Immunosuppressive therapy with cyclosporine for cardiac transplantation

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CARDIAC TRANSPLANTATION has been primarily limited in its clinical implementation by the almost uniform occurrence of vigorous allograft rejection responses. The clinical course of heart recipients differs from that of kidney recipients in three regards. First, the heart recipient appears to display less endogenous, native immunosuppression and therefore experiences more virulent rejection crises. Not only are these rejection episodes difficult to control with conventional immunosuppressive agents, but also the need for combinations of powerful drugs results in a proclivity toward infection. Second, due to the absence of the adequate extracorporeal support that dialysis provides to the renal transplant recipient, there is less tolerance of rejection episodes by cardiac allograft recipients. This limitation has resulted in a more vigorous approach to the diagnosis of allograft rejection, for example by weekly endomyocardial biopsies, and a lower threshold for the treatment of putative rejection episodes. Third, in contradistinction to renal recipients who display relatively well-defined functional abnormalities as possible intermediate end-points of allograft rejection, there are presently no widely accepted reproducible functional criteria that can discern early attack on the cardiac transplant. Atrial arrhythmias, hypotension, and/or congestive heart failure indicate not early, but rather serious, allograft damage.

In the setting of cardiac transplantation, there is clearly a need for an effective agent to prevent the development of host immunity. The widely used immunosuppressive combination of azathioprine, a competitive inhibitor of nucleic acid biosynthesis, and corticosteroid (prednisone), although manipulated with exquisite attention to detail, was inevitably unsuccessful. A narrow therapeutic index was noted due to severe depression of proliferation (by azathioprine) and function (by corticosteroids) of nonspecific host immune elements, namely polymorphonuclear leukocytes and monocytes/macrophages, to infection with pathogens not only normally controlled by T cells (viruses, fungi, and protozoans) and nonspecific resistance and B cells (bacteria). Due to the low therapeutic index of the azathioprine-prednisone combination, rejection prophylaxis was rarely achieved, and high-dose steroid therapy and/or antilymphocyte serum (ALS) were required for immunosuppression. Treatment with ALS resulted in further depression of T cell immunity with the almost uniform occurrence of cytomegalovirus infection, as well as potentiation of the reduction in circulating granulocyte. Thus, although in the 1970s cardiac transplantation clearly held promise, success was realized at only a few centers at which patience and attention to detail salvaged patients to an impressive return to health.

The entry of cyclosporine (CsA) into therapy accompanying heart transplantation occurred in the United States in 1981 with the initial trial at Stanford, followed the next year at our institution (The Texas Heart Institute) and at the University of Pittsburgh. There were several early lessons from the initial experience: First, when CsA is used in conjunction with the extensive immunosuppressive regimen previously employed, there is a high risk of lymphomas. Second, in some patients CsA effectively affords prophylaxis against rejection. These patients display a much more benign posttransplant course than ever observed with the use of previous immunosuppressive regimens. Third, CsA therapy tends to mask early clinical signs of allograft rejection, increasing the dependence on the endomyocardial biopsy not only for diagnosis but also for prognosis of the outcome of antirejection therapy. Fourth, the overall result of transplantation with CsA is improved by 25% from 50% to at least 75% 1 year patient survival. Finally, because of the improved efficacy of CsA, cardiac transplantation can be readily extended to patients beyond 50 years of age.1 However, at present what would constitute optimal use of CsA is unclear: there is no consensus concerning the time of initiation, dosage, or need for combination therapy with other immunosuppressives to optimize prophylaxis of allograft rejection.
The agent. CsA is a neutral, lipophilic endecapeptide derived from Tolypocladium inflatum Gams and purified chromatographically to a white, crystalline powder that is insoluble in water, but dissolves rapidly in ethanol and more slowly in lipids, such as olive oil, after being heated at 60°C for 2 hr. CsA has three structural characteristics: (1) a highly aliphatic nature with seven of the 11 amino acids being N-methylated, (2) a precise steric conformation maintained by cross-ring H bonding, and (3) a unique β-hydroxy, unsaturated, 9-carbon amino acid at position 1 that contains an ethylene bond. Chemical modification studies suggest that the active site is the hydrophilic region, containing amino acids 11, 1, 2, and 3.

The commercially available oral solution, which has a clear, yellow, oily appearance, contains CsA (100 mg/ml) in an olive oil and pegicologie 5 oleate (Labrafil M 1944CS) vehicle with 12.5% alcohol. The oral solution should be stored in the original container at a temperature less than 30°C; refrigeration should be avoided.

Commercially available injectable CsA is a clear solution, containing drug (250 mg/5 ml) in polyoxyl 35 castor oil (Cremaphor EL) with 32.9% alcohol. For infusion a concentration of 0.5 to 2.5 mg CsA/ml achieved by dilution with 0.9% sodium chloride or 5% dextrose solution is stable for 12 to 24 hr at 25°C. The use of glass bottles for administration is recommended due to leaching of phthalates from commercial plastic bags. Intravenous administration has been the preferred parenteral route, because CsA absorption after intramuscular delivery in myoglobin is slow and unreliable. The oral/intravenous biologic equivalence of drug concentrations is 3:1.

Although the intravenous route effectively delivers CsA to the systemic circulation, there are some reservations about its use due to anaphylactoid reactions, which did not occur with orally administered CsA. In addition to anaphylactoid reactions after bolus administration of CsA, the high drug levels produced thereby may exacerbate renal dysfunction, causing oliguria in newly engrafted, as well as native, kidneys. Even slow intravenous delivery of CsA may carry some risk. Administration via a central, rather than a peripheral, venous line produced an apparent pulmonary “capillary leak” syndrome in two of 12 liver transplant recipients, and Powles et al attributed convulsions in bone marrow recipients to intravenous CsA administration. The toxic reactions are probably attributable to the solvent Cremaphor (20% weight/volume), which has been shown to be responsible for other drug reactions; there is no evidence implicating CsA per se.

Methods of measurement of CsA concentration. Because of its unique chemical structure, CsA content can be estimated by a variety of techniques. On the one hand, at the present time the most specific technique to quantitate CsA is high-performance liquid chromatography (HPLC). The HPLC method has the sole advantage that it estimates parent CsA separate from drug metabolites. Although the HPLC technique has the potential to individually estimate levels of drug metabolites, this has not yet been exploited in the clinical arena. On the other hand, even though the initial HPLC problems of analytic instability and low sensitivity have been overcome, some limitations remain: (1) sample preparation is laborious, (2) efficiencies are variable extraction even from plasma, (3) this complex instrumentation requires special technician training, (4) per sample analysis time is relatively lengthy, and (5) elevated temperatures are necessary for chromatographic analysis, resulting in a reduced column lifetime and column failure after as few as 30 injections.

In general, methods requiring minimal preparation of the plasma sample demand complex analytic techniques, and those requiring extensive sample preparation have simpler methods of analysis. The variable extraction efficiency is compensated for by exogenous addition of the internal standard, structural analog cyclosporine D (CsD).

Present day clinical practice tends to depend on CsA estimates by the second technique, competition radioimmunoassay (RIA) by a kit distributed by Sandoz, which detects not only CsA, but also a group of cyclosporine-related metabolites; this group of compounds may be referred to as Cs. The polyclonal sheep antibody detection reagent is raised toward cyclosporine C that has been hemisuccinylated at its threonyl-position 2 to guinea pig gamma globulin. Nonradioactive CsA in patient samples competes for antibody binding with a constant amount of 3H-dihydro-CsA. Charcoal then selectively removes the Cs not bound to the antibody. After sedimentation of the charcoal, the radioactivity in the antibody-bound supernatant fraction is determined by liquid scintillation counting. For uniformity of results several important performance variables must be controlled: temperature and timing of sample storage, separation, and transport; method and frequency of measurement of the standard curve; mode of preparation of charcoal; and type of scintillation fluid and counting vials. Computer analysis of RIA results yields iterated, weighted log-log plots with linear regression values calculated by the method of least squares. RIA has several advantages: (1) the possibility to rapidly process large numbers of
samples, (2) simplicity, and (3) ready standardization and computer analysis of data.

