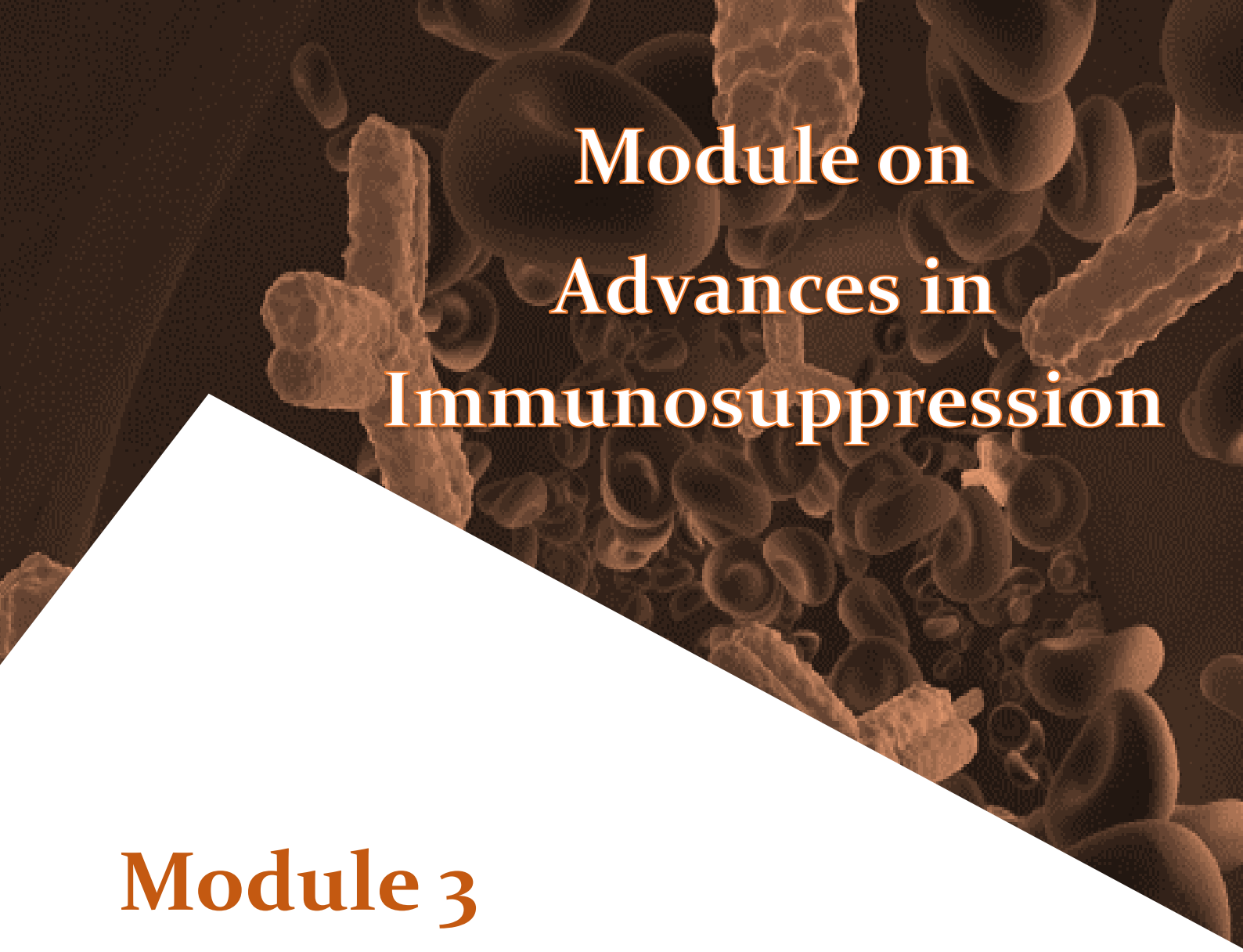

Module on Advances in Immunosuppression

Module 3

Understanding the Immunosuppression of Mycophenolate

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Introduction

Discovered in 1896 from a penicillium culture, Mycophenolic acid, previously known as RS-61443, serves as the foundation for Mycophenolate mofetil, which is the morpholinoethyl ester of the acid. Mycophenolic acid initially showcased a range of properties, including antineoplastic, antibacterial, antifungal, and antiviral effects, with its immunosuppressive potential later recognized. Mycophenolate mofetil was developed to improve the bioavailability of this compound. Building upon encouraging animal studies, Sollinger et al. initiated groundbreaking human trials involving kidney transplant recipients. Consequently, the FDA granted approval for mycophenolate mofetil's use in preventing rejection among patients with allogeneic renal transplants. Currently, ongoing research delves into its potential as a rescue therapy for organ transplant patients experiencing rejection despite standard immunosuppressive measures.

Pharmacokinetics

Absorption. Mycophenolate mofetil is rapidly absorbed upon oral ingestion, quickly converting into mycophenolic acid, its active metabolite, through presystemic metabolism. This metabolite further undergoes conversion to mycophenolic acid glucuronide (MPAG), which lacks pharmacological activity. Not directly measurable in plasma after oral administration, mycophenolate mofetil is extensively absorbed in healthy volunteers, with a mean relative bioavailability of mycophenolic acid reaching 94%. Peak plasma concentration (Cmax) typically occurs about two hours post-ingestion, with mycophenolic acid concentrations remaining below 2.5 µg/mL within 12 hours post-administration. Enterohepatic circulation leads to secondary peaks in plasma levels, although the extent in humans remains uncertain.

