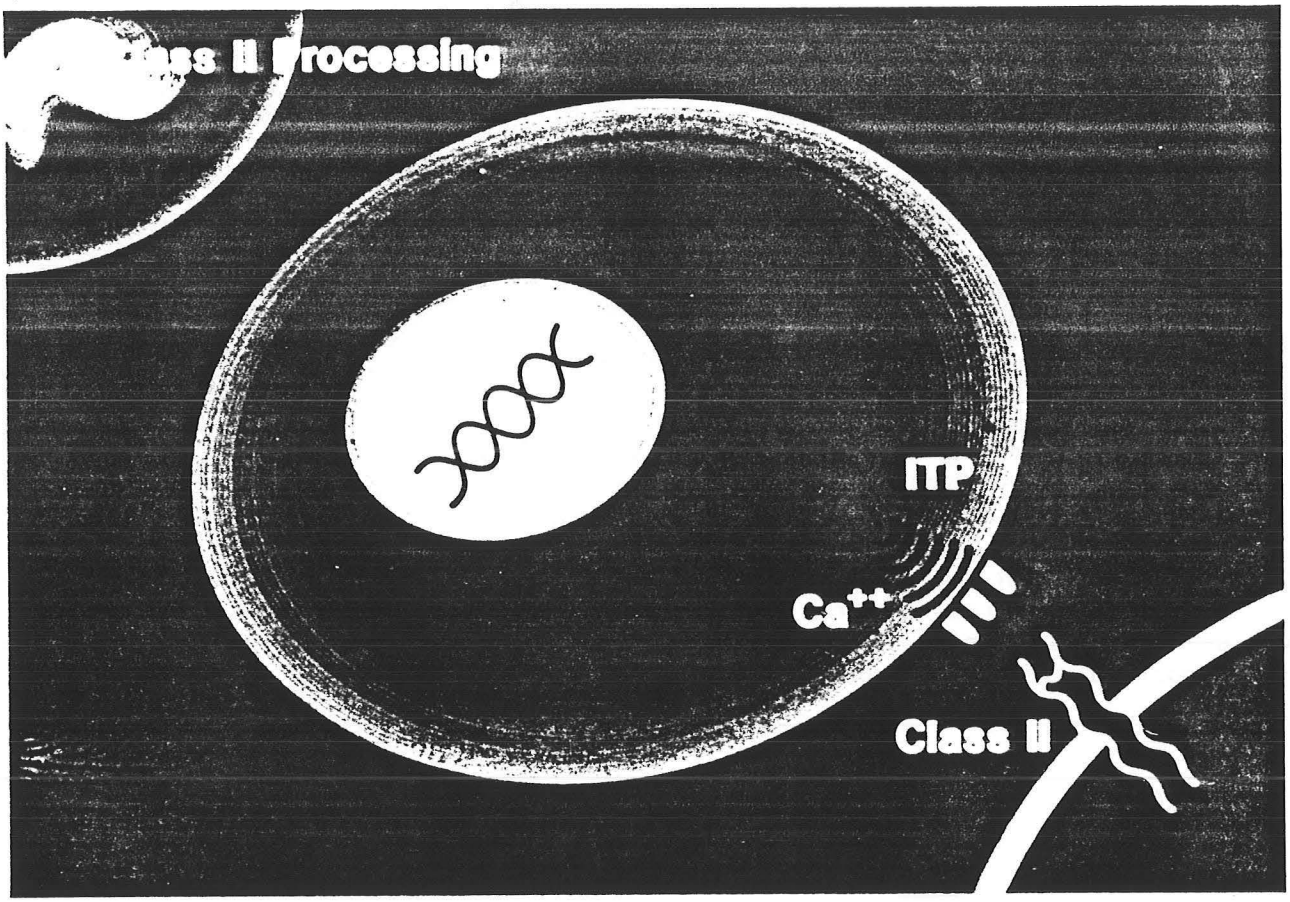
A general working understanding of the mechanisms by which cyclosporine functions as an immunosuppressive agent, its pharmacology, and its complication profile will be essential for the clinician in Medicine in the next several years. It is, therefore, my purpose to review the most important complication as yet identified for cyclosporine, nephrotoxicity. Will this series of complications reduce the usefulness of this agent which holds such promise? Is cyclosporine a miracle medicine or a medicine of illusion? It is the purpose of this grand rounds to answer these two questions.

II. BACKGROUND

A. IMMUNOSUPPRESSIVE MECHANISMS OF CYCLOSPORINE

In order to explicate the mechanisms by which cyclosporine and other immunosuppressive drugs are helpful in organ transplantation, one must review the manner in which an alloantigen sensitizes the host. Although covered in great detail in the previous grand rounds, a brief review of this topic is necessary. The immune event is initiated by the encounter of various immunocompetent cells with alloantigen displayed on the various cell types present in a given organ transplant. The afferent antigenic recognition wing is initiated by the encounter of clonaltypic helper T-cells bearing appropriate antigen receptors with specific class II HLA molecules on the cell surface of the transplant (Fig. 1). Such encounter stimulates the change of the helper cell from a quiescent, non-activated cell in the G_0 phase of the lymphocyte cell cycle, a time at which the content of cellular RNA and DNA is diploid and intracellular energy metabolism is quite low, to an actively metabolizing cell. The helper lymphocyte enters the G_{1a} phase of the cell cycle which is characterized by the stimulation of transcription of new messenger RNA for an array of proteins many of which will ultimately be displayed on the cell surface as "activation receptors". RNA content of the cell increases while DNA content remains the diploid amount. Simultaneous to this activation of helper lymphocytes is the stimulation of monocytes responding to a similar alloantigen. Although the monocyte will not traverse the same cell cycle pathway as will the activated lymphocyte, de novo synthesis of monokines is stimulated, an important example of which is the stimulation and ultimate secretion of the monokine interleukin-I (Il-I). The temporal sequence by which the activated T-helper cell obtains its array of activation receptors has been worked out in a number of laboratories including our own; receptors which appear early after activation include the Il-I receptor and the interleukin-II (Il-II) receptor.

FIGURE 1



As shown in figure 2, the activated lymphocyte with its receptors is now poised to receive additional signals which will permit the cell to continue its movement through the phases of the cell cycle. At this point, a second signal is required by the activated lymphocyte for it to complete its clonal expansion. This signal is provided, in many models, by the activated monocyte. In this depiction of the process, the monocyte-derived signal is the Il-I molecule which now binds into the Il-I receptor newly synthesized on the activated helper lymphocyte. By a process which, taken together with antigen recognition, involves second messengers that may involve calcium related signals and signals of high energy phosphate compounds such as ITP, the genome of the activated lymphocyte is stimulated further to transcribe new message for a host of lymphokines, one of the earliest of which is the lymphokine Il-II (Fig. 3). Il-II will serve as an autocoid, a substance made by this cell which will bind into a receptor on the surface of the same cell and alter its function. Engagement of the activation Il-II receptor by this newly synthesized Il-II is the signal required by the lymphocyte to push it through the next two phases of the cell cycle, G_{1b} and S phase. During the S phase, DNA is doubled, the cell is poised to enter metaphase, and cell division will ensue, all prerequisites for clonal expansion of the cells which are capable of responding to a given allograft.

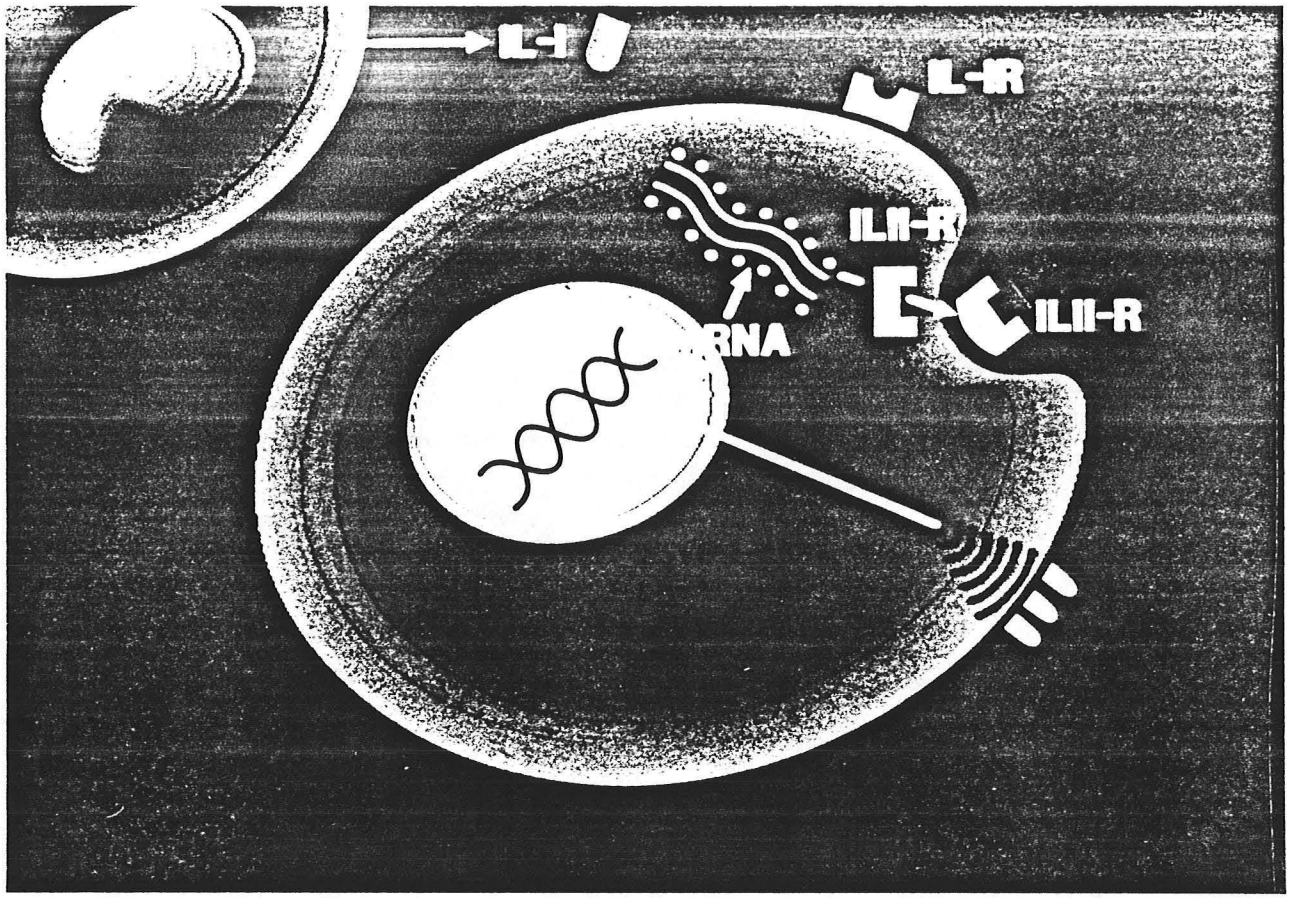


Fig. 2

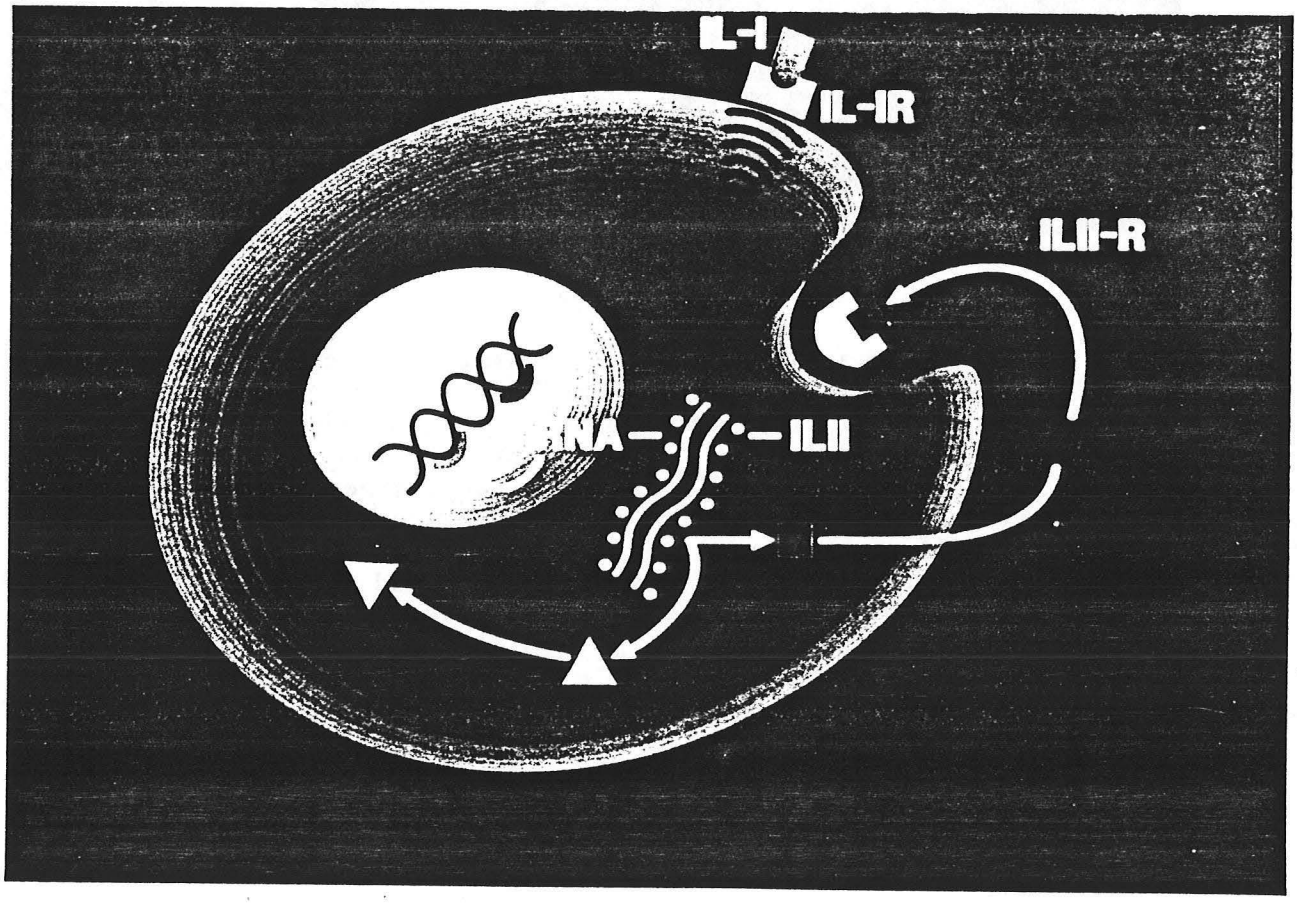
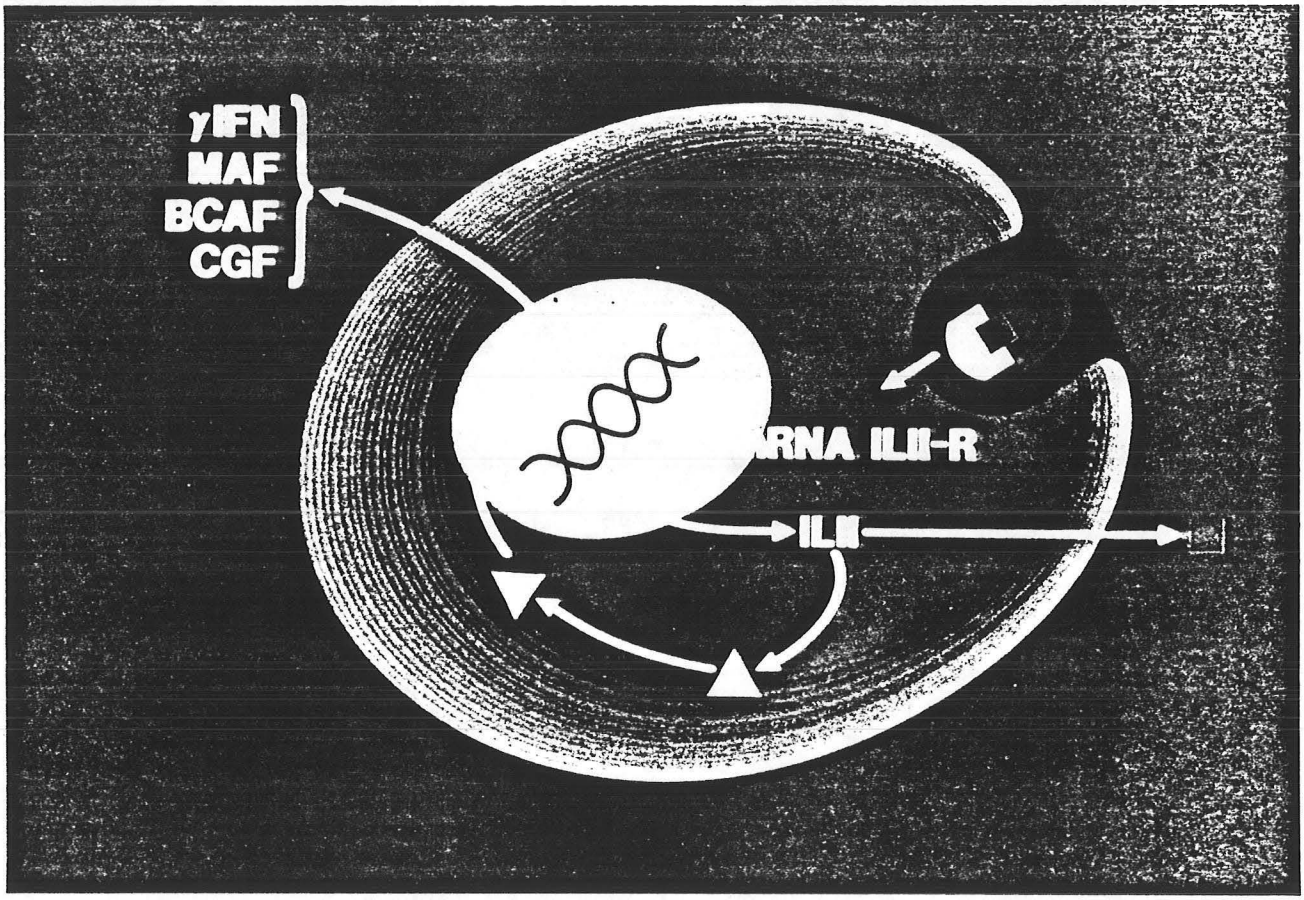


Fig. 3

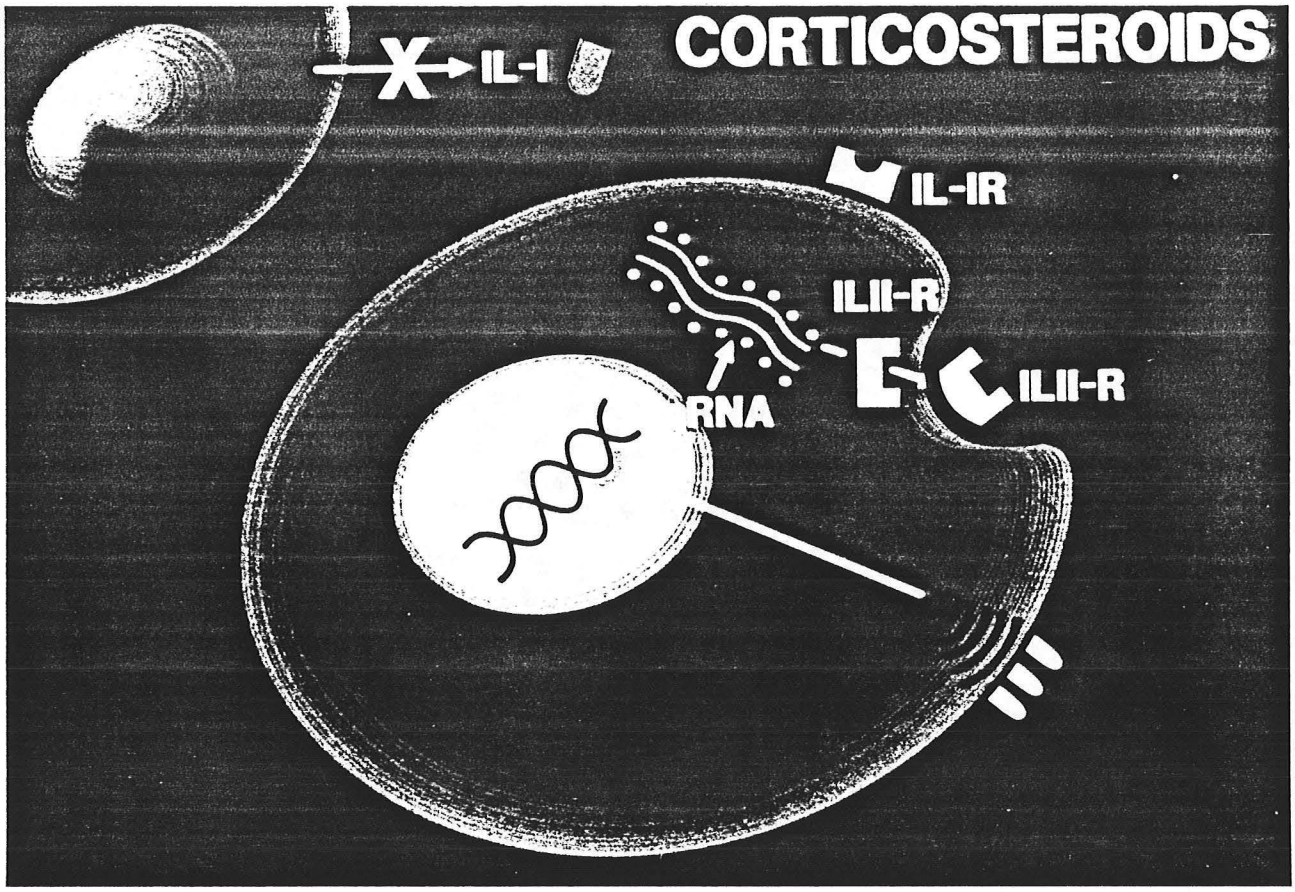
Simultaneous to the activation of the helper pedigree of lymphocytes is the activation of the cytotoxic lymphocyte by its encounter with class I HLA antigens that may be displayed on the surface of the transplant. The cytotoxic cell will traverse a similar pathway of cell activation, but requires additional signals from the helper lymphocyte in order to complete any effective clonal expansion event. The help is provided, in part, by the release of Il-II by the activated lymphocyte and by certain other lymphokines such as cytotoxic activating factor (CAF) (Fig. 4).

FIGURE 4



With this schema in mind, it is posssible to examine the mechanisms by which the various immunosuppressive medicaments prevent allograft rejection. As shown in figure 5, corticosteroids, agents which have a multiplicity of actions in higher mammals, by virtue of stabilization of membranes, reduce the release of the monokine Il-I by the activated monocyte. If the flow diagram that we have established for alloimmunity is correct, then this primary action of steroids will have several secondary effects. Without Il-I binding into its receptor on activated lymphocytes, the second signal to antigen required to complete the clonal expansion of the helper cells would not be provided. Il-II synthesis secondarily will be diminished, the movement of the helper cell into S phase of the cell cycle would be reduced, cell division events diminished. Because steroids in doses which are otherwise clinically "safe"

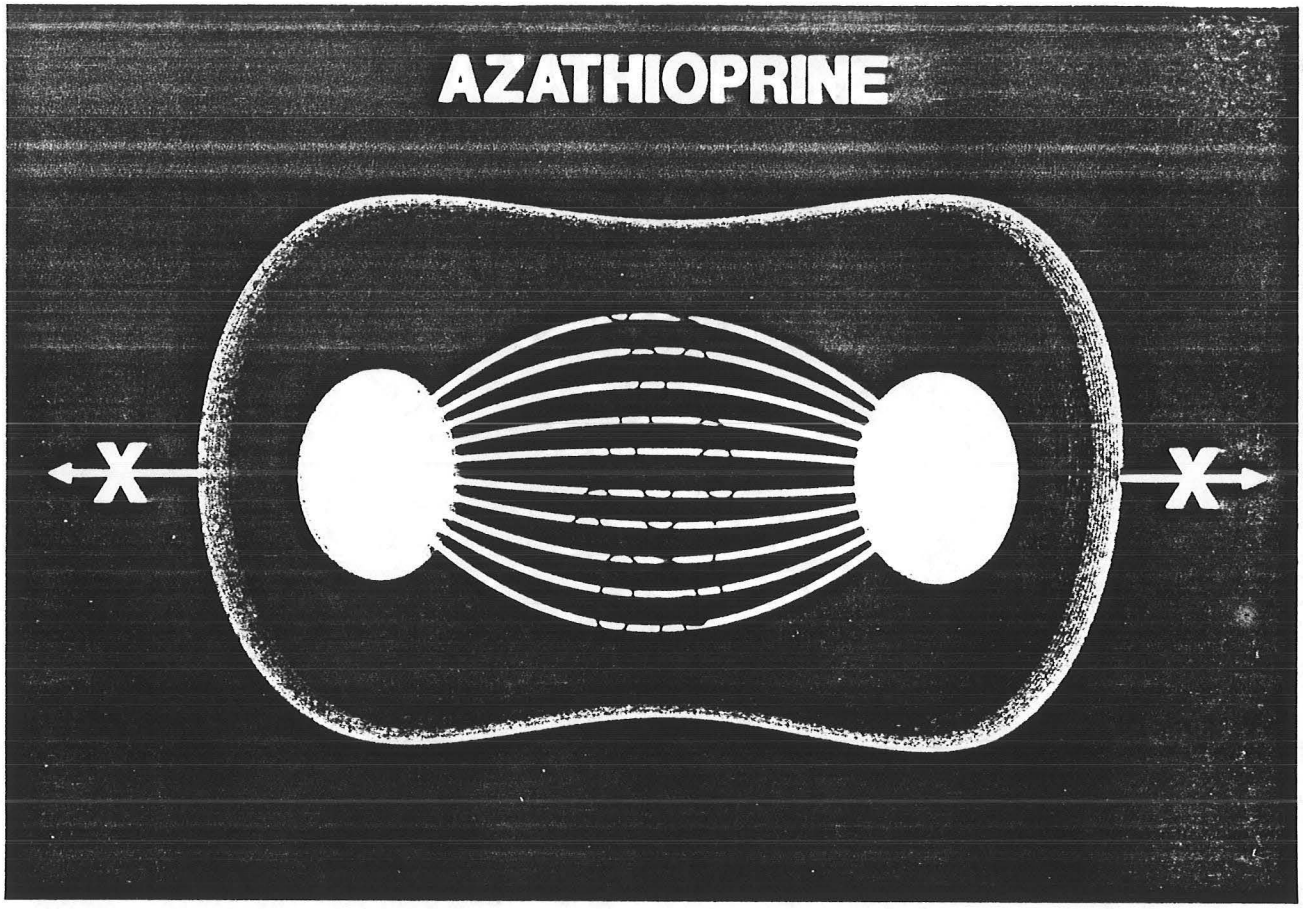
FIGURE 5



cannot completely abrogate the elaboration of Il-I, it is reasonable to hypothesize that corticosteroids alone could not prevent entirely clonal expansion; rejection may ensue. This assumption is supported by clinical data.

A second group of medicines, often used in combinations with steroids to prevent allograft rejection, are the cytotoxic drugs exemplified by azathioprine. Azathioprine is an imidazole derivative of 6-mercaptopurine. The derivatization of this drug allows it to be orally administered and thus more clinically useful in a practical sense. Azathioprine is "activated" in the liver by the removal of the derivative and the production of 6-mp which inhibits the conversion of inosine monophosphate (IMP) to either AMP or GMP thus reducing the precursors required for RNA and/or DNA synthesis. As shown in figure 6, this group of medicines is immunosuppressive during the clonal expansion event when cells are undergoing DNA synthesis and require enhanced amount of precursor. In the absence of such enhanced precursor molecules, the cells will have aborted clonal expansion and will die. Thus, the cytotoxic drugs work at a distal point in the afferent alloantigenic recognition wing. When one employs cytotoxic drugs, one is balancing the capacity of the drug to inhibit cellular division in cells important for rejection while diminishing the same events in normal cells such as cells of the blood forming elements.

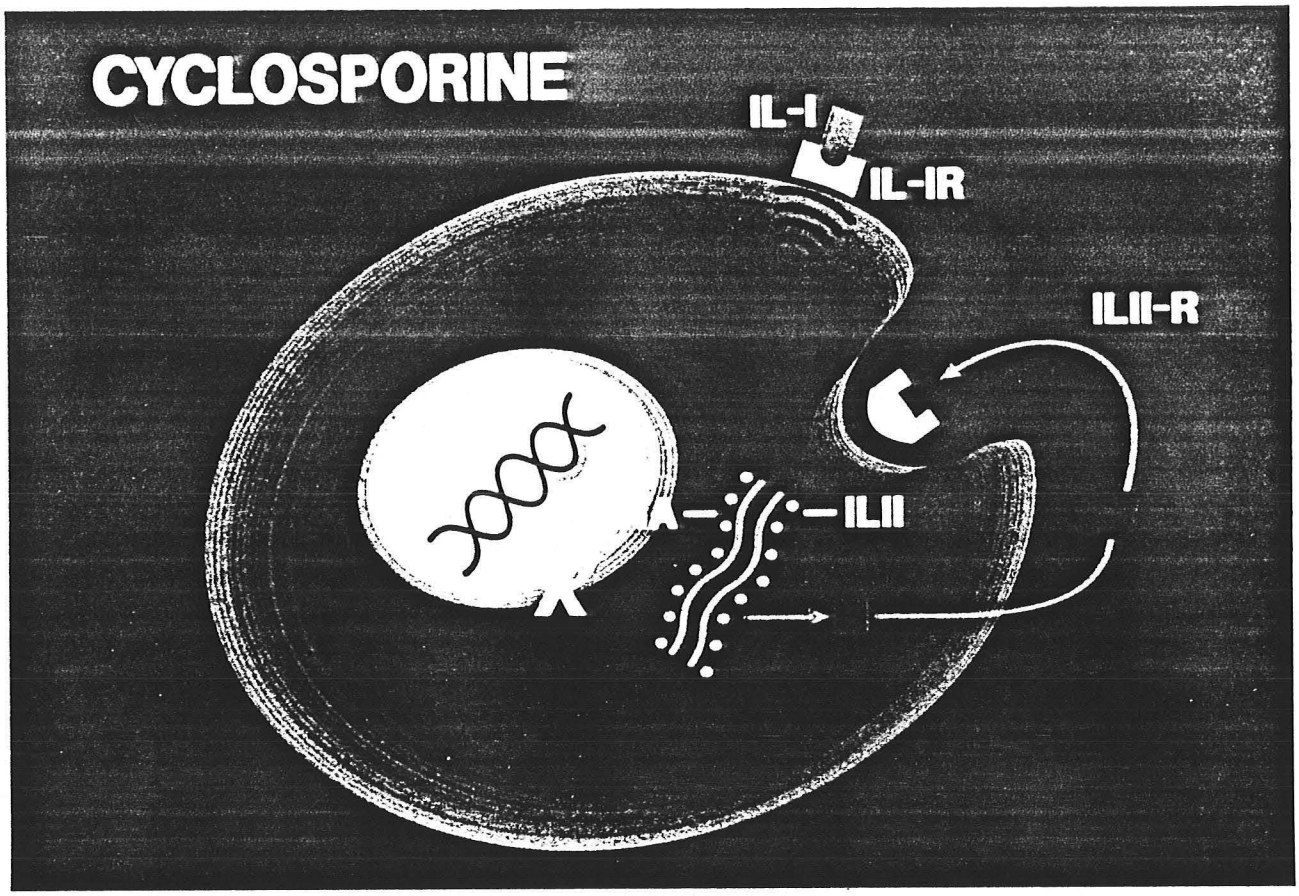
FIGURE 6



Therapies designed to completely eliminate cellular division can induce a state of total tolerance of an allograft or tumor in exchange for marrow suppression. In the balance of therapeutic benefit to toxicity, it is clear that all cellular division cannot per force be inhibited and azathioprine will not be completely successful in eliminating rejection episodes.

The most recent thinking concerning the mechanisms by which cyclosporine functions as an immunosuppressive agent within the context of this schema deals with the capacity of the drug to specifically inhibit the transcription of new messenger RNA for the lymphokine Il-II. An array of secondary events may flow from this single inhibition: 1) diminished clonal expansion of helper cells; 2) inhibition of lymphokine production consequent to Il-II binding; 3) diminished helper activity for cytotoxic T-cell activation; 4) diminished monocyte activating factor (MAF)-reduction in Il-I production by monocytes; 5) reduction in γ interferon production-diminished synthesis of class II antigens on organ specific parenchymal cells. There is preliminary evidence that the stimulation of Il-II production is accompanied by the elaboration of a molecule which is an intracellular signal molecule which regulates the continued de novo synthesis of the Il-II receptor (Fig. 3). Cyclosporine is felt to alter this regulatory peptide so that in time the cell not only does not make Il-II but also ceases to synthesize the Il-II receptor (Fig. 7).

FIGURE 7



Taken together lymphocyte activation is aborted in G_{1a}, never traverses the remaining phases of the cell cycle, and the lymphocyte returns ultimately to its quiescent state as afferent recognition does not lead to clonal expansion of sensitized lymphocytes. Effector cells are not produced, effector mechanisms are not set in motion, rejection does not occur.

Although contradictory material exists in the literature that might render a single coherent picture of this mechanism in question, the mechanism just described for the immunosuppressive activity of cyclosporine seems to fit most of the observed experimental data. Lafferty and colleagues reviewed the experimental data and came up with the following observations that seem to be a common thread in the majority of studies:

1) clonal expansion of activated T-cells is interrupted;

2) presentation of Il-II to antigen pulsed lymphocytes has its normal effect;

3) cyclosporine appears to interrupt the antigen delivered activation signal at some point;

4) primary T-cell activation is inhibited;

5) cyclosporine does not alter cytotoxic T-cell capacity to kill target;

6) cyclosporine does not interrupt the capacity of Il-II to enhance cytotoxic T-cell function.

All of these effects can be explained by the mechanism proposed above, to wit cyclosporine inhibits the production of Il-II but not the Il-II receptor. Additional experimental observations are also generally supportive of the proposal described here. In a general sense, cyclosporine has been demonstrated to prevent S phase transformation of T-lymphocytes discerned either by morphologic transformation or in terms of enhanced incorporation of DNA percursors during mitosis. The drug clearly inhibits the production of Il-II and a variety of other lymphokines after antigenic stimulation. Interestingly, it has been shown by Waldmann's group to prevent the activation of the Il-II gene by phorbol ester in cultured human T-cell lines. Although cyclosporine may inhibit the synthesis of activation-induced receptors which occur temporally after the Il-II receptor, there has been little evidence that the synthesis of the Il-II receptor itself is inhibited by the drug.

The bulk of evidence, then, supports the holistic hypothesis that cyclosporine inhibits the synthesis of Il-II but not its receptor. To review cells activated by antigen in the presence of cyclosporine are responsive to exogenously administered Il-II synthesized from recombinant DNA. Cytotoxic lymphocytes are also amenable to Il-II derived signals once synthesized by antigen. Miyawaki et al have shown that cyclosporine does not prevent expression of the TAC antigen, the Il-II receptor identified by an unique monoclonal antibody, after mitogen stimulation of human T-lymphocytes. That same group demonstrate, however, that other activation receptors that appear on the lymphocyte consequent to appearance of the Il-II receptor are inhibited by cyclosporine further supporting the notion that a block in the production of the lymphokine Il-II will freeze the cells in early G_{1a} of the cell cycle and prevent the attainment of the later activation receptors. More recently, Gauchat et al have found contradictory evidence with respect to the impact of cyclosporine on the synthesis of the Il-II receptor protein. Their data suggests that cyclosporine may inhibit the activation by lectin of the Il-II receptor gene, but once the gene has been activated, cyclosporine would have had no effect on translation of the message and ultimate insertion of a new peptide into the cell membrane. This single set of experiments stands in contrast to most of the extant data and suggests that the unique model chosen by those investigators may uncover a potential effect of cyclosporine that is not generally observed in vivo.

In summary, on can generate a list of do's and dont's concerning the immunosuppressive effects of cyclosporine.

DO'S OF CyA

1. Reduces Monocyte Production of IL-1
2. Inhibits Production of IL-II by Activated Lymphocytes
3. Consequent to Above Abrogates Clonal Expansion of Helper T Cells and Final Differentiation of TC Cells

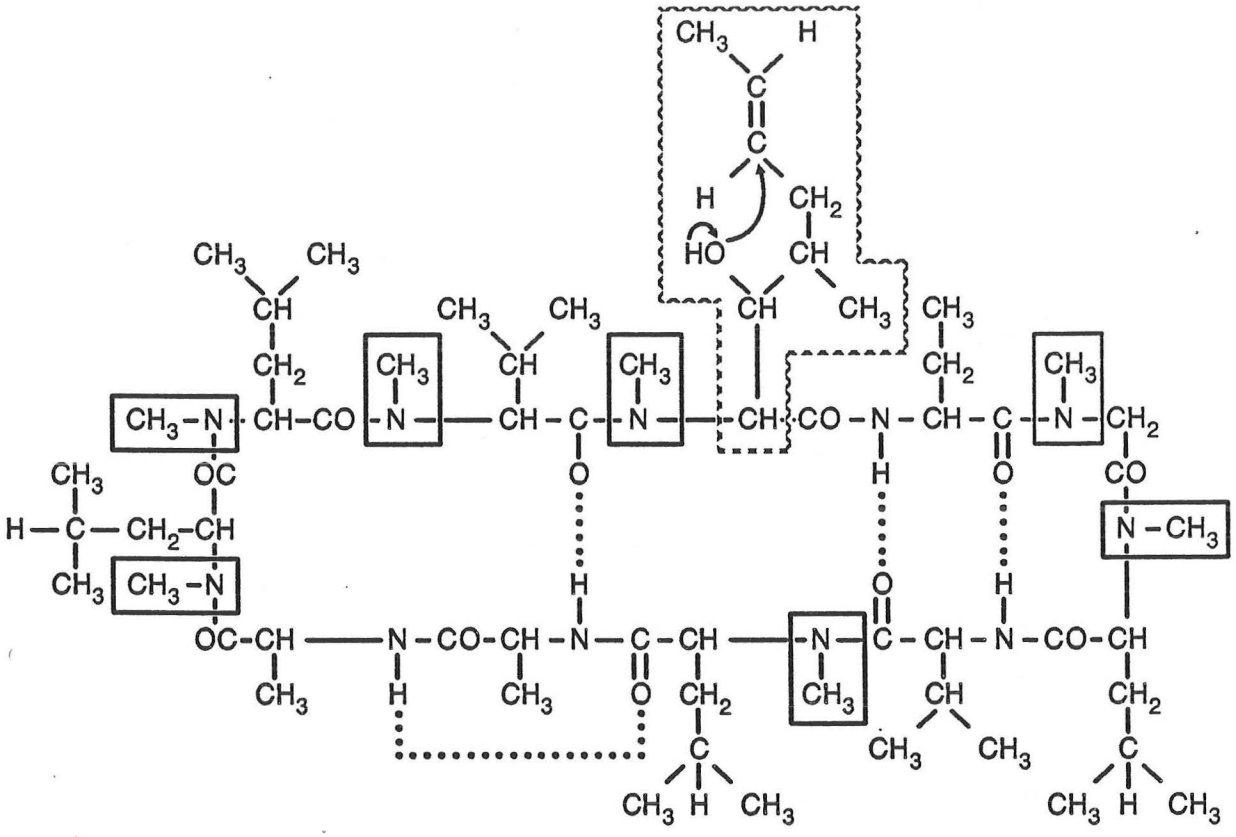
DON'T'S OF CyA

1. Does not Block IL-II Receptor Synthesis
2. Does not Reduce the Capacity of Activated TH or TC to Respond to Exogenous IL-II
3. Does not Delimit the Action of TC Once Generated

B. PHARMACOKINETICS

Having reviewed the manner in which cyclosporine functions as an immunosuppressive drug, it is essential that the pharmacologic principles that underline the distribution of cyclosporine in patients be reviewed in order to understand the toxic potential in general and to understand nephrotoxicity in particular.

Cyclosporine A is a neutral lipophyllic endecapeptide extracted from the fungus tolypocladium inflatum gams which is soluble in ethanol and in lipids. It is an unique molecule as recently reviewed by Kahan. In his formulation, the molecule has three structural characteristics which may explain its pharmacodynamics, pharmacokinetics and toxic potential (see figure).
At present, the drug is available as an oral solution in an olive oil base with 12.5% alcohol. It can also be obtained in a formulation for intravenous administration. More recently there has been a great deal of research concerning the nature of the lipid base fruits of which have led to the development of a gelatin capsule eliminating the need for consumption of distasteful liquids. The 1200 dalton peptide is a highly lipophilic molecule which is mainly bound to lipoproteins. Binding to non-lipoproteins in plasma such as albumin is relativley unimportant. The molecule reaches the plasma membrane of cells as part of lipoprotein fractions and is available for cell accumulation in this manner. Since the lipoprotein bound drug is the form which is tissue available, total plasma or even whole blood measurements are more instructive than free unbound measurements in plasma water.



STRUCTURAL CHARACTERISTICS OF CyA

1. 7 of The Total of 11 Amino Acids are N Methylated Which Explains the Aliphatic Nature of the Drug
2. A Ring Structure with Precise Steric Conformation
3. A Unique 9 Carbon AA with an Ethylene Bond Not Previously Encountered in Nature

The lipophilic properties of cyclosporine themselves explain the large volume of distribution for this drug and its limited dialysance. Although absorption of an oral dose is highly variable, absorption half-life ranges between 1-2 hrs with a peak blood concentration achieved in about 4 hrs (range 1-8 hrs). Absorption of the drug is highly dependent on adequate gastric emptying; patients with disorders in gastric emptying have great difficulty absorbing the drug. In clinically relevant terms, the diabetic patient, often with gastroparesis, has been very difficult to manage because of the delayed gastric emptying and poor absorption of the medication. Kahan has calculated that bioavailability of orally administered drug is a approximately 30%, a calculation which has led to the recommendation to administer intravenous doses at 1/3 of the usual oral dose when this route of administration is thought to be essential.

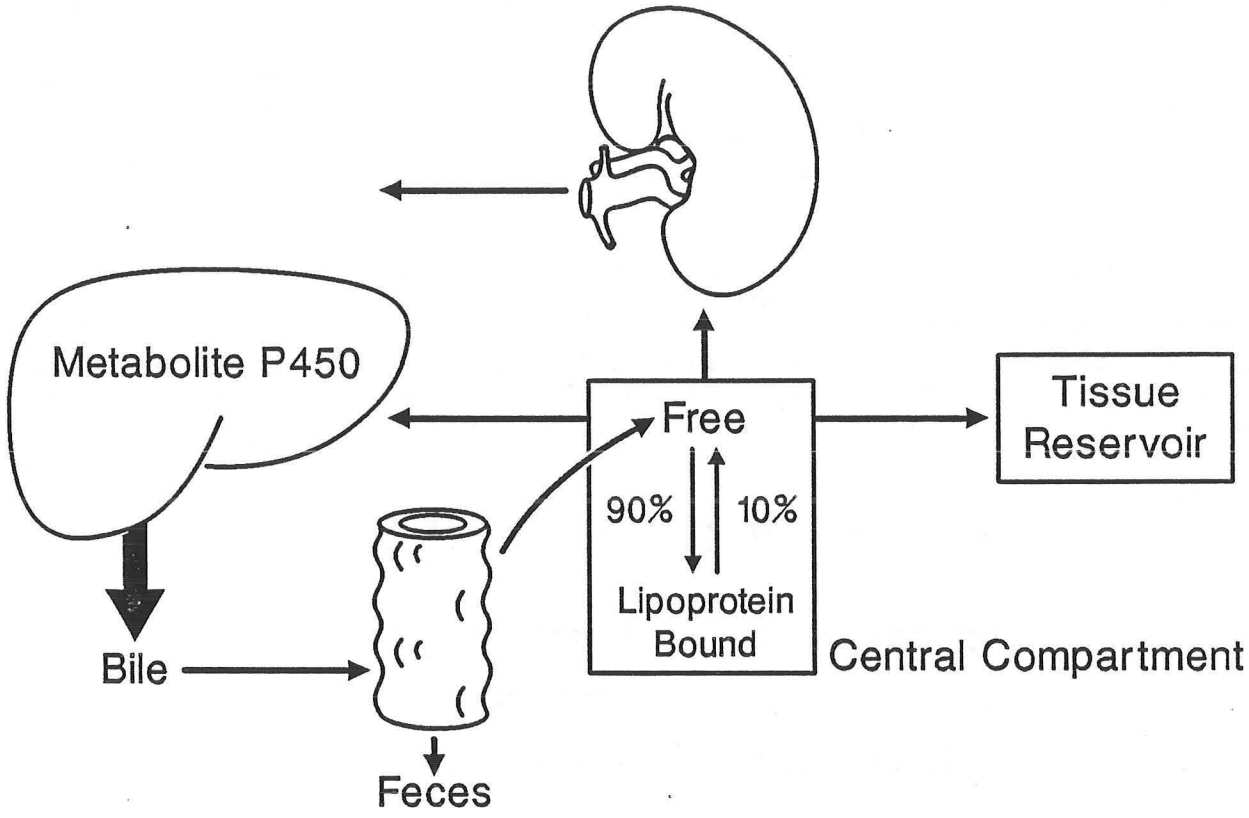
The drug is absorbed as a lipid through the lacteals of the small bowel reaching the blood compartment by way of the thoracic duct. The lipophyllic nature of the drug as well as the site of drug metabolism predicts the organ distribution. As shown in the figure, the liver, the organ which synthesizes the majority of the metabolites formed, is the organ of greatest concentration, followed by the pancreas and the body fat. Indeed, the fat serves as a depot for the drug so that blood levels often underestimate the total body burden. The volume of distribution for the parent compound alone is approximately 3½-4 L/kg, similar to other highly bound drugs such as the digitalis glycosides. At clinically applicable doses, cyclosporine A is eliminated with first order kinetics, generally by conversion to inactive moieties predominantly by the liver followed by excretion through the entero-hepatic circuit. Almost all of the metabolic reactions represent oxidative hydroxylation or demethylation steps employing the cytochrome P450 system of the liver. This fact permits one to predict an array of drug-drug interactions.

DRUG INTERACTIONS WITH CyA

Increase Levels	Decrease Levels
Ketaconazole	Dilantin
Hi Dose Lasix	Phenobarbital
Erythromycin	
Acyclovir	

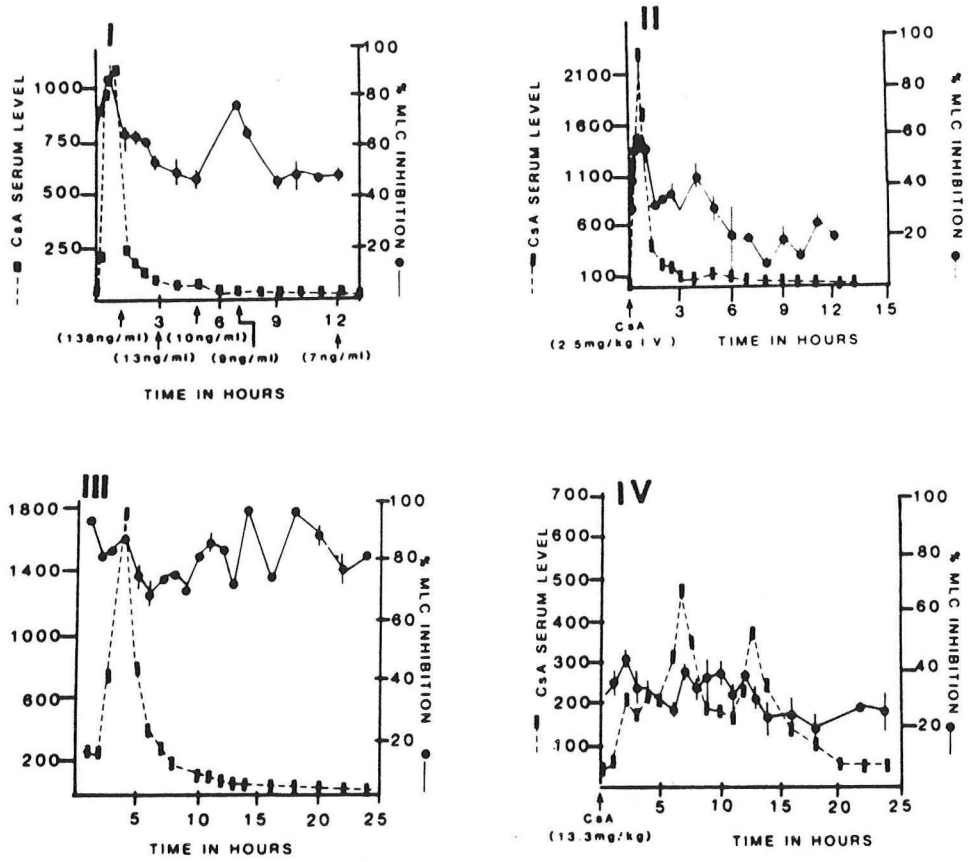
Those drugs which enhance the P450 system contribute to the rapid elimination of cyclosporine and the potential for under immunosuppression of transplant recipients; those drugs which inhibit the P450 system diminish metabolic elimination of cyclosporine and lead to potential overdosage and observed clinical toxicity.

Newburger and Kahan believe a two compartment open model best describes the pharmacokinetics of cyclosporine with a rapidly equilibrating central compartment represented by the blood and a slowly equilibrating tissue compartment. Taken all factors together, the calculated and reported T½ approximates 10 hrs with a clearance rate of about 12-15 ml/min/kg. The majority of the metabolites are excreted through the entero-hepatic circulation with little renal clearance. Most believe that there is no requirement to adjust dosage for renal failure. When initiating therapy it is reasonable to assume that by 2 days (between 4-5 half-lives) a constant cyclosporine level will be achieved on a steady dosage regimen. Although once a day dosing achieves a reasonable drug and therapeutic steady state for most patients, Rogers and co workers highlight the variations in absorption and pharmacodynamics within patients suggesting individualization of dosing based on four specific patterns of bioeffectiveness of the drug. In pattern I, despite quite low serum levels of parent compound, there seems to be two distinct peaks of immunosuppressive activity. In pattern II there seems to be a single peak of immunosuppression commensurate with the measured levels of drug. A third pattern emerges in which there is persistent immunosuppressive



activity of the drug unrelated to the measured amount in the serum. Lastly, the fourth pattern is characterized by a lack of immunosuppressive activity despite adequate levels of drug in serum. If these four patterns are correct, one must per force hypothesize an active metabolite to account for these findings. Such was the interpretation of Rogers et al, although Borel, the scientist who discovered the drug at Sandoz Company, cannot find any immunosuppressive activity of the various metabolites described so far. To the relief of most transplant physicians, the numbers of patients who fall into patterns 1, 3, and 4 seem, apparently, very low so that once a day dosing, having been shown to be clinically beneficial in the bulk of transplant recipients, remains the standard.

An understanding of these pharmacologic properties will help in the construction of strategies to avoid toxicities once described and help in an understanding of the difficulties in developing these strategies. The highly lipophyllic nature of the drug, the failure to understand the relationship between dose and drug concentration in blood compartments, the absence of a tight relationship between immunosuppressive efficacy and toxicity, the important role played by the P450 system, the large volume of distribution, all must be taken into account in developing programs to eliminate or reduce cyclosporine toxicity.



III. CLINICAL NEPHROTOXICITY

A. PRIMARY NON-FUNCTION

Case Study

TS, a 39 y/o full blooded American Indian from El Paso, underwent uncomplicated cadaver renal transplantation. He was prepared immunologically by a preoperative intravenous infusion of 5 mg/kg cyclosporine A administered over 4 hrs. On the first 3 post-operative days 5 mg/kg IV cyclosporine was given on each day. When gut function became normal, he was switched to 15 mg/kg oral cyclosporine. Residual urine flow rate had been 200 cc/day. In the first week the patient had between 50 cc and 200 cc of urine flow a day. There was no demonstrable clearance, the serum creatinine remained elevated, the patient required hemodialysis every other day. Cyclosporine whole blood levels by RIA (the method used at PMH at that time) were slightly elevated; the oral dose was adjusted downwards toward 13 mg/kg. At two weeks of

non-function a core renal biopsy was obtained revealing acute tubular necrosis. When non-function reached four weeks duration, a repeat core biopsy was performed with the same diagnosis. TS was sent home for continued hemodialysis on cyclosporine 12 mg/kg·d and prednisolone 30 mg d to continue hemodialysis. Renal function was never exhibited by TS. At three months his nephrologist discontinued cyclosporine and prednisolone. Two weeks after discontinuance of the immunosuppressive regimen the patient developed fever of 102° orally and a tender iliac in the area of the transplant surgery. He was returned to PMH where he underwent an uncomplicated transplant nephrectomy. The pathologic specimen revealed acute rejection at that time.

The case of TS represents what is called in the transplant business primary non-function while receiving cyclosporine. In the earlier experience with cyclosporine there was a vigorous debate concerning the role that cyclosporine played in the development of, severity of, and the prognosis of primary non-function. The University of Pittsburgh group headed by Starzl and Hakela argued that the incidence of primary non-function was no greater in patients receiving cyclosporine than was their historical experience under the umbrella of conventional immunosuppression. Their review of the published information sets forth the early debate. More recently, the deleterious effect that pre- and perioperative cyclosporine may have in renal transplant outcome with special reference toward primary non-function is recognized by most programs. Professor Roy Calne recognized this from his anecdotal experience as early as 1978 when he observed the induction of several cases of acute renal failure in patients with working allografts attendant to intravenous administration of high dose cyclosporine. He counselled that cyclosporine not be given pre-, or perioperatively, but be started only after renal function had been exhibited by the given transplant. The same implicit argument could be made when one examines the results of the Canadian multi-center trial of cyclosporine conducted by the research team headed by Dr. Calvin Stiller. This group demonstrated that the therapeutic advantage offered by cyclosporine for improvement of transplant graft survival at one year was significant only for groups of patients who received their transplant within 24 hrs of organ harvest. Older kidneys faired less well. Upon analysis of the explanation of these findings, the Canadian authors suggested that primary non-function or acute tubular necrosis rates were higher in the kidneys preserved over a longer periods of time. The use of cyclosporine coupled to prolonged preservation times led to the abrogation of the cyclosporine graft survival advantage.

PRIMARY NONFUNCTION AND CyA

ATN	Conventional 27%	CyA 33%
Dialyses	3 Days	6 Days
Oligoanuria	6 Days	12 Days

Simmons et al Trans Proc 18: 19, 1986

RELATIONSHIP BETWEEN PRIMARY NONFUNCTION AND OUTCOME IN CyA TREATED RECIPIENTS

ONE YEAR GRAFT SURVIVAL

Group I	Group II	Group III
0-1 Dialyses	2-5	>5
88%	53%	38%

Rocher et al Clin Transpl 1: 29, 1987

There are three distinctly different questions involved with the role that cyclosporine may play with primary non-function of the renal transplant. The first issue is whether the incidence of acute tubular necrosis for primary non-function is actually increased by the drug. Most recent analyses by individual transplant centers all agree with the finding that the rate of occurrence of acute tubular necrosis in the cyclosporine treated patient is not increased over the previous experience when one takes in account similar means of organ preservation. Representative data is shown in the table from the University of Minnesota's large program. The historical rate of ATN in this program has been variably reported as 30-33%. Simmons et al performed a study in which the conventional immunosuppressive regimen was compared to cyclosporine and prednisone with the cyclosporine given perioperatively. ATN incidence was 27% in 75 patients receiving allografts in the prospective study compared to 33% of 83 patients receiving cyclosporine values statistically different. Our own analysis is quite similar at PMH. Our rate of ATN is reasonably low, 21%, which is actually lower than our previous experience an outgrowth of the adoption of the perioperative fluid regimen characterized by colloid rather than crystalloid infusion designed by Dr. Ingemar Dawidson. An additional factor reflects the preponderance of kidneys which we harvested locally by primary transplant surgeons in the Dallas area.

In contrast to the failure to define a cyclosporine effect on an increased rate of primary non-function occurence, is the experience with the effect of the drug in prolonging the ATN. The number of dialysis treatments required and the number of days spent by the patient in ATN are both greater. Returning to the prospective University of Minnesota study which did not show an increase in the rate of ATN, one can see both of the described findings. Dialysis treatments required were doubled when cyclosporine was used (from 3-6 treatments). The days of oligo-anuria was also doubled from 6±1 to 12±1 days. The number of kidneys which never functioned also increased from an event which was quite rare to an event now which occurs predictably each year in most large transplant centers.

The fate of the kidney which demonstrates primary non-function under the aegis of cyclosporine treatment has also been studied recently by many groups. I have selected only one set of data from the published material, that of Gonwa et al. At 18 months, Gonwa finds the graft survival in patients treated with cyclosporine who demonstrated good initial function to be 77% to be contrasted to the 43% graft survival exhibited by cyclosporine treated patients whose initial function was poor. Our own data is even more striking. Examining the fate of first transplant recipients, the one year actuarially computed graft survival for patients receiving a kidney in 1986 who exhibit initial excellent function was 95% as compared to less than 50% for those patients who did not exhibit initial good function when cyclosporine was used. One can conclude from these and other similar reports that the greatest impact that perioperative cyclosporine has is to prolong and increase the severity of primary non-function which taken together with the use of a other potential nephrotoxins and the ischemia a consequence of organ harvest leads to poor graft survival.

There has been a major attempt to assign individual risk factors to explain the cyclosporine effect on primary non-function. Since the occurrence of cyclosporine-induced prolongation of primary non-function is relatively uncommon, it is reasonable to look at those features which might be present to

MULTIVARIATE ANALYSIS OF POTENTIAL RISK FACTORS FOR PRIMARY NONFUNCTION

STATISTICALLY SIGNIFICANT FACTORS

Donor Related
- Warm Ischemia Time
- Shipped
- Perfusion

CyA Related
- IV Use
- Mean Day 3 Level

Kahan et al Transplantation 43: 65, 1987

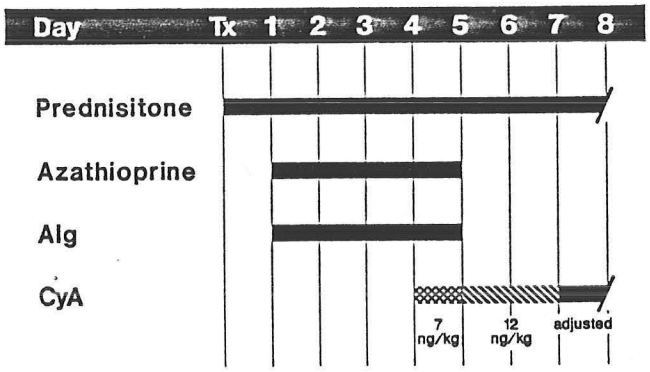
explain the potential increased toxicity of the drug. The data from the multi-center Canadian cooperative trial provides the first hint as to potential risk factors. As reported by Stiller, early cyclosporine nephrotoxicity appeared in the group in which donor kidneys had been preserved more than 24 hrs pointing to factors related to the organ harvesting event. More recently Kahan's group at the University of Texas at Houston has had opportunity to examine risk factors by multivariate analysis. Two distinctly different groups of risk factors were identified. The first group were entirely related to the circumstances surrounding donor harvest. Those factors which were statistically important in predicting primary non-function in the cyclosporine treated patient included prolonged warm ischemia time, the use of pulsatile perfusion as the method of preserving the tissue as opposed to simple cold storage, and whether the kidney was shipped from a different harvesting team to the transplanting team. The latter observation has important clinical and political consequence. The present political climate on Capitol Hill with respect to transplant issues is to foster greater cooperation in organ-sharing among the various transplant programs. The scientific rationale offered to support this political and medical view deals with the statistical likelihood of identifying donor and recipient pairs with high degrees of tissue matching. The scientific presumption is that this increased matching may improve graft survival, a notion which at best is remains to be proven in the cyclosporine era. Carried to its logical consequence, then, a great deal of kidneys would be shipped from the harvesting team to the transplanting team in order to obtain excellent tissue matches. Not only would kidneys be shipped routinely, but the law, as presently conceived, would require such shipping. Yet shipping of kidneys, increasing potentially the time between harvest and surgical placement, increasing the potential for ischemic injury to the kidney, using kidneys harvested by a wide range of harvesting teams of varied skill all would establish the setting most conducive for the cyclosporine effect on primary non-function, which we have already shown leads to poor graft survival results. When Kahan's group analyzed recipient features which may contribute to primary non-function in the cyclosporine era, no statistically significant factors were observed independent of cyclosporine use. Matching did not explain the data. The implicit conclusion that one can reach from these data is that early placement of locally harvested kidneys is a much more important predictor of graft outcome than is excellent tissue matching.

The second group of risk factors identified by Kahan are those related to the amount and route of cyclosporine used in the peri-operative period. Statistically significant, albeit weak, is the pre- or perioperative intravenous use of cyclosporine. More important in the hands of Kahan is the concentration of drug in the blood of patients on the third day after transplantation. His group argues that the concomitance of IV use and toxic levels along with ischemic injury attendant to the harvesting process taken together lead to the cyclosporine nephrotoxic effect that is clinically observed as the prolongation and worsening of primary non-function.

These clinical studies suggest that cyclosporine injury is synergistic to the potentially nephrotoxic environment a consequence of organ harvest and graft placement. There is now accumulating experimental evidence to support this view derived from the clinical studies. Experimentally various assays which assess the presence of and severity of tissue injury are worsened when renal slices, renal cells in tissue culture, or perfused kidneys are rendered ischemic prior to the provision of a dose of cyclosporine which by itself is not nephrotoxic as judged by the endpoints used. These observations lend credence to the clinical impressions that examine the management of organ harvest, the strategies to avoid nephrotoxity and the recommendations for tissue matching and organ shipping.

Some of the therapeutic strategies to circumvent transplant damage related to cyclosporine in freshly harvested and placed kidneys in the perioperative period are obvious and grow out of the clinical and experimental studies detailed above. The transplant team should make every effort to place the kidney within 24 hours. Acceptance of tissue with extended warm ischemia times is no longer routine at our program. The Transplantation Team at Parkland prefers the use of organs harvested locally, within the Southwest Organ Bank Procurement Organization system. Other strategies have been recently described which take advantage of the concept that early cyclosporine may be detrimental rather than helpful. All of these strategies take as their central point the delay of the first use of cyclosporine, as did Professor Calne empirically in the early use of the drug. In this early period, when the allograft initiates the sensitization of the host, a crucial or vulnerable period for prolonged graft survival, an alternate immunosuppressive regimen must be used. The goal of the alternate regimen is to prevent sensitization without the use of cyclosporine, to prevent the movement of the activated lymphocyte through the lymphocyte cell cycle, and to prevent the release of lymphokines such as γ interferon in order to prevent the entire allograft from attaining class II HLA antigens on parenchymal cells thereby enhancing antigenicity of the graft. A large multicenter cooperative study headed by Dr. Ron Ferguson of the Ohio State Medical School employs a treatment regimen he calls "sequential therapy" in which prednisone and azathioprine are begun pre-transplant followed by the institution of antilymphoblast globulin (ALG) for the first five postoperative days. At this point, the azathioprine and ALG are stopped, prednisone is tapered and cyclosporine begun at 10 mg/kg·d. Using this strategy, Sommer et al report the rate of ATN at 10%, significantly less than their earlier experience, with no increase in the number of days of oliguria or hemodialysis. Equally heartening was the graft survival for first transplant recipients at two years of 85% for and 72% for second, numbers similar to those that we obtained at Parkland Hospital.

QUADRUPLE INDUCTION THERAPY



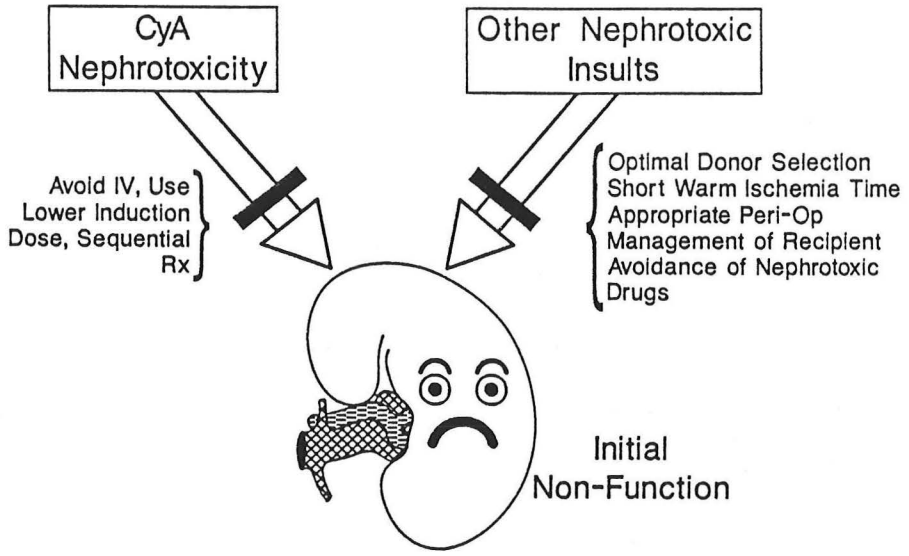
QUADRUPLE INDUCTION THERAPY

ATN Rate	24%
sCREAT	ATN 2.2 +/- 0.4 nl 1.7 +/- 0.2
Gft Survival	86%
Rejection	23%

Sagalowsky et al Abs ASN '86

The UT-Parkland Hospital program has adopted a similar but slightly different program that we have dubbed "quadruple induction therapy", the strategy of which is to prevent early rejection, to prevent the early use of cyclosporine when kidneys are most vulnerable, to achieve therapeutic blood levels of cyclosporine without acute cyclosporine nephrotoxicity in the working allograft. A combination of ALG, prednisone, and azathioprine is also used for the first five postoperative days. In our protocol, rather than discontinuing abruptly ALG and azathioprine, we overlap one or more days with cyclosporine at 7 mg/kg. The differences between the Ferguson protocol and the PMH version are not necessarily slight. In a previous prospective trial, abrupt cessation of one variety of immunosuppressive for cyclosporine was associated with a 53% incidence of acute transplant rejection in the first few days after switch. We argued that an overlapping period was necessary in order to achieve an adequate tissue level of drug, recognizing the pharmacologic properties of cyclosporine as a lipophilic agent. We also used a higher dose of CyA after the switch because we found, upon blood level monitoring, that many patients did not achieve a therapeutic blood level even on this present dose. In our initial analysis of 20 consecutive patients, graft survival was 95% (one year actuarial). Six instances of acute renal failure occurred without graft loss. The mean creatinine of 1.7 ± 0.2 mg/dl for the group without ATN was not different from that for those with (2.2 ± 0.4). In this initial group only two of 20 experienced any rejection episodes while 18 of 20 were rejection-free. We now have treated more than 100 patients with this protocol. Although our experience with the rejection episode is not as fantastic as our initial examination, it is still superb. Approximately 23% of individuals will have a rejection episode that can be temporally related to the switch, a figure which is markedly reduced from the pre-cyclosporine era of 98% rejection episodes or to the 53% rejection episodes with our previous use of cyclosporine.

	1983	1986
CyA	Periop	Sequential
ATN	33%	21%
Losses	50%	37%
HD Rx	9	4



In summary, cyclosporine is associated with a hyperacute variety of nephrotoxicity characterized by a prolongation of and deeping of primary non-function. This form of nephrotoxicity is related to the presence of a multiplicity of other nephrotoxic injuries, early high-dose use, and intravenous administration. Therapeutic strategies which reduce primary ischemic injury to the kidney which avoid the early use of cyclosporine while preventing rejection with an alternative drug regimen, have been highly successful in reducing substantially this variety of cyclosporine nephrotoxicity.

B. ACUTE, DOSE-RELATED NEPHROTOXICITY

Case Study

SJ received a 1 DR matched cadaveric renal allograft 3/27/84 at PMH. The allograft functioned immediately in recovery room and dropped the serum creatinine to 2.7 mg/dl by the 12th hospital day. Treatment included perioperative intravenous cyclosporine followed by two days of IV cyclosporine then high-dose oral cyclosporine (15 mg/kg) coupled to prednisolone at a tapered schedule. At discharge he was taking cyclosporine 850 mg/d and prednisolone 45 mg/d. In 1984 PMH used measurements of whole blood cyclosporine levels accomplished through the kind hospices of Dr. Barry Kahan at The University of Texas at Houston by an IRA method which took four days to return. SJ was seen several times in the clinic with stable renal function at the discharge creatinine. On 4/24/84 creatinine in the clinic was noted to be 3.8 mg/dl. The patient was asymptomatic, the physical examination was entirely normal, there was no hypertension. The patient had experienced no fever in the few days before the clinic visit nor had he noted a reduction in his urine flow rate or an increase in his weight. The graft was described on physical exam as small, firm, and non-tender. A bruit in the iliac fossa was not detected. A renal nuclide scan was

obtained with excellent initial upstroke of the blood flow portion of the curve, although total renal function was diminished. Ultrasonographic analysis of the graft region failed to reveal a urine leak, obstructive nephropathy or other mechanical catastrophe to explain the elevation in creatinine. His maintenance cyclosporine dose was 700 mg/dl. A diagnosis of acute, dose-related cyclosporine nephrotoxicity was made and the cyclosporine dose was halved immediately. Within three days his serum creatinine returned to the baseline 2.7 mg/dl. Later that week RIA measurements confirmed an elevated blood level of the immunosuppressive medication. Subsequently, cyclosporine has been tapered so that his maintenance until quite recently was 300 mg/d or about 3.2 mg/kg·d. His serum creatinine ultimately declined to 1.8 mg/dl where it has been until recently when a bout of severe chronic aggressive hepatitis has altered his requirements for immunosuppression.

In clinical terms, this variety of cyclosporine nephrotoxicity is exemplified by renal dysfunction in a patient treated with cyclosporine in the first days or weeks of therapy. In the kidney transplant recipient, this form of toxicity presents as a diagnostic dilemma between the entities of renal transplant rejection, which of course may cause renal dysfunction, surgical and mechanical difficulties, and dysfunction due to the immunosuppressive drug per se. The strong immunosuppressive properties of cyclosporine has made this diagnostic dilemma more difficult as an array of lymphokines and monokines which are responsible in great measure for the constitutional symptoms that are clinically associated with allograft rejection are inhibited. Rejection, therefore, is often symptomatically milder and more difficult to appreciate at the bedside. In those instances in which patients experience fever, allograft swelling, perigraft tenderness, hypertension, and/or other more non-descript constitutional symptoms such as myalgias or arthalgias, a diagnosis of rejection is easily made. Unfortunately, the absence of this panoply of constitutional symptoms does not allow one to dismiss the diagnosis of rejection to explain a declination in GFR. There has developed a rather large body of literature attempting to deal with the laboratory analysis that would allow the physician to discern the appropriate diagnosis. The least controversial laboratory test used to try and understand the genesis of renal dysfunction is the ultrasound which examines the potential for mechanical problems attendant to the surgical procedure but does not really address the rejection or cyclosporine toxicity issues. Radionuclide scanning using such reagents as Tc-99m DTPA or I^{131}-hippuran has been variably successful in distinguishing between nephrotoxicity and rejection. Findings compatible with the diagnosis of cyclosporine nephrotoxicity on serial scans include disruption of the nuclide pattern consistent with progressive tubular functional abnormalities without alteration in the pattern of renal perfusion. Transplant rejection, on the other hand, has been characterized by diminished renal perfusion and delay in nuclide transit time. Kim et al compared nuclide scanning with histologic findings in 25 patients of which 15 had documented nephrotoxicity by virtue of retrospectively discerned response to therapy. In 12 of the 15 cases, the scanning criteria just elaborated was adequate to make the appropriate diagnosis. Unfortunately, this optimistic view of the use of the scan has not been the general experience. Cases of milder rejection often have little or subtle changes in perfusion which may lead to a misdiagnosis. The absence of severe constitutional symptomatology in the rejecting patient

reflects the diminution in episodes characterized by a great deal of graft swelling or edema thus raising the potential for milder scanning changes in rejection and the capacity to misdiagnose cyclosporine nephrotoxicity. On the other hand, the most severe varieties of cyclosporine nephrotoxicity have been shown to be associated with diminished renal perfusion related to intense vasoconstriction which would ultimately be seen on the scan and be misdiagnosed as an acute rejection episode. The relative nonspecificity and lack of sensitivity of the scanning test has reduced its role substantially.

More recently Danovitch et al have espoused the use of nuclear magnetic resonance imaging (NMR) as a non-invasive radiographic means of making the appropriate diagnosis. Again advantage is taken of the potential for acute rejection to markedly swell the kidney with inflammatory edema and hemorrhage which in a classic case blurs the distinct margin that may be visible by NMR imaging between the cortex and the renal medulla. Instances of renal dysfunction in which the cortical-medullary junction is well preserved were shown to be related to cyclosporine nephrotoxicity in a substantial number of cases, while blurring of this margin was related most often to rejection. Objections can be lodged for the NMR studies similar to those for the nuclide scanning techniques. Severe cases of rejection, those most easily diagnosed at the bedside, provide "classical" imaging. Unfortunately in the cyclosporine era, rejection is often much more subtle than under previous immunosuppressive regimens so that the absence of severe rejection abnormalities can no longer be taken as prima facie evidence for the opposite diagnosis. With both of these noninvasive studies, the physician is in the position of making a diagnosis in the absence of certain findings, without definite proof or evidence to support the diagnosis made.

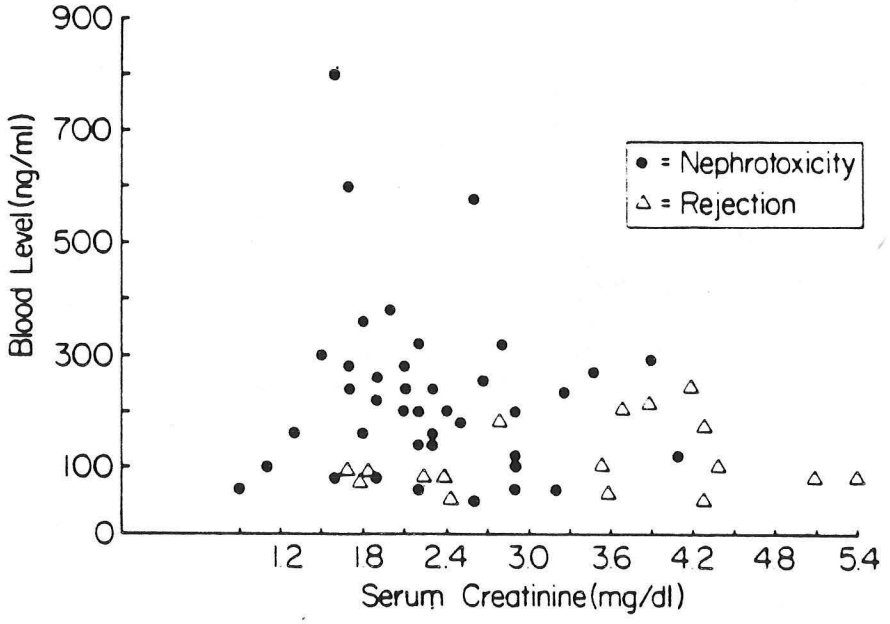
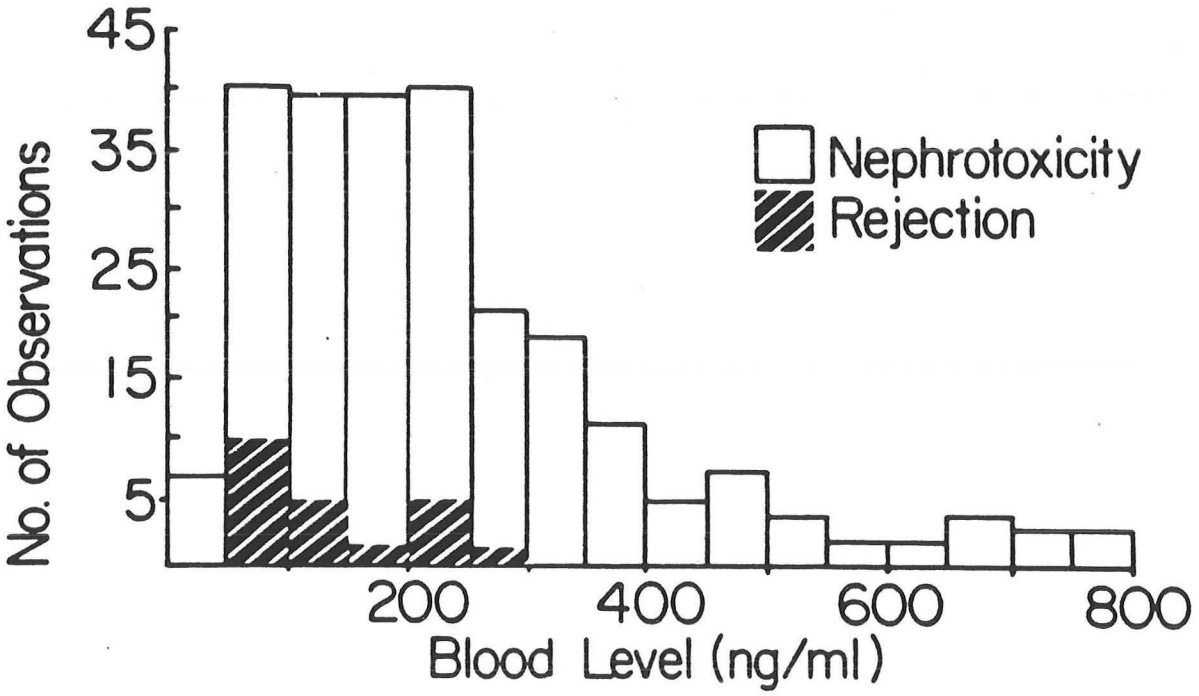


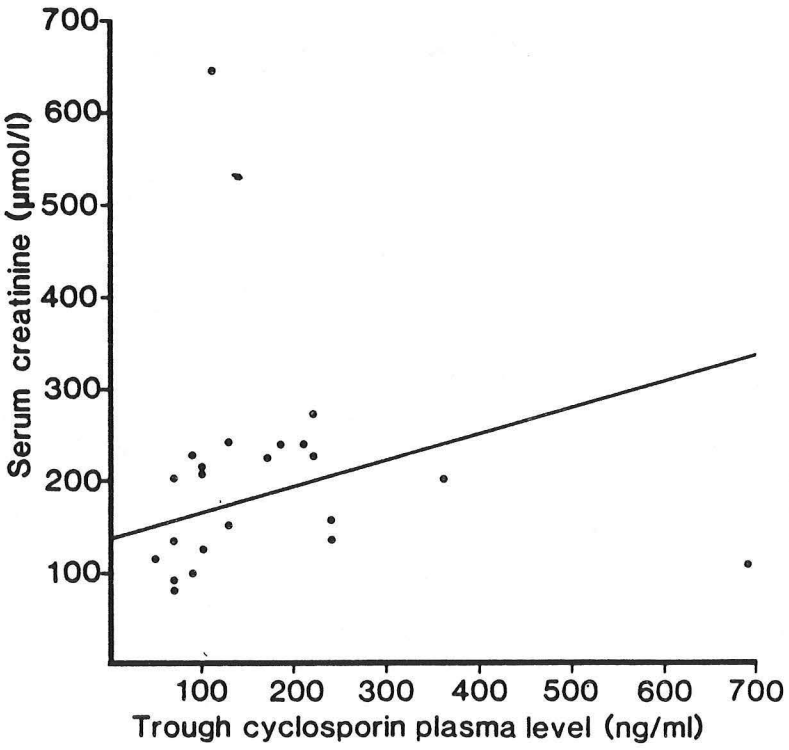
Fig 1. Relationship of whole blood trough CsA whole blood levels plotted against serum creatinine performed at the time of onset of the first episode of renal dysfunction.

It would be most helpful if assays of cyclosporine content in blood or tissues would correspond to clinical toxicity. The pharmacokinetics of the drug would argue, a priori that blood or serum measurements would not be the most accurate means of accessing tissue toxicity. Additionally, the so-called therapeutic window for cyclosporine has been determined by virtue of the capacity of the drug to inhibit the synthesis and release of the lymphokine Il-II in tissue culture. The target level that we seek at PMH is 100-200 ng/ml, a value which ranges between 3-5 times the minimum dose necessary to completely inhibit Il-II production as determined by western blot analysis. Since toxicity is mechanistically unrelated to the therapeutic mechanisms that under pin the drug and since targeted levels relate to immunosuppressive rather than tissue toxicity, blood level monitoring to diagnosis or avoid nephrotoxicity has not been very helpful. These issues aside, it is generally true that instances of renal dysfunction which are not easily diagnosable are more often related to cyclosporine nephrotoxicity when blood levels of the drug are very high and more often related to rejection when blood levels of the drug are below those deemed adequate for immunosuppression.

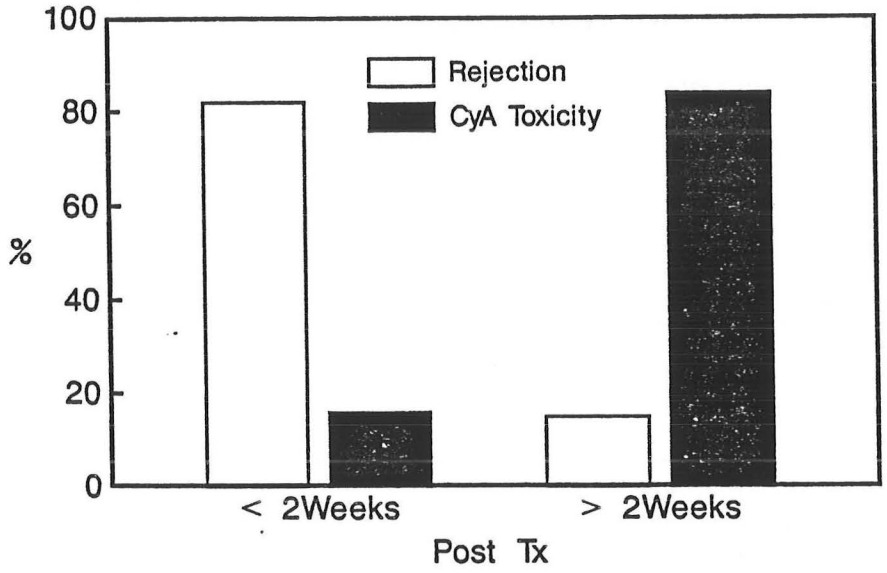


It has been easier to prove a statistical correlation between cyclosporine trough levels and rejection. Unfortunately, there was a great deal of overlap in the large series reported by Kahan and by Keown so that any single determination was found to be inadequate alone as a diagnostic criterion. Ferguson from Ohio State could demonstrate that rejection episodes rarely occurred when cyclosporine levels are quite high; again single measurements

were unable to distinguish between the two most important diagnostic choices. Early after transplant, for example, only three of eight patients had a blood level within the normal range when nephrotoxicity was felt to exist. Five of the eight patients had values that did not overlap with patients with normal function. Interestingly, the diagnostic utility of the cyclosporine blood level rapidly declines with time after surgery so that after two months posttransplantation the degree of overlap between patients with normal function and nephrotoxicity is great. When patients approach one year after transplantation blood levels completely lose their capacity to distinguish between patients with clinical toxicity and normal function. As we will see later in this section, this probably reflects the imposition of a different variety of cyclosporine nephrotoxicity, a more chronic, not dose-responsive variety. Although blood level maintaining may only hint at the etiology for renal dysfunction, there is a general relationship between the serum creatinine that is achieved by a given renal transplant and the amount of cyclosporine in the blood.



As shown in the figure there is a statistically significant relationship between creatinine and plasma cyclosporine level although the r value of of 0.41 suggests that there is multiplicity of other factors which contributes to the variance observed. A similar relationship exists in the experimental animal in which on can observe a linear relationship between the dose of cyclosporine provided to the beast and the creatinine. To complicate the use of blood level monitoring further, Ferguson has demonstrated a temporal relationship between the time after transplantation and the likelihood of a given diagnosis. Not only is the likelihood that monitoring is less predictive of abnormal status with time is the temporal relationship that governs the chances of a given reason for renal dysfunction.



Ferguson 1985

As shown by Ferguson's analysis of 63 patients from 0-13 weeks after transplantation, rejection is much more likely a diagnosis early (< 2 weeks), while cyclosporine nephrotoxicity was most likely the diagnosis late (> 4 weeks). 82% of all episodes of renal dysfunction within two weeks was casued by acute allograft rejection, while the adverse obverse was true; that is 85% of renal dysfunction after two weeks were related to cyclosporine toxicity. With respect to blood level monitoring, then, one may conclude that analysis of cyclosporine levels are mere guides to correct diagnosis. In the early postoperative period it is possible to use the level with more confidence, while measurements at one year or greater are worthless. Very high levels are most often associated with toxicity, very low levels with the potential for rejection. The great deal of overlap does not allow one to use the level alone to make an appropriate diagnosis. Moreover levels within a great range of so-called therapeutic are completely unhelpful. Taken together, the evidence supports the view that lavish attention paid to cyclosporine levels is a clear mistake. One can use the levels early only as guidelines to diagnosis taken together with a wide range of tests that are being performed. Certainly it cannot be taken as a gold standard and should be used wisely. The appropriate utility of blood monitoring may be to follow patients with oral absorption problems, to ensure that a therapeutic dose adjustment has had its expected effect, to monitor the impact on drug distribution when other medications are needed which have potential drug-drug interactions.

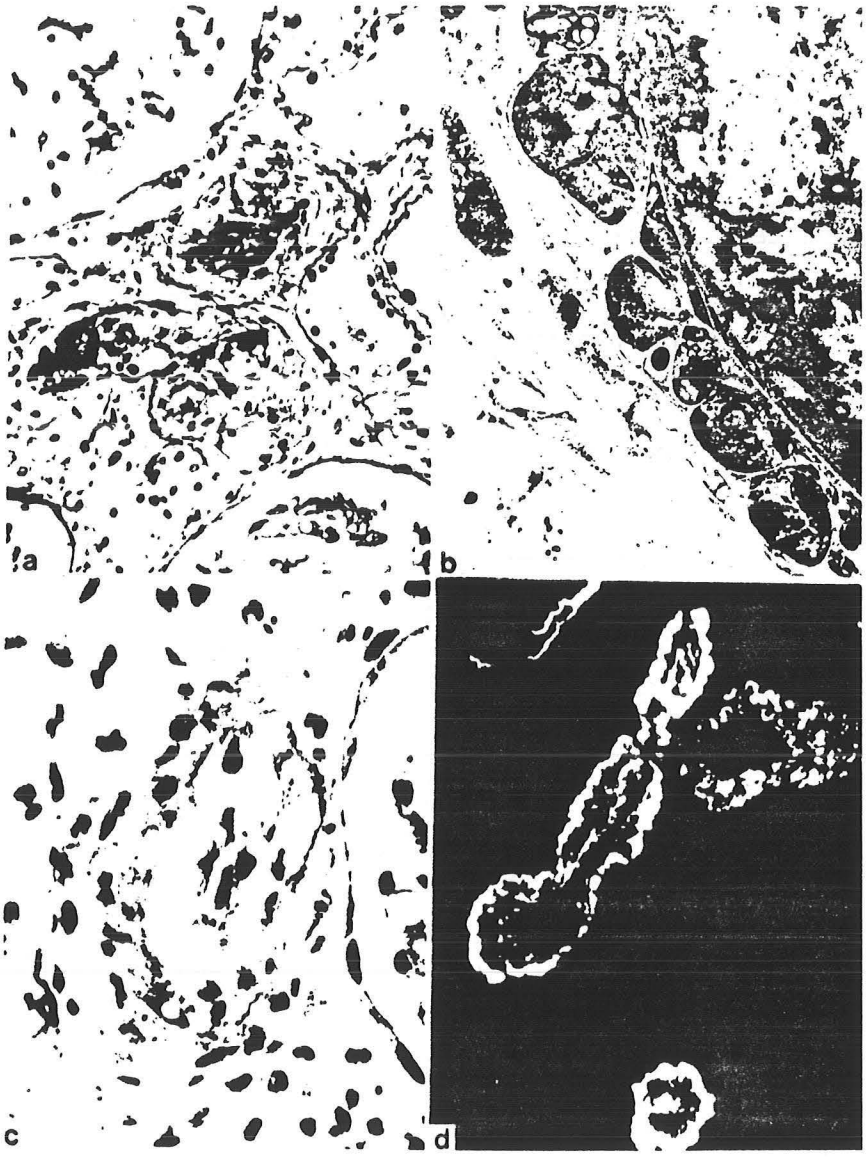
If nuclide scanning, NMR imaging or blood level monitoring are all imperfect means of solving the diagnostic dilemma between rejection and cyclosporine nephrotoxicity, pathologic diagnoses fares better. The utility of the renal biopsy in this regard is based on the distinct morphologic patterns described by Professor M.J. Mihatsch and his colleagues at the University of Basel, who believe it is possible to make a definitive diagnosis of rejection and to make an informed guess about cyclosporine toxicity as well from biopsy material. In their hands, core renal biopsies of the acute, reversible variety of cyclosporine induced nephrotoxicity may have one of four distinct patterns.

MORPHOLOGIC FINDINGS IN ACUTE NEPHROTOXICITY OF CyA

1. Toxic Tubulopathy
2. Peritubular Capillary Congestion with Mononuclear Cell Infiltrate
3. Both
4. Normal Microscopy

Mihatsch et al 1985

The first pattern they call toxic tubulopathy and is characterized by inclusion bodies in tubular epithelial cells which correspond to giant mitochondrial, often best seen by electron microscopy, by isometric tubular vacuolizes which do not contain drug and occasionally by tubular microcalcification. The giant mitochondria are found predominantly in the proximal tubule at the rate of one per cell. In contrast, the isometric vacuolization is found in the straight portion of the proximal tubule, is morphologically distinct from the kind of irregular vacuolization often seen in ischemic renal injury, and may be seen at the light microscopic level. A second pattern in acute cyclosporine toxicity relates to peritubular capillary congestion with dilatation of the capillary lumens often filled with mononuclear cell infiltrate without interstitial infiltrates or edema. It is the presence of mononuclear cells in capillary lumens which is often confusing in that the presence of such infiltrates in general have been the sine qua non of acute cellular rejection. For Mihatsch, the location in the biopsy and the amount of the infiltrates are diagnostically distinguishing, a distinction which has not been universally accepted. Further complicating the matter, mononuclear cell infiltrates are cleared from renal biopsies after high-dose steroid therapy so that the use of infiltrates as a diagnostic criterion requires one to know when the biopsy was obtained vis a vis treatment. Most often biopsies performed in cases of cyclosporine nephrotoxicity contain evidence for both toxic tubular changes and the congestive changes including luminal mononuclear cell infiltrates. Further limiting the ability of the core biopsy to provide positive evidence of nephrotoxicity is the fact that toxicity can be present with significant renal impairment without any light



A. Giant mitochondria as inclusions-
   light microscopy
B. Giant mitochondria by EM
C. Vacuoles
D. Microcalcification

microscopic changes. Most pathologists, then, use the biopsy to define the presence of findings consistent with acute cellular rejection, the absence of which suggests the diagnosis of cyclosporine nephrotoxicity to explain renal dysfunction.

Other investigators have been less enthusiastic with respect to specific morphologic changes related to acute cyclosporine nephrotoxicity than Mihatsch and colleagues. Cameron's group from Guys Hospital in London examined biopsies routinely performed at one week and one month after transplant as well as with each episode of renal dysfunction in 60 consecutive allograft recipients. Rejection and cyclosporine toxicity were defined by response to therapy in a retrospective manner. Findings from these 60 biopsies were compared with 20 biopsies performed in patients with stable function. In 32 of 35 cases of rejection, a disfuse interstitial mononuclear cell infiltrate or frank arteritis was found. In contrast focal mononuclear cell infiltrates were found in patients with normal function or with patients with nephrotoxicity at equal rates. Indeed there were no important differences between biopsies from patients with nephrotoxicity and those with stable function. Cameron concluded that core biopsy can provide evidence for the absence of rejection in the face of renal dysfunction which point to cyclosporine nephrotoxicity. Even more damaging to the Mihatsch point of view have been the findings of Morris and his group from Oxford who performed a prospective study of 107 biopsies from patients receiving cyclosporine and 106 biopsies from patients receiving conventional drugs read so-called "blind". The study pathologist could find no glomerular, tubular, vascular, or interstitial changes which could distinguish between a transplant with "normal" function and cyclosporine toxicity. Although the transplant physician may be closer to being able to appreciate positive evidence for cyclosporine nephrotoxicity on the core renal biopsy, the number of pathologists who feel capable of discerning such subtle changes related to the acute variety of cyclosporine toxicity are few. The story will be quite different when chronic cyclosporine nephrotoxicity and the renal biopsy is discussed. Suffice to say, the most appropriate use for the renal biopsy for acute cyclosporine toxicity at this time is to provide a negative piece of datum. Renal dysfuction after transplantation in a patient on cyclosporine whose core biopsy does not reveal acute cellular rejection is more likely to be related to cyclosporine toxicity. Taken together with other pieces of information which, alone, are nondiagnostic such as an elevated blood level, the time after transplantation, a normal flow scan, maintenance of the cortico-medullary junction on NMR imaging, one can attempt a diagnosis and alter therapy accordingly.

More recently a new variety of transplant biopsy has been characterized, validated, and established in the United States by our own transplant program, the fine needle aspiration biopsy technique. This technique holds out promise for the solution to the diagnostic dilemma under discussion. Whereas isometric vacuolization of the straight portion of the proximal tubule has been described by Mihatsch to occur ocassionally when core biopsies are evaluated by light microscopy, it is almost uniformally seen in parenchymal cells aspirated from kidneys in patients with cyclosporine toxicity proven by response to dose adjustment. Furthermore, the diagnosis of acute cellular rejection is the most easily made by the fine needle aspiration biopsy technique in a highly accurate, highly sensitive, and highly specific manner (> 90% specificity and sensitivity). Interestingly, in contrast to the general statistical trend, Häyry and von Willebrand have found a few instances

in which cyclosporine toxicity occurred concomitant with allograft rejection, a finding which can only be made with the fine needle technique in which the state of maturity of infiltrating immunocompetent cells reveals the presence of acute rejection while parenchymal cell vacuolization uncovers cyclosporine excess. Although promising, final prospective confirmation of the utility of the fine needle aspiration biopsy technique in identifying cyclosporine toxicity has yet be made. It is still possible that the morphologic pattern related to cyclosporine toxicity by FNAB may merely reflect high-dose cyclosporine use, which does not always correspond to tissue toxicity. More careful laboratory and clinical observation must be made before a final conclusion can be made concerning FNAB. One, however, may conclude from the available data that FNAB may offer a major addition to the capacity of one to diagnose acute cyclosporine toxicity with positive data.

While Mihatsch and colleagues can describe morphologic changes in intracellular organelles which may suggest that cyclosporine has primary tubular toxicity of the magnitude encountered with tubular poisons such as aminoglycosides, most investigators have generally found that tubular function remains intact or is only slightly altered during acute cyclosporine nephrotoxicity. Humes and colleagues found that cyclosporine could inhibit mitochondrial respiration in a dose dependent manner when renal cortical mitochondria were isolated from solubilized cells and placed in solution. The drug also concentrated in the lipid bylayer, of the cell wall, as would be suggested by its lipophilic nature. Thus, Hume argues, there is potential for renal tubular damage caused directly by this drug. When screened more specifically for renal cell injury, however, Hume found basal and uncoupled respiratory rates, intracellular tubular potassium and calcium levels, and adenine nucleotide contents of isolated rabbit tubules perfused with cyclosporine to be similar to sham controls. They concluded that, despite the potential for direct tubular harm, there was no direct evidence for actual cyclosporine-induced toxic renal tubular cell injury. They hypothesized that the acute cyclosporine nephrotoxic effect was entirely a consequence of alterations in intrarenal blood flow patterns. In their experimental model, they reasoned that diminished renal blood flow accounted for the observed elevations in BUN and in serum creatinine, and for the diminution in isotopically measured glomerular filtration rates. In their model, cyclosporine was shown to reduce total renal blood flow by a mean of 36% (by a radioactive microsphere technique) which could account *per se* for all observed functioned changes.

PROPOSED MECHANISM FOR ACUTE CyA NEPHROTOXICITY

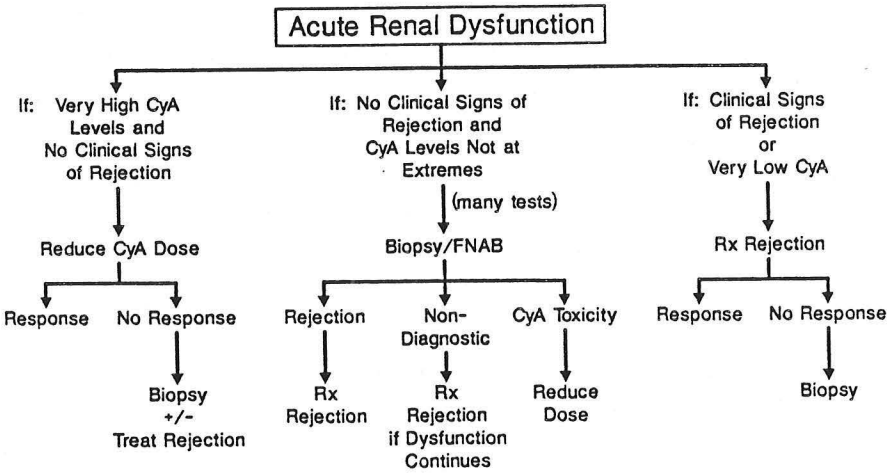
1. Little Evidence for Tubular Subcellular Damage When Cell Intact
2. RBF and Cortical Blood Flow Acutely Reduced
3. Acutely Renin, Angiotensin II, Elevated; Prostacyclin, PGE and Bradykinin Reduced
4. By Micropuncture: Increases in Efferent Arteriolar Resistance and Decreases in Filtration Surface Area
5. Verapamil, Enalapril, and Fish Oils Protective

The findings of Shore and his group support the notion that acute cyclosporine nephrotoxicity is a consequence of altered renal blood flow. Employing glomerular micropuncture techniques originally described by Brenner and colleagues, Shore demonstrated that reduction of GFR is causally related to marked increases in efferent arteriolar resistance coupled to reduction of the glomerular filtration surface area (K_f), all the consequence of elevations in intrarenal angiotensin-II levels. Shore demonstrated that the reductions in renal blood flow, in glomerular filtration rate, and in urine flow could be blocked by a specific angiotensin-II converting enzyme inhibitor. The mechanism by which cyclosporine induces angiotensin-II elaboration acutely in patients is not clear and may involve changes in renin, bradykinin, and/or prostaglandin synthesis. Fruitful research in this area may ultimately uncover a therapeutic approach which reduces the chances for, or severity of cyclosporine nephrotoxicity by restoring to normal the intrarenal signal peptides which control in part of the glomerular filtration rate.

THERAPEUTIC APPROACH TO ACUTE CyA NEPHROTOXICITY

1. Rule Out Mechanical Dysfunction with Sonogram
2. Perform an Array of Tests to Make the Best Diagnostic Guess: Scan, Blood Level, Renal BX, Rejection
3. Search for Positive Evidence for Rejection
4. Reduce the Dose If CyA Toxicity Felt Present - by 100 mg, by Half, Skip a Day
5. Response Must be Seen within 3 Days - If Not Consider Treating for Rejection

DIAGNOSTIC AND THERAPEUTIC ALGORITHM FOR POSSIBLE ACUTE CyA NEPHROTOXICITY



In conclusion that cyclosporine is a drug which has improved our immunosuppressive control of transplants immensely thereby enhancing graft survival rates while toxic to the very organ transplanted, is a paradox which has led to no small amount of clinical insecurity. Cyclosporine nephrotoxicity is difficult to diagnose with definitive evidence so that when renal dysfunction of the transplant is encountered an expensive array of tests are performed searching for the appropriate clues to permit an educated guess as to the correct diagnosis. Even then, when all the clues are available, an educated guess constructed and a therapeutic change instituted, confirmation of the diagnosis can only be made retrospectively by response to the therapeutic change made. It is not uncommon for a therapeutic change based on the complements of clinical studies to be ineffective generating a clinical response exactly opposite to that first taken. Since acute cyclosporine toxicity appears to be completely reversible and appears to be related to blood flow changes rather than to direct tubular toxic changes, I believe that it is most important that the diagnosis of allograft rejection be searched for assiduously and treated briskly when present. Positive evidence by various biopsy techniques is available for this diagnosis, the consequences of misdiagnosis in this direction may be grave and irreversible, and a false positive diagnosis can be rapidly remedied. It is my view, then, that one should err on the side of potentially over-diagnosing acute rejection. Further research into the mechanisms which casue acute cyclosporine nephrotoxicity will lead to therapeutic approaches which may lessen the rate of occurrence of this all too common side effect of this extraordinarily potent and important new immunosuppressive medicine.

C. ARTERIOLOPATHY

Case Study

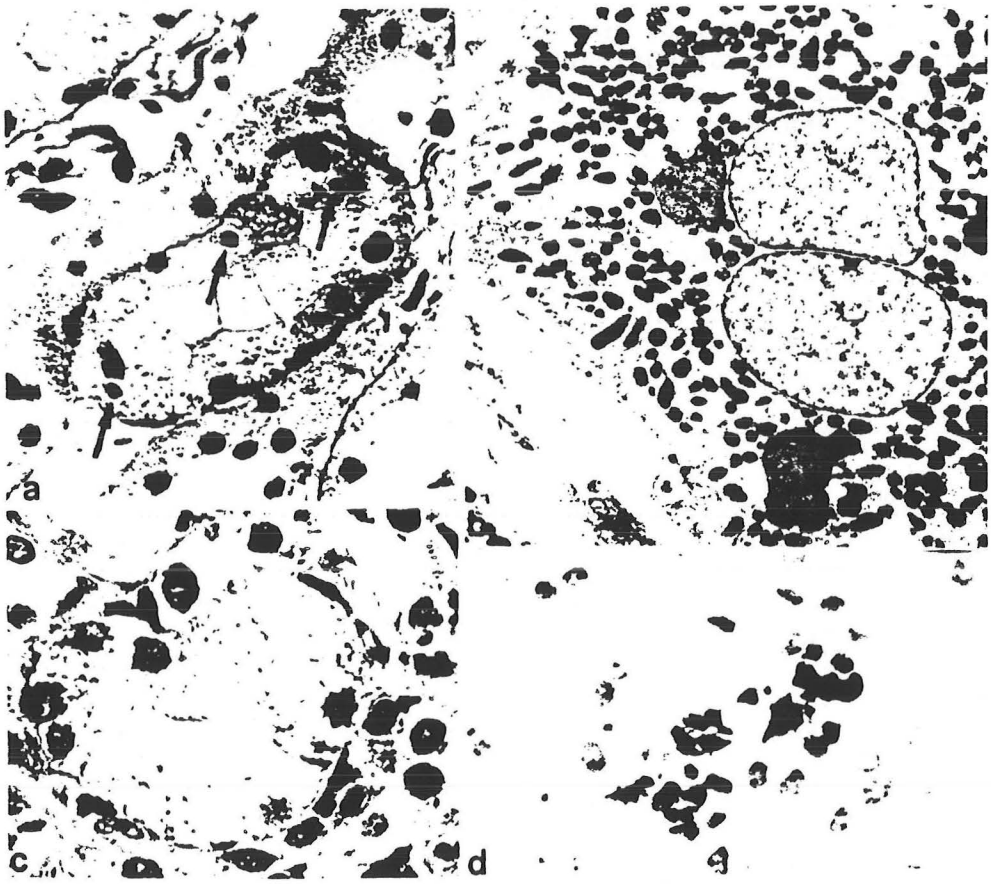
LB, a 28 year old man with type I diabetes mellitus, underwent an uncomplicated cadaver renal allograft procedure on 3/25/85. He was immunosuppressed with prednisolone and cyclosporine begun orally on the first postoperative day at a dose of 14 mg/kg d. He made urine in the recovery room and dropped his creatinine from 7.6 to 1.2 mg/dl reached on the fifth postoperative day. On the sixth postoperative day the creatinine rose briskly to 3.9 mg/dl. An ultrasound study failed to reveal mechanical problems. Despite the absence of constitutional symptoms, the markedly elevated creatinine and the decline in urine to virtual oliguria was taken to support the diagnosis of acute cellular rejection. Fours doses of Bolus steroids at 1 g d without altering the downhill course. Anti-lymphocyte globulin (ALG) was begun. Because of the failure of anti-rejection medicine, a fine needle aspiration biopsy, a procedure just begun at that time at PMH, was performed. Analysis of the aspirated cells revealed total necrosis of all parenchymal cells suggesting occlusion of the renal artery. An arteriogram was performed later that day and revealed total occlusion of the renal artery distal to the anastomosis ruling out a surgical complication. The patient was returned to hemodialysis and prepared for nephrectomy. On the 15th hospital day the transplant was removed. Pathologic evaluation of the transplant failed to reveal evidence of cellular or humoral rejection. A clot in the

main renal artery was found quite distal to the surgical anastomosis, which was patent. All the intrarenal pathologic findings were secondary to ischemic necrosis.

This case study is representative of a variety of cyclosporine nephrotoxicity which is uncommon but devastating. Intrarenal arteriolopathy caused by cyclosporine has been described by a multiplicity of workers, but has been a controversial lesion with respect to the immunosuppressive agent under review. The capacity of cyclosporine to alter intravascular hemostatis was first suggested by Shulman and the Seattle group of bone marrow transplant physicians. In a series of patients who underwent bone marrow transplant, who therefore had normal native kidneys, a clinical syndrome resembling hemolytic-uremic nephritis was observed in three patients. In this series, cyclosporine was given at high dose (15 mg/kg) along with a number of other immunosuppressive agents. All of the three cases had normal renal function prior to the use of the immunosuppressive cocktail which included cyclosporine. On pathologic review of the materials presented to the pathologist in a coded fashion, a vascular injury pattern was observed in the cyclosporine treated patients with arteriolar and glomerular capillary thrombosis accompanied by a tubule interstitial fibrotic pattern which will be discussed in the next section at greater length. Later an Australian group reported what they felt to be recurrence of hemolytic-uremia syndrome (HUS) in a patient receiving a transplant for that preimary disorder under the umbrella of cyclosporine. The authors suggested that cyclosporine may trigger recurrence of HUS. More likely than leading to recurrent disease or causing a specific arteriolar entity, cyclosporine initiates alteration of clotting factors de novo which leads to a similar pattern of clot formation within the entire renal arterial tree. A frank picture of microvascular injury was reported in additional groups of patients receiving a bone marrow transplant in which the pattern resembled that of thrombotic thrombocytopenic purpura. J. Stewart Cameron's group, with the bone marrow experience before them, prospectively biopsied all patients receiving cyclosporine for maintenance of a renal allograft. Biopsies were performed routinely one and four weeks after transplantation and during periods of renal dysfunction. In eight renal biopsy specimens obtained from seven renal allograft recipients, glomerular thrombi were discovered without evidence of rejection. Some of the thrombus material obstructed glomerular capillary lumens. In biopsy material obtained from patients using prednisolone and azathioprine as the immunosuppressive regimen, such a pathologic finding was not encountered in the absence of acute vascular rejection. The authors concluded that cyclosporine must be associated alone with this variety of vascular injury.

The propensity for high dose cyclosporine to cause arterial thrombotic lesions was an outgrowth of bedside observations which required a pathophysiologic explanation. Mihatsch and Thiel have approached the problem of cyclosporine toxicity in a more investigatory manner. The pathology unit at the University of Basle has been involved with prospective analysis of tissue injury related to cyclosporine use from the beginning of the investigations by the Sandoz Company, first in animals and then in treated men. The approach in their hands has been to analyze in a carefully controlled manner, renal biopsy material at defined time periods and access the possibility that observed potential lesions which might be peculiar to cyclosporine use. These grand rounds have already commented upon the findings of this group with respect to acute tubular nephropathy and discussed the

possibility that many of those findings are either very subtle or relatively nonspecific. In contrast to those subtle nonspecific findings are those of this group concerning vascular lesions of cyclosporine. Mihatsch describes a specific lesion characterized by mucoid thickening of the arteriole intima wit proliferation of the muscle cells and arteriole hyalinosis which leads to obliterative arteriolopathy at the level of arcuate vessels and afferent arterioles.



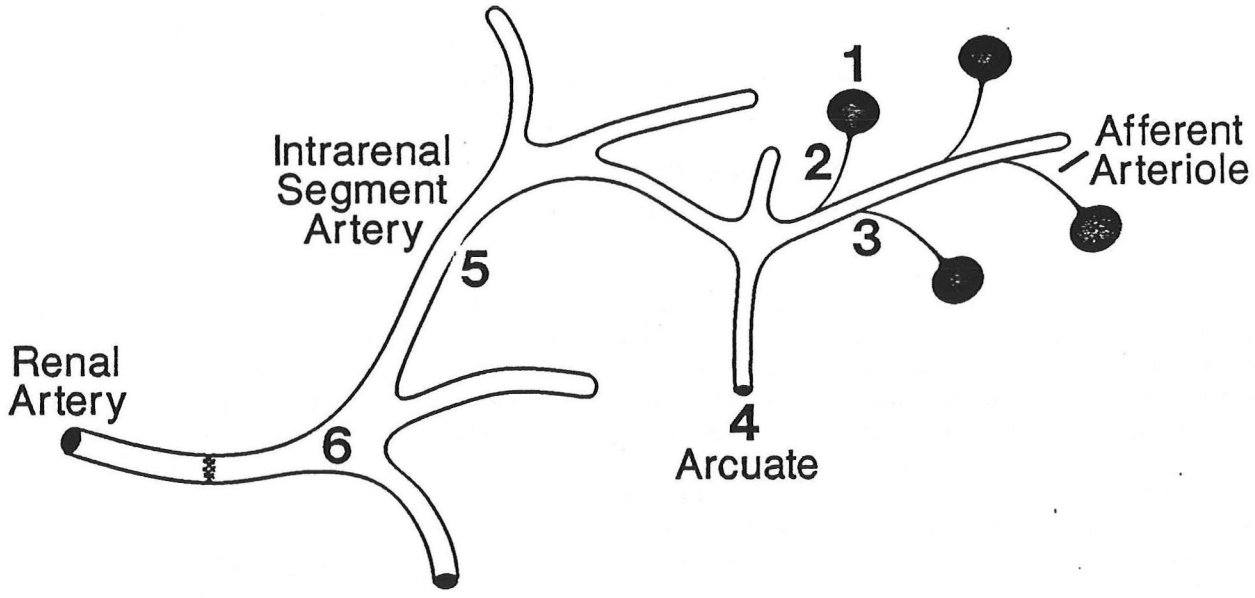
A. Arteriolosclerosis and necrosis with protein deposits
B. Massive protein deposits in vessel wall
C. Mucoid thickening of intima
D. IF of deposits

This lesion was reasonably common, often associated with primary nonfunction or graft dysfunction unrelated to immune injury in the first month of therapy, and was more common when high dose cyclosporine was used. These prospective pathologic findings coupled to the clinical observations and to interventionist biopsy material are persuasive pieces of evidence to support

the relationship between cyclosporine and an arterial lesion which itself can lead to renal graft loss on the one hand or destruction of native kidneys when cyclosporine is used for other disorders on the other.

Although this evidence is indubitable, a question has remained as to the anatomic site in the arterial tree which can be affected by cyclosporine in this matter. I have already shown thrombotic events occurring in the glomerular capillary bed, the afferent arteriole and the arcuate arteries within the kidney. There is additional clinical evidence that even larger intrarenal artery occlusion may also occur. Thrombosis of the main renal artery away from the anastomosis, thus belying a surgical complication, has also occurred. Recently, the UT Houston transplant group reviewed 325 consecutive cases receiving the cyclosporine prednisone combination from 1984-1986. This group encountered six distinct cases of thrombosis of the main renal artery which occurred between 4-12 days after transplantation. In each case, rejection was ruled out by the clinical course and by a renal biopsy. No other etiologic factors were discerned. Because the catastrophe was recognized in several cases early enough, revascularization was attempted and was successful in a few. An overlapping group of 297 patients treated with the previous conventional immunosuppressive regimen had no cases of main renal artery thrombosis. It is clear, then, that the entire renal vascular tree is susceptible to this variety of cyclosporine nephrotoxicity, a variety which is devastating and leads to acute graft loss in a similar matter to the unfortunate event in our patient.

SITES OF ARTERIOLOPATHY



At this time, the mechanism by which cyclosporine A alters hemostatic integrity is merely conjecture. It is clear that monocyte derived factors, including interleukin-I, may be potent inducers of tissue procoagulant factors which can condition the endothelial walls as thrombogenic surfaces. Cyclosporine has also been shown to directly inhibit monocyte released procoagulant factors generated by allogeneic stimulation in the mixed-lymphocyte culture. Helin and Edgington interpret their results as a inhibition of helper T-cell function by cyclosporine required for stimulation of monocyte release of procoagulant activity. Interestingly, these effects of the drug would lead to a diminished capacity to clot rather than the converse. This effect may explain why one fails to obtain measurements of a hypercoaguable state when screening plasma of patients with severe intrarenal clotting phenomena related to cyclosporine. The present best hypothesis to explain the intrarenal clotting events is related to the role played by cyclosporine in inhibiting prostacyclin (PGI_2) in the kidney. Prostacyclin is a potent endogenous inhibitor of platelet aggregation, reduction of which leads to interstitial cell damage, capillary wall necrosis and possible capillary thrombosis. Cyclosporine inhibits prostacyclin stimulating plasma factors leading to a diminished synthesis and release of PGI_2 by vascular endothelial cells either in culture or in situ in the kidney. A great deal more research is necessary in this area to generate a cogent hypothesis which might lead to therapeutic intervention to prevent or treat this devastating variety of cyclosporine nephrotoxicity.

CYCLOSPORINE AND THROMBOGENESIS

DECREASED THROMBOGENESIS

Inhibition of Monocyte Derived
Platelet Activating Factor
(dec ILII = dec ILI = dec PAF)

INCREASED THROMBOGENESIS

Diminished Prostacyclin Leading
to Increased Thrombogeneic
Endothelial Surfaces

In contrast to the clear relationship between cyclosporine use and arterial thrombotic events in the kidney has been the story interrelating cyclosporine use to venous thrombosis. Anecdotally, a wide variety of venous thromboses have been described in cyclosporine treated patients. It was assumed that the venous lesions were causally related to drug therapy in these reports. The initial report examining this issue was from the University of Leuven in Belgium in which 90 consecutive cadaver renal allograft recipients from 1983-84 were analyzed. In this group, 13 patients experienced 17 thromboembolic complications including 10 cases of pulmonary emboli and one case of renal vein thrombosis. This high incidence of venous thromboembolism was extraordinary for this group and certainly did not reflect their experience in patients treated with azathioprine. In analysis of the etiologic features that might have explained the thromboembolic phenomena,

only cyclosporine appeared to be a variable not present in the previous group that did not experience the complication. Sensitized by the Leuven report, many other transplant groups examined their own data for the presence of venous thromboembolic complications. The Oxford group reported that thromboembolism was not particularly uncommon after renal transplantation in general regardless of the nature of the immunosuppressive regimen. When they aggressively pursued the potential of venous thromboembolism with angiography and venography, the incidence of venous thrombosis actually was found to be smaller in patients treated with cyclosporine than in those with azathioprine. They concluded that venous thrombosis was a common complication of surgery in general, particularly when prolonged bedrest was encountered. Cyclosporine, by reducing hospital stay, often associated with early and excellent renal function, by reducing time spent in bed, reduced, not increased, venous thromboembolic phenomena. Cameron from Guy's also found fewer venous thromboemboli in cyclosporine treated patients, although glomerular and arteriole thrombi were countered in his series out of proportion to previous experience. The American experience as reported by Ferguson from a consortium of Midwestern transplant programs, which include the University of Minnesota and Ohio State University, also revealed no effect of cyclosporine in producing venous thromboembolism. The University of Texas at Houston program, the Pittsburgh experience, and the Dallas experience all corroborate the low incidence of venous thromboembolism after cyclosporine use. One concludes from these series that cyclosporine alters arterial but not venous hemostatic integrity.

Cyclosporine arteriolopathy is an uncommon but devastating complication. It has only been recently recognized and confirmed so that prospective treatments strategies to abrogate its occurrence have not yet been generated. It appears clear that this complication is associated with early high dose use and is seen generally within the first three months of transplantation. Severe arterial tree complications associated with cyclosporine toxicity must be added to the events which must be diagnostically sought for when acute allograft dysfunction occurs within the perioperative period. It appears that the incidence of cyclosporine arteriolopathy is declining as transplant programs, generally for other purposes, have voided early cyclosporine use and have reduced induction cyclosporine dose schedules. This is the most appropriate strategy at present to avoid the arterial lesions. Analysis of the role that prostacylin inhibition plays as an etiologic factor in this complication may be the basis for construction of newer approaches which attempt to stimulate the synthesis of prostaglandins in the kidney early in the perioperative period.

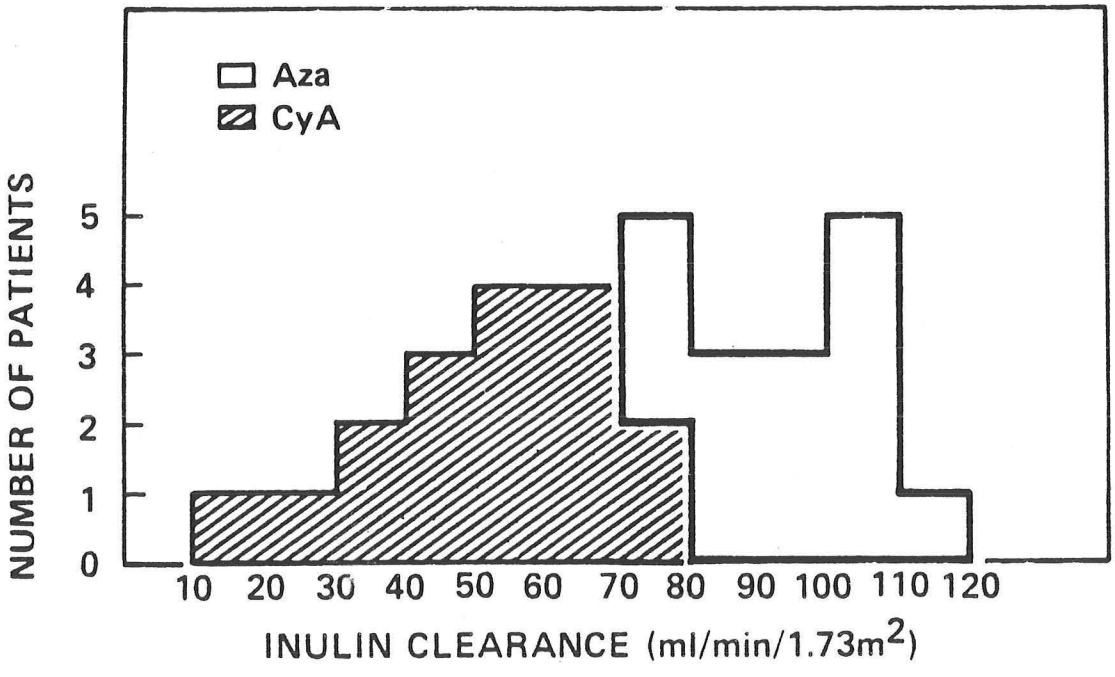
D. CHRONIC CYCLOSPORINE NEPHROTOXICITY

Case Study

MB, a 42-year-old computer expert for a firm in the Silicon Valley was in excellent shape until the winter of 1984. He prided himself on his athletic prowess, often winning local 10 K races. That fatal winter he was striken with a viral myocarditis which left him in congestive heart failure. His exercise tolerance was progressively diminished to the point he no longer could continue to work. When he was ultimately confined to his bed and chair, he

underwent successful cardiac transplant at the Stanford Transplant Program. Although he had two mild rejection episodes, his graft worked well so that by 6 months after transplantation he was able to resume part time work and by one year after transplantation he was able to return to fulltime work. He was also able to play a vigorous set of tennis. Serum creatinine prior to his viral illness was 1.1 mg/dl. It rose during the period of heart failure to 1.8 mg/dl. In the immediate post-transplant period when his heart was experiencing excellent function his creatinine fell to the baseline period but resumed a higher value during cyclosporine therapy, 1.6-1.8 mg/dl. By 6 months after transplant, his creatinine was stable at 2.1 mg/dl. Serume creatinine at the 1 year visit had risen to 2.5 mg/dl. Because of the concern relating cyclosporine to nephrotoxicity, GFR by isotopic clearance measurement was performed and revealed 22 ml/min. By 20 months post-transplantation frank renal failure ensued, clinical uremia was present, and dialysis was instituted. Heart transplant function was excellent, cardiac output was 6 liters/min.

This case report is a perfect illustration of the most difficult clinical problem that has been encountered with cyclosporine, its propensity to induce a chronic, inexorable renal injury which can ultimately lead to loss of native or transplant function. It was the Stanford Heart Transplant Program which first alerted the medical fraternity to this important complication which has caused all of the transplant world pause. The relationship between cyclosporine and chronic nephrotoxicity had been merely a clinical anecdote when Myers instituted his careful analysis of renal function in heart transplant patients receiving cyclosporine. Part of the difficulty was that it was unclear that there were four distinct clinical syndromes of nephrotoxicity so that the sporadic reports of isolated cases of nephrotoxicity of one or another variety could not constitute a prima facie case for a relationship of the drug with renal injury. Myers argued that the heart transplant recipients constituted a group of individuals who could more directly test for a direct relationship of drug use to nephrotoxicity. In all of the cases, the patients had heart transplants without renal transplants so that there was no problem with the difficult diagnostic dilemma between renal immunologic or drug related toxicity.



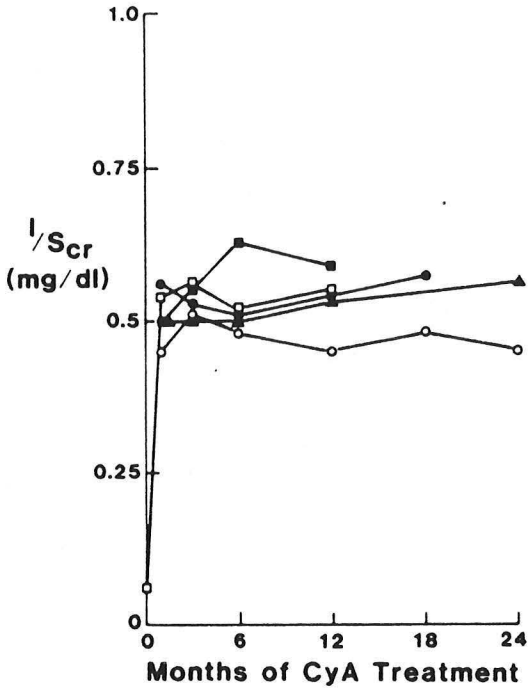


Fig 1. Plot of the reciprocal of serum creatinine in mg/dL ($^1/S_{cr}$) against months of cyclosporine treatment in a series of reported patients following renal transplantation. ○ Kahan et al,[5] ● Ferguson et al,[6] □ Thiel et al,[7] ▲ Sutherland et al,[8] ■ Barry et al.[13]

Seventeen heart transplant recipients were followed for at least one year on cyclosporine were compared to a control group of 15 individuals who had received successful heart transplants using conventional immunosuppression. The control and cyclosporine-treated groups had equivalent cardiac outputs and heart transplant function. Despite only modest changes in serum creatinine in the cyclosporine-treated group at one year, inulin clearance was markedly diminished. Indeed, there was virtually no overlap in the glomerular filtration rate measured in native kidneys in patients receiving cyclosporine or azathioprine. No single azathioprine-treated patient had a clearance less than 70 ml/min·1.73 m^2 while no cyclosporine-treated patient had a GFR of greater than 80 (see figure). The decline in renal function with time was progressive and could be described as a linear function with the 1/creatinine vs time mathematical construct In a larger cohort of 32 heart transplant recipients followed for at least 12 months, end-stage renal failure ensued in several, two requiring hemodialysis. Biopsy material revealed a tubulo-interstitial pattern of injury characterized primarily by bland fibrosis. More specific forms of the lesion will be described later in these rounds. Glomerulosclerosis was also encountered. In all of the cases the patients had received intravenous, perioperative cyclosporine or high-dose oral drug at 17.5 mg/kg·d which was tapered to no lower than 10 mg/kg·d. In later studies Myers and his group made two additional contributions to the delineation of and understanding of chronic cyclosporine nephrotoxicity. When first published, the Myers data was looked at with hearty skepticism since

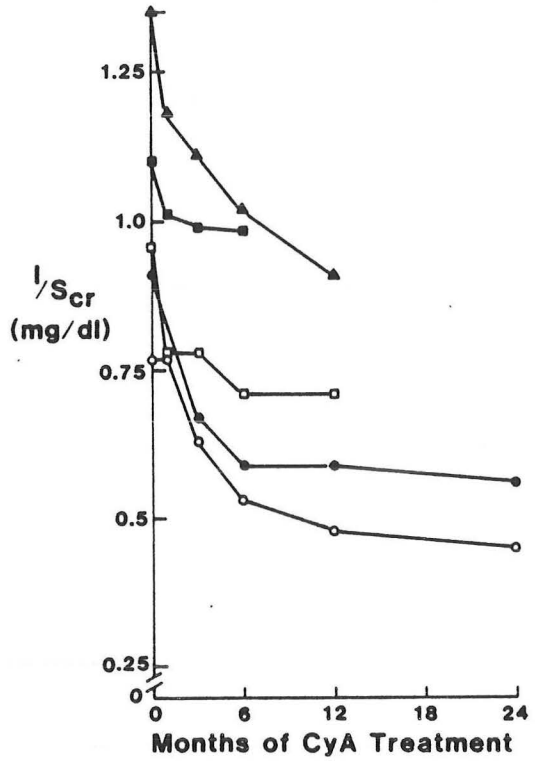


Fig 2. Plot of the reciprocal of serum creatinine in mg/dL ($^1/S_{cr}$) against months of cyclosporine treatment in a series of reported patients following nonrenal transplantation or autoimmune disease. ○ Moran et al,[4] ● Iwatsuki et al,[14] □ Palestine et al,[15] ▲ Stiller et al,[10] ■ von Graffenried and Harrison.[16]

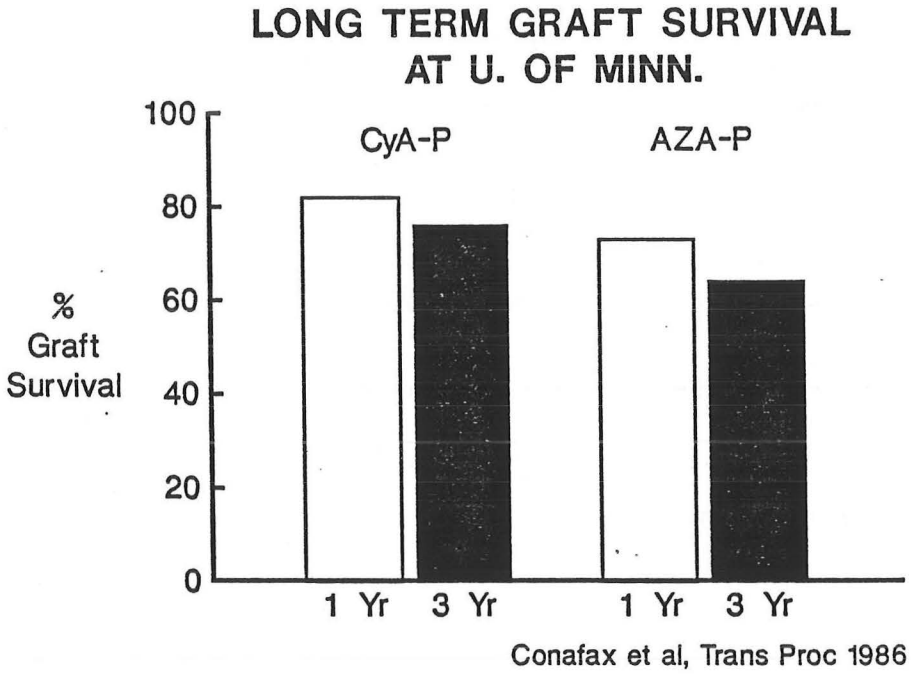
analysis internally of the serum creatinines at one and two years post-renal transplantation failed to reveal an important increase in serum creatinine. Stanford was criticized for using doses which were clearly too high and for failing to taper the cyclosporine to a lower stable value. If cyclosporine nephrotoxicity could ensue, it was argued, it was the result of inappropriate dosing schedule which was avoided by a more rapid taper to doses below 10 mg/kg·d. Re-analysis of the Stanford cohort by Myers as cyclosporine continued to be tapered to 7 mg/kg·d uncovered further diminution in native renal function despite this lower dose. Moreover, Myers demonstrated that the serum creatinine was an inappropriate and inaccurate measurement of renal function in the cyclosporine treated patient. Tubular secretion of creatinine has been shown to be enhanced by cyclosporine so that total serum creatinine no longer reflects accurately glomerular filtration rate as revealed by measurements of GFR by inulin clearance. As inulin clearance, the gold standard in the laboratory, is technically difficult and too cumbersome for routine clinical practice, an accurate alternative means of assessing GFR was described by Myers. They showed that various isotopic clearance methods are easy, accurate, and circumvent the problem with respect to creatinine and nephrotoxocity in the cyclosporine-treated patient. Parenthetically, on the

strength of the Myers data, many transplant programs have taken to following transplant function using various isotopic clearance methods. At Parkland Hospital we have established the us of the clearance of radioactive iodothalamate (glofil), since early in our transplant history, which places us in excellent position to be able to most accurately follow the cyclosporine-treated patient.

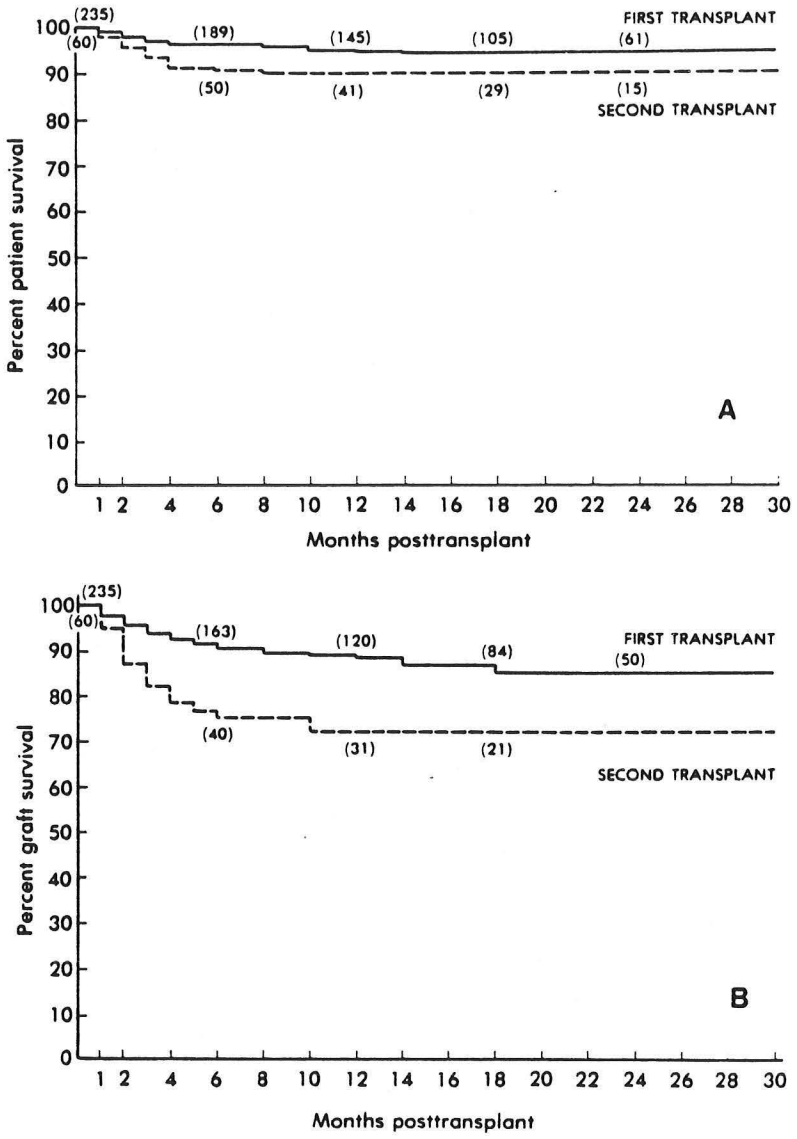
Taking the lead of the Myers group, the National Institutes of Health carefully analyzed renal function and renal morphology in patients receiving cyclosporine for the treatment of ocular inflammatory disorders of presumed autoimmune origin. Twenty-six patients with this disease, generally characterized by posterior uveitis, received cyclosporine as the sole agent with doses which began at 10 mg/kg·d orally. In clinical non-responders the dose was raised to a maximum of 15 mg/kg·d. In the face of nephrotoxicity, if observed, the dose was adjusted downward. It was clear from their data that the dose of the drug is associated with chronic, potentially irreversible renal dysfunction. Virtually every patient experienced an elevated serum creatinine after cyclosporine was initiated, reflecting diminution in native kidney function. The average cyclosporine dose per patient ranged from a low of 4.2 mg/kg to as high as 15.4 and cummulative dosage ranged from 1.8 g up to as high as 16.7 g in the time of study. There was a statistical relationship between these doses and the observed reduction in GFR. The NIH group studied a cohort of 17 of the treated patients who agreed to a kidney biopsy after an average of 2 years of therapy more carefully. At the time of biopsy in this cohort, the glomerular filtration rate measured by inulin clearance had fallen to a mean of 69 ml/min·1.73 m^2 with one patient as low as 19. All patients had had normal renal function at the time that cyclosporine was begun. On biopsy a glomerular and tubulo-interstitial fibrotic pattern was clearly present. There could be no argument that the morphologic changes in any way were related to immunologic activity since the primary disease did not involve the kidney, there was no transplantation of the kidney, and only cyclosporine was part of the treatment protocol. Recently, Austin from this NIH group reported repeat biopsies in a smaller group of the 17 studied patients initially. In each of these patients the cyclosporine dose was markedly reduced when renal function had declined to a target value below basal. What was found was generally heartening in that the morphologic lesions did not progress when cyclosporine was either stopped or markedly reduced in dose in the majority of the patients . Unfortunately, a few of the biopsies worsened commensurate with a decline in renal function. There was no clinical parameter that would allow one to predict who would have a salutory effect from dose reduction or discontinuance of the drug at some point after chronic cyclosporine toxicity had ensued. In no case did renal morphology actually improve.

These two sets of studies clearly demonstrate the impact that cyclosporine exerts on the kidney to induce a chronic fibrotic lesion which can itself lead to renal failure. The renal transplant literature also has direct evidence for the induction of cyclosporine related chronic nephrotoxicity. In all programs, the mean serum creatinine in patients treated with cyclosporine is statistically greater than in the azathioprine era. At PMH, the mean serum creatinine at one year for successful allografts in 1982 was 1.7 mg/dl while in 1985 was 2.1 mg/dl. On the other hand, despite the dire predictions of the Myers and NIH groups, graft survival rates over time in the first few years of analysis seem not to be greatly affected by

cyclosporine. The rate of decline in graft survival from 1 to 3 years after surgery was even slower than with conventional drugs as revealed by Najarian in his analysis of the University of Minnesota data and by Kahan in his analysis of the UT Houston group. Unfortunately, the definition of graft survival utilized the absence of return to hemodialysis or the stability of serum creatinine which has the problems already described as criteria for lack of nephrotoxicity. These kinds of data could hide progressive declines in renal function related to potential chronic nephrotoxicity. Only recently has Myers lesson been learned by many programs and isotopic glomerular filtration rate measurements have been made. Hearteningly recent treatment programs have been associated with stable GFR measured isotopically as well.



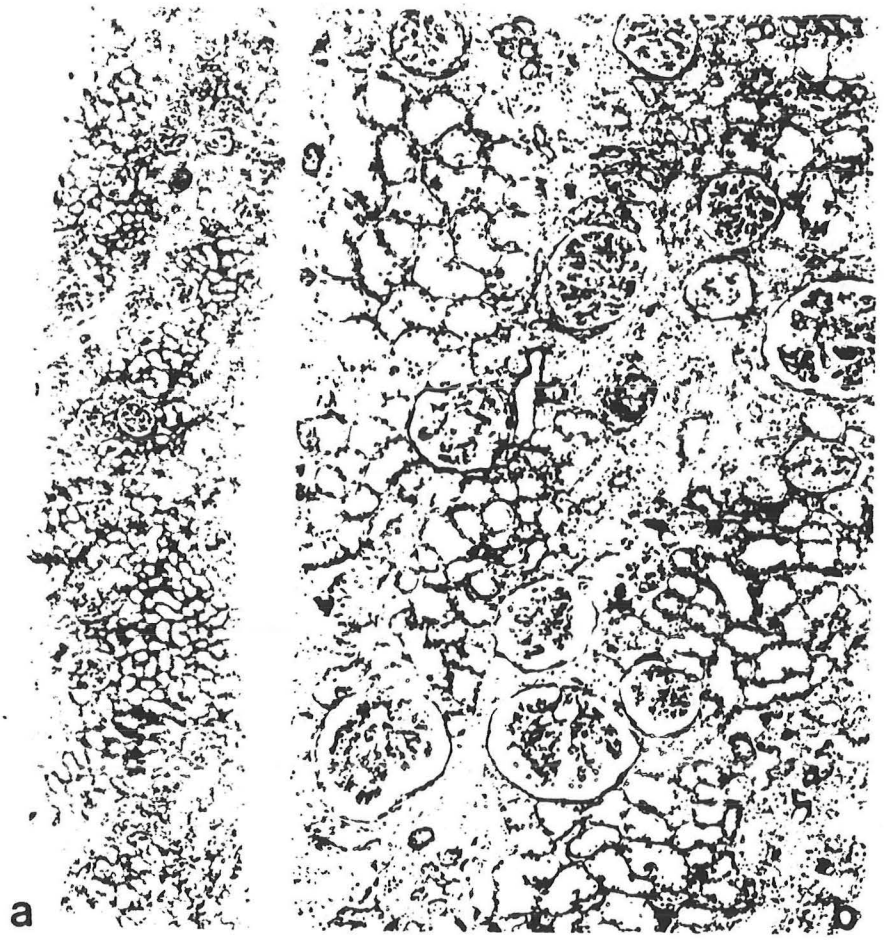
It is now clear that there is a reasonably distinct morphologic lesion associated with the chronic cyclosporine nephrotoxicity. Mihatsch reported a cyclosporine-associated lesion which is distinct; to wit, irregularly distributed areas or stripes of interstitial fibrosis and atrophic tubules found in the renal cortex. Interstitial fibrotic lesions are not uncommon in transplant biopsies and have generally been associated with an entity called chronic rejection. The form of fibrosis found with chronic regjection is more global, is not found in a striped pattern, and often has a major mononuclear cell infiltrate as part of the picture. The striped pattern of fibrosis reported by Mihatsch seems to be reasonably unique to the cyclosporine-associated lesion and is rarely accompanied by a major interstitial infiltrate. Many authors also report the concomitance of important focal or global glomerulosclerosis associated with the striped interstitial fibrosis in chronic cyclosporine nephrotoxocity. Although the striped fibrosis is peculiar to cyclosporine, this glomerular lesion is non-specific as it can also be found in chronic rejection. The accompanying striped fibrosis is felt to tip the morphologist off as to the genesis of the glomerular lesion as well. After the description of this cyclosporine-associated lesion in renal transplant recipients was made, the identical entity was found in the heart transplant recipients at Stanford and



UT-HOUSTON

in the ocular inflammatory disorder patients at the NIH in settings in which immune injury in the kidney was unlikely. It is therefore clear that this morphologic lesion is directly associated with cyclosporine and can be used as evidence for this form of nephrotoxicity.

If chronic cyclosporine nephrotoxicity is inevitable, intractable, and inexorably leads to total loss of renal function, cyclosporine as an immunosuppressive medicine would be a drug of illusion. On the one hand, early graft survival is spectacularly improved, on the other hand long term graft survival would be unexpected. Clinical observations suggest that this pessimistic view of the role of the drug in the future is incorrect. Although most programs have had no more than five years of experience with cyclosporine, prospective cohorts have been followed for as long as 10 years without the inevitable renal failure and return to dialysis being visualized



STRIPED FIBROSIS

A. Low Power

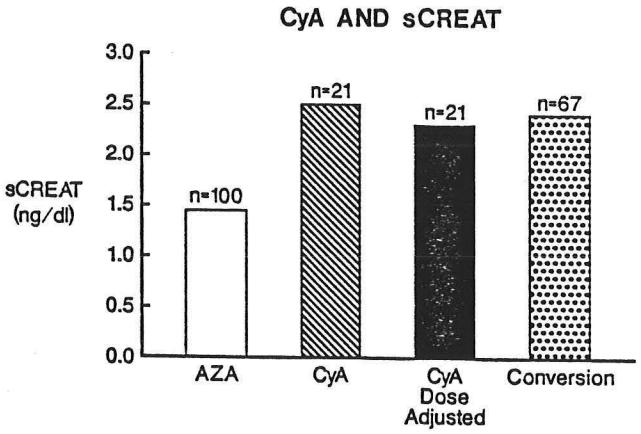
B. High Power

as a common outcome of long-term use. To many patients, five years of successful renal transplant function is a worthwhile payment for ultimate loss of the graft to the medicine. Lastly, treatment strategies have been designed which may abrogate or lessen this form of cyclosporine nephrotoxicity.

To design prospectively strategies to avoid chronic nephrotoxicity of cyclosporine one has to know more precisely the pathogenesis which underpins this lesion. One also must understand the clinical correlates that define this syndrome. At this time it is not understood whether early high dose use, cummulative dose, or prolonged use of high dose cyclosporine or a mixture of these three is causative. Most programs have adopted a lower dose for induction of graft tolerance with initial doses averaging 10-12 mg/kg/d rather

than 15-20 as had been used at the beginning of the cyclosporine era. Many programs have adopted the sequential therapy described earlier in these rounds to avoid early use of high dose cyclosporine. Most programs have adopted a more rapid taper of cyclosporine in patients who were stable several months after transplantation, targeting 5-7 mg/kg d as a maintenance dose at one year for renal transplant patients and a somewhat higher value for patients with transplants of other organs.

One interesting strategy to avoid chronic cyclosporine nephrotoxicity, conversion from cyclosporine to azathioprine of stable patients, has had mixed results. One can construct an immunologic argument that states that graft tolerance can be induced by inhibiting response to class II HLA antigens presented by passenger leukocytes and preventing gamma interferon induction of class II HLA antigen synthesis on parenchymal cells by cyclosporine. In time a reduced amount of immunosuppression may be necessary to prevent lymphocyte sensitization. Ultimately, one can return to the conventional drugs which gave a 50% graft survival when used initially without loss of the cyclosporine effect on enhancement of graft outcomes, one can perform a conversion protocol. Initial results attempting to convert patients at four months, the Morris experience, had a dismal outcome with a high percentage of acute rejection ensuing at the time of conversion.



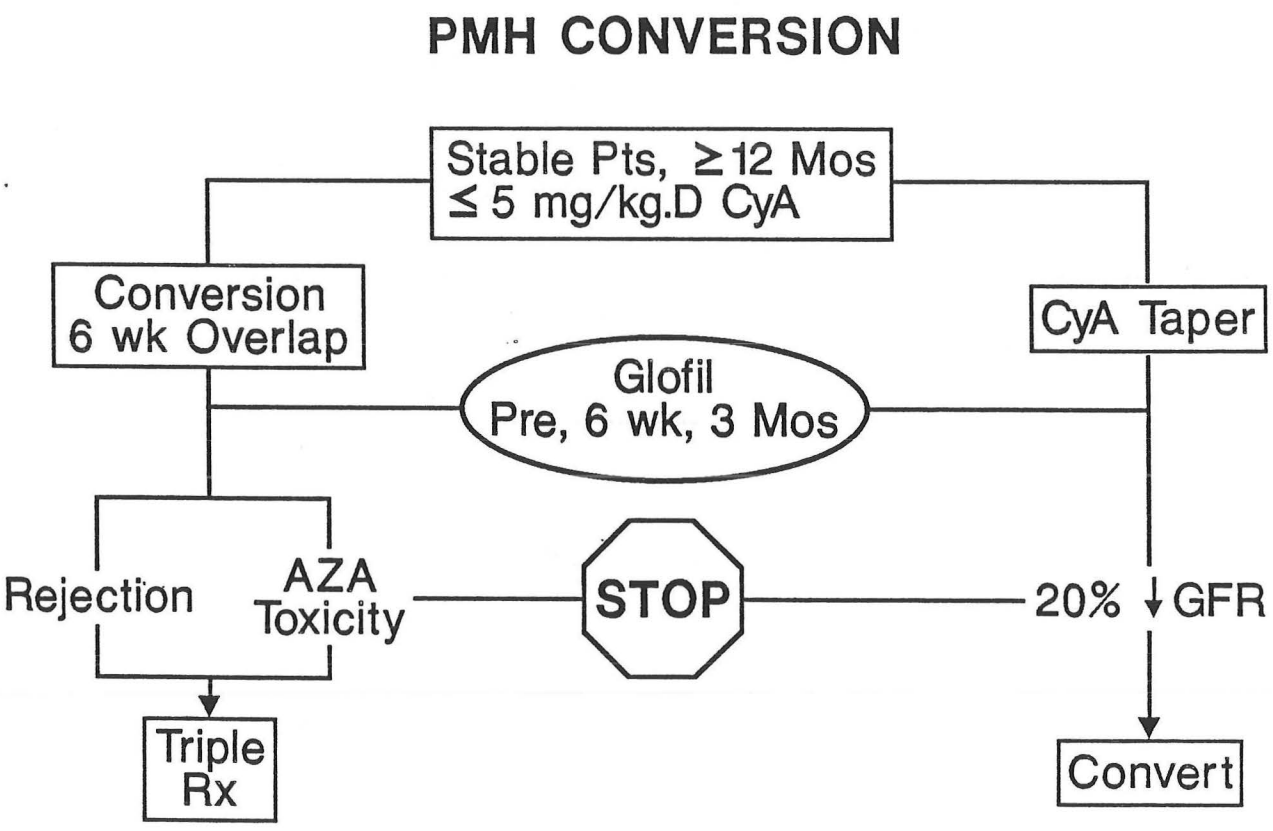
The Brigham experience as reported by Tilney has also been less than spectacular. Abruptly converting patients from prednisone/cyclosporine to azathioprine/cyclosporine at 4-6 months was associated with a 28% incidence of acute rejection. Although only six grafts were lost of 58 patients converted in this manner, the morbidity of anti-rejection therapy and the loss of stable grafts was disheartening. Of those patients who could successfully undergo conversion, however, maintenance serum creatinine fell to 20% of the cyclosporine value at 6 months and chronic nephrotoxicity did ensue.

CyA CONVERSION AT PBBH

Who:	Stable Pts at 4-6 Mos
How:	Change to Pred/Aza
Rejections:	28%
Graft Survival:	84%
Graft Loss:	16%
Serum CREAT:	20% Decrease

Tilney et al Trans Proc, 1986

PMH has completed its own prospective trial of conversion. Our treatment goals were to convert only stable patients who had been tapered to low doses of cyclosporine at one year and to accomplish conversion in a gradual rather than abrupt fashion. Patients at one year with no rejection episodes within the past three months and stable renal function on cyclosporine doses at 5 mg/kg•d or below were randomized to be converted over 6 weeks period to azathioprine/prednisolone or chosen to remain in the cyclosporine/prednisolone treated group. Renal function was followed carefully by the isotopic measurement of the glomerular filtration rate.



Of the 10 patients successfully converted in our initial study, the post-conversion serum creatinine fell from 2.2 to 1.9 mg/dl and the glomerular filtration rate increased by 20%. Unfortunately, 7 required a return to cyclosporine. Six patients experienced acute rejection episodes necessitating therapy culminating in one graft loss. Hyperbilirubinemia and severe leukopenia were other complications requiring discontinuance of the conversion protocol. Of the nine patients remaining on cyclosporine, renal function remained stable without rejection. Serum creatinine fell in this group as cyclosporine dose continued to be diminished; glomerular filtration rate did not decline over the next 12 months follow-up period. We made two conclusions from our prospective trial and from the review of the literature. First, it is clear that conversion as an option for the patient with stable renal function is associated with an unacceptable rate of acute rejection episodes.

CONVERSION AT PMH

	Conversion	Low CyA
N	10	9
Rejections	6	0
Losses	1	0
sCREAT at 0	2.2	2.1
sCREAT at End	1.9	1.9
GFR	Down 20%	Down 20%

The possibility of rejection after converting stable patients exists whether conversion occurs early or late after the transplant event. Abrupt or gradual cessation of cyclosporine and institution of conventional immunosuppression had no relationship to the propensity to induce acute rejection at the conversion point. On a more optimistic note, we concluded that avoidance of perioperative cyclosporine, lower induction dose schedules, and a more rapid taper toward 5 mg/kg·d is associated with renal functional stability and the absence of chronic nephrotoxicity. We have followed our patients an additional year and a half and can confirm the salutory effect of this protocol employing the maintenance of low dose cyclosporine/prednisone over many years duration.

IV. CONCLUSION

Cyclosporine A is a potent new immunosuppressive agent which has dramatically altered therapeutic approaches to transplantation in general and to renal transplantation in particular. When initially introduced into clinical practice there was anticipation that cyclosporine would be a miracle drug. Like many other success stories, increasing experience raised spectres of major problems. As the cyclosporine nephrotoxicity story began to unfold, there was a fear that the new "miracle" drug would be a drug of illusion. It was not until the cyclosporine nephrotoxicity problem was described, recognized, and even acknowledged by the transplant fraternity that therapeutic strategies could be designed and tested which could delimit the clinical problem and maintain the extraordinary advance made by this medicine. At this time, the recommendations for the use of cyclosporine include:

1. Avoidance of intravenous cyclosporine.

2. Sequential induction therapy that utilizes a cadre of immunosuppressive agents without the use of cyclosporine in the initial period post-op when ischemia is common.

3. Lower dose induction therapy (12 mg/kg·d).

4. The use of a wide range of diagnostic tests taken together to help discern the difference between acute rejection and acute cyclosporine nephrotoxocity, including frequent resort to core or fine needle aspiration biopsy.

5. Assiduous attention to taper the cyclosporine dosage schedule aiming for 5 mg/kg·d at one year.

6. Careful estimate of the glomerular filtration rate even in so-called stable patients by isotopic clearance methods.

7. Avoidance of conversion to conventional drugs within one year in completely stable patients.

These outlined approaches to cyclosporine nephrotoxicity have already diminished the clinical importance of this side effect and have heightened the enthusiasm with which the transplant world envisions cyclosporine. As we learn at the experimental level in greater detail the nature of the pathophysiologic mechanisms at play which cause the different forms of nephrotoxicity, more specific and targeted therapeutic strategies may be designed to further reduce the chances of cyclosporine toxicity.

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