The antibody used in the assay reacts with the immunologically inactive groups at positions 4 to 10 because the conjugation at position 2 buries the active site of CsA into the guinea pig gamma globulin carrier molecule. Thus, this method has the primary disadvantage that it cannot distinguish between parent compound and some CsA metabolites: for example, metabolite 21 has 77% cross-reactivity (CR); metabolites 1, 8, and 12% CR; and metabolite 17, 32% CR. Parent CsA compound accounts for 73% of the RIA peak value after drug administration, whereas it accounts for only 38% of the trough value. Ratios of RIA/HPLC whole blood CsA values in renal transplant patients are 3 to 1:1; values of up to 15:1 can be observed in liver transplant recipients, due to impaired excretion of drug metabolites. Monoclonal antibodies raised toward and specific for the parent CsA compound have been developed by use of a conjugate linked on the immunologically inactive portion of the ring. Initial clinical trials suggest a good correlation with CsA levels measured by HPLC.

In spite of the theoretical desire to measure CsA alone, at the present time there is neither definitive information that a metabolite measured by RIA is not useful to quantitate, nor a simple, widely used, uniform, HPLC technique that is standardized and verified for routine clinical application. However, it is not yet clear that there is an advantage to monitoring drug levels by monoclonal compared with polyclonal reagents. Indeed, the data show the superiority of monitoring drug levels by RIA over HPLC to either avert rejection in renal transplant recipients or to prevent nephrotoxicity. This, due to its overall simplicity and flexibility for clinical application, its 25 ng/ml lower detection limit, and its similar precision and accuracy compared with HPLC in detection of spiked CsA, the RIA method has been extensively applied in the clinical arena.

Therapeutic drug monitoring. There is considerable controversy regarding the appropriate matrix for determinations of CsA concentrations. The majority of CsA in blood is cell-associated, primarily with erythrocytes. Lysis of erythrocytes preloaded with CsA has shown more than 80% of the drug to be associated with the cell contents. Column chromatography of cells saturated with H-CsA has shown moderate binding to hemoglobin, but not to carbonic anhydrase. Although CsA binds to human lymphocytes and other leukocytes, from a pharmacokinetic standpoint this binding capacity is small relative to erythrocytes. Binding to plasma proteins is independent of concentration between 0.02 and 20 μg CsA/ml of plasma, but does vary with temperature: 73% of the drug is bound at 4°C, 94% at 20°C, and 97% at 37°C. The influence of temperature on plasma protein binding is independent of its distribution among blood components. There is low binding to albumin, an unsaturable binding of moderate degree to α1-acid glycoprotein in the concentration range of 0.01 to 0.6 μg CsA/ml. Ultracentrifugation reveals that more than 80% of CsA is associated with high- (57%) and low-density (25%), but not with very low-density (2%) or chylomicron lipoprotein fractions, via noncovalent forces in which form CsA is delivered to plasma membranes by rapid exchange reactions. In various patients between 5% and 15% of CsA is not associated with proteins. Mraz et al. demonstrated exchange/transfer of CsA among the different serum lipoprotein classes, and from albumin to lipoproteins. It is unclear whether the “free” nonlipoprotein-associated fraction better reflects effective drug concentrations than bound materials, since the lipoprotein association is assumed to limit neither tissue concentrations nor target interactions. Thus, although there may be considerable variation in lipoprotein concentrations within the patient population and important interindividual differences in the overall quantity of plasma binding of CsA, the clinical relevance of this is unclear.

Measurement of CsA in plasma or serum has been the generally recommended matrix, because the drug concentration in extracellular fluid appears to be in equilibrium with plasma water. The major problem with use of plasma or serum as the matrix is the observation that the distribution of drug between plasma and blood cells is temperature dependent. When temperature is lowered from 37°C to 21°C or 4°C, the drug diffuses rapidly from plasma into blood cells, reaching equilibrium after 2 hr. On the other hand, to avoid the potential experimental vicissitudes if time and temperature are not stipulated, the manufacturer of the RIA kits has recommended they be used on whole blood samples. However, in addition to the increased pipetting error with whole blood, there are four complications. First, estimation of the cell-associated fraction in whole blood may reflect a “bound” fraction not in ready equilibrium with tissue sites and not available for immunosuppression. Second, the amount of CsA in whole blood determinations may reflect the hematocrit, which is known to increase markedly in renal recipients during the first three posttransplant months. Third, because many metabolites are associated with erythrocytes, the use of whole blood amplifies the de-
viation of the RIA from the HPLC value. Plasma or serum levels, particularly when appropriate sample dilutions are used to perform the assay under optimal conditions, probably reflect circulating “free” CsA levels already in equilibrium with the intercellular space and available for therapeutic or toxic effects.

The optimal drug concentrations of CsA are not precisely known, and vary according to assay method, matrix sample, combined immunosuppressive regimen, and time after transplantation. By examining the RIA trough values of renal transplant patients suffering toxic complications of CsA therapy, principally nephrotoxicity, a serum trough value of 250 ng/ml was established as the upper limit of the putative therapeutic window.9, 17 Similarly, Griffith et al.18 found a 1000 ng/ml whole blood CsA level estimated by RIA to represent a toxic threshold for nephrotoxicity. The lower limit of the therapeutic window was assumed to correspond to the exogenous CsA concentration necessary to cause 50% inhibition of a normal mixed lymphocyte reaction in vitro, namely 100 ng/ml.9, 17 There was a correlation between the incidence of treated rejection episodes and the number of days required after transplant for the patient to achieve a level of 100 ng/ml: the incidence of rejection episodes was 10% when it took 5 days, 20% when it took 10 days, and 33% when it was more than 15 days. Furthermore, patients with renal dysfunction who displayed a CsA trough level of less than 100 ng/ml suffered from allograft rejection significantly more often than from nephrotoxicity.17 Gluckman et al19 noted that patients with low levels of CsA (due to inadequate drug dosages) experienced a 65% incidence of severe acute graft-vs-host reactions as compared with the 14% incidence in individuals receiving adequate drug doses. Therefore, the apparent therapeutic window for trough serum CsA levels by RIA in the immediate posttransplant period is 100 to 250 ng/ml. The therapeutic range in whole blood by RIA is 300 to 800 ng/ml. The therapeutic range in whole blood by HPLC is 100 to 150 ng/ml.20 Presumably because of saturation of the peripheral compartment, it seems safe after 60 days of therapy to reduce the therapeutic window for trough RIA serum concentrations to 75 to 150 ng/ml21 and whole blood RIA values from 400 to 800 ng/ml to 200 to 600 ng/ml, with further reduction to 50 to 100 ng/ml serum and 150 to 400 ng/ml whole blood after 6 months.* However, it appears that the therapeutic window differs among patients, probably due to variable individual CsA pharmacodynamics, the presence of simultaneous drugs heightening renal target organ sensitivity to the nephrotoxic injury (e.g., amphotericin, bacitracin, and nonsteroidal anti-inflammatory drugs), or idiosyncratic reactions. In addition, the window is imperfect: some patients experience rejection episodes in spite of apparently adequate drug levels; others show nephrotoxicity in the face of low blood values. These inadequacies of monitoring CsA therapy by trough values alone led to the concept of individualizing therapy through a pharmacokinetic approach.

Pharmacokinetics. There are significant differences in pharmacokinetic variables in patients treated with CsA. The oral bioavailability of CsA varies from 1% to 67%,22 with an average of 29%. CsA appears to be absorbed both into portal blood and into the lacteals, wherein it is transported via the thoracic duct to the vascular tree. The limited bioavailability of CsA has been attributed to poor gastrointestinal absorption, as well as to a “first pass” effect due to obligatory passage of portal venous blood through and thus extensive metabolism of the oral dose by the liver. Significant variables affect the CsA absorptive process: gastrointestinal disorders such as ileus, diarrhea, or liver disease; gut incompatibility with the olive oil vehicle or diluents such as milk; bile salt composition; drug solubility in intestinal chyme; and intestinal transit time.