In renal transplant patients receiving doses ranging from 100 to 3500 mg of mycophenolate mofetil daily, serum concentrations and the area under the plasma concentration–time curve (AUC) increase proportionally with the dose. Early post-transplant, plasma mycophenolic acid concentrations and AUC are approximately 50% lower compared to stable renal transplant patients. However, there is a significant increase in maximum plasma mycophenolic acid concentrations and AUC between post-transplant days 1 and 20 in patients receiving

mycophenolate mofetil 1750 mg twice daily. Steady-state serum concentrations are typically reached by day 7, though there is interpatient variability. While most pharmacokinetic studies focus on renal transplant patients, variations are possible in recipients of other solid organs. Food intake reduces the Cmax of mycophenolic acid but does not affect the AUC.

Distribution

Mycophenolic acid's binding to plasma albumin is concentration-dependent, resulting in an increase in the plasma concentration of unbound mycophenolic acid as the dose increases. The elevation in total plasma mycophenolic acid concentrations correlates with a rise in the fraction of free mycophenolic acid. Despite the presence of medications such as cyclosporine, prednisone, tacrolimus, warfarin, digoxin, or phenytoin at supratherapeutic concentrations, the binding of mycophenolic acid remains unaffected. However, increased plasma concentrations of MPAG, which may occur shortly after renal transplantation, do raise the free fraction of mycophenolic acid. While high concentrations of furosemide minimally impact free plasma mycophenolic acid, high doses of aspirin resulting in salicylate concentrations exceeding 250 mg/L may displace mycophenolic acid from serum albumin. Moreover, a decrease in serum albumin concentration from 41.4 g/L to 20.7 g/L results in a 2.2-fold increase in the free fraction of mycophenolic acid. Inhibition of IMPDH rises proportionally with escalating concentrations of unbound mycophenolic acid, with increases in serum albumin concentrations in vitro requiring a higher mycophenolic acid concentration for 50% inhibition of IMPDH.

The binding of mycophenolic acid to α1-acid glycoprotein is minimal. Mycophenolic acid exhibits minimal binding to plasma lipoproteins in a concentration-independent manner. Both mycophenolic acid and MPAG are predominantly present in plasma, with minimal distribution into cells. For analyzing mycophenolic acid concentrations, plasma rather than whole blood should be used.

Metabolism

After oral intake, mycophenolate mofetil undergoes hydrolysis to generate mycophenolic acid. This compound then proceeds to form an inactive phenolic glucuronide along with three other inactive metabolites: N-(2-carboxymethyl)morpholine, N-(2-hydroxyethyl)-morpholine, and

the N-oxide of N-(2-hydroxyethyl)-morpholine. Notably, alcoholic cirrhosis does not seem to notably impact the hepatic metabolism of mycophenolic acid to MPAG.

Excretion:

Mycophenolic acid is predominantly excreted through the kidneys, with more than 90% of a given dose being eliminated in the urine as MPAG. The excretion of MPAG occurs via both glomerular filtration and tubular secretion. Changes in plasma concentrations of mycophenolic acid and MPAG have been noted in individuals with renal insufficiency. In a study involving a single dose, patients with severe renal impairment exhibited nearly a twofold increase in the mean mycophenolic acid plasma AUC, while the MPAG AUC increased threefold to sixfold. Conversely, Cmax was reduced by approximately one third in the same patient group. Hemodialysis did not seem to significantly affect plasma concentrations of mycophenolic acid or MPAG. A minimal amount of mycophenolate mofetil was eliminated through fecal excretion.

Efficacy in renal transplantation

Forty-nine patients who underwent renal allografts were randomly assigned to one of eight mycophenolate mofetil dosage regimens, ranging from 100 to 3500 mg/day. Additional immunosuppressive treatment included Minnesota antilymphocyte globulin, cyclosporine (initiated once serum creatinine levels were below 3 mg/dL), and prednisone. Unfortunately, one patient passed away due to acute myocardial infarction on the first postoperative day and was replaced. Five other patients did not complete the trial, with reasons unrelated to mycophenolate mofetil therapy in four cases. Mycophenolate mofetil was discontinued in one patient due to hemorrhagic gastritis suspected to be linked to the drug. Overall, mycophenolate mofetil was well tolerated, with no observed bone marrow suppression, hepatotoxicity, or nephrotoxicity. Patients receiving higher dosages (>2 g/day) experienced fewer severe rejection episodes, and those occurring with higher dosages were less severe and more easily reversible.

Subsequently, 43 of the original 49 patients participated in a long-term follow-up trial lasting 14–26.5 months. Patient and graft survival rates at 18 months were 100% and 95%, respectively, with no withdrawals from the trial. Five patients experienced adverse effects necessitating a reduction in mycophenolate mofetil dosage. Four episodes of acute rejection were observed in four patients more than four months post-surgery. Rejection episodes were successfully reversed in two patients, while two others had their mycophenolate mofetil dosage increased to 3 g/day to manage rejection.

In a separate randomized, placebo-controlled, double-blind trial, Sollinger and the U.S. Renal Transplant Mycophenolate Mofetil Study Group32 investigated the efficacy of mycophenolate mofetil in preventing acute rejection in 449 patients who had received cadaveric renal transplants. Over the first six months post-transplantation, patients were administered mycophenolate mofetil at doses of 2 g/day (n = 167) or 3 g/day (n = 166), or azathioprine at 1–2 mg/kg/day (n = 166). Additional immunosuppressive treatment included antithymocyte globulin, cyclosporine, and prednisone. Four patients (two from each of the mycophenolate mofetil and azathioprine groups) did not receive the study medication and were therefore excluded from the analysis. The primary study endpoints included biopsy-proven rejection,

treatment failure (defined as graft loss), and withdrawal from the study for any reason despite a lack of biopsy-proven rejection. The incidence of rejection and treatment failure was 47.6% in the azathioprine group, 31.1% in the mycophenolate mofetil 2-g/day group, and 31.1% in the mycophenolate mofetil 3-g/day group (with p-values of 0.0015 for azathioprine versus mycophenolate mofetil 2 g/day and 0.0021 for azathioprine versus mycophenolate mofetil 3 g/day). There was no significant difference in the percentage of patients with rejection managed by clinical judgment alone among the treatment groups (6% for azathioprine, 4.8% for mycophenolate mofetil 2 g/day, and 5.4% for mycophenolate mofetil 3 g/day). Kaplan-Meier estimates revealed graft loss rates of 8.6%, 1.8%, and 6.7% for the azathioprine, mycophenolate mofetil 2-g/day, and mycophenolate mofetil 3-g/day groups, respectively, with graft loss mostly attributable to rejection.

Adverse effects and opportunistic infections were documented across all three treatment groups. Among the adverse effects, anemia, hypertension, and diarrhea were the most commonly reported for mycophenolate mofetil. Diarrhea and other gastrointestinal complications, including esophagitis, gastritis, and gastrointestinal hemorrhage, were more prevalent in the mycophenolate mofetil groups compared to the azathioprine group. Moreover, a higher incidence of adverse effects was noted in the mycophenolate mofetil 3-g/day group compared to the 2-g/day group. Opportunistic infections were present in all treatment groups, with cytomegalovirus (CMV) being the most frequently identified pathogen. Tissue-invasive CMV infection was notably more common in recipients of mycophenolate mofetil (6.1%, 9.1%, and 10.8% in the azathioprine, mycophenolate mofetil 2-g, and mycophenolate mofetil 3-g groups, respectively). While two azathioprine recipients developed Pneumocystis carinii pneumonia (PCP), this complication was not observed in the mycophenolate mofetil groups. Additionally, three patients receiving mycophenolate mofetil 3 g/day developed aspergillus or mucor infections, which were not reported in either the 2-g group or the azathioprine group. There were no reports of hepatotoxicity, and neutropenia and thrombocytopenia occurred infrequently.

The European Mycophenolate Mofetil Cooperative Study, a randomized, double-blind, placebo-controlled trial, also investigated the effectiveness of mycophenolate mofetil in patients with first or second cadaveric renal transplants. In this study, patients were assigned to receive either placebo (n = 166), mycophenolate mofetil 2 g/day (n = 165), or mycophenolate mofetil 3 g/day (n = 160). Additional immunosuppressive treatment included cyclosporine and

prednisone, while antibody induction therapy was not administered, and azathioprine therapy was prohibited during the trial. The primary endpoints were similar to those in the U.S. trial. Control patients exhibited a significantly higher incidence of biopsy-proven rejection, presumed rejection, or treatment failure compared to patients in either of the mycophenolate mofetil groups ($p < 0.001$).

A notable difference between the placebo group and the mycophenolate mofetil groups was observed within the initial six months regarding the frequency of biopsy-proven rejection or treatment failure ($p < 0.001$). Corticosteroids or antilymphocyte agents, or both, were administered for rejection within the first six months post-transplantation to 172 patients (86 [50%], 47 [27.3%], and 39 [22.7%] individuals in the placebo, mycophenolate mofetil 2-g/day, and mycophenolate mofetil 3-g/day groups, respectively). Forty-two patients (17 [41%] in the placebo group, 11 [26%] in the 2-g/day group, and 14 [33%] in the 3-g/day group) experienced graft loss or mortality within the initial six months, primarily due to rejection. Fourteen of the 15 deaths were not linked to mycophenolate mofetil therapy. One death, attributed to hemorrhagic pancreatitis, possibly was.

In total, 151 patients opted to withdraw prematurely from the trial due to adverse effects or unsatisfactory responses (34.9% in the placebo group, 22.4% in the 2-g group, and 35% in the 3-g group). Adverse effects prompting the discontinuation of study medication were more prevalent among patients receiving mycophenolate mofetil therapy. Discontinuation of study medication due to an unsatisfactory response was more common in the placebo group.

Gastrointestinal adverse effects, including severe events like large-bowel perforation, were more frequently observed in the mycophenolate mofetil groups. Hematologic toxicity, such as leukopenia and anemia, occurred more often in patients treated with mycophenolate mofetil.

Tissue-invasive CMV infection was more prevalent in patients receiving mycophenolate mofetil 3 g/day compared to the other groups. However, a similar rate of CMV viremia was observed across all study groups. Other viral infections were more frequent in the mycophenolate mofetil groups than in the placebo group. Four placebo recipients developed PCP, and one experienced a fungal infection, which were not observed in the mycophenolate mofetil groups.

Following the promising results of mycophenolate mofetil as a maintenance therapy for renal transplant recipients, Sollinger and colleagues conducted a multicenter pilot study to evaluate

its efficacy in treating acute allograft rejection. Seventy-five patients with biopsy-proven rejection, unresponsive to muromonab-CD3 or antilymphocyte globulin, were enrolled in this open-label study. Treatment with mycophenolate mofetil at doses of 2 or 3 g/day was initiated within 48 hours after biopsy. The patient cohort included individuals with transplants from living relatives (n = 11) or unrelated donors (n = 3), as well as those with primary (n = 50) or secondary (n = 11) cadaveric transplants. Renal function showed improvement or stabilization in 52 out of 75 patients (69%). Success rates were higher among patients with serum creatinine concentrations below 4.0 mg/dL (79%) compared to those with concentrations exceeding 4.0 mg/dL (52%). Nineteen patients discontinued therapy due to treatment failure, and an additional 11 patients stopped treatment due to complications, with adverse effects of mycophenolate mofetil directly attributed to discontinuation in four of these cases (pancreatitis, CMV-associated colitis, hemorrhagic gastritis, and other gastrointestinal issues).

Efficacy in liver transplantation

Currently, limited information exists regarding the utilization of mycophenolate mofetil in liver transplant recipients, with only a few documented case reports outlining its role in maintaining immunosuppression. Freise et al. described administering mycophenolate mofetil alongside prednisone in four liver transplant recipients who had previously experienced complications induced by cyclosporine. Despite being on therapy for over a year, none of these patients showed signs of rejection.

Similarly, Klintman et al. utilized mycophenolate mofetil to manage 23 liver transplant patients who were already receiving cyclosporine, prednisone, and azathioprine for immunosuppression maintenance and persistent acute rejection. These patients had confirmed rejection via biopsy despite undergoing treatment with high-dose corticosteroids and muromonab-CD3. Mycophenolate mofetil was introduced 4–44 weeks post-liver transplantation, and the observation period ranged from 5 to 11 months. Of the participants, 91% responded positively, with 14 experiencing complete resolution of rejection and 7 showing improvement. Sixteen patients remained on mycophenolate mofetil therapy. However, four patients exhibited underlying chronic rejection, which was unresponsive to mycophenolate mofetil treatment.

In conclusion, the available data on mycophenolate mofetil in liver transplant recipients are scarce. Large-scale, randomized, double-blind trials are necessary to gain deeper insights into its potential efficacy and impact in this patient population.

Efficacy in heart transplantation

Clinical trials assessing the primary immunosuppressive effects of mycophenolate mofetil in heart transplant recipients have not been conducted. However, the drug has been investigated as a rescue therapy for rejection resistant to other medications in patients who have undergone heart transplantation. In a study by Ensley et al., the efficacy of mycophenolate mofetil was examined in dosages ranging from 500 mg to 3 g per day, which were assigned non-randomly, in 30 patients with mild acute rejection (classified as International Society of Heart and Lung Transplantation [ISHLT] grade 1b or 2) occurring more than 28 days after surgery. Before enrollment, these patients had experienced an average of 2.4 rejection episodes. The Utah Transplantation Affiliated Hospitals Cardiac Transplantation Program (UCTP) rejection grading system was utilized by the researchers; UCTP grade 3 rejection corresponds to an ISHLT grade of 1b or 2. Throughout the eight-week duration of the study, concurrent immunosuppressive therapy (prednisone and cyclosporine) remained unchanged.

Complete resolution of acute rejection (ISHLT grade, 0) was observed in 20 (66%) of the patients within four weeks. Upon completion of the trial, the mean ISHLT rejection score significantly decreased (1.8 at eight weeks versus 3.0 at baseline, $p < 0.001$). Throughout the study period, corticosteroid dosages and cyclosporine levels remained unchanged. Mycophenolate mofetil was discontinued in eight patients—four due to continued mild rejection and four due to progression to moderate rejection (ISHLT grade 3A or 3B). Although higher dosages were associated with a nonsignificant reduction in the occurrence of progression to moderate rejection (2 [33%] of 6 patients receiving 500 mg/day versus 2 [8%] of 24 patients receiving 1 g/day or more), the difference was not statistically significant. Mycophenolate mofetil therapy was continued as long-term prophylaxis in the 20 responders for an average of 430 days. During long-term follow-up, the rate of reported moderate rejection was 0.24 episodes per patient-year. The therapy was well tolerated, with discontinuation necessary in only one patient (due to gastrointestinal complaints). No hepatotoxicity or

nephrotoxicity was observed during the initial eight-week trial period. The rate of major infectious complications was 0.2 episodes per patient-year during the long-term follow-up.

In a similar trial, Kirklin et al. assessed the efficacy of mycophenolate mofetil in managing rejection in 17 heart transplant patients with persistent (n = 5), refractory (n = 1), or recurrent (n = 11) rejection. The drug was administered at 3 g/day for two months, with seven patients having their dosage increased to 3.5 g/day five days after starting therapy. Patients were also eligible for enrollment in a long-term follow-up study. The overall frequency of rejection decreased from 0.67 episodes per month in the six months before therapy to 0.27 episodes per month in the six months after therapy initiation ($p < 0.0001$). This reduction was observed irrespective of the time elapsed since transplantation. However, a more substantial reduction in rejection frequency was noted within six months post-surgery (1.18 and 0.35 episodes per month before and after mycophenolate mofetil therapy, respectively, $p = 0.0002$) compared to beyond six months post-surgery (0.33 and 0.18 episodes per month before and after therapy, $p = 0.4$). (Rejection frequency typically peaks in the early post-transplant period and decreases over time.)

Two patients died after commencing therapy. One patient, who started mycophenolate mofetil therapy six days after retransplantation, succumbed to CMV sepsis 68 days later. The second patient, already experiencing multiple organ system failure before initiating mycophenolate mofetil therapy, passed away due to pulmonary dysfunction 72 days later.

After the initial two-month study period, mycophenolate mofetil therapy was continued in 14 out of the 17 patients. One patient discontinued mycophenolate mofetil treatment due to severe gastrointestinal adverse effects. Additionally, eight patients experienced gastrointestinal symptoms that resolved after temporarily reducing the dosage. Seven patients did not exhibit adverse effects that could be attributed to mycophenolate mofetil therapy. There were no clinically significant changes in hepatic and renal function observed, and bone marrow suppression was not detected. Furthermore, infection rates remained unchanged before and after initiating therapy.

Kobashigawa et al. detailed their findings on 15 cardiac transplant recipients treated with mycophenolate mofetil for persistent or refractory rejection. Azathioprine was discontinued upon rejection diagnosis, and mycophenolate mofetil therapy commenced at dosages of 2–3 g/day. Among the nine patients with moderate rejection, all showed improvement upon repeat

biopsy (mean time to re-biopsy: 16 days). Six out of these nine patients experienced complete resolution of rejection approximately 39 days after starting mycophenolate mofetil therapy. In the case of the six patients with documented mild rejection, all demonstrated improvement on repeat biopsy conducted after an average of 19 days of therapy. Five of these six patients achieved complete resolution after an average of 47 days following initiation of mycophenolate mofetil therapy. No adverse effects were reported.

Taylor et al. conducted monitoring of 33 patients from the aforementioned trials to evaluate mycophenolate mofetil as chronic maintenance therapy. Prior to the initiation of mycophenolate mofetil therapy, these patients had experienced an average of 2.8 episodes of acute rejection each. The mean time from transplantation was 295 days, with follow-up averaging 23.7 months (range, 2–37.4 months).

Sixteen patients were discontinued from treatment: four due to rejection, four due to adverse drug reactions, and eight for reasons unrelated to the study medication. Thus, 17 patients underwent mycophenolate mofetil therapy for an average duration of 33 months. Throughout the study period, 37 rejection episodes were recorded, comprising 28 instances of mild rejection (ISHLT grade 1B or 2) and 9 cases of moderate rejection (grade 3A or 3B). Among the 28 mild rejection episodes, 19 were initially managed by increasing the mycophenolate mofetil dose (average increase: 818 mg). Resolution of rejection was observed upon repeat biopsy at day 13 in 13 out of these 19 cases. No alterations were made to additional immunosuppressive therapy during these episodes. Five patients continued to experience persistent rejection, which was resolved with high-dose oral corticosteroids. One patient progressed to moderate rejection, and resolution occurred following administration of intravenous corticosteroids. Thus, increasing the mycophenolate mofetil dosage successfully treated 13 (68%) out of 19 episodes of mild rejection. All nine episodes of moderate rejection required brief courses of intravenous corticosteroids, with six patients showing resolution at the first biopsy. Three out of the nine patients were transitioned to azathioprine—two at the initial diagnosis of rejection and one after documented persistent rejection. Rejection occurred on average 240 days after initiating mycophenolate mofetil therapy. The mean corticosteroid dosage decreased from 15.5 mg/day at enrollment to 6.8 mg/day at the time of rejection. Gastrointestinal complaints were the most frequently reported adverse effects during the 782 patient-months of mycophenolate mofetil therapy, with transient leukopenia occurring in 15% of the patients.

Mycophenolate mofetil demonstrates effectiveness in managing cardiac transplant recipients with biopsy-proven rejection despite triple-drug immunosuppressive therapy. In such cases, mycophenolate mofetil replaces azathioprine in the immunosuppressive regimen. The initiation of mycophenolate mofetil therapy may be considered for managing biopsy-proven rejection as an alternative to conventional therapy. However, randomized trials comparing mycophenolate mofetil with azathioprine for primary immunosuppression are imperative. Data from ongoing clinical trials are necessary before recommending mycophenolate mofetil in primary immunosuppressive regimens immediately after cardiac transplantation.

Bone marrow suppression

Initial reports cast doubt on the association between mycophenolate mofetil therapy and myelosuppression, considering the concurrent administration of other agents with myelosuppressive properties. Reports of leukopenia, thrombocytopenia, and anemia in psoriatic patients treated solely with mycophenolic acid suggest a potential link between mycophenolate mofetil and bone marrow suppression. Moreover, the European Mycophenolate Mofetil Cooperative Study noted a higher incidence of leukopenia in patients receiving mycophenolate mofetil compared to those on placebo. Notably, the percentage of patients receiving concomitant antilymphocyte agents did not significantly differ between the mycophenolate mofetil group and the placebo group during the study period.

Myelosuppression linked to mycophenolate mofetil therapy encompasses anemia, leukopenia, and thrombocytopenia. While neutropenia is uncommonly documented, its occurrence may be associated with the simultaneous use of ganciclovir. The incidence of myelosuppression in large randomized trials involving renal transplant recipients varied from 7% to 35%, with anemia and leukopenia being the most prevalent presentations. Rare occurrences of pancytopenia and agranulocytosis were reported. Typically, myelosuppressive effects manifested within 30 to 180 days post-transplantation. In most instances, these effects improved approximately one week following the cessation of mycophenolate mofetil therapy. Similar rates of myelosuppression were observed with azathioprine.

Malignancies: Immunocompromised individuals face heightened susceptibility to lymphomas and specific other malignancies. Nonmelanoma skin cancer incidents have been documented in patients using mycophenolate mofetil, with no apparent correlation to dosage. Lymphomas

and lymphoproliferative disorders have been observed more frequently in groups treated with mycophenolate mofetil compared to those receiving placebo or azathioprine, albeit the overall occurrence rate was less than 2%.

Long-term monitoring of mycophenolate mofetil therapy in transplant recipients is still relatively scarce. However, in a particular study, 6 out of 85 psoriasis patients (7%) developed malignancies over an extended period of mycophenolic acid administration, spanning up to 13 years. The treatment regimen for these psoriasis patients consisted solely of mycophenolic acid as the immunosuppressive agent. It's plausible that a higher occurrence of malignancies could be observed with prolonged use of mycophenolate mofetil in transplant patients who are also receiving additional immunosuppressive therapy.

Patients undergoing immunosuppressive therapy face an elevated risk of developing infectious complications. The types of opportunistic infections seen with mycophenolate mofetil therapy are similar to those associated with azathioprine. Among these, cytomegalovirus (CMV) infection, encompassing both tissue-invasive disease and viremia, stands out as the most frequently observed, occurring in 186 out of the 910 patients examined in various studies. Additional infections noted during mycophenolate mofetil treatment include herpes simplex (14%), herpes zoster (5%), and candidal infections (1.9%). While cases of Pneumocystis pneumonia (PCP) and other fungal complications are rare, the risk of infection may correlate with the dosage of mycophenolate mofetil.

Data from large U.S. and European studies indicate that herpes simplex and tissue-invasive CMV infections were more prevalent at a dosage of 3 g/day compared to 2 g/day. Additionally, an increased risk of CMV infection has been associated with higher levels of 12-hour mycophenolic acid area under the curve (AUC). Notably, the 3-g/day dosage did not exhibit a definitive increase in other opportunistic infections such as herpes zoster and candida in these trials. However, patients concurrently receiving mycophenolate mofetil and muromonab-CD3 may face an elevated risk of infection.

Purine biosynthesis involves two primary pathways. In the de novo pathway, active in T and B lymphocytes, 5-phosphoribosyl-1-pyrophosphate (PRPP) is transformed into inosine monophosphate, which is then converted to guanosine monophosphate (GMP) by the rate-limiting enzyme inosine monophosphate dehydrogenase (IMPDH). Guanosine triphosphate (GTP) is subsequently generated, participating in DNA synthesis. Mycophenolic acid acts as a potent, selective, and reversible noncompetitive inhibitor of IMPDH. This inhibition leads to a depletion of intracellular guanosine nucleotide pools while leaving adenosine triphosphate (ATP) pools unaffected. The decrease in GTP production slows down the attachment of saccharide moieties to glycoproteins expressed on certain adhesion molecules, which in turn recruit monocytes and lymphocytes to sites of inflammation and graft rejection. Additionally, GMP can be produced outside lymphocytes through a salvage pathway involving PRPP and guanine, which are converted to GMP via hypoxanthine-guanine phosphoribosyltransferase.

Furthermore, mycophenolic acid inhibits the proliferation of T and B lymphocytes and dampens humoral immune responses by B lymphocytes. Notably, mycophenolic acid does not hinder the production of cytokines such as interleukin-1 and interleukin-2 in humans.

Effects of MMF on T lymphocytes

MPA exhibits a cytostatic effect by inhibiting the proliferation of human T and B lymphocytes, which is approximately fivefold more potent on lymphocytes compared to fibroblasts and other cell types. This effect aligns with MPA's inhibitory actions on the two isoforms of IMPDH. MMF, as a consequence, suppresses the generation of cytotoxic T lymphocytes and the elimination of allogeneic cells, making it a commonly used medication for preventing allograft rejection.

Moreover, MMF is anticipated to suppress T lymphocyte-mediated immunopathogenetic processes in conditions like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and similar disorders. For instance, IL-15, a significant T cell growth factor expressed in RA synovial tissue, prompts T-cells to activate cells of monocyte-macrophage lineage, leading to the production of TNFa. Another cytokine, IL-17, derived from T cells, exhibits pro-

inflammatory effects and contributes to joint erosion in arthritis mouse models. While this cytokine is found in the synovial fluid of RA patients, its specific role in the pathogenesis of arthropathy in RA or SLE remains uncertain.

There is emerging evidence suggesting that T cell proliferation and their products play a role in the pathogenesis of inflammatory skin conditions. For example, IL-15 is implicated in psoriasis, while the T cell-derived cytokine IL-31 is associated with atopic and non-atopic dermatitis. It is also suspected that T lymphocyte proliferation and mediator production may contribute to the development of malar and discoid rashes in SLE, although further investigation is needed to confirm this hypothesis.

Effects of MMF on B lymphocytes

Antibodies directed against donor antigens play a pivotal role in graft rejection. In the context of systemic lupus erythematosus (SLE), autoantibodies represent a hallmark feature and can manifest years before the onset of clinical symptoms. Over time, these autoantibodies reach a critical threshold, precipitating the development of clinical disease. In addition to producing autoantibodies, B cells possess the ability to internalize and present antigens to T cells. Even in scenarios where B cells fail to generate antibodies, they remain essential for the emergence of an SLE-like syndrome in mice with a genetic predisposition.

The enduring involvement of B cells in established autoimmune rheumatic diseases is underscored by the therapeutic efficacy of interventions targeting these cells. Encouraging early findings have been observed with a mouse/human monoclonal antibody directed against CD20, a B cell-specific antigen, in rheumatoid arthritis (RA). Combinations of rituximab with high-dose steroids, along with either methotrexate or cyclophosphamide, have yielded a notable 50% response rate according to the American College of Rheumatology (ACR) criteria in seropositive RA patients. Moreover, in patients with SLE, the administration of rituximab alongside high-dose steroids and cyclophosphamide has demonstrated improvements in overall lupus activity for most individuals.

MPA exerts inhibitory effects on the proliferation of human B cells, as illustrated in Figure 2, and suppresses the in vitro formation of specific antibodies by human spleen cells. Notably, MPA demonstrates greater efficacy in suppressing secondary antibody responses compared to cyclosporin A. Therapeutic doses of MMF in rodents significantly dampen primary antibody

responses, as depicted in Figure 3. While the extent of MMF-induced suppression of ongoing human antibody production is not fully elucidated, there is evidence suggesting its occurrence.

In a subset of organ graft recipients, chronic rejection is correlated with the presence of anti-HLA donor-specific antibodies. Notably, combined therapy involving MMF and tacrolimus has been shown to substantially reduce the production of donor-specific antibodies. The impact of MMF treatment on the levels of autoantibodies in systemic lupus erythematosus (SLE) is detailed in reports by other contributors to this supplement.

MMF suppresses the recruitment of lymphocytes and monocytes into sites of inflammation

As outlined previously, therapeutic levels of MMF lead to the depletion of GTP reserves in human lymphocytes and monocytes. The recruitment of leukocytes to inflammatory sites involves multiple stages, with firm attachment to endothelial cells (EC) being crucial, a process facilitated by selectins recognizing fucose-containing oligosaccharides. The significance of fucosylation is underscored by studies demonstrating that in mice lacking /(1→3) fucosyltransferase (due to genetic inactivation), selectin ligands are absent, impairing lymphocyte homing. Fucose and mannose are transferred to oligosaccharides via GDP-sugar intermediates. Depletion of GTP due to MMF treatment impedes this process, resulting in decreased adhesion of lymphocytes and monocytes to activated EC. This phenomenon was initially demonstrated in our laboratory and further substantiated by subsequent studies. Blaheta et al. developed sophisticated assays enabling the independent measurement of each stage in human lymphocyte recruitment, including attachment to different receptor molecules and allogeneic EC, as well as their penetration through EC. MPA was found to significantly suppress both the adhesion and penetration rates of CD4+ and CD8+ T cells in a dose-dependent manner. Maximal inhibition was achieved with 10 mM MMF, a concentration attainable therapeutically. MMF exhibited strong suppression of VCAM-1, E-selectin, and P-selectin expression, whereas cytokine-induced upregulation of ICAM-1 was only mildly reduced. Additionally, in another study, MMF was observed to inhibit human monocyte attachment to EC.

Evidence suggests that MMF reduces the recruitment of lymphocytes and monocytes to inflammatory sites in vivo, such as in cases of kidney allograft rejection. It is plausible to

hypothesize that MMF treatment would impede the infiltration of lymphocytes and monocytes into inflamed joints, kidneys, skin, blood vessels, and the brain of patients with SLE. MMF has been shown to diminish monocyte recruitment and mitigate glomerular injury in various rat models of renal disease, including experimental diabetic nephropathy.

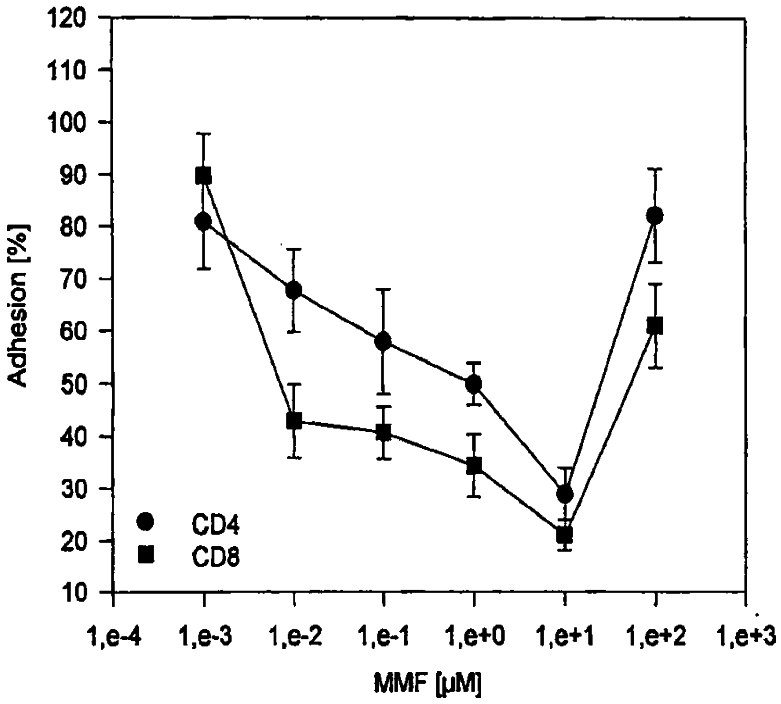

Figure 4 Effect of MMF on adhesion of T cells to activated endothelial cells.27

Effects of MMF on cytokine production

MMF is anticipated to reduce the production of pro-inflammatory cytokines TNFα and IL-1β, as cells of monocyte-macrophage lineage, which are major cytokine producers, are suppressed in their recruitment to sites of inflammation by MMF. Moreover, MMF prompts the terminal differentiation of macrophages, leading to decreased IL-1 production and increased secretion of the IL-1 receptor antagonist. In a rat model of chronic renal allograft rejection, MMF was observed to lower the expression of cytokine genes derived from lymphocytes and monocytes. While it is likely that MMF exerts similar effects on the kidneys, joints, and other tissues in SLE, this aspect has not been thoroughly investigated yet.

Monitoring

Patients prescribed mycophenolate mofetil should undergo regular monitoring for signs of organ rejection, infection, malignancy, and common adverse effects. Those experiencing gastrointestinal discomfort should be advised to take the medication with food, or alternatively, the dosing regimen can be adjusted to three times daily. In cases of gastrointestinal reactions, dosage reduction may be necessary.

Leukopenia occurs in 23.2% to 34.5% of patients receiving mycophenolate mofetil. The risk of leukopenia may be heightened when mycophenolate mofetil is administered concurrently with ganciclovir or other myelosuppressive agents. Additionally, there may be other bone marrow-suppressive effects. Therefore, regular complete blood counts are recommended to monitor for leukopenia and other hematological abnormalities.

Changes in plasma albumin levels have been linked to alterations in the concentration of unbound mycophenolic acid. The immunosuppressive efficacy of mycophenolate mofetil is directly correlated with the level of unbound mycophenolic acid, as inhibition of IMPDH activity increases with higher levels of free mycophenolic acid. Therefore, routine assessment of albumin levels is recommended for transplant patients receiving mycophenolate mofetil as part of their immunosuppressive regimen.

Mycophenolate mofetil is contraindicated in individuals with known hypersensitivity to the drug or mycophenolic acid. Although animal studies have not indicated an adverse effect on spermatogenesis with mycophenolic acid, there have been reports of failed implantation in female animals. Due to teratogenic effects observed in animal studies, women of childbearing age should use effective contraception while taking mycophenolate mofetil. Additionally, animal data suggest that mycophenolate mofetil may be excreted in breast milk, so women receiving the medication should be informed about potential risks to nursing infants. Currently, there is insufficient data on the effects of mycophenolate mofetil in pediatric patients to recommend its use in this population.

Dosage and administration

Mycophenolate mofetil, marketed as Cellcept by Roche Laboratories, is supplied in 250-mg capsules for oral administration. The manufacturer recommends an initial dose of 1 g twice daily. The average daily wholesale cost of this regimen is approximately $15.43. Mycophenolate mofetil therapy is typically used alongside cyclosporine and prednisone. Clinical trials have shown the effectiveness of mycophenolate mofetil at dosages of up to 3 g per day. However, higher doses have not demonstrated superior efficacy and may heighten the risk of adverse effects. Therefore, such doses are not recommended for patients with severe renal dysfunction (glomerular filtration rates below 25 mL/min). Presently, there are no specific guidelines regarding mycophenolate mofetil dosages for patients with moderate or severe renal dysfunction.

Although the ingestion of food may reduce peak plasma levels of mycophenolic acid, it does not affect the overall exposure to the drug (AUC). Therefore, mycophenolate mofetil can be taken with or without food. If neutropenia occurs, it may be necessary to interrupt mycophenolate mofetil therapy or adjust the dosage accordingly.

MMF is not fibrogenic

Understanding the effects of drugs used to prevent allograft rejection and treat systemic disorders like systemic lupus erythematosus (SLE) on collagen formation is crucial, given their impact on conditions such as atherosclerosis and nephropathies.

TNF-alpha (TNFα) and interleukin-1 (IL-1) are involved in recruiting fibroblasts and stimulating their proliferation, while transforming growth factor-beta (TGFβ) induces differentiation, including the expression of procollagen genes, in these cells. By reducing the recruitment of monocyte-macrophage lineage cells into affected tissues and organs, mycophenolate mofetil (MMF) is expected to decrease the production of TNFα and IL-1.

Calcineurin inhibitors like cyclosporin A and tacrolimus promote the expression of TGFβ in various cell types and in grafted kidneys, potentially contributing to fibrogenesis over time. However, MMF does not induce the expression of TGFβ. Progressive glomerulosclerosis observed in various types of renal diseases is associated with the activation of mesangial cells and excessive production of collagen and other matrix proteins. Mycophenolic acid, the active

metabolite of MMF, inhibits such activation of cultured human mesangial cells, potentially mitigating the progression of glomerulosclerosis.

Prevention of vascular disease by MMF

Calcineurin inhibitors are associated with adverse effects such as hypertension and increased cholesterol levels in the bloodstream, both of which are recognized risk factors for cardiovascular disease. Additionally, sirolimus treatment may lead to hypertriglyceridemia, a condition associated with diabetes mellitus. Conversely, hypertension and elevated serum lipid levels are not typically observed in patients undergoing treatment with mycophenolate mofetil (MMF).

Proliferative arteriopathy, a common manifestation of chronