Immunosuppression in Liver Transplantation
Beyond Calcineurin Inhibitors

John Fung, Dympna Kelly, Zakiyah Kadry, Kusum Patel-Tom, and Bijan Eghtesad

Although calcineurin inhibitors (CNIs) remain the mainstay of immunosuppression in liver transplantation (LTX), their long-term toxicity significantly contributes to morbidity and mortality. The elucidation of mechanisms of alloimmunity and leukocyte migration have provided novel targets for immunosuppression development. The toxicities of these agents differ from that of the CNI and act additively or synergistically. CNI avoidance protocols in LTX have not been achieved routinely; however, pilot trials have begun to delineate the limitations and promises of such approaches. CNI-sparing protocols appear to be much more promising in balancing the early need for minimizing rejection while tapering doses and minimizing long-term toxicity. (Liver Transpl 2005;11: 267–280.)

Until the routine application of tolerogenic approaches in liver transplantation (LTX) are successful, “optimal” iatrogenic immunosuppression will be defined as the level of drug therapy that achieves graft acceptance with least suppression of systemic immunity. In this way, the spectrum of toxicities will be minimized, although not entirely eliminated. The approach to achieving this goal is varied—the timing, dosing, and selection of immunosuppressive agents differ widely. In addition, a variety of new agents is being introduced with mechanisms of action that target novel pathways. Current protocols use multiple drugs, each directed at a discrete site in the T-cell activation cascade.1 Most immunosuppressive regimens combine drugs, often with different modes of action and toxicities, which allows lower doses of each drug. Induction therapy refers to the use of antibody therapy in the perioperative period, usually as part of sequential drug therapy, which delays the introduction of maintenance therapy. This practice is based on the concept that early incapacitation of the immune system may reduce the likelihood of subsequent rejection and avoid early toxicity of calcineurin inhibitors (CNIs). In general, cyclosporine (CsA) or tacrolimus (TAC), both CNI agents, forms the backbone of most maintenance immunosuppressive regimens being used today. However, the long-term use of such agents has been associated with increased risk of renal failure2–3 and de novo malignancies.4 Thus, recent attempts have emphasized the approach of CNI minimization or even CNI avoidance.

Overview of Alloimmunity

Foreign antigens are presented to lymphocytes by antigen-presenting cells (APCs), which are epitomized by dendritic cells. In LTX, alloantigens are shed after reperfusion into the circulation and are presented in secondary lymphoid organs, such as the spleen and regional lymph nodes. Naïve lymphocytes home to these secondary lymphoid organs via specific receptors. There they encounter APCs, which enzymatically process foreign proteins and load them onto major histocompatibility complex molecules, which are displayed on the cell surface to T cells. The T-cell receptor is the antigen-recognition unit on the T-cell surface that determines antigen specificity. It is associated with “accessory” molecules, including CD3 and either CD4 or CD8. The T-cell receptor–CD3 complex interacts with the peptide fragment carried by the major histocompatibility complex molecule of the APC, is stabilized by the CD4 or CD8 molecule, and results in “Signal 1” of T-cell activation, a calcium-dependent pathway. Signal 1 is necessary but insufficient to activate naïve T cells—other signals are required also. “Signal 2” is calcium independent and results from binding of the costimulatory family of molecules on T cells (CD28 receptor) with their cognate ligands found on APCs, B7.1 (CD80), B7.2 (CD86), CD40/CD40L (CD154), and other newly described receptor/ligand pairs. Both Signals 1 and 2 are required for naïve T-cell

References


activation, which is primarily mediated by calcineurin; protein kinase C; and zeta-associated protein – 70 activation of NF-AT, NF-κB, and AP-1, respectively. These factors translocate to the nucleus and bind various gene promoters [most notably interleukin-2 (IL-2)] associated with T-cell activation and proliferation, initiating the G0 to G1 transition in the cell cycle. "Signal 3" of T-cell activation results from autocrine and paracrine cytokine-mediated signaling via specific cytokine receptors. The IL-2 receptor family includes receptors for IL-2, IL-4, IL-7, IL-12, and IL-15. This receptor family shares a common γ-chain, which differs in the composition of the α and β chains. In T cells, the most important cytokine is IL-2, which binds to the IL-2 receptor γ-chain and activates the Janus kinases (JAK) 1 and 3. This triggers a cascade of intracellular signaling pathways including signal transducers and activators of transcription (STAT) 5, Ras-Raf-MAP kinase, and mTOR/P13-K/p70 S6 kinase activation.

Generation of effector cells, such as antibody-secretting B cells, cytotoxic T cells, and activated macrophages, is facilitated by cytokines generated by activated T helper cells. Activated and effector T cells upregulate cell adhesion molecules, such as CD2, LFA-1, and VLA-4, while downregulating L-selectin; this results in alteration of their pattern of migration in recognition of chemokines and altered endothelial adhesion signals. In addition, effector T cells mediate damage via several pathways, including secretion of tumor necrosis factor-α (TNF-α), TNF-β (also known as lymphotoxin), and Fas ligand expression, as well as cytotoxins, such as perforin and granzymes, a family of serine proteases.

T-cell activation is downregulated during the process of an immune response via expression of regulatory proteins. Specifically, the CD28 receptor binds to the negative regulatory protein, CD152 (CTLA4), which is upregulated on APC, delivering a suppressive signal to T cells and inducing a phenomenon known as activated immune cell death. Finally, the role of regulatory T cells, CD4+, CD25+, CTLA4+, in alloimmunity is unknown at this time.

### Overview of Immunosuppressive Agents

Currently available immunosuppression applied in clinical transplantation can be categorized into 2 types: pharmacologic and biologic. Pharmacologic immunosuppression consists of corticosteroids, cytokine suppressive agents, and cell cycle inhibitors. Biologic immunosuppression consists of monoclonal and polyclonal antilymphocyte antibodies and anticytokine receptor antibodies.² Immunosuppressive drugs also

<table>
<thead>
<tr>
<th>Immunosuppressive Agent</th>
<th>Target Pathway(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pharmacologic</strong></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>(a) Selective lysis of immature cortical thymocytes (b) Blockade of cytokine gene transcription in APC</td>
</tr>
<tr>
<td>Cyclosporin (Sandimmune, Neoral, Gengraf)</td>
<td>Signal 1 transduction via TCR</td>
</tr>
<tr>
<td>Tacrolimus (Prograf)</td>
<td>Signal 1 transduction via TCR</td>
</tr>
<tr>
<td>Rapamycin/Sirolimus and Everolimus/SiDZ RAD (Rapamune and Certican)</td>
<td>Signal 3 transduction via IL-2 receptor</td>
</tr>
<tr>
<td>Azathioprine (Imuran)</td>
<td>Inhibition of purine metabolism and DNA synthesis</td>
</tr>
<tr>
<td>Mycophenolic acid (CellCept, Myfortic)</td>
<td>Inhibition of purine metabolism and DNA synthesis</td>
</tr>
<tr>
<td><strong>Biological</strong></td>
<td></td>
</tr>
<tr>
<td>Anti-CD3 pan-T cell (Orthoclone OKT3)</td>
<td>(a) Causes depletion and receptor modulation in T cells (b) Interferes with Signal 1</td>
</tr>
<tr>
<td>Anti-thymocyte globulin (ATGAM, Thymoglobulin)</td>
<td>(a) Causes depletion and receptor modulation in T cells (b) Interferes with Signal 1, 2 and 3 (c) Inhibits lymphocyte trafficking</td>
</tr>
<tr>
<td>Anti-IL-2 α-chain receptor (Zenapax, Simulect)</td>
<td>Inhibits T cell proliferation to IL-2 (Signal 3)</td>
</tr>
<tr>
<td>Anti-CD52 (Campath 1-H)</td>
<td>Causes depletion of thymocytes, T cells, B cells (not plasma cells), monocytes</td>
</tr>
</tbody>
</table>

**Abbreviations:** APC, antigen-presenting cells; TCR, T-cell receptor; IL, interleukin.
can be classified by the pathway that they interrupt, as shown in Table 1. Inhibitors of the Signal 1 pathway include agents that affect T-cell recognition of alloantigen and signal transduction via the calcium-dependent calcineurin pathway. Signal 2 inhibitors inhibit costimulatory pathways, and Signal 3 inhibitors inhibit cytokine-driven proliferation. Finally, other agents inhibit a variety of other points in the immune system (e.g., antimetabolites that interfere with RNA and DNA replication) or lymphocyte trafficking, and there are agents whose mechanism of action has not yet been determined. This is particularly true for agents that are still considered investigational (Table 2).

**Use of Immunosuppressive Agents in Clinical LTX in the United States**

The nature of immunosuppressive agent use in LTX in the United States was recently reported by the Scientific Registry of Transplant Recipients, which analyzed the United Network of Organ Sharing database. As shown in Table 3, the use of CNI was reported in 97% of patients discharged from the hospital after LTX in the United States in 2002. Corticosteroids use was reported in more than 90% of LTX, although for antimetabolite therapy, mycophenolate mofetil (MMF) was noted in nearly 48% and azathioprine (AZA) was noted in approximately 4% at discharge. Rapamycin (RAPA) use was noted in nearly 7% of LTX at discharge. Induction antibody use was noted in 18% of LTX, the majority of antibody use being the anti-IL-2 receptor antibodies and the remainder being antithymocyte globulins. It is clear that the overwhelming majority of programs view the use of CNIs as essential to the success of LTX, both in early and later phases after LTX. It is on this basis that application of CNI-minimization and CNI-avoidance protocols are being considered.

**CNIs**

Both CsA and TAC are CNIs by virtue of their shared property of binding to their specific immunophilins, which leads to inhibition of calcineurin activity. Both immunophilins have peptidyl-prolyl cis-trans isomerase activity, although this activity is not thought to be linked to the immunosuppressive activity of the CNI-immunophilin complex. This complex in turn, inhibits transcription of several genes, including IL-2, IL-3, and IL-4; granulocyte-macrophage colony stimulating factor; interferon-γ; and TNF-α. Thus, CNIs act by interfering with Signal 1 T-cell signal transduction.

The routine application of CNIs to LTX has dra-

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**Table 2. Investigational Immunosuppressive Agents in Clinical Testing and Target Pathways**

<table>
<thead>
<tr>
<th>Immunosuppressive Agent</th>
<th>Target Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>FK778</td>
<td>Interferes with pyrimidine metabolism and DNA synthesis</td>
</tr>
<tr>
<td>WHI-P-154</td>
<td>Signal 3 transduction via Jak3/STAT5</td>
</tr>
<tr>
<td>LEA29Y</td>
<td>Signal 2—also known as CTLa4-Ig and inhibits B7/CD28 interaction</td>
</tr>
<tr>
<td>FTY720</td>
<td>Inhibits naïve T cell homing to the high venule endothelial cell on secondary lymphoid tissue</td>
</tr>
</tbody>
</table>

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**Table 3. Use of Immunosuppressive Agents in Liver Transplantation in the US Data from the 2002 Scientific Registry of Transplant Receipts* Annual Report†**

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
<th>Agent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction antibody</td>
<td>17.6%</td>
<td>ATG</td>
<td>0.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OKT3</td>
<td>0.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thymoglobulin</td>
<td>4.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zenapax</td>
<td>4.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Simulect</td>
<td>8.0%</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>90.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNIs</td>
<td>97%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclosporin</td>
<td>10.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tacrolimus</td>
<td>86.7%</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>51.3%</td>
<td>AZA</td>
<td>3.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMF</td>
<td>47.7%</td>
</tr>
<tr>
<td>RAPA</td>
<td>6.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>89.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNIs</td>
<td>&gt;100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclosporin</td>
<td>18.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tacrolimus</td>
<td>88.8%</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>53.2%</td>
<td>AZA</td>
<td>4.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMF</td>
<td>50.1%</td>
</tr>
<tr>
<td>RAPA</td>
<td>16.7%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: RAPA, rapamycin; AZA, azathioprine.

* Refers to data from year 2002
† Maintenance figures are based on one year follow-up (cited figures are from 2001)—figures may reflect multiple agents during the first posttransplant year.
matically reduced (1) rejection, (2) morbidity associated with treatment of rejection and graft loss, and (3) death caused by rejection. The incremental improvement in patient and graft survival seen with the introduction of CsA in LTX was far more dramatic than the improvement seen with the introduction of TAC in LTX. However, 3 separate prospective randomized trials have revealed a significantly lower incidence of rejection under TAC and no significant difference in 1-year patient or graft survival. The side effects of TAC and CsA overlap and include nephrotoxicity, neurotoxicity, diabetogenicity, increased susceptibility to opportunistic infections, and certain de novo malignancies. It has been suggested that the inherent CNI properties of TAC and CsA are linked to 1 of their most important side effects (nephrotoxicity), although recent data suggest that the inhibition of the peptidyl-prolyl cis-trans isomerase activity may account for this nephrotoxicity. The impact of the variety of metabolic and physiologic alterations in LTX with the use of CNIs is an increased risk of deaths caused by cardiovascular complications.

Alternatives to CNIs in LTX

Although a large number of non-CNI immunosuppressive agents are available, their use in LTX has been primarily adjunctive to the use of CNIs. These protocols have attempted to reduce the overall exposure to CNIs and have included CNI-minimization by augmenting low levels of CNIs with less nephrotoxic agents. In some reports, CNI-freedom has been achieved by considering late conversion from CNI-based immunosuppression to non-CNI maintenance. CNI-avoidance protocols have been reported in a small number of LTX series, although these have been primarily limited to patients with defined perioperative conditions (e.g., renal failure). A brief review of the non-CNI agents currently available will help in understanding the rationale for CNI-minimization or CNI-avoidance protocols.

Corticosteroids

As shown in Table 3, by far the most used non-CNI agents in LTX are represented by corticosteroids. Corticosteroids have been shown to prolong skin graft survival in rabbits. Starzl et al. and Murray et al. in 1963 independently showed the benefit of combining corticosteroids with another immunosuppressive agent, AZA, to obtain meaningful survival after allogenic renal transplantation in humans. Steroids continue to be used to control acute episodes of rejection and for prophylaxis in preventing rejection.

Unfortunately, acute and chronic dosing of corticosteroids are associated with side effects, including hypertension, hyperglycemia, delayed wound healing, osteoporosis, glaucoma, suppressed growth, hyperlipedemia, increased risk of gastrointestinal ulceration, risk of fungal infections, and suppression of the pituitary–adrenal axis. Thus, attempts to reduce or eliminate corticosteroid use have required the use of other non-CNI immunosuppressive agents.

Antibody Induction

As noted earlier, antibody induction therapy has been limited to the perioperative period as a means to reduce early exposure to CNIs or to obviate the need for large doses of perioperative corticosteroids. Antibody therapy can be depleting, receptor modulating, or both. With the use of depleting antibody preparations, a phenomenon known as “first dose effect,” related to intravascular release of cytokines by lymphocytes, can occur. The symptoms, including fever, chills, tachycardia, gastrointestinal disturbances, bronchospasm, and fluctuations of blood pressure, can be blocked by pretreatment with corticosteroids, diphenhydramine hydrochloride, and acetamenophen.