From the vascular tree CsA is widely distributed throughout the body. The uptake of Cs by the liver is the highest in the body, probably related to its role in drug metabolism and excretion.22, 23 The pancreas contains the second highest amount of Cs, possibly due to uptake of this cyclic endecapeptide by the mechanism that extracts the small cyclic peptide somatostatin. Because of its lipophilic nature, fat is the third major depot of Cs. The vascularized organs (heart, lung, brain, and kidney) have intermediate tissue concentrations, and muscle and spinal cord have a low content.

The volume of distribution (Vd) of a drug represents the size of the compartment necessary to account for the total amount of drug in the body if it were present at the same concentration as in the plasma. A large Vd is expected for CsA due to its lipid solubility, extensive tissue binding properties, and low level of tight binding to plasma proteins. The average Vd determined in 221 renal transplant recipients by RIA with use of a serum matrix is 8.7 liters/kg,24 suggesting a distribution space of about 600 liters, which is comparable to that for digoxin (700 liters) and greater than that for amphetamine (300 liters). CsA extraction is estimated as the fraction of drug presented to the eliminating organ that is cleared after a single pass through that organ. Liver blood flow is 24 ml/min/kg and it is

*Woningkeit K: Personal communication.

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probably the primary organ of extracton, so CsA clearance values yield the estimate of 50% hepatic extraction, a value similar to that observed with the narcotics morphine and meperidine. Because CsA elimination follows first-order kinetics with a constant fraction of drug eliminated per unit time, and because CsA clearance and Vd appear to remain constant over time and are independent of dose, it is possible to alter the administered CsA dosage in proportion to the desired change in plasma concentration. A deviation in first-order kinetics only occurs when doses greater than 6 mg/kg/hr are rapidly infused intravenously. In this case the kinetics become zero-order, i.e., a constant amount of the drug is eliminated per unit time, presumably due to saturation of the hepatic uptake mechanism. At the point of saturation, small dosage increments result in marked elevations in circulating CsA levels with severe nephrotoxicity and general malaise.

HPLC analysis of metabolites has revealed them to be more polar than CsA, which has been observed to display the longest retention time on 10 μm Li Chromsorb RP18 (Spectra Physics, Basel) columns. The characterized, organic phase metabolites, all of which are slightly more hydrophilic than, but retain the cyclic structure of, CsA, represent region-specific oxidative (phase I) reactions of hydroxylation and/or demethylation at positions 1, 4, 6, and/or 9. The liver appears to be the major site of CsA metabolism. The cytochrome P450 system has been circumstantially implicated in CsA metabolism for three reasons. First, hydroxylation and demethylation reactions are usually catalyzed by these enzymes. Second, some drugs that interact with this system have been shown to alter CsA disposition. On the one hand, drugs that induce cytochrome P450 activity have been shown to accelerate CsA elimination. Included in this group are rifamycin, which specifically increases the N-demethylation pathway and reduces CsA levels; phenytoin, which causes a 30% to 50% reduction in the area under the concentration-time curve (AUC) for CsA and increased total body clearance; phenobarbital; and Arocol 1254. On the other hand, substances competing for cytochrome P450 activity decrease CsA elimination. Included among these are ketoconazole; high-dose furosemide, which causes a 300% reduction in P450 activity and 50% reduced N-demethylation in the mouse; and erythromycin. The reported effect of cimetidine in increasing CsA concentrations has not been confirmed, but rather its adverse effect on renal function may relate to altered creatinine secretion by proximal tubules. In addition, consistent with the important role of competition for cytochrome P450 sites, CsA itself reduces prednisolone clearance from 2.6 ± 0.5 to 1.9 ± 0.5 ml/min/kg. Similarly, prednisone reduces CsA clearance; patients receiving higher doses of corticosteroid show a prolonged half-life (t½) of CsA. The capacity of CsA to be a cytochrome P450 inducer is expected because of its ability to penetrate the plasma membrane and presumably combine with cytoplasmic receptors controlling positive (as well as negative) induction regulatory control sites on gene action. Third, an unpublished study from Sandoz notes that incubation of human liver microsomal preparations in vitro with CsA causes NADPH-dependent degradation, generating metabolites 1, 17, and 21. The process is susceptible to inhibition by carbon monoxide, ketoconazole, and SKF 525A.

It is not presently possible to predict the rate of CsA metabolism with the use of standardized probes: direct assessment of drug clearance is necessary due to the observed 20-fold interindividual difference. Just as inadequate drug effects are noted in the presence of rapid drug elimination, toxicity occurs when patients cannot eliminate the drug and it begins to accumulate. The appreciable interstrain difference in the susceptibility of experimental animals to CsA toxicity observed by Whiting et al. may be attributed to the differential content of various hepatic cytochrome P450 isozymes.

Following intravenous administration, CsA pharmacokinetics measured by HPLC were described by Follath et al. using a three-compartment model, but Yee et al. described it using a two-compartment model. After oral administration a two-compartment open model is appropriate, including a rapidly equilibrating central volume of blood and organs with high blood flow, and a second tissue compartment. The t½, which is calculated by the elimination rate after completion of the absorption and distribution phases, represents the time required for elimination of half the concentration of a drug present in the system if no additional drug is administered. The normal t½ of Cs in renal transplant recipients is about 9 hr by RIA and it has been determined by HPLC to be 21 to 28 hr in one bone marrow allograft recipient and 10.7 hr in kidney recipients.* Comparison of pretransplant (CsA alone) vs posttransplant (CsA plus corticosteroid) t½ showed a slight increase from 7.24 ± 2.80 to 9.27 ± 3.39 hr (p = NS). According to the RIA results, a clinical steady state would be achieved after four t½ or about 2 days of constant CsA dosage. Clearance reflects the rate of replacement necessary to maintain a given plas-

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*Ptachinski R: Personal communication.
ma concentration once the patient has been “loaded” with drug and it indicates the volume of serum, plasma, or blood cleared per unit of time. Using whole blood HPLC, Beveridge et al. reported a drug clearance of 15 ml/min/kg in one bone marrow transplant recipient with normal hepatic and renal function who received 5 days of continuous CsA infusion. Yee et al. noted a clearance of 12.8 ml/min/kg in bone marrow recipients. The rate of drug clearance by the RIA method after intravenous administration of CsA is 12.3 ml/min/kg. Children show a 40% higher drug clearance than adults, necessitating more frequent and higher doses. Cs clearance in man is affected by age: patients over the age of 45 years show a 30% decrease in clearance, with a concomitant 50% increase in AUC, presumably because of decreased hepatic perfusion or function. However, because older patients also show a reduced Vd, the t½ remains the same. Thus, older patients require less drug but the same dosing frequency.

Using serum as the matrix, Yee et al. reported a reduced drug clearance in 10 patients with serum bilirubin greater than 2 mg/dl. The impendment was more evident by RIA than HPLC measurements (4.8 vs 8.3 ml/min/kg), suggesting that hepatic impairment markedly interfered with metabolite elimination. Thus, the use of RIA in the setting of altered hepatic function grossly underestimates the CsA dosage required to achieve usual levels of parent compound. By demographic analysis with RIA, it has been shown that patients with impaired hepatic function clear the drug one-third more slowly, leading to an increased AUC. Thus, it is important to adjust dosage schedules in these patients by reducing the actual amount and/or increasing the interdose interval.

The liver appears not only to be the primary metabolic site, but also the primary excretion site via biliary secretion and fecal elimination. Renal excretion is modest, so hepatic conjugation with glucuronic amino acids or sulfate (phase II biotransformation reactions) is unlikely to represent a major metabolic pathway. Only 60% of the total urinary radioactivity can be assigned to known metabolites (1.1% as metabolite 17% and 0.5% as metabolite 18), the remainder being associated with several small, polar peaks. The major components in bile are metabolites 8 and 17. The majority of CsA is excreted via the feces, although precise quantitative estimates of fecal CsA and its metabolites are not available.

Mechanism of action. CsA acts relatively selectively and reversibly on T, rather than B, lymphocytes. It does not inhibit antibody production toward lipopoly-

saccharide in nude mice; therefore T cell–independent B cell responses are intact. There are only modest effects of CsA on polymorphonuclear, macrophage/monocyte, and natural killer cells. The drug does not delete T cell precursors. Mixed lymphocyte reactions in vitro show inhibited T cell proliferation, but intact generation of suppressor cells. Hess et al. showed that concentrations of CsA as low as 10 to 20 ng/ml reduce interleukin-2 (IL-2) synthesis, and concentrations of 100 ng/ml inhibit the maturation of cytotoxic T lymphocytes, but even 1000 ng/ml has no detectable effect on T suppressor cells. CsA has a lesser effect on the responses of primed lymphocytes, but does inhibit lymphokine release from sensitized cells. Furthermore, polyclonal cytolytic T cell function is not inhibited by CsA. CsA does not act by inhibiting IL-2 receptor expression.

The immune response depends on amplification by growth factors (or lymphokines) to recruit and activate cellular elements. The effects of CsA to inhibit lymphokine production affects a number of subpopulations of T cells, including T helper, T cytotoxic, and T delayed hypersensitivity cells. Since there is neither a protein store nor constitutive synthesis of lymphokines, their production is exquisitely sensitive to inhibitors. Granelli-Piperno et al. have noted that the effect of CsA to block the release of lymphokines from T lymphoblasts is selective: neither the total protein synthesis, as detected by trichloroacetic acid–precipitable material nor the one-dimensional SDS-PAGE pattern is altered. Kronke et al. found that CsA inhibited the nuclear transcription of IL-2, but not of HLA, IL-2 receptor, or HT-3, mRNA in a dose-dependent manner at concentrations up to 1 μg/ml. IL-2 has been used as a prototype to dissect the effect of CsA, because it serves as an important signal to both T and B cell pathways. In addition, there is some evidence that separate sets of CsA-sensitive T helper cells produce IL-2 and (γ-interferon) (γ-IFN) on the one hand, and B cell growth factor on the other. γ-IFN stimulates macrophages to produce interleukin-1 (IL-1), the second signal accompanying the first signal antigen to stimulate T helper cell activation. There is as yet little evidence that CsA directly affects IL-1 generation by macrophages, save for the observation of Bunjes et al. in experimental animals. Rather it appears likely that reduced IL-1 generation is a secondary effect of inhibition of γ-IFN synthesis. In addition, CsA does not block concanavalin A–stimulated mouse macrophages from producing plasminogen activator.

The tissue receptor site for CsA inhibition of lymphokine generation is unclear. Ryffel et al. initially pro-
posed a specific plasma membrane receptor site. However, LeGrue et al.\textsuperscript{13} noted that not only T but also B lymphocytes, kidney cells, and cholesterol/phosphatidylcholine liposomes displayed the same “receptor” properties. CsA may block the transduction of the membrane signal to cytoplasmic activation for the cellular response. The observation of Truneh et al.\textsuperscript{49} that IL-2 stimulation bypasses steps necessary for antigen or mitogen activation suggests that distinct biochemical mechanisms may discern CsA-sensitive from CsA-insensitive phases of the immune response. Lymphocyte activation pathways include voltage-gated or receptor-operated Na\textsuperscript{+}, K\textsuperscript{+}, or Ca\textsuperscript{2+} channels; the phosphatidylinositol pathway; and cyclic nucleotide-dependent protein kinases, including Ca\textsuperscript{2+} calmodulin–activated and Ca\textsuperscript{2+} phospholipid–dependent protein kinase C. CsA is known to not affect the influx of calcium after activation by mitogen or calcium ionophore\textsuperscript{50} or by the binding of OKT3 (Orthodone, Ortho, Raritan, NJ) monoclonal antibody.\textsuperscript{51} Thus, while CsA may alter plasma membrane transduction of the activation signal,\textsuperscript{14} it appears more likely that after passive entry into the cell by partitioning into the lipid bilayer, CsA inhibits key enzymes in the activation cascades by competitively binding to hydrophobic sites, the second proposed mechanism of action.

The Ca\textsuperscript{2+} binding protein calmodulin mediates many cellular processes requiring Ca\textsuperscript{2+}. Calmodulin, a 17 Kd protein, is localized just below the cap formed upon activation of lymphoid cells. Calmodulin may participate both in ligand-induced redistribution of specific receptors to produce capping, and in ligand-cytoskeletal interactions associated with the initiation of lymphocyte activation via microfilaments, microtubules, coated vesicles, and intermediate filaments. Furthermore, calmodulin has been implicated in regulation of the cell cycle and initiation of DNA synthesis and mitosis, chromosome-to-pole microtubules, and assembly and disassembly of microtubules in the mitotic apparatus. Calmodulin is known to act in the nucleus via a calmodulin-dependent inducible mRNA. Trifluoperazine, a calmodulin antagonist, inhibits both receptor-ligand endocytosis and the early signals for the mitogenic responses of T and B cells.\textsuperscript{52} The immune response may be modulated by alteration of the effect of calmodulin on adenylate cyclase and/or phosphodiesterase.

Colombani et al.\textsuperscript{53} reported that CsA binds to and inhibits calmodulin. Precipitation of radiolabeled CSA-treated cells with anticalmodulin antibody sedimented CSA. Using a fluorescent dansylated derivative of CsA that allows determination of binding by flow cytometry, these workers showed on the one hand that association with CsA increases the fluorescence of dansyl calmodulin when it binds to its ligand calcium. On the other hand, dansyl CsA displayed increased fluorescence on binding to calmodulin only in the presence of calcium. The role of calmodulin binding has been recently questioned.\textsuperscript{*} First, active and inactive cyclosporins show equivalent binding to calmodulin without stereospecificity. Second, 100-fold greater concentrations (10\textsuperscript{-5}M) than the immunosuppressive dose (10\textsuperscript{-7}M) are required to inhibit the binding of calmodulin to its own isolated acceptor proteins and the activation of the calmodulin-dependent enzyme assay cyclic nucleotide phosphodiesterase. Furthermore, at a concentration of 10\textsuperscript{-5}M, none of the cyclosporins attenuates calcium mobilization during isolated smooth muscle excitation, a predicted effect if binding to calmodulin affects activation of myosin light-chain kinase, leading to light-chain phosphorylation and muscle contraction.

The second independent major activation pathway in T cells involves the hydrolysis of phosphatidylinositol-4,5-biphosphate, a very minor constituent of membranes, to diacylglycerol (DAG), which is normally almost absent from membrane but is transiently produced in response to extracellular signals, and inositol triphosphate, which activates protein kinase C (PKC), a Ca\textsuperscript{2+} and phospholipid-dependent enzyme. PKC is a single polypeptide chain of 77 Kd with a hydrophobic domain that binds to membranes, and a hydrophilic domain that is the catalytic active center independent of DAG, Ca\textsuperscript{2+}, or phospholipid. Under resting conditions PKC is largely present in soluble, inactive form; on activaton it tightly associates with cell membranes. DAG increases the affinity of PKC for Ca\textsuperscript{2+}, thereby fully activating the enzyme even in the absence of changes in Ca\textsuperscript{2+} levels. Concomitant with the receptor stimulation that produces inositol phospholipid breakdown, there is mobilization of Ca\textsuperscript{2+} that acts synergistically with PKC to elicit the full response of lymphocytes including proliferation in response to phylohemagglutinin (PHA)\textsuperscript{54} and to concanavalin A.\textsuperscript{55} PKC phosphorylates seryl and threonyl, but not tyrosyl residues of many endogenous proteins. Although the precise substrate in lymphocytes is unknown, PKC can phosphorylate microtubule-associated proteins, ribosomal 5S protein, and initiation factor 2 (β-subunit) for protein synthesis in other cells. In addition to generation of PKC activation, inositol phospholipids yield arachidonic acid via two consecutive reactions: one

\*LeGrue SJ: Personal communication.
catalyzed by phospholipase C and DAG lipase, and the other by release of phospholipase A2 from phosphatidylycholine and ethanolamine. After receptor stimulation both reactions act in concert. PKC therefore is a pivotal switchpoint in the activation cascade. However, the action of CsA on PKC is controversial.

A third proposal by Handschumacher and his colleagues that CsA binds to cytosolic receptors was based on the identification of a CsA binding cytoplasmic protein, which they called cyclophilin, of 15 Kd molecular mass and isoelectric point at pH 9.6. Cyclophilin activity was assayed based on the capacity of cytoplasmic fractions to bind to (3H)-CsA, resulting in the drug’s elution from Sephadex LH columns in the void volume. Cyclophilin may represent a molecule necessary for lymphokine gene activation possibly related to a gene “derepressor,” or a binding sink to inactive CsA.

Inhibition of release of lymphokine gene derepressor substances or masking of regulatory genes that determine lymphokine expression may be invoked as two additional mechanisms to explain the action of CsA to selectively inhibit lymphokine gene derepression. It is this action that prevents generation of lymphokine messenger RNA, the step in the activation cascade that has been documented to be inhibited by CsA therapy. Although one cannot exclude additional effects of CsA on mRNA processing, stability, or translation, it appears unlikely that the drug’s major action is on posttranslational protein modification or secretion. Rapid utilization or destruction of secreted lymphokine seems equally improbable.

Thus, the exact site of action of CsA is unknown. Definition of the CsA tissue binding site that determined inhibition of lymphokine generation may not only allow selective pretransplant bioassays that may predict relative susceptibility to CsA immunosuppression, but also postoperative assessment of relative saturation of binding sites. At present only the final outcome of CsA inhibition, namely reduced lymphokine generation, can be used as a pharmacodynamic tool to assess the immunosuppressive effect of the drug on the body.

Pharmacodynamics

Enumeration of lymphocyte subsets. Since T helper cells represent an important (although not the sole) source of lymphokines, serial enumeration of the OKT4+ compartment has been used to assess CsA effect. CsA-treated patients with biliary cirrhosis display a decreased OKT4/OKT8 ratio, a finding confirmed in transplant recipients. There are difficulties in the use of cell enumeration as an index of immunosuppression. A variety of cells carry the OKT4 marker, including not only putatively CsA-sensitive T helper cells, but also CsA-resistant inducers of suppressor cells and CsA-resistant T helper for some B cell responses, as described by Klaus and Kunkl. Furthermore, both the OKT4 + T helper and OKT8 + CTL produce IL-2 and γ-IFN and would be expected to be inhibited by CsA. Thus, a specific reduction in OKT4 + or augmentation of OKT8 + cells would not be expected. In spite of these limitations, in the majority of patients treated with CsA there is a shift from an OKT4/OKT8 ratio greater than 1.0 to a ratio less than 1.0 after transplantation. The shift is primarily due to decreased numbers of OKT4+ cells with a slight increase in OKT8+ cells. In addition, Kerman et al. have observed in 152 renal transplant patients with OKT4/OKT4 ratios of less than 1.0 a lower mean serum creatinine level (1.8 ± 0.7 mg/dl) than that in 43 patients with ratios of less than 1.0 (2.3 ± 0.6 mg/dl). Since patients in both groups received similar mean doses of CsA and had similar trough levels, the depressed OKT4/OKT8 ratio appears to reflect a favorable pharmacodynamic effect of CsA on the immune response.

IL-2 generation: lymphocyte performance tests. Since CsA displays a relatively selective immunologic action to inhibit lymphokine generation, IL-2 generation is reduced in CsA-treated kidney recipients compared with that in normal individuals, dialysis patients, or azathioprine-prednisone–treated recipients. The difference between IL-2 production in normal individuals and those treated with CsA only (no corticosteroid) is highly significant. In addition, there is no significant difference between inhibition of IL-2 generation in patients treated solely with CsA and that in those treated with CsA-prednisone. By 1 week after inception of CsA immunosuppressive therapy, IL-2 generation is inhibited 55%; the inhibition is durable at 1 month after transplant. By 2 months after transplant, IL-2 generation has substantially improved. Just before initiation of high-dose steroid therapy for a putative rejection episode, diagnosed both by clinical criteria and by renal biopsy, patients display an inferior pharmacodynamic effect, with uninhibited generation of IL-2.

γ-IFN generation: lymphocyte performance tests. Because of the cumbersome nature of the IL-2 bioassay and because of the availability of both a murine monoclonal antibody recognizing γ-IFN and an enzyme-linked immunoassay, patient peripheral blood lymphocytes were assayed for the pharmacodynamic effect of CsA with respect to inhibition of both IL-2 and γ-IFN synthesis on each supernate after PHA stimulation. There was marked inhibition of γ-IFN genera-
tion by 180 samples from 30 patients at various times after transplantation. For about 2 months after transplant there was severe inhibition of γ-IFN generation, which was effectively nil for the first month. Therefore, assay for γ-IFN generation appears to be a more sensitive, rapid, and flexible method than the IL-2 bioassay. Serial monitoring of γ-IFN inhibition with 750 samples from 112 recipients of cadaver and 33 recipients of living renal allografts from relatives revealed initially severe and thereafter modest to moderate, but variable, inhibition of the generation of γ-IFN over a 2 year time frame after transplantation.

Pharmacokinetic considerations in pharmacodynamic assays. To assess the pharmacodynamic efficacy of CsA, the mixed lymphocyte reactions (MLR) provides a convenient index in vivo of alloresponsiveness in vivo. Keown et al.\(^4\) showed that serum samples from CsA-treated patients inhibit the MLR responses of normal cells toward third-party stimulators. Analysis of 79 patients by Rogers et al.\(^6\) revealed four distinct patterns of serum inhibition of MLR: two peaks of MLR inhibition (pattern I, occurring in 55% of recipients), one peak (pattern II, in 15%), continuous high inhibition (pattern III, in 15%), and no inhibition (pattern IV, in 15%). There were no differences in serum trough and peak CsA levels (by RIA) or in measured pharmacokinetic variables among patients displaying various pharmacodynamic patterns. Furthermore, pre transplant MLR inhibition profiles corresponded to posttransplant profiles in 85% of patients. Patterns II and IV were associated with a significantly higher risk of allograft rejection episodes, a finding recently confirmed in heart recipients.\(^6\) The MLR type also correlated with the incidence of cardiac allograft rejection: 0.10 ± 0.11 rejections/month for type I (n = 11), 0.15 ± 0.12 for type III (n = 4), and 0.40 ± 0.10 (n = 2) and 0.83 (n = 1) for types II and IV, a difference significant at the p < .005 level by chi-square analysis.\(^6\)

The immunosuppressive component mediating peak II is similar to CsA with respect to titration kinetics of inhibition of MLR and cell-mediated lympholysis; resistance to boiling, freezing, and thawing; and its hydrophobic nature, although it is slightly less hydrophobic than cyclosporine.\(^6\) The hypothesis proposed to explain the finding of recrudescent MLR inhibition after waning of blood CsA levels measured either by HPLC or RIA cites the generation of an active drug metabolite. Comparisons of HPLC profiles of serum samples obtained during peak II with those obtained at earlier time points demonstrate enrichment of a component eluting at 19 min, designated metabolite E, which displays one-fourth the MLR inhibitory capacity of the parent CsA. According to this hypothesis type II patients fail to generate the metabolite, while type III patients biodegrade the active material with difficulty. Thus, insights into active metabolites may afford new tools to assess immunosuppressive efficacy in vivo.

Clinical immunosuppressive efficacy for prophylaxis of allograft rejection. In spite of the fact that a number of CsA-based drug regimens have been introduced, there appears to be little difference among them with respect to patient survival or even the frequency of allograft rejection episodes. The raison d’être of these regimens appears to be a reduction in CsA-induced nephrotoxicity, particularly in the early posttransplantation phase, when by virtue of preexistent renal dysfunction due to cardiac disease, the extracorporeal bypass phase, and the adverse effects of anesthesia, other medications, and surgical stress, the host’s kidneys may be exquisitely sensitive to the nephrotoxic action of CsA.

Single-drug regimen. The single-drug regimen of CsA alone beginning at 25 mg/kg/day was recommended by Calne\(^7\) as a solution to the problem of the high frequency of neoplasms observed in patients receiving the same dose of CsA with combined cyclophosphamide-prednisone in usual doses. Because most kidney recipients experience rejection episodes on single-drug therapy and because less than 30% of patients can be maintained on CsA alone, this regimen has been regarded to be of insufficient potency for patients undergoing cardiac transplantation.

Double-drug regimen. The most widely used double-drug regimen is CsA and prednisone. In our center, the CsA regimen for renal transplantation is begun with continuous infusion at 2.5 mg/kg/day to achieve steady-state plasma levels of 150 ng/ml for 3 days before oral therapy is begun. Oral therapy begins at a dose of 14 mg/kg, and is thereafter tapered to 10 mg/kg at 2 weeks and 7 mg/kg at 2 months, with an aim of keeping trough serum levels between 100 and 250 ng/ml, as determined by RIA. The steroid regimen begins at 120 mg orally daily, and the oral dose is tapered to 30 mg at day 6 and 20 mg by day 60. In contrast to the previous use of azathioprine-prednisone immunosuppression, which resulted in an overall 51% 1 year graft survival, CsA-prednisone used in 300 consecutive kidney recipients yielded an overall 1 year graft success rate of about 80% and patient survival of 94.7%. However, the lower rate of success with the former regimen was due in part to unrestricted acceptance of recipients between the ages of 2 and 70 years who displayed strong immune responsiveness, were
rarely splenectomized or transfused, and who were never treated with ALS.

For the patient undergoing cardiac transplantation, the regimen is modified to use higher doses of CsA to maintain the serum trough value between 200 and 400 ng/ml. The steroid regimen begins with 120 mg by mouth daily, and is tapered to 20 mg by 8 months. The 1 year patient survival is 74% under conditions of unrestricted transplantation in 132 recipients between 0.5 and 62 years of age, regardless of transfusion status and without splenectomy. However, 81% of patients experience infectious episodes (60% of these infections are not fatal) and 75% experience rejection episodes. The average patient experiences 1.9 rejection episodes, and receives at least one high-dose (500 mg) bolus of corticosteroids followed by a high oral dose (120 mg) regimen with a gradual tapering of dose. The steroid-resistant or recurrent episodes of rejection that occur early in about 40% of cardiac recipients are generally treated with intravenous equine antilymphocyte or murine monoclonal OKT3 antibody. With the use of this regimen the overall length, and thereby cost, of the initial admission has been reduced from $91,000 to $52,000.

An alternative double-drug regimen in which CsA and azathioprine are used has been studied by Yacoub et al. in 188 cardiac recipients, with 81% 1 year and 77% 2 year patient survival. Use of corticosteroids was restricted to antirejection therapy in an effort to reduce the overall morbidity associated with corticosteroid therapy.

**Triple-drug regimen.** An attempt to use CsA-prednisone-based immunosuppression at Stanford University led to a high incidence of nephrotoxicity as well as the need for long-term dialysis therapy in several patients in whom transplantation was otherwise successful. This adverse outcome probably resulted from the excessively high, prolonged CsA doses used at Stanford. To allow reduction of the CsA dose, this institution advocates triple-drug therapy with CsA (8 mg/kg), azathioprine (23 mg/kg), and prednisone, a regimen that has reduced the incidence of renal dysfunction and afforded 75% 1 year graft survival.

**Quadruple-drug regimen.** Because of the risk factors associated with the immediate posttransplant phase, Simmons et al. recommended the use of ALS for the initial 5 to 10 day postoperative period to spare the newly transplanted kidney from the possible adverse effects of CsA. This approach has been used for cardiac transplantation; however, a recent evaluation by Schuler et al. suggests that the initial 4 days of ALS-azathioprine-prednisone therapy in the absence of CsA is associated with decreased patient survival. They now advocate beginning CsA 24 rather than 96 hr postoperatively.

**Clinical immunosuppressive efficacy for therapy of established allograft rejection.** Because of the potency of high concentrations of CsA to inhibit the responses of mature T cells, a trial was conducted to compare the efficacy of supplemental intravenous CsA with intravenous bolus doses of corticosteroids to reverse moderate cardiac rejection. Moderate rejection was defined by endomyocardial biopsy findings of interstitial mononuclear cells with mild-to-moderate cardiac myocyte degeneration. After diagnosis patients received, in addition to their oral dose of CsA, a supplemental infusion of 2.0 mg/kg/day for 7 days. Oral doses of corticosteroids were not altered. Of 21 patients treated with intravenous CsA, 16 rejection episodes resolved. The other five episodes resolved with subsequent steroid therapy. This 76% rate of resolution of acute rejection episodes was similar to the 81% reversal rate observed in 32 recipients treated with high-dose (7 mg/kg) methylprednisolone. Of significance was the observation that none of the CsA-treated patients displayed acute or chronic nephrotoxicity or hepatotoxicity. Furthermore, infectious complications occurred in only 23% of CsA- vs 50% of the steroid-treated patients. These observations suggest that patients demonstrating a poor pharmacodynamic reaction to CsA as evidenced by allograft rejection may benefit from increased drug doses to heighten the immunosuppression.

**Complications of CsA therapy.** Excessive immunosuppression, a major complication in transplant patients, presents acutely as infection and chronically as failure of immune surveillance and emergence of malignancy. Since the drug has little effect on T cell-independent, B cell-mediated antibody responses to complex antigens bearing repeating epitopes, patients treated with 10 to 15 mg/kg CsA display a reduced incidence of bacterial and fungal infections compared with those receiving azathioprine-prednisone.

Whereas 15% of patients on azathioprine-prednisone show evidence of pulmonary infection, the most common site, only 1% of those on CsA-prednisone patients are similarly afflicted. The use of CsA has little effect on decreasing the incidence and severity of herpes virus diseases, which occurs in 10% of recipients during the first posttransplant month. Cytomegaloviral infections are observed in only 3% of recipients during the first 2 months, a figure significantly reduced from 15% noted for patients on azathioprine-prednisone, possibly due to a direct toxic effect of CsA on the
of immunosuppression is malignancy, especially those types with a likely viral cause. Non-Hodgkin’s lymphoma, which occurs most commonly during the first year after transplantation procedures, occurs with 49 times greater incidence in immunosuppressed than in untreated patients. Although CsA per se is not teratogenic in animals or in man, its selective effect on T cells may impair resistance to Epstein-Barr virus–induced B cell proliferation. Thus Calne et al.77 using high doses of CsA-cyclophosphamide-prednisone, observed five lymphomas in their first 23 patients. Similarly, the CsA-ALS-azathioprine-prednisone combination caused an increased incidence of lymphoproliferative syndromes, accompanied by dysgammaglobulinemias.78 Starzl et al.79 documented regression of lymphomas in CsA-treated patients after they discontinued or drastically reduced the drug dose. With the present use of more modest drug doses, the incidence of malignancy with CsA-prednisone is now no greater than with other agents. A survey of 3000 patients, including 1800 renal, 130 heart, 850 bone marrow, 170 other organ graft recipients, as well as 100 patients not receiving grafts, documented 15 lymphomas, i.e., an incidence of 0.5% in contradistinction to the 2% to 11% rate previously reported in patients receiving conventional immunosuppressive agents.80 However, in a series of almost 500 renal and 52 cardiac transplants, there was only a single case of Kaposi’s sarcoma reversed by reduction of the CsA dose. Thus, judicious attention to maintaining only modest levels of immunosuppression may avert both infectious and neoplastic complications.

**Neuroectodermal toxicity**

*Central nervous system.* Due to the high lipid solubility of CsA in plasma membranes,14 it is to be expected that the drug might affect central nervous system function. Neurologic manifestations of involuntary hand tremors, seizures (rare), and, occasionally, burning paresthesias of the palms and soles, may occur during the first 3 months of therapy and generally respond to dose reduction. Involuntary fine tremors are the most common early neurologic complication, observed in 22% of kidney transplant recipients, and serving as a valid measure of drug overdosage. These tremors slowly respond to dose reduction, and are rarely seen after 12 months of therapy or at doses below 5 mg/kg. Convolutions, which occur both de novo and in patients with a previous history of these disorders, vary from grand mal to petit mal and in our experience generally represent motion rather than sensory disorders. In bone marrow transplant recipients seizures have been attributed to concomitant bolus methylprednisolone therapy or hypomagnesemia, presumably due to renal wasting.

Treatment of the convulsive disorders with phenytoin (and/or phenobarbital) raises additional problems, because this drug induces production of a cytochrome P450 isozyme that enhances CsA degradation. Therapy with divided doses of CsA twice or three times a day is then necessary to maintain trough blood levels within the therapeutic range for CsA. Rarely patients complain of lethargy and somnolence, symptoms that can be controlled by bedtime drug administration.

*Hypertrichosis.* Hypertrichosis, occurring on the face and upper trunk, is distinguishable from hirsutism, or hair growth in the male pattern, which is caused by steroids. The drug increases existent, but does not engender new, hair growth in almost half of the patients who receive it. The degree of hypertrichosis tends to be moderate unless CsA therapy is combined with potentiating agents such as minoxidil. Save for in a limited group of female and pediatric patients, this complication is rarely significant and frequently responds to treatment with depilatory creams.

*Gingival hyperplasia.* Gingival hyperplasia, akin in appearance to that observed with phenytoin, is rarely evident before the third posttransplant month, and tends to occur in patients with poor dental hygiene. Institution of a rigorous dental program has been noted to improve all but the most severe cases. Twice in our experience gingivectomy was required.

**Hepatotoxicity.** The liver dysfunction frequently noted in kidney transplant recipients even before the introduction of CsA has been attributed to azathioprine and/or other concomitant drug therapy, or to viral infection with cytomegalovirus, hepatitis, or herpes virus. In Calne’s initial series of allograft recipients, hyperbilirubinemia, usually accompanied by elevated serum alkaline phosphatase and aspartate aminotransferase levels, was observed in virtually all recipients treated with 25 mg/kg CsA. Klintmalm et al.81 confirmed the finding of hepatotoxicity in kidney allograft recipients receiving 20 mg/kg/day CsA. In a series of 400 recipients treated at our center with a dose beginning at 14 mg/kg, there was a 48% incidence of hepatotoxicity, generally presenting as an increased SGPT level, and frequently accompanied by elevations in
SGOT and bilirubin. There were three patterns observed: elevated bilirubin level only (generally greater than 1.5 mg/ml and in one instance to 7 mg/dl), bilirubin level, SGOT, and SGPT levels all increased and abnormal transaminase levels only. Among the patients displaying elevated transaminase levels, the most common abnormality was an elevated SGPT value. Interestingly, there was no significant difference in serum bilirubin or liver enzyme levels in patients treated with CsA-prednisone and in those receiving azathioprine-ALS-prednisone in the Minnesota randomized trial.\(^{82}\) Changes in alkaline phosphatase appear to be related not to the liver but rather to bone due to 1,25 hydroxycholecalciferol production by the renal allograft, as previously noted by Rodger et al.\(^{83}\) The 12% of patients who display concurrent renal and hepatic impairment illustrate a clinical syndrome of drug toxicity.\(^{17}\)

An increased incidence of associated pancreaticobiliary complications has been noted,\(^{84}\) and these include cholelithiasis attributed to the high content of CsA metabolites in bile altering its lithogeneity, and acute pancreatitis attributed to the accumulation of CsA in the pancreas and/or biliary-induced abnormalities.

In most cases hepatic dysfunction responds promptly to dose reduction, although in some instances there is a slow return to normal values. One patient in the CsA arm of our randomized series required conversion to azathioprine because of the hepatotoxicity, which nevertheless reappeared 1 month later and was only reversed by subsequent change of therapy to cyclophosphamide. The last maneuver produced an inferior level of immunosuppression and graft loss in the second year. CsA hepatotoxicity may be potentiated by concomitant therapy with other hepatotoxins. For example, marked increases in hepatic dysfunction have been observed upon concomitant azathioprine treatment of patients for chronic nephrotoxicity. In sum, hepatic toxic reactions are generally reversible and responsive to reduction of the CsA dose and thus do not represent a significant problem in clinical practice.

**Hematologic reactions.** In contradistinction to azathioprine, which is administered in sufficient dosage to produce myelosuppression in practically every patient, CsA only rarely causes this effect. Less than 1% of kidney transplant recipients in our series experienced leukopenia or thrombocytopenia, and in these the condition improved upon reduction of the CsA dose. Hemolytic reactions with reticulocytosis, anemia, and abnormal peripheral blood smears have been encountered in 3%. In many instances they produce de novo hemolytic uremic syndrome,\(^{85}\) but in others they present as purely hematologic phenomena responsive to reduction of the CsA dose. Because of the possible contribution of concomitant infections and our inability to demonstrate altered erythrocyte osmotic fragility or the presence of circulating serum factors that increase erythrocyte lysis, an unequivocal association between CsA and hemolysis has not been proven. Furthermore, there is a question of increased coagulability due to the occurrence of renal artery thromboisosis, as noted in seven of our first 300 renal transplant recipients.\(^{86}\) Also, Vanrenterghem et al.\(^{87}\) have suggested an increase in thrombotic events.

**Nephrotoxicity**

*Clinical presentation.* Kidney function is altered in almost all transplant patients treated with CsA, including kidney, heart, liver, and bone marrow recipients. Furthermore, patients receiving CsA for autoimmune disease, including diabetes mellitus,\(^{88}\) uveitis,\(^{89}\) and collagen diseases,\(^{90}\) experience about a 33% decrease in glomerular filtration rate, which is reversible within 2 to 6 months of discontinuing the drug. The characteristic picture of CsA nephrotoxicity is a reduced creatinine clearance with elevation of serum creatinine and disproportionate increases in blood urea nitrogen (BUN), hyperkalemia, hypertension, hyperuricemia, and hyperkalemic hyperchloremic type intravenous renal tubular acidosis with preserved urine volume and sodium reabsorption. Suppressed plasma renin activity and tubular insensitivity to aldosterone, i.e., a hyporeninemic hypoaldosteronism,\(^{91}\) are two factors contributing to the hyperkalemia. The suppression of plasma renin activity may result from extracellular fluid expansion, inhibition of renal prostaglandin synthesis, or other causes. In kidney transplant recipients the incidence of clinically significant nephrotoxicity requiring aggressive investigation and dose reduction varies from 30%\(^{82,84}\) to 74%,\(^{92}\) and thus is presumably related to different strategies of drug management. The lower incidence is observed when there is a fixed schedule to taper CsA doses, while the latter incidence is typical at centers that only decrease the dose when nephrotoxic complications occur.

*Mechanism.* Each of three major causes of decreased glomerular filtration rate have been implicated in the CsA-induced injury: increased intraproximal tubular pressure activating glomerulotubular feedback, decreased filtration coefficient, and vasoconstriction. The initial hypothesis of the nephrotoxic mechanism suggested that CsA causes tubular damage, leading to functional impairment because of back pressure along nephrons blocked by damaged tubules and a feedback
mechanism whereby angiotensin lowers glomerular flow. The minimal structural damage observed microscopically in the proximal tubule straight segment in the rat and in the convoluted segment in man includes isometric vacuolation (due to swelling of the endoplasmic reticulum), eosinophilic inclusion bodies, giant mitochondria, intracellular calcification, and increased numbers of lysosomes. Snez et al. have reported three findings suggesting that the tubular effect is not the primary pathogenetic mechanism. First, tubular vacuolization has been observed in the absence of overt renal dysfunction. Second, CsA must be administered at high doses for prolonged periods to produce these changes. Third, sole administration of the lipid vehicle without CsA to animals caused formation of vacuoles.

The mechanism of the proposed tubular injury is unclear. One possibility is a direct effect due to increased serum drug levels. There is evidence that patients with increased drug levels tend to have a greater incidence of nephrotoxicity. A demographic study revealed nephrotoxic patients to have the stigma of an increased AUC/dose without a change in disposition variables, suggesting that these patients have an increased total body CsA content. Second, since the kidney, particularly the S3 segment of the proximal tubule, contains the cytochrome P450 system, a nephrotoxic metabolite might be locally generated causing direct toxicity. However, the toxicologic activities of metabolites in man are unknown with respect to whether they are effective in inducing or alternatively ameliorate the organ toxicity caused by parent CsA. Unfortunately, there is neither a suitable animal preparation to test nephrotoxic properties, nor a sufficient quantity of CsA metabolites to assess their differential properties. A third possibility is specific deposition of CsA in the tubule, von Willebrand and Hayry, using the polyclonal Sandoz antibody in immunofluorescence assays, noted CsA deposition in tubular cells in aspiration cytology; however, tissue levels have failed to demonstrate appreciable renal drug accumulation such as that observed of gentamicin in the lysosomes of proximal tubular cells and of cis-platinum in the S3 segment of the proximal tubule.

There is an important contribution of other nephrotoxic drugs to augment the CsA-induced injury. Interactions at the level of the kidney with known nephrotoxins have been documented for amphotericin B, the aminoglycoside gentamicin, melphalan, and trimethoprim. Drugs that may affect the creatinine secretion process, such as cimetidine, also appear to increase the nephrotoxic effect of CsA in some patients. Since patients with creatinine clearance in the range of less than 40 ml/min are secreting as much creatinine as they are filtering and because nephrons are lost, the proximal tubule secretes more creatinine, so that patients suffering the decreased glomerular filtration rate associated with CsA nephrotoxicity are particularly vulnerable to tubular toxins.

The possibility of a pure glomerular toxicity of CsA was suggested by the findings of Myers et al. in cardiac transplant recipients who developed progressive, diffuse, tubulointerstitial fibrosis and focal-to-total glomerular sclerosis, reflecting either direct toxic effects or compensatory maladaptive changes in single-nephron hemodynamics. The reduced glomerular filtration rate with a low glomerular capillary ultrafiltration capacity and altered filtration fraction resulted in glomerular obsolescence. Since injuries producing decreased filtration tend to progress with time, it was not unexpected that a follow-up report would note continued loss of glomerular surface area in the second posttransplant year. While the sophisticated renal function assays strongly documented a renal injury, its marked severity and progress in this patient series compared with patients from other centers has been attributed to the high initial and maintenance doses of CsA that particularly sensitized the kidney to injury from other nephrotoxic drugs. There are no findings supporting a direct toxicity of CsA on the glomerulus, but rather present evidence suggests that renovascular ischemia with or without arterial hypertension is the proximate cause of the glomerulosclerosis.

The hypothesis of Baxter et al. proposes that CsA alters renal hemodynamics and consequently causes tubular hypoxia progressing to interstitial fibrosis. Indeed, the rapid improvement in clinical condition observed on reduction of the CsA dose is consistent with an important role of altered renal hemodynamics. Compromised renal blood flow would reduce the glomerular filtration rate and cause a proximal tubular defect. Using radioactive microspheres, Humes et al. demonstrated decreased total renal blood flow sufficient to explain the decreased glomerular filtration rate and suggested that CsA produces prerenal failure. By estimating effective renal plasma flow with 131I-hippurate, Tegzess et al. noted improved blood flow in CsA-treated recipients whose therapy was changed to azathioprine.

There are three possible hemodynamic mechanisms of reduced blood flow: vasoconstriction, perhaps via stimulation of the renin-angiotensin-aldosterone system; inhibition of prostaglandin-mediated vasodilatation, as seen with nonsteroidal anti-inflammatory
drugs; or efferent arteriolar vasoconstriction mediated by angiotensin II, which would be sensitive to angiotensin converting–enzyme inhibitors. In animal preparations Paller and Murray103 and Sullivan et al.104 have shown a 48% decrease in blood flow, increased renal vascular resistance, and increased plasma renin activity after intravenous infusion of 20 mg/kg (or even lower doses) of CsA, suggesting that CsA is an α-agonist. Renal blood flow was not restored by angiotensin converting–enzyme blockade or by prostacyclin inhibition, but was ameliorated by the α-antagonist phenoxybenzamine or by renal denervation. CsA-induced activation of the sympathetic nervous system would enhance tubular reabsorption of sodium and other solutes. Reduced distal delivery and sodium retention would contribute to hypertension, hyperkalemia, and hyperuricemia. While α-agonism may play a role in acute nephrotoxicity, the hyporeninemic state suggests that it may not be the primary mechanism in later phases of the injury.

Another possible mechanism of altered blood flow is arteriolopathy caused by reduced prostacyclin production secondary to decreased synthesis of prostacyclin-stimulating facor. Mihatsch et al.104 have described histopathologic findings of arteriolopathy including endothelial cell swelling and disruption without proliferation or cellular infiltration, arteriolar hyalinosis or necrosis, and increased numbers of lysosomes and protein deposits in necrotic myocytes, suggesting that these lesions lead to the histopathologic picture of striped interstitial fibrosis. Prostacyclin is practically the only metabolite of arachidonic acid produced by renal cortical arteries and afferent arterioles. Indeed, there is a similarity between CsA-induced renal failure and the decreased blood flow, diminished glomerular filtration rate, salt and water retention, and hyperkalemia produced by inhibitors of prostaglandin synthesis, nonsteroidal anti-inflammatory drugs, which themselves markedly potentiate CsA nephrotoxicity. In addition, the urine shows increased thromboxane A2, a potent renal vasoconstrictor, which may be produced by renal tissue locally in response to ischemia or released by platelets or mononuclear cells accumulating in the graft. Whether the changes in prostaglandin production are the cause or the result of the disorder is unclear. Although prostacyclin is believed to play only a minor role in physiologic regulation of renal hemodynamics, in the presence of increased vasoconstrictor influences such as would occur with α-agonism, synthesis of prostaglandin is normally enhanced and renal perfusion is rendered increasingly dependent on prostaglandin. Indeed, prostacyclin produced in response to decreased cortical blood flow serves as an important local hemodynamic regulator. The action of CsA on prostacyclin-stimulating factor might either be an independent effect, or alternatively prostacyclin-stimulating factor might be a lymphokine produced by T helper cells. Indeed, Rossi et al.105 reported that IL-1, a lymphokine whose release from murine monocytes has been reported to be depressed by CsA, induces the synthesis of prostacyclin by endothelial cells. The latter, unitary hypothesis would explain both CsA-mediated immunosuppression and nephrotoxicity.106,107

Conclusions. Cardiac transplantation under CsA therapy can be regarded as a major therapeutic approach to the patient with intractable heart failure. Improvement of allograft survival to 75% with reduced morbidity and mortality from infection has accompanied the use of CsA, an agent with an excellent immunosuppressive index for inhibition of alloresponsiveness while sparing nonspecific host resistance. However, CsA-induced nephrotoxicity presents a major complication, and a significant cause of immediate and long-term postoperative renal dysfunction. Because of the nephrotoxicity, the trend is toward a reduction of the dose of CsA used, with anticipated lower immunosuppression. Thus, the optimal CsA regimen is not known. Marked interindividual differences in the pharmacokinetic variables of absorption, distribution, metabolism, and elimination combined with a lack of consensus about the analytic method, matrix, and target drug concentrations have led to physicians taking an empiric approach to CsA therapy. Furthermore, differences in pharmacodynamic sensitivity to CsA therapy as evidenced by immunologic tests in vitro further complicate the correlation between pure pharmacokinetic variables and clinical outcome. Clearly, CsA has reduced the severity, and to a lesser extent the incidence, of allograft rejection episodes. Combination immunosuppressive regimens in which CsA, prednisone, azathioprine, ALS are used are being widely applied to achieve better rejection prophylaxis in spite of a lack of documented clinical efficacy in randomized trials or a firm scientific basis. Future progress will depend on results of controlled trials to discern the optimal mixture of immunosuppressive agents that achieves synergistic activity with low toxicity.

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