Early mythology depicts two saints in a life-and-death struggle to save a limb from a foregone fate. Saints Cosmos and Damien replaced a cancerous leg with a limb from a deceased Moor. This illustration was mere fantasy until the latter part of the previous century with the seminal surgeries performed by Dr. Joseph Murray on identical twins and, later, Dr. Starzl’s pioneering orthotopic liver transplant (OLT). Immunosuppression itself has come from the dark ages to where it stands today: a careful balancing act between toxicity and rejection. This article is designed as an overview of current immunosuppression in OLT and will not delve into areas such as basic science or pipeline preview.

The first attempts at whole liver grafting were carried out in 1955 with auxiliary grafts. This involved insertion of an extra liver at an ectopic location. This approach left the diseased liver intact. Early results, however, were disappointing. This early experience in the animal model and later attempts in humans led to the development of the OLT model. This was first attempted in 1963 in humans and today is the primary model for liver transplantation.

Early immunosuppression was suboptimal at best. Corticosteroids and azathioprine were used in combination by Starzl et al. in his first 5 transplants. The majority of the Colorado series from 1963 to 1976 received corticosteroids, azathioprine, and antilymphocyte globulin. A total of 170 patients were transplanted, and approximately 50% were children. Patients were divided into 3 time-based series. Series 1 (1963-1976, 111 patients) had a 1-year survival rate of 28.8%, Series 2 (1976-1978, 30 patients) had a 1-year survival rate of 50%, and the Series 3 (1978-1979, 29 patients) had a 1-year survival rate of 34.5%. Variations on these approaches included thoracic duct drainage or cyclophosphamide, though neither produced significant advantage. The Cambridge University group reported on a series from 1968 to 1980 with a total of 93 patients, with a 1-year survival rate of 23.7%. The lower survival rate may have been due to the lower rate of pediatric patients transplanted by the Cambridge group.

Dawn of a New Era

The early 1970s saw the introduction of a new class of immunosuppressants, the so-called “calcineurin inhibitors” (CNIs). These compounds form the backbone of immunosuppression in liver transplantation recipients. Currently, 2 CNIs are available: CyA and tacrolimus (TAC).

CyA was discovered by Jean-Francois Borel in 1973 from a soil fungus, *Tolypocladium inflatum*. Food and Drug Administration approval was obtained in 1983. CyA causes selective suppression of cell-mediated immunity via inhibition of T-cell activation. After forming a complex with its cytoplasmic receptor protein (cyclophilin), CyA binds to and inhibits the calcium and calmodulin-dependent phosphatase calcineurin (Fig. 2). It is believed that calcineurin plays a vital role in the transcriptional process by which interleukin (IL) 2 and other cytokines are activated. The production of these substances by T helper cells is a vital component of the immune response and is central to the graft rejection process.

Two formulations of CyA are presently available. The standard formulation (Sandimmune) was the first to come to market and requires an emulsification step prior to digestion and subsequent release of the CyA. The step is heavily dependent on food intake, biliary flow, and gastrointestinal motility and therefore subject to unpredictable bioavailability, with values ranging from 1% to 89% with a mean of 30%. The newer formulations are microemulsion preconcentrates (Neoral, Gengraf), which consist of the drug in a lipophilic solvent. These formulations produce more consistent bioavailability and are less dependent on biliary flow. CyA is metabolized primarily in the liver via...
cytochrome P450-3A pathway. Drug interactions are common (Tables 1A and 1B).

Nephrotoxicity is one of the main side effects of CyA therapy. This can be both an acute and a long-term complication, inducing a post-OLT rate of renal failure up to 20%. Common metabolic abnormalities include hyperkalemia, hypomagnesemia, hyperlipidemia, and, to a lesser extent, hyperglycemia. Hypertension, gingival hyperplasia and hirsutism are also a common occurrence. Between 10% and 28% of patients have neurological manifestations that range from mild tremor and peripheral
neuropathy to psychoses, hallucinations, motor weakness, and seizures (Table 2).10

CyA initial dosage ranges from 10 to 15 mg/kg/day divided into 2 doses. Adjustment of the oral dose is based on trough level measurement, usually within 24 hours of starting CyA. Target trough levels vary widely. Commonly used dose-targeted ranges in liver transplantation would be 250-350 ng/mL during weeks 1-2, 200-300 ng/mL during weeks 3-4, 150-250 ng/mL during weeks 5-24, and 100-200 ng/mL during weeks 25-52.11,12 More recently, dose adjustments based on blood concentration at 2 hours after dose has been shown to more closely correlate total exposure vs. C0 monitoring (trough). One example of this would be target blood concentration levels of 850 to 1400 ng/mL at 2 hours after dose from 0 to 3 months posttransplant.13

In 1984, a soil sample containing the fungus Strep-
tomyces tsukubaensis was discovered from near Mt. Tsukuba in Japan. Two years after this discovery, TAC was isolated.14 TAC is 100 times more potent than CyA and exerts its action by binding to FK binding protein (FKBP12). This complex then inhibits calcineurin, which is responsible for transcription of IL-2, IL-3, IL-4, IL-8, and various chemotactic factors.

TAC absorption occurs in the duodenum and jejunum. Unlike CyA, TAC absorption is not influenced by presence of bile, which is advantageous in cholestatic patients or those with biliary diversion or ileus. Food reduces bioavailability, and TAC should be taken on an empty stomach. Metabolism occurs in the liver via the cytochrome P450-3A. Coadministration of medications that inhibit or induce cytochrome P450 may significantly affect blood levels of TAC (Tables 1A and 1B). Side effects are similar to CyA, including nephrotoxicity, neurotoxicity, posttransplant diabetes mellitus, and hyperkalemia.15

In general, the difference between the 2 CNIs can be summarized as follows: TAC has a higher rate of posttransplant diabetes mellitus, while CyA predisposes to more hypertension, dyslipidemia, hirsutism, and gum hyperplasia (Table 3). Initial dose guidelines for TAC range from 0.1 to 0.15 mg/kg/day orally. Dosages are modulated based on trough levels. Factors affecting adjustments are disease state, renal function, age of the patient, and other concomitant medications. Therapeutic goals for TAC early after surgery would be from 10-15 ng/mL. Multi-center trials have shown OLT patients receiving TAC-based immunosuppression compared to CyA have a lower rate of rejection within the first year, and patients infected with hepatitis C have longer graft survival.16 A large percentage of OLT programs use TAC-based regimens.17

### Table 1A. Drugs That May Increase Tacrolimus and Cyclosporine Blood Concentrations

<table>
<thead>
<tr>
<th>Calcium Channel Blockers</th>
<th>Antifungal Agents</th>
<th>Macrolide Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem</td>
<td>Fluconazole</td>
<td>Clarithromycin</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>Itraconazole</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Ketoconazole</td>
<td>Troleandomycin</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Voriconazole</td>
<td>Azithromycin</td>
</tr>
<tr>
<td></td>
<td>Clotrimazole</td>
<td>Telithromycin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prokinetic Agents</th>
<th>Miscellaneous Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisapride</td>
<td>Amiodarone</td>
</tr>
<tr>
<td>Metaclopramide</td>
<td>Cimetidine</td>
</tr>
<tr>
<td></td>
<td>Methylprednisolone</td>
</tr>
<tr>
<td></td>
<td>Omeprazole</td>
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<tr>
<td></td>
<td>Protease inhibitors</td>
</tr>
<tr>
<td></td>
<td>Nefazodone</td>
</tr>
<tr>
<td></td>
<td>Ethinyl estradiol</td>
</tr>
</tbody>
</table>

### Table 1B. Drugs That May Decrease Tacrolimus and Cyclosporine Blood Concentrations

<table>
<thead>
<tr>
<th>Anticonvulsants</th>
<th>Antibiotics</th>
<th>Herbal Preparations</th>
<th>Miscellaneous Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Rifabutin</td>
<td>St. John’s Probufol</td>
<td></td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Rifampin</td>
<td>Wort Terbinafine</td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Rifapentine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fosphenytoin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Common Side Effects of Cyclosporine

- Hypertension
- Renal dysfunction
- Hirsutism
- Hyperkalemia
- Gingival hyperplasia
- Hypomagnesemia

### Table 3. Common Side Effects of Tacrolimus

- Posttransplant diabetes mellitus
- Nausea, vomiting, diarrhea
- Hyperkalemia
- Tremor
- Hypertension
- Hypomagnesemia
- Headache
- Renal dysfunction
Corticosteroids

As mentioned earlier, corticosteroids have been a mainstay since early days of liver transplant. It is by far the most heavily utilized non-calcineurin inhibitor in liver transplant. Corticosteroids exert their most critical immunosuppressive effect by blocking T-cell–derived and antigen-presenting cell-derived cytokine expression. This includes IL-1, IL-2, IL-3, and IL-6. Corticosteroids continue to be used in reversing acute rejection and in maintenance therapy.

Side effects are numerous and include hypertension, hyperglycemia, osteoporosis, hyperlipidemia, increased risk of gastric ulcers, risk of fungal and bacterial infections, and suppression of HPA axis (Table 4).

Dosing varies widely but can be summarized as follows: a bolus dose of methylprednisolone just prior to surgery, i.e., 500 to 1,000 mg, followed by rapid taper over next few weeks to minimal dose, i.e., 25-50 mg/day. The vast majority of programs will try to wean corticosteroids off within the first year except in cases of autoimmune. The percentage of patients receiving corticosteroids prior to hospital discharge decreased from 91% in 2002 to 82% in 2003. In part this can be attributed to the raising concern over negative impact of this class of medication in the recurrence of hepatitis C.17

Antimetabolites

Azathioprine was the first antimetabolite used in liver transplant but its use has since decreased dramatically over time. Azathioprine is an imidazolyl derivative of mercaptopurine and antagonizes purine metabolism. The result is an inhibition in synthesis of DNA, RNA, and proteins. Current use stands at <5% of U.S. transplant centers.17 This has been primarily due to the side effect profile, which includes significant myelosuppression and hepatotoxicity (Table 5). Typical dosage is 1 to 2 mg/kg/day.

Mycophenolate mofetil (MMF, CellCept) and mycophenolic acid (MPA, Myfortic) are the most recent additions to the antimetabolite arena, with MMF approval in 1995 and MPA in 2004. MPA is a delayed-release product in contrast to MMF, which is immediately released. Both formulations inhibit the de novo purine nucleotide synthesis via abrogation of the inosine monophosphate dehydrogenase and the production of guanosine nucleotides. This action leads to a blockage of DNA replication in T and B lymphocytes that are unable to use alternate salvage pathways. Studies have shown a large variation in MMF pharmacokinetics in liver transplantation related to fluctuations in serum albumin concentrations, changes not seen in the renal transplant population. Liver dysfunction impairs MPA conjugation and prolongs MPA half-life. Furthermore, TAC has been shown to augment the bioavailability of MPA, resulting in higher exposure to MPA (Tables 6A and 6B).19 The incidence of adverse effects (nausea, gastritis, abdominal pain, diarrhea, and neutropenia) requiring dose reduction or withdrawal is high, ranging from 24% to 57% (Table 5).20

The most recent data on usage shows a continuing trend upward with >50% of the U.S. transplant centers reporting use within the first year of transplant.37 Initial dosage ranges from 2-3 g daily for MMF and 720 to 1,440 mg daily for MPA, divided into 2 doses. To

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**Table 4. Common Side Effects of Corticosteroids**

<table>
<thead>
<tr>
<th>Hypertension</th>
<th>Mental status changes</th>
<th>Lipid abnormalities</th>
<th>Impaired wound healing</th>
<th>Hyperglycemia</th>
<th>Cushinoid syndrome</th>
<th>Ulcers</th>
<th>Myopathy</th>
<th>Osteoporosis</th>
<th>Fluid retention</th>
<th>Cataracts</th>
</tr>
</thead>
</table>

**Table 5. Common Side Effects of Mycophenolate Mofetil, Mycophenolic Acid, and Azathioprine**

<table>
<thead>
<tr>
<th>Nausea, vomiting, diarrhea</th>
<th>Anemia</th>
<th>Leukopenia</th>
<th>Weight loss</th>
<th>Thrombocytopenia</th>
</tr>
</thead>
</table>

**Table 6A. Drugs That May Decrease Mycophenolate and Azathioprine Blood Concentrations**

<table>
<thead>
<tr>
<th>Antacids</th>
<th>Cholestyramine</th>
<th>Iron preparations</th>
</tr>
</thead>
</table>

**Table 6B. Drugs That May Increase MMF and Azathioprine (AZA) Blood Concentrations**

<table>
<thead>
<tr>
<th>Allopurinol (AZA)</th>
<th>Probenecid (MMF)</th>
<th>ACE inhibitors (AZA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate (AZA)</td>
<td>Tacrolimus (MMF)</td>
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</tbody>
</table>
reiterate, dose reductions are common and in some patients removed completely, especially when combined with other myelosuppressive medications, such as sirolimus.

**Antibody Induction**

Antibody therapy has been used as a means of delaying the introduction of maintenance therapy and/or helps facilitate the removal of an immunosuppressive agent, particularly corticosteroids. Antibody therapy can be seen as depleting or receptor modulating or both.

**Antithymocyte Globulin (ATG)**

Polyclonal antilymphocyte antibody preparations are heterologous preparations. Animals (rabbits and horses) are immunized with human T cells and thymocytes. Antisera are then collected. A purified gamma globulin fraction (ATG) is used to reduce the likelihood of serum sickness. The ATG preparations approved by the Food and Drug Administration are ATGAM (of equine origin) and Thymoglobulin (of rabbit origin). These polyclonal preparations are directed at multiple different epitopes on the T cell (CD2, CD3, CD4, CD8, CD28, and the T-cell receptor) as well as CD16 found on natural killer cells and macrophages. These antibodies cause depletion of T cells by apoptosis, antibody mediated cytolysis and internalization of the cell surface receptors. The biologic effects of the depleting antibodies are profound and last longer than the presence of heterologous antibody. Side effects can include a "first-dose" effect (cytokine release syndrome) and is related to the myriad of cytokines released by these lymphocytes upon their demise. The symptoms typically include fever, chills, tachycardia, gastrointestinal disturbances, bronchospasm, and fluctuations of blood pressure, which all can be ameliorated by pretreatment with corticosteroids, diphenhydramine, and acetaminophen.20

Usage in the United States has doubled from 3% in 2002 to 6% in 2003, primarily due to new immunosuppressive strategies as outlined later in this manuscript.17 Dosing ranges from 1.5 to 5 mg/kg as a single infusion usually over 4 to 6 hours for 3-5 days, depending on the indication.

**Monoclonal Anti–T-Cell Antibodies**

Muromonab-CD3 (OKT3) is a murine-derived antibody directed to a specific portion of T cells. It exerts its activity by binding to the CD3 antigen on the surface of T lymphocytes. This binding inactivates the adjacent T-cell receptor, which is critical for activation of T lymphocytes. The end result is a rapid fall in the number of mature lymphocytes. OKT3 was first used in liver transplantation in 1987 for prophylaxis against acute cellular rejection and later to reduce CNI exposure and treatment of steroid-resistant rejection.20 More recent data has shown a significant drop-off in favor of IL-2 receptor antibodies and polyclonal preparations.17

A cytokine-release syndrome is more frequently associated with the first dose as compared to the polyclonal antibody preparations and starts 1-3 hours following administration. Reactions can be quite severe and can range from flu-like symptoms (pyrexia, chills, dyspnea, chest pain, and tightness) to severe and life-threatening shock-like reactions (pulmonary edema). These symptoms can be mitigated with the administration of a corticosteroid, diphenhydramine, and acetaminophen just prior to dose administration. Reexposure to OKT3 may result in lower efficacy due to antimurine antibodies that may form. In the early transplant period when the incidence of acute cellular rejection was as high as 71% and steroid-resistant rejection was more common, OKT3 was the mainstay in the treatment of steroid-resistant rejection with high salvage rates.21,22 Currently, steroid-resistant rejection rates are much lower, thanks to improved immunosuppressive agents and strategies. OKT3 use today is much lower, with many centers preferring treatment regimens for acute cellular rejection to increasing TAC blood levels and then adding corticosteroid boluses if rejection is still present.17,23

FDA approved dosage is 5 mg intravenously daily for 10 to 14 days. Dosage adjustment or drug discontinuation may be necessary if there is a reduced T-cell clearance (increase in CD3-positive T cells) or low plasma OKT3 plasma levels.

**IL-2 Receptor Antibodies**

Two products are currently marketed: basiliximab (Simulect) and daclizumab (Zenapax). Daclizumab is a humanized product while basiliximab shows chimeric properties. Both bind to the IL-2R-α-chain, which is upregulated on the surface of activated T lymphocytes. Immunosuppression is achieved by competitive antagonism of IL-2-induced T-cell proliferation. Although the half-lives of both drugs are decreased in liver transplant patients when compared to kidney recipients, the immunosuppressive effects extend well into the third week for basiliximab and up to 10 weeks for daclizumab.20

Side effects are generally mild for both agents and comparable to placebo.24 Although neither agent is FDA approved for use in liver transplantation, usage appears to be increasing. Typical dosing for basiliximab is 20 mg given intravenously on the day of surgery, prior
to engraftment. A second dose of 20 mg is given 4 days later. Relevant dosage for daclizumab is 1 mg/kg every 14 days for a total of 5 doses. A number of variations to the daclizumab regimen have been reported in literature over the past few years and will be discussed later in Steroid Avoidance.

Miscellaneous

The following agents are used at different stages in liver transplant, from induction to conversion.

Rapamycin

Sirolimus (Rapamune, RAP) is a macrocyclic triene antibiotic (structurally related to TAC) with immunosuppressive, antitumor, and antifungal properties, although the latter 2 are not clinically significant. RAP binds to the immunophilin FKBP12 and blocks the response of T- and B-cell activation by cytokines, which prevents cell-cycle progression and proliferation; in contrast, TAC and CyA inhibit the production of cytokines. Interestingly, although RAP binds to the same immunophilin (FKBP12) as TAC, it has a very different mechanism of action—i.e., blockage of cell-cycle progression at the juncture of G1 and S phase.25 Because of this, many refer to the binding site for RAP as the “target of RAP.”

Early excitement in the liver transplant arena was generated from a single-center study out of Halifax, Nova Scotia. A small number of liver transplant patients were given combination therapy of TAC and RAP with targeted levels of 7 ng/mL and 5 ng/mL, respectively. Acute cellular rejection rates were 14% with an average follow-up of 23 months.26,27 Since that time, multiple serious side effects have limited its use early on in liver transplantation. This includes leukopenia, thrombocytopenia, elevated serum cholesterol and triglyceride levels, anemia, lymphocele, wound dehiscence, and oral ulcerations (Table 7). The most serious side effect has been the issue of hepatic artery thrombosis and includes the following black box warning in liver transplantation: “The safety and efficacy of Sirolimus (Rapamune) as immunosuppressive therapy have not been established in liver transplant patients, and therefore such use is not recommended.” Drug interactions are common and can be profound (Tables 8A and 8B). A report from Colorado compared 170 RAP-treated liver transplantation patients to 180 historic controls using RAP and low-dose CNIs in a corticosteroid avoidance protocol. All patients had a minimum 1-year follow-up. No significant difference was seen between the 2 groups in relationship to hepatic artery thrombosis.20 If this potential hurdle can be cleared, the potential benefit of RAP with low nephrotoxicity is very appealing.

Current Therapeutic Strategies

Steroid Avoidance

Interests in corticosteroid abstention stems from the well-known side effects of osteoporosis, hyperglycemia, cushingoid features, hypertension, as well as the deleterious effects on recurrence of hepatitis C.
The first randomized study concerning complete steroid avoidance was published by the Eason et al. The first group of 36 patients received TAC, MMF and a 2-dose regime of rabbit antithymocyte globulin (Thymoglobulin, rabbit ATG) 1.5 mg/kg on day 0, and 1. The second group received TAC, MMF, and corticosteroids with no induction. Median follow-up was ~1.5 years. Graft survival in each group was 89%. The biopsy-confirmed rejection rate in the rabbit ATG group was 20.5% vs. 32%. TAC blood levels were elevated and all patients in the thymoglobulin group responded; 64% in other group required additional steroids for treatment, and that percentage was statistically significant (P < 0.05). The incidence of recurrent hepatitis C was 50% in the rabbit ATG group and 71% in the steroid group (P = not significant). Details about surveillance and recurrence of hepatitis C were not expounded upon.

A similar study using daclizumab induction without MMF and both groups receiving TAC showed a reduction in biopsy-confirmed steroid resistant rejection in the induction group (P < 0.027). While the overall adverse events were similar, the incidence of diabetes mellitus and cytomegalovirus infections were higher (P < 0.001 and P = 0.002, respectively) in the TAC/steroids group. Follow-up was relatively short at 3 months. While these are encouraging results, larger randomized trials with longer follow-up are needed.

Renal Sparing Protocols

The sentinel articles by Ojo et al. and Gonwa et al. brought a stark realization about the true incidence of renal failure after transplant. Up to 21% of patients developed chronic renal failure within 5 years after receiving a nonrenal transplant. Thirteen years post–liver transplant, 18.1% of patients were diagnosed severe renal dysfunction, a significantly higher percentage when compared to controls. Both articles alluded to a direct role of CNIs.

Different strategies have been developed to help in this ongoing struggle. One approach has examined adding MMF and reducing the dose of the calcineurin inhibitor or eliminating the calcineurin inhibitor altogether. These studies have produced some encouraging results with up to 50% of patients showing at least a >15% improvement in renal function. A number of studies have shown an increased risk of rejection when the CNI is completely removed.

Conversion from CNI to Sirolimus

Recent studies with small numbers looked at the impact of switching patients with chronic renal impairment of CNI to RAP. Twenty-eight patients with creatinine >1.8 post-transplant were eligible for conversion. Mean time to conversion is approximately 2 years. Sirolimus was initiated at 2 mg/day, and doses were adjusted to maintain levels of 4 to 10 ng/mL. Seven did not tolerate RAP and 6 progressed to end-stage renal disease. The subset of 14 patients (50%) who did tolerate conversion had a decline in creatinine that persisted to week 48. Questions still remain on the optimal time to conversion and whether it can be used in the early posttransplant period. Large randomized trials are ongoing in an effort to answer these questions.

Calcineurin Inhibitor Avoidance

Very few studies have looked at complete calcineurin inhibitor avoidance. The latest data from the scientific registry of transplant recipients database show ~99% of patients are discharged on either TAC (majority) or CyA. The concept of using agents without nephrotoxicity in solid-organ-transplant recipients where rejection has less of a negative impact on the long-term survival has sparked some enthusiasm, but to date little has been published to demonstrate success with this strategy.

Individualization of Drug Therapy

Tailoring medication therapy in the transplant patient is a concept that has seen a dramatic evolution over time and yet still has a long way to go. Early literature talks of “immunologic monitoring” as a means to individualize therapy through dissecting mechanisms of action, rejection, immunosuppressive medications, and graft facilitation. This is still true today. The quandary is how far to take individualization. There exists a need to individualize patient-specific regimens, and yet standardization of these regimens helps programs to quantify outcomes and strengthen cost containment.

Today we are able to assign patients to protocols that best fit their needs: for example, delaying the introduction of CNIs to allow for renal function to improve, corticosteroid avoidance for people with osteoporosis or hepatitis C, or hepatitis B immunoglobulin for patients infected with hepatitis B. Within each protocol, allowances are made for discretion.

With the advent of unlocking human DNA, new avenues have opened to true individualization based on drug metabolism. Pharmacogenetic typing offers the possibility of significant improvement in the individualization of immunosuppressive drug prescribing with reduced rates of rejection and toxicity.

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