The Impact of Novel Immunosuppressive Agents on Infections in Organ Transplant Recipients and the Interactions of These Agents with Antimicrobials

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Several of the new immunosuppressive agents that are used to treat transplant recipients possess in vitro activity against specific pathogens, enhance the activity of antimicrobial agents, or have unique drug interactions with antimicrobial agents. Mycophenolate mofetil may have a protective effect against *Pneumocystis carinii*; it also enhances the activity of ganciclovir and has strong antiviral activity against human immunodeficiency virus type 1. High doses of mycophenolate mofetil have been associated with a higher frequency of tissue-invasive cytomegalovirus disease but not with asymptomatic cytomegalovirus infection. Rapamycin exhibits potent in vitro fungicidal activity against *Cryptococcus neoformans* and several pathogenic fungi in transplant recipients; however, it is not known whether its immunosuppressive effect in organ transplant recipients outweighs its antifungal activity. Recognition of the unique characteristics of these agents and the evolving spectrum of opportunistic infections has implications for the differential diagnosis, management, and prophylaxis of infections in organ transplant recipients in the modern immunosuppressive era.

The goals of modern immunosuppressive treatment of organ transplant recipients are no longer limited to controlling episodes of transplant rejection but now also include achieving decreases in associated toxic side effects, long-term metabolic complications, and the potential for malignancy. In this regard, the advances in immunosuppression that have been made in recent years have been truly revolutionary. For nearly 2 decades, calcineurin inhibitors (e.g., cyclosporine and tacrolimus) have been the mainstay of immunosuppressive therapy. However, nephrotoxicity and neurotoxicity are significant therapy-limiting toxicities of these drugs.

Rapamycin (or sirolimus) is a novel immunosuppressant with a mechanism of action that is distinct from that of calcineurin inhibitors [1]. Mycophenolate mofetil (MMF) has emerged as a promising alternative to azathioprine because of its lesser potential for inhibition of erythropoiesis and myelopoiesis, compared with azathioprine [2–5]. Finally, the novel monoclonal antibodies inhibit the proliferation of activated, but not resting, T cells [6] and thus offer selective immunosuppression with fewer side effects.

In the present report, we review the infectious complications associated with the use of novel immunosuppressants for transplant recipients, and we discuss the drug interactions between immunosuppressants and antimicrobial agents. Although a number of chemical and biological immunosuppressants are in development, this review focuses on the agents that currently are either in use or in the advanced stages of clinical trials.
METHODS

Articles that pertain to clinical trials, cohort studies, case series, practice guidelines, and reviews of the use of novel immuno-suppressive agents for organ transplant recipients were identified in a search of the MEDLINE database. Search terms included “mycophenolate mofetil,” “rapamycin,” “monoclonal antibodies,” “daclizumab,” “basiliximab,” and “transplantation.” To evaluate drug interactions, a search of TOXLINE was done using the key words “mycophenolate mofetil,” “mycophenolic acid,” “rapamycin,” and “interactions.” The manufacturers of MMF (Roche) and rapamycin (Wyeth-Ayerst) were contacted and were asked whether any unpublished reports of relevant interactions existed. Bibliographies of the articles identified, in addition to abstracts presented at the annual meetings of the American Society of Transplantation and the Interscience Conference on Antimicrobial Agents and Chemotherapy for the period from 1999 through 2001, were also searched for relevant studies.

MMF

MMF (Cellcept; Roche) is an ester prodrug of mycophenolic acid produced by the mold Penicillium brevicompactum [7]. MMF is converted to mycophenolic acid, which is a noncompetitive inhibitor of inosine monophosphate dehydrogenase, a key enzyme in the de novo pathway of purine synthesis [2]. The antiproliferative action of MMF is primarily directed toward lymphocytes, because these cells cannot efficiently use the salvage pathway for the synthesis of guanosine nucleotides. In clinical trials, MMF has been proven superior to azathioprine in reducing the number of episodes of transplant rejection in heart, kidney, liver, and lung transplant recipients [8–13].