Antithymocyte globulin

Polyclonal antilymphocyte antibody preparations are heterologous preparations. Animals (including rabbits and horses) are immunized with human T cells and thymocytes, and antisera are collected. A purified gamma globulin fraction (antithymocyte globulin) is used to reduce the likelihood of serum sickness. The US Food and Drug Administration approved antithymocyte globulin preparations are ATGAM of equine origin (Pfizer, New York, NY) and thymoglobulin of rabbit origin (Genzyme, Boston, MA). The limitations of the antithymocyte globulin preparations are related to variability in potency; reactivity to formed blood elements leading to leukopenia, thrombocytopenia, or anemia; and serum sickness. Characterization of the antigen specificity of these preparations suggests that multiple cell surface molecules are recognized by the polyclonal antibody preparations, and the varying specificities may account for the variations in their clinical efficacy. As summarized in Table 4, although there are representative antibodies directed to the major T-cell surface molecules (CD2, CD3, CD4, CD8, CD28, and the T-cell receptor), there are often antibody specificities to
Immunosuppression in LTX

Table 4. Relative Strengths and Specificities of Various Clinically Utilized Polyclonal Antilymphocyte Antibody Preparations Toward Defined Lymphocyte Antigens*

<table>
<thead>
<tr>
<th>Source</th>
<th>CD2</th>
<th>CD3</th>
<th>CD4</th>
<th>CD5</th>
<th>CD8</th>
<th>CD11</th>
<th>CD18</th>
<th>CD28</th>
<th>CD45</th>
<th>TCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATGAM</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Merieux (rabbit)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Merieux (horse)</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Fresenius</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Thymoglobulin*</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>RATG (Carolina‡)</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

* Determination made by inhibition of binding of specific monoclonal antibody binding by the test polyclonal antibody sample.
‡ Determined by monoclonal antibody inhibition and immunoprecipitation/gel electrophoresis.

For other leukocyte specificities (CD20 and CD40 found on B cells, CD16 found on NK cells and macrophages, common adhesion molecules LFA-1 and LFA-3), ubiquitous cell surface antigens (major histocompatibility complex Class I and Class II antigens), and activated T-cell specificities. The antibodies cause depletion by apoptosis, antibody mediated cytolysis, and internalization of the cell surface receptors. The biological effect of the depleting antibodies are profound and last longer than the presence of heterologous antibody.

Thymoglobulin has been used as an induction agent in LTX to reduce or eliminate corticosteroids use, minimize the use of CNIs, delay exposure to CNI in patients with preexisting renal failure, and explore the possibility of eliminating maintenance immunosuppression. Eason randomized 119 LTX patients to receive thymoglobulin 1.5 mg/kg on day 0 and 1, versus methylprednisolone 1 g followed by a steroid taper over 3 months, combined with maintenance treatment of TAC and MMF for 3 months. Patient and graft survival and incidence of acute cellular rejection (ACR) were similar in both groups. Patients in the corticosteroid group had more severe ACR, which required additional steroid treatment (50% of patients with ACR compared with 7% in the thymoglobulin group). Thymoglobulin induction also was associated with a reduced incidence of recurrent HCV, posttransplantation diabetes mellitus, and CMV infection; there was no increased risk of infectious complications.

When investigators at Indiana University used thymoglobulin induction combined with delayed low-dose TAC treatment, they demonstrated a 1-year graft and patient survival of 92% and 96% respectively, combined with a low incidence of ACR (6%) and a reduced incidence of renal complications. The most common adverse effects were fever, rigors, and tachycardia.

Tchervenkov et al. compared thymoglobulin induction combined with CNIs and corticosteroids to a regimen of CNIs and steroids and either MMF or AZA. Freedom from ACR was higher in the thymoglobulin-treated group (72% vs. 50%). In a subgroup of patients with preexisting renal failure (creatinine > 1.5 mg/dL), thymoglobulin induction was associated with improved early patient survival and rejection-free graft survival and a significant recovery in serum creatinine in the first posttransplantation month.

Starzl et al. reported a strategy of using high-dose thymoglobulin, 5 mg/kg, (premedicated with 1 g methylprednisolone) administered preoperatively, followed by low-dose TAC monotherapy starting on postoperative day 1. This protocol is based on the strategy of depleting recipient reactive lymphocytes before LTX, followed by post-LTX low-dose immunosuppression, which allows for some degree of immune activation, a factor thought to be critical for long-term graft acceptance. With a minimum 1-year follow-up, the maintenance requirements for CNIs were significantly reduced, with the dose frequencies of TAC being every other day (n = 2), 3 doses per week (n = 4), 2 doses per week (n = 2), and 1 dose per week (n = 3). Although this protocol to date has not eliminated CNIs, it has significantly reduced daily and weekly requirements of these drugs and eliminated the use of corticosteroids.

Monoclonal Anti–T-Cell Antibodies

The application of hybridoma technology allowed Kung et al. to produce mouse monoclonal antibodies directed against T-cell surface antigens. One monoclonal antibody, muromonab-CD3 (OKT3), has defined specificity to the CD3 receptor. OKT3 reacts with more than 95% of peripheral, postthymic T cells without affecting immature thymocytes. OKT3 was first used in LTX in 1987 for prophylaxis against
ACR and later to reduce CNI exposure. The development of human antimouse antibodies in 60% of LTX patients may reduce efficacy and prohibits its reuse.

Further refinements in monoclonal antibody development came with the ability to splice mouse antibody DNA encoding either the entire variable region (chimeric) or only the complementarity-determining regions in the hypervariable loop (humanized) into a human antibody molecule. This significantly lowers the antigenicity of the resultant antibody, while retaining specificity to the target antigen.

**IL-2 Receptor Antibodies**

Two IL-2R monoclonal antibodies are currently in clinical use: daclizumab (Zenapax, Hoffman-La Roche, Nutley, NJ) and basiliximab (Simulect, Novartis, Basel, Switzerland). Daclizumab and basiliximab are humanized and chimeric IgG1 monoclonal antibodies, respectively. Both bind to the IL-2R 

\[ \alpha \text{-chain (referred to as CD25 or T-cell activation antigen), which is upregulated on the surface of activated T lymphocytes, resulting in internalization of the IL-2R-chain, rendering the IL-2 receptor able to bind to IL-2 but at low affinity.} \]

The half-life of both drugs is lower in LTX recipients compared with renal TX recipients because of the higher volume of distribution in patients with ascites and increased immunoglobulin breakdown because of hypersplenism. The receptor suppression effects of basiliximab last for 3–4 weeks; receptor suppression effects can last up to 10 weeks for daclizumab.

In LTX, a trial of OKT3 versus a rat antihuman IL-2R 

\[ \alpha \text{-chain monoclonal antibody (LO-Tact-1) with CsA, AZA, and corticosteroids showed that administration of LO-Tact-1 was associated with less steroid-resistant ACR, similar to OKT3, but with fewer CMV infections. The 2 humanized/chimeric monoclonal IL-2R 

\[ \alpha \text{-chain antibodies were introduced into clinical practice to reduce or delay the use of CNIs, particularly in patients with renal insufficiency or to eliminate corticosteroids. The combination of daclizumab induction with delayed low-dose TAC is particularly effective in improving renal function with low rates of rejection compared with patients who receive OKT3 induction followed by TAC and compared with patients receiving lower dose TAC throughout the posttransplantation period. Other studies have reported safe and effective use of daclizumab induction combined with delayed, low-dose CNIs in LTX recipients with renal impairment. Its use without CNIs has been less successful. Hirose et al. at the University of California, San Francisco, compared daclizumab, corticosteroids, and MMF in 1 group of patients with a second group receiving daclizumab, MMF, and delayed CNI. The study was halted after enrollment of 7 patients because of an unacceptably high rate of ACR—100% in the group without CNI. Four patients developed steroid-resistant ACR requiring OKT3. The pharmacokinetic profile of daclizumab in these LTX recipients was different than the profiles seen in renal TX recipients, in which the half-life is 3–4 weeks. The elimination of drug in ascites may have contributed to the short half-life and the subtherapeutic drug levels seen within hours of the first dose and within 4–6 days of the second dose.

Basiliximab combined with CsA, AZA, and corticosteroids or used in combination with CsA and corticosteroids is associated with an ACR rate of 35%. Kwekkeboom et al. suggested that ACR occurred in this group because of T cells that bypass CD25 blockade, because in situ immunohistochemical staining showed sufficient coating of infiltrating T cells. When used in combination with low-dose TAC, MMF, and perioperative steroids, basiliximab induction is associated with reduced TAC associated morbidities (new onset diabetes, CMV infection), a low incidence of opportunistic infections, and a reduced rate of biopsy proven ACR (6% vs. 27%), compared with the standard regimen of TAC and corticosteroids.

The role of IL-2R 

\[ \alpha \text{-chain monoclonal antibodies in LTX immunosuppressive regimens is unclear. They are well tolerated with few side effects but add significantly to the cost of posttransplantation medications. Their current role is probably in the management of patients with pre-LTX renal impairment.}

**Alemtuzumab**

Alemtuzumab or campath-1H (C-1H) is a humanized, recombinant anti-CD52 monoclonal antibody. It targets antigen CD52, a cell surface glycoprotein, expressed on more than 95% of peripheral blood lymphocytes, thymocytes, monocytes, and macrophages. C-1H produces profound depletion of circulating lymphocytes for approximately 1 month, and lymphocyte recovery, particularly CD4 cells, is slow. The rationale for its initial use was to facilitate lower doses of maintenance immunosuppression. Later, higher doses of C-1H were used to induce tolerance and eliminate maintenance immunosuppression. The use of C1H alone produced profound lymphocyte and monocyte depletion but did not prevent the development of ACR.

The University of Miami reported an experience with C-1H induction combined with low-dose TAC...
alone in adult LTX recipients.\textsuperscript{51} This group was compared with patients receiving standard doses of TAC and corticosteroids. C-1H induction resulted in significantly improved renal function compared with the control subjects. The incidence of ACR was significantly lower with C-1H induction in the first 2 months (15\% in the study group vs. 46\% in the control group) after LTX, but the median time to rejection was longer, with the majority of ACR episodes occurring after 2 months when lymphocyte counts began to recover. The overall incidence of ACR was not significantly different between the 2 groups (46\% in the C-1H group vs. 55\% in control subjects). C1H-related side effects were minimal.\textsuperscript{51} Further studies have focused on its use in combination with other low-dose immunosuppressive therapy; however, CNIs appear to be an essential element of these regimens.\textsuperscript{52}

**Mycophenolic Acid**

Mycophenolic acid (MPA) (Myfortic, Novartis) and the pro-drug, MMF (RS 61443, CellCept, Hoffman LaRoche) a semisynthetic derivative of MPA, are immunosuppressive agents that inhibit the \textit{de novo} purine nucleotide synthesis by inhibiting inosine monophosphate dehydrogenase and the production of guanosine nucleotides. They block DNA replication in T and B lymphocytes, which are unable to use alternate salvage pathways.\textsuperscript{53} MPA also reduces recruitment of leucocytes to inflammatory sites. MMF is rapidly absorbed after oral administration and is hydrolyzed to MPA. The bioavailability of MPA is approximately 90\%. The drug is glucuronidated in the liver to the inactive MPA glucuronide and is excreted primarily by the kidneys. Jain et al. demonstrated large variations in MMP pharmacokinetics in LTX recipients\textsuperscript{14} related to fluctuations in serum albumin concentrations, changes not seen in renal transplantation recipients. Liver dysfunction impairs MPA conjugation, which prolongs MPA half-life. The incidence of adverse effects (nausea, anorexia, gastritis, abdominal pain, diarrhea, neutropenia) requiring dose reduction or withdrawal is high, ranging from 24\% to 57\%.\textsuperscript{55,56}

Before the availability of MMF, AZA was used as an adjuvant immunosuppressive agent but was associated with significant myelosuppression, gastrointestinal toxicity, and hepatotoxicity and was not useful in the treatment of ACR. MMF acts by a similar mechanism to AZA but is more selective, has fewer myelotoxic and hepatotoxic side effects,\textsuperscript{57} and is a more effective immunosuppressive agent. When MMF is used in combination with TAC and steroids, the dose of TAC required is lower, and renal function is improved.\textsuperscript{58} A prospective randomized trial of TAC and corticosteroids vs. TAC, corticosteroids, and MMF reported similar patient and graft survival, with fewer ACR episodes, fewer steroids, and improved renal function in the MMF group.\textsuperscript{59} The favorable effect on ACR stopped after withdrawal of the drug. MMF was withdrawn in 37\% of patients, emphasizing that the use of antiproliferative agents may be limited in LTX recipients with an inherently high rate of infections and preexisting leukopenia, thrombocytopenia, and hypersplenism.

MMF, as monotherapy after CNI withdrawal, has been associated with an unacceptably high incidence of ACR,\textsuperscript{59} severe ductopenic rejection requiring retransplantation, and severe steroid-resistant ACR.\textsuperscript{60} Although MMF is not suitable for all LTX candidates, it does have a role as a CNI-sparing agent, particularly in patients with renal dysfunction and neurotoxicity. It can be safely added to the current immunosuppressive regimens without increasing infectious complications.\textsuperscript{56}

**RAPA**

RAPA is a macrolide antibiotic structurally related to TAC. It binds to the immunophilin, FKBP12, but does not inhibit cytokine gene transcription in T cells. Rather, RAPA blocks signals transduced from a variety of growth factor receptors to the nucleus by acting on phosphatidylinositol kinases called mammalian targets of RAPA (mTOR).\textsuperscript{61} It also inactivates 70-kD-S6 kinase, resulting in selective inhibition of the synthesis of new ribosomal proteins, prolonging cell cycle progression from the G\textsubscript{1} to the S phase. Platelet-derived growth factor activates 70-kD-S6 kinase in fibroblasts, a critical step in the initiation of tissue injury, fibroblast migration, and proliferation, with synthesis of collagen and wound repair. The addition of RAPA is associated with diminished fibroblast activity,\textsuperscript{62} which interferes with wound and tissue healing, suggested in several reports in liver,\textsuperscript{63} kidney,\textsuperscript{64} and lung\textsuperscript{65} transplantation. The pivotal prospective-controlled trials in kidney transplantation that led to US Food and Drug Administration approval in 1999, delineated the efficacy and side effects of RAPA, which included leukopenia, thrombocytopenia, elevated serum cholesterol levels, anemia, gastrointestinal disturbances, lymphocoele and wound infections, oral ulcerations, and triglyceride levels.\textsuperscript{66,67} An increased incidence of “pneumonitis” and “aseptic pneumonia” has been reported, which in most cases is reversible with RAPA discontinuation; however, it can be fatal.\textsuperscript{64,68} Two multicenter phase II/III trials of RAPA in primary LTX have been conducted comparing CNI and...
corticosteroids to a RAPA, CNIs, and corticosteroids. The Wyeth Study 211 examined the safety and efficacy of RAPA/CsA/corticosteroids compared with standard TAC/corticosteroids in LTX. Wiesner et al. reported for the Rapamune Liver Transplant Study Group that the 60-day efficacy failure was 39% for RAPA/CsA vs. 48% for TAC and respective ACR rates were 30% and 50%. The 6-month graft survival was 85% RAPA/CsA vs. 90% for TAC, and the corresponding 6-month patient survival was equivalent for both groups (90% and 92%, respectively). There were higher rates of wound complications in the RAPA/CsA and a higher incidence of vascular thrombosis, but technical and donor factors were thought to play a role.

A subsequent phase II/III controlled, randomized study compared RAPA/reduced dose TAC/corticosteroids to standard TAC/corticosteroids in primary LTX (Wyeth Study 220). An interim analysis revealed reduced incidence of ACR in RAPA/TAC group, but higher efficacy failure at 30 days, because of higher graft loss compared with TAC group. There were 6 cases of HAT in RAPA/TAC and 1 in TAC. Although the incidence of HAT did not differ significantly from the estimated 4% historical incidence of HAT, and only 3 cases were thought to be possibly related to RAPA/TAC, study enrollment was halted. Ultimately the US Food and Drug Administration, based on the findings summarized in Table 5, mandated the “Black Box” warning: “The safety and efficacy of Rapamune (sirolimus) as immunosuppressive therapy have not been established in liver transplant patients, and therefore, such use is not recommended.”

Table 5. Adverse Events in TAC Versus RAPA/TAC in Liver Transplant Trial

<table>
<thead>
<tr>
<th></th>
<th>TAC</th>
<th>RAPA/TAC</th>
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<tbody>
<tr>
<td>Deaths</td>
<td>6 (5.4%)</td>
<td>16 (14.5%)</td>
</tr>
<tr>
<td>Graft Loss</td>
<td>4 (3.6%)</td>
<td>11 (10.0%)</td>
</tr>
<tr>
<td>Combined</td>
<td>10 (8.9%)</td>
<td>25 (22.7%)</td>
</tr>
<tr>
<td>HAT/PVT</td>
<td>2 (1.8%)</td>
<td>9 (8.2%)</td>
</tr>
</tbody>
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Abbreviations: TAC, tacrolimus; RAPA, rapamycin; HAT, hepatic artery thrombosis; PVT, portal vein thrombosis.

Table 5 shows the adverse events in TAC versus RAPA/TAC in liver transplant recipients. The table compares the number of deaths, graft loss, combined events, and hepatic artery thrombosis (HAT) and portal vein thrombosis (PVT) in the TAC and RAPA/TAC groups. The data indicate a significantly higher number of deaths and combined events in the RAPA/TAC group, suggesting that RAPA/TAC may have a higher risk of complications compared to TAC alone.

Continued in several patients because of side effects or complications. The group from the Dalhousie University reported on 25 LTX patients, and later 56 LTX recipients. RAPA levels were targeted at 7 ng/mL, and TAC levels were targeted at 5 ng/mL. ACR rates were low (14%), and the overall survival was 93% with an average 23-month follow-up. Only 1 case of HAT was noted, and wound complications occurred in 12%.

Investigators in Colorado compared 170 RAPA-treated LTX patients with 180 historic LTX control subjects with a minimum of 1-year follow-up using RAPA as primary immunosuppression along with low-dose CNIs in a corticosteroid avoidance protocol. They noted a reduction in the incidence of ACR, and 5.3% of the RAPA LTX patients developed HAT compared with 8.3% of the historical control subjects. There was no difference in the incidence of wound complications between the 2 groups. The RAPA-treated group also included 16 living donor LTX recipients in whom no HAT was seen, and liver regeneration was not affected (J. Trotter, personal communication, October 2002 even though RAPA in vitro is a potent inhibitor of hepatocyte regeneration. The doses of RAPA in both the Edmonton and Colorado experience were modest, and levels were low.

The question of the most appropriate CNI to use with RAPA was indirectly addressed by Trotter. Trotter found that a normal serum creatinine was present in only 6% of patients receiving the combination of RAPA/CsA compared with 67% of patients receiving RAPA/TAC. Suggested reasons for this increased nephrotoxicity included an increase in the intrarenal concentration of CsA or a drug interaction causing an increased exposure to CsA because of decreased clearance through competitive inhibition of the activity of cytochrome P4503A4 or p-glycoprotein.

Further clinical trials are warranted to delineate the optimal use of RAPA. In addition, the antifibrogenic property of RAPA may be a useful characteristic in LTX.
for diseases in which fibrosis may cause graft dysfunction.63,79

A similar analog to RAPA is SDZ-RAD (40-O-[2-hydroxyethyl] RAPA, Everolimus, Certican) developed by Novartis, which differs only slightly from RAPA in its bioavailability. Phase 3 kidney transplantation studies are nearing completion,80 and SDZ-RAD also has been used in LTX. Levy et al. presented results from a randomized controlled, double blind trial with patients who were randomized to 1 of 3 doses of SDZ-RAD (1 mg/day, 2 mg/day, 4 mg/day) or placebo along with CsA and corticosteroids.81 ACR rates were lower in the SDZ-RAD groups compared with the placebo group, and serum creatinine was slightly higher in the SDZ-RAD group. Higher doses of SDZ-RAD were associated with increased dropout rates.

**Future Prospects for CNI-Free Immunosuppression**

Thus far, the majority of immunosuppressive drug regimens in LTX call for CNI use. There are a growing number of protocols in which CNI use is minimized, and there are a few ongoing attempts to establish CNI-free protocols. These efforts will be facilitated by the introduction of novel immunosuppressive agents (Table 2). The following are examples of unique classes of immunosuppressive agents that may have some future application in CNI-free LTX immunosuppression.

**FK778—A Leflunomide Analog**

FK778 (Fujisawa, Osaka, Japan) is a synthetic malononitrilamide derived from an active metabolite of leflunomide, A77 1726. It has immunosuppressive and antiproliferative properties. It binds to the enzyme, dihydro-orotate dehydrogenase, thereby inhibiting de novo pyrimidine biosynthesis, blocking T- and B-cell proliferation, and strongly suppressing IgG and IgM antibody production.82 It possesses antiviral properties83 and can inhibit polyoma virus replication in vitro. A vasculoprotective effect with inhibition of neointima formation, thought to be associated with tyrosine kinase inhibition, was demonstrated in animal studies84 and adds to the interest in this drug. Phase 1 (United States) and phase 2 (Europe) renal transplantation trials have been completed.85 When administered in high and low doses with TAC and steroids, it is pharmacologically active with a reduced rate of ACR. The main adverse effect is anemia. Ongoing studies on LTX patients are being conducted in Europe.

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**Figure 1. Overview schematic of immune response. Abbreviations: APCs, antigen-presenting cells; MHC, major histocompatibility complex; TCR, T-cell receptor; LCK, lymphocyte-specific cytoplasmic tyrosine kinase; NFAT, nuclear factor of activated T cells; JAK, Janus kinases; STAT, signal transducers and activators of transcription; MAPK, mitogen-activated protein kinase.**
JAK 3/STAT Inhibition

JAKs are vital intermediaries between cytokine receptors and STAT, which result in activation of the immune cells. JAK-1 and JAK-2 are activated by a broad range of cytokines that use gp130. JAK-3 is activated only by cytokines that bind to γ-chain-containing receptors. Stimulation of JAK-3, a cytoplasmic tyrosine kinase, leads to dimerization of STAT 5 transcription factor (Fig. 1). This action is specific and selective for the IL-2 family of cytokines, making it a useful target for immunosuppressive drugs. A number of JAK inhibitors, with varying degrees of specificity for JAK-3, are under investigation. Specific JAK-3 inhibitors, which have demonstrated immunosuppressive activity in small animal models, exhibit fewer of the hematological side effects seen with those agents that also inhibit JAK-2.

FTY720—Disruption of Lymphocyte Homing

FTY720 represents a new class of immunosuppressive agent that alters cellular homing patterns. It is a synthetic analogue of sphingosine, isolated from the ascomycete *Isaria sinclairii*. It decreases the number of circulating blood lymphocytes, and lymphocyte counts reach less than 30% of baseline value within 72 hours of treatment. FTY720 acts as a high affinity agonist at the G-protein-coupled sphingosine-1-phosphate receptor-1 on thymocytes and lymphocytes inducing aberrant internalization of the receptor. This renders the cells unresponsive to the serum lipid sphingosine-1-phosphate receptor-1, depriving them of a signal to leave lymphoid organs; lymphocytes are unable to recirculate to peripheral inflammatory tissues and graft sites but remain functional in the lymphoid compartment. Its major side effect identified in phase 1 and 2 trials was a negative chronotropic effect, possibly a direct effect of the drug on the sinus node, and exacerbated in the presence of beta-blockade. Phase 3 clinical trials are under way to establish FTY720's clinical utility. There are data that suggest that FTY720 may provide a level of protection from ischemia/reperfusion injury in livers.

Costimulatory Signal Blockade

Blockade of the costimulatory Signal 2 prevents T-cell activation and clonal expansion, rendering T cells immunologically unresponsive and achieving a state of peripheral tolerance in small animals. Blockade of T-cell/antigen-presenting—cell costimulatory pathways mediated via CD40-CD154 have been accomplished using the anti-CD154 monoclonal antibody, although the CD28-CD80/CD86 pathway is inhibited with the fusion protein, CTLA4-Ig. Both agents have reached early clinical testing. Results in animal models demonstrate long-term survival of renal, cardiac, and skin allografts. Clinical trials with antibodies against CD-154 were halted because of reports of thromboembolic phenomena, presumably because of the presence of CD154 on activated platelets; however, the utility of costimulatory blockade has been demonstrated in kidney transplants using LEA29Y (CTLA4-Ig). In a multicenter phase 2 study in kidney transplant recipients receiving induction therapy with basiliximab, MMF and corticosteroids patients were randomized.
1:1:1 to 3 groups, with “high-intensity” LEA29Y, with “low-intensity” LEA29Y, and with CsA. Patient and graft survival and incidence of ACR were similar, but metabolic complications (hypertension, cholesterol, diabetes) were lower with LEA29Y treatment. Some studies have suggested that concomitant administration of CNI or steroids antagonizes the tolerogenic effect of costimulatory blockade. However, this remains somewhat controversial.

Concluding Remarks

Although continued attempts to replace CNI have demonstrated that this approach is promising, the use of CNI remains the backbone of immunosuppressive protocols in LTX. The long-term efficacy of CNI minimization and CNI avoidance has yet to be determined, but the immunologic privilege enjoyed in the liver allograft should make these trials relatively safe. CNI-free protocols appear to be promising in renal transplantation. The benefits of reducing long-term cardiovascular, metabolic, and oncologic risks as side-effects of CNI use appear to justify these attempts.

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