Impact on cytomegalovirus (CMV). A higher incidence of tissue-invasive CMV disease (but not asymptomatic CMV infection) has been documented in MMF-treated patients, particularly in those receiving >2 g of MMF per day [10, 14]. The incidence of CMV infection and disease among renal transplant recipients receiving 2 g of MMF per day was comparable with the incidences of CMV infection and disease among those who received either placebo or azathioprine in 3 large, randomized, double-blind trials [9, 11, 15]. However, the Tricontinental Study documented a higher incidence of CMV disease among patients who received 3 g of MMF per day, compared with patients who received smaller doses [10]. In that study, CMV disease consisted predominantly of gastrointestinal disease [10]. A higher incidence of gastrointestinal side effects among patients in the high-dose MMF group may have led to more-frequent performance of endoscopic procedures and, therefore, to a greater likelihood of diagnosis of CMV disease. In a historical case-control study, the MMF group had a higher incidence of CMV disease than did the control group that received cyclosporine and corticosteroids [16]. Although the overall incidence of CMV disease was higher among renal transplant recipients who received azathioprine, CMV organ involvement occurred more frequently in patients who received MMF than in those who received azathioprine (58% vs. 18%; P = .03) [14].

The mechanism by which MMF may enhance the risk of CMV infection or disease remains poorly understood. MMF depletes the intracellular guanosine triphosphate and deoxyguanosine triphosphate pools in lymphocytes, thus decreasing their proliferation [17]. Because recovery from CMV infection is dependent on the expansion of natural killer cells and activated virus-specific T lymphocytes [18], depression of the T lymphocyte response by MMF may be involved [19]. Although humoral immunity is considered to play a less important role in host defense against CMV, MMF has been shown to impair humoral responses in vivo and in vitro studies [17, 20, 21]. Clinical trials of liver, heart, and lung transplant recipients receiving MMF have failed to show an increase in the incidence of CMV infection or disease [8, 12, 22–26].

In concentrations achievable in plasma, MMF and mycophenolic acid have been shown to potentiate the antiviral activity of acyclovir, penciclovir, and ganciclovir by up to 350-fold [27, 28]. Synergy has also been documented for thymidine kinase–deficient strains of herpes simplex virus and varicella-zoster virus [27]. The type of CMV prophylaxis used appears to influence the incidence of CMV disease in transplant recipients who receive MMF. In the absence of CMV prophylaxis or with the use of acyclovir in dosages of ≤2.4 g/day, the incidence of CMV disease was higher among patients who received MMF than among patients who did not receive MMF [14, 16, 29, 30]. However, the use of acyclovir in dosages of >2.4 g/day [23, 31] was associated with comparable rates of CMV disease among patients who received MMF-based immunosuppression and those who did not. None of the studies of ganciclovir prophylaxis documented a significant difference between the incidence of CMV disease among patients who received MMF and the incidence among those who did not [12, 22, 26, 32]. The potential for toxicity when acyclovir or ganciclovir are used with MMF has not been documented in organ transplant recipients.

Impact on other viral infections. The incidence of herpes simplex virus infections in renal transplant recipients was comparable in patients who received MMF and those who did not receive MMF [9–11]. In a retrospective study of pediatric renal transplant recipients, however, the use of MMF was associated with a significantly higher rate of varicella-zoster virus infection (P = .026) [33]. A study of heart transplant recipients also documented a higher frequency of herpes zoster in association with MMF treatment (P = .049) [8].

The use of MMF for heart transplant recipients was asso-
associated with a higher rate of acute cholestatic hepatitis due to hepatitis C virus (HCV) and a decreased rate of survival among patients with de novo HCV infection and acute cholestatic hepatitis [34]. Among HCV-infected patients who received MMF, a significant increase in the rate of fibrosis progression after transplantation was noted at 2 years of follow-up [35]. Other studies of liver transplant recipients that had a comparable duration of follow-up have documented no impact of MMF on the rate or severity of HCV recurrence [36–40]. MMF has been shown to inhibit hepatitis B virus replication in culture of human hepatocytes [41, 42]. Its effect on hepatitis B virus infection in the clinical setting remains to be determined.

MMF possesses strong antiviral activity against HIV type 1 (HIV-1) [43, 44] at concentrations far below those used for immunosuppression in organ transplant recipients [45]. MMF and abacavir synergistically inhibited HIV-1 replication in stimulated peripheral blood mononuclear cells and in monocyte-derived macrophages [45]. Clinical trials to assess the efficacy of MMF as antiretroviral therapy are under way.

Impact on Pneumocystis carinii and other infectious agents. MMF possesses potent activity against P. carinii in animal models [46]. The likely mechanism of MMF’s activity against P. carinii involves inhibition of inosine monophosphate dehydrogenase. In 4 randomized, controlled trials of MMF in renal transplant recipients, none of the 1068 patients who received MMF developed P. carinii infection, compared with 10 (1.8%) of 563 patients who did not receive MMF (P = .00006) [10, 47, 48]. It may be too premature, however, to recommend discontinuation of P. carinii prophylaxis for patients receiving MMF. No effect of MMF on bacterial or fungal infections in organ transplant recipients has been documented.

Drug interactions. MMF undergoes complete presystemic metabolism to mycophenolic acid by hepatic esterases [2, 49, 50]. The pharmacokinetic parameters of MMF that were noted after a single dose was given to patients with alcoholic cirrhosis were not significantly different from those noted in healthy volunteers [51]. MMF is primarily excreted, by glomerular filtration and active tubular secretion, as mycophenolic acid and its glucuronide [52]. In patients who were undergoing dialysis, the duration of clearance of MMF was 5 times longer than that of subjects with normal renal function [53].

Coadministration of 1 g of MMF and 800 mg of acyclovir to healthy volunteers did not alter the pharmacokinetic parameters of MMF and acyclovir but, rather, resulted in a significantly higher area-under-the-curve value for mycophenolic acid glucuronide, compared with the effects of MMF administered alone [54]. The clinical significance of this observation, however, is not known. No significant interaction between a single dose of MMF, 1.5 g, and intravenously administered ganciclovir, 5 mg/kg, was noted in renal transplant recipients [55, 56]. Close observation of the patient’s condition and adjustment of the dosage of the antiviral agent and MMF may be warranted.

Trimethoprim-sulfamethoxazole, 160 mg and 800 mg, did not interact with MMF in healthy volunteers [52]. However, a reduction in the dose of trimethoprim-sulfamethoxazole is necessary if creatinine clearance is $<30 \text{ mL/min}$ [52]. No drug-drug interactions have been reported between MMF and $\beta$-lactam antibiotics, glycopeptides, macrolides, aminoglycosides, quinolones, rifamycins, tetracyclines, azole antifungal agents, amphotericin B, lipid formulations of amphotericin B, fluocytosine, nystatin, terbinafine, or protease inhibitors.

RAPAMYCIN

Rapamycin is a macrolide derived from the bacterium Streptomyces hygroscopicus. The drug was originally discovered when tested for antimicrobial activity against Candida albicans and later was determined to have potent immunosuppressive activity [57, 58]. After cell entry, rapamycin binds to the immunophilin FKBP-12 [59, 60]. The mammalian target of FKBP-12-rapamycin is “target of rapamycin” (mTOR). mTOR regulates the translation of mRNA required for cell division and thus inhibits progression from the G1 to the S phase of the cell cycle [1]. Rapamycin has been shown to act synergistically with cyclosporine [61].

Impact on cytomegalovirus and other viruses. Most studies of rapamycin have been done in combination with cyclosporine. The use of rapamycin has been associated with a decrease in the incidence of acute rejection without accrual of an additional risk of CMV infection [62–66]. The incidences of CMV infection and disease noted in US and global studies of renal transplant recipients were comparable among the various study groups [63]. A randomized European trial also concluded likewise [65]. A significantly lower incidence of CMV infection was documented among renal transplant recipients who received rapamycin (5%) than among those who received cyclosporine (21%; P = .045) in a study that included MMF and corticosteroids in both treatment arms [62].

In one study, a trend toward a higher incidence of herpes simplex virus infection was noted in association with receipt of rapamycin [65]. The frequency of herpes zoster infection was comparable in the groups that received rapamycin and cyclosporine [64, 65]. On the basis of studies published thus far, it is not known whether rapamycin influences the incidence or severity of HCV infection in transplant recipients [66, 67].

Impact on fungal infections. Rapamycin exhibits potent in vitro fungicidal activity against Cryptococcus neoformans [68]. The antifungal action of rapamycin is mediated via FKBP-12-rapamycin’s inhibition of TOR1 in the yeast. Rapamycin also inhibits other pathogenic fungi, such as C. al-
bicans, Aspergillus fumigatus, Aspergillus flavus, and Fusarium oxysporum.

Whether the in vitro antifungal activity of rapamycin translates into a beneficial clinical effect remains to be determined. In an outbreak of invasive aspergillosis that occurred in a renal transplant unit when construction activity was being done, the combination of rapamycin and MMF was associated with an increased risk of invasive aspergillosis (RR, 37.1; 95% CI, 2.22–621.5); the risk persisted even when patients were stratified according to receipt of corticosteroids [69].

**Drug interactions.** Rapamycin is a substrate for both cytochrome P-450 3A4 (CYP3A4) and P-glycoprotein. Inhibitors of CYP3A4 may decrease metabolism and result in an increase in blood levels of rapamycin, whereas inducers of this isoenzyme may increase metabolism, resulting in a subsequent decrease in rapamycin levels (table 1).

There are structural similarities between rapamycin and the macrolides. Indeed, patients with known hypersensitivity to macrolide antibiotics have been excluded from clinical trials of rapamycin. [73–75]. Macrolide antibiotics with a 14-membered ring, such as erythromycin, clarithromycin, and troleandomycin, have been shown to inhibit CYP3A4 [71, 76–77]; erythromycin also inhibits P-glycoprotein [71]. However, azithromycin, which has a 15-membered ring, did not induce or inactivate the hepatic cytochrome P-450 system in Sprague-Dawley rats [78]. Although pharmacokinetic studies of interactions between rapamycin and the aforementioned macrolides have not been conducted, in 2 renal transplant recipients who were treated with rapamycin and who received erythromycin for suspected legionellosis, a 5-fold increase in the rapamycin levels in blood was noted [79]. It may be prudent to monitor rapamycin levels in blood during administration of macrolide antibiotics.

Use of rifampin and, to some extent, rifabutin results in an increase in metabolism and a decrease in rapamycin levels [70, 71]. For 14 healthy volunteers, pretreatment with rifampin, 600 mg/day for 14 days, followed by a single 20-mg dose of rapamycin increased the clearance of rapamycin by 5.5-fold [80]. If the use of rifampin is deemed necessary in patients receiving rapamycin, a 5-fold increase in the oral dose of rapamycin should be considered [80].

Azole antifungal agents ( clotrimazole, fluconazole, itraconazole, and ketoconazole) are potent inhibitors of CYP3A4 and could potentially result in an increase in the level of rapamycin. Concurrent administration of multiple doses of rapamycin and ketoconazole resulted in a 10.9-fold increase in the area-under-the-curve value for rapamycin [72]. Monitoring of rapamycin levels in blood is suggested for patients who are receiving clotrimazole, fluconazole, and itraconazole. Therapeutic monitoring and close observation are recommended when protease inhibitors (indinavir and ritonavir) are used concurrently to treat patients who are receiving rapamycin. Trimethoprim-sulfamethoxazole (80 mg/400 mg) did not interact with rapamycin in 15 renal transplant recipients [72].

**EVEROLIMUS**

Everolimus (Certican; Novartis Pharma AG), a 40-O[2-hydroxyethyl] derivative of rapamycin, has a shorter half-life and achieves a steady-state concentration more quickly than does rapamycin [81]. Like rapamycin, it binds to FKBP-12 [82]. Only limited clinical experience with this drug exists thus far. For 103 renal transplant recipients who were randomized to receive 1, 2, or 4 mg of everolimus with cyclosporine, the overall rate of CMV infection was 3% and did not differ between the 3 groups [83]. A randomized trial among kidney transplant recipients documented a lower incidence of CMV infection among patients who received everolimus than among those who received MMF [83]. Of note, everolimus has been shown to have potent inhibitory activity on posttransplant lymphoproliferative disorder–like human Epstein-Barr virus–positive lymphoblastoid B cell lines both in vitro and in vivo [84]. Everolimus inhibited in vitro proliferation of these cells and arrested their cell cycle progression at the early G0/G1 stage [84]. In vivo, it markedly delayed or completely inhibited the growth of Epstein-Barr virus–positive B cells xenotransplanted into SCID mice [84]. In some instances, everolimus was able to eradicate the established tumor [84].

**Table 1. Drug interactions between rapamycin and antimicrobial agents.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Potential interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolide antibiotics</td>
<td>Structurally similar to rapamycin and potential for increased rapamycin level [70]</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Induction of rapamycin metabolism and decrease in rapamycin levels; 5-fold increase in oral dose of rapamycin is suggested when used with rifampin [71]</td>
</tr>
<tr>
<td>Azole antifungal agents</td>
<td>Inhibition of rapamycin metabolism and increase in rapamycin level [72]</td>
</tr>
<tr>
<td>Indinavir and ritonavir</td>
<td>May induce rapamycin metabolism and decrease rapamycin levels [72]</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>No interaction [72]</td>
</tr>
</tbody>
</table>

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Table 2. Impact of novel immunosuppressive agents on major infections or pathogens in organ transplant recipients.

<table>
<thead>
<tr>
<th>Immunosuppressive agent</th>
<th>CMV</th>
<th>Other herpesviruses</th>
<th>HCV</th>
<th>Fungal infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycophenolate mofetil</td>
<td>Higher incidence of tissue-invasive CMV disease but not asymptomatic CMV infection [15]; potentiation of activity of ganciclovir against CMV [27, 28]</td>
<td>Higher rate of varicella-zoster virus infections [33]</td>
<td>Higher rate of acute cholestatic hepatitis due to HCV [34]; delayed allograft fibrosis due to HCV [39]</td>
<td>Potent activity against Pneumocystis carinii and a lower incidence of P. carinii infection [46]</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td>Basiliximab</td>
<td>No effect [90–92]</td>
<td>Lower rate of herpes simplex virus infections [91]</td>
<td>No data</td>
</tr>
<tr>
<td></td>
<td>Daclizumab</td>
<td>Lower incidence of CMV infection [96]</td>
<td>No effect [96–99]</td>
<td>No effect [100, 101]</td>
</tr>
</tbody>
</table>

**NOTE.** CMV, cytomegalovirus; HCV, hepatitis C virus.
MONOCLONAL ANTIBODIES

Nonspecific immunosuppression associated with the earlier monoclonal antibodies, such as OKT3, has potentially significant toxicity and may result in life-threatening opportunistic infections. A selective target for more-specific immunosuppression is the IL-2 receptor (IL-2R) system, responsible for the proliferation of T cells [6, 85]. The α chain of IL-2R (CD25) is critical in the formation of functional receptors and the mediation of T cell proliferation [85]. Use of monoclonal antibodies against IL-2R (CD25) therefore offers a novel strategy to achieve selective yet effective immunosuppression [86–89].

Two monoclonal antibodies, basiliximab (Simulcet; Novartis) and daclizumab (Zenapax; Roche), recently have been developed. Basiliximab is a chimeric monoclonal IL-2R antibody (murine/human) with human IgG1 constant heavy chain regions and a κ light chain. Daclizumab is a molecularly engineered human IgG1 IL-2R antibody that is 90% human and that retains the original 10% murine component in the hypervariable segments for binding specificity. A reduction in the incidence of allograft rejection with the use of basiliximab and daclizumab has been amply documented among kidney, heart, liver, lung, and kidney-pancreas transplant recipients [90–99].

Impact on CMV and other herpesvirus infections. In 3 randomized, double-blind, placebo-controlled trials, the efficacy of basiliximab in kidney transplant recipients did not differ from that of placebo [90, 91]. The efficacy of daclizumab has been assessed in 2 randomized, controlled trials of kidney transplant recipients. A US study [97] found comparable rates of CMV disease, whereas a European trial [96] noted a lower incidence of CMV disease. Fewer episodes of acute rejection that required use of antithymocyte globulin and corticosteroid therapy in the daclizumab group may have accounted for the lower incidence of CMV infection among patients who received daclizumab, compared with those who received placebo, in the European study [96]. Controlled studies of heart, liver, lung, and pancreas transplant recipients have documented similar rates of CMV disease between the daclizumab and control groups [93–95, 98, 99].

The significantly lower incidence of herpes simplex virus infection noted in transplant recipients in the basiliximab group, compared with that noted in the placebo group (2.9% vs. 9.2%), was believed to reflect greater OKT3 and corticosteroid use to treat acute rejection in the placebo group [91].

Impact on HCV infection. Studies documenting the recurrence of HCV infection in patients who receive daclizumab have yielded conflicting results [100–102]. Two studies of liver transplant recipients failed to show any impact of daclizumab use on the recurrence of HCV infection [100, 101], whereas others have noted a higher rate of recurrence in liver transplant recipients who received daclizumab [102, 103].

Impact on bacterial and fungal infections. To date, a difference in the incidence of bacterial and fungal infections in patients who have received basiliximab or daclizumab, compared with placebo, has not been documented [90–99].

CONCLUSIONS

Table 2 summarizes the impact of novel immunosuppressive agents on major infections in organ transplant recipients. Improved outcomes with the novel immunosuppressive agents are notable, because a reduction in the number of episodes of rejection has been accomplished largely without a significant increase in life-threatening infections. The long-term sequelae of these agents, however, remain to be discerned. Potent in vitro antimicrobial activity of several immunosuppressive agents against specific pathogens and the potential for synergy with antimicrobial agents have implications relevant for antimicrobial prophylactic approaches and for the development of immunosuppressive agents that retain antimicrobial activity in vivo. A number of new immunosuppressive agents are under development or are on the horizon; these include novel inhibitors of T cell proliferation (e.g., tyrosine kinase inhibitors), antibodies to adhesion molecules (e.g., anti-CD11-α), and agents that alter the recruitment of immune cells responsible for rejection (e.g., chemokine receptor blockers). It is hoped that the emerging repertoire of novel immunosuppressive drugs with highly specific sites of action will allow maximal immunosuppression to be achieved with minimal risk of infectious morbidity.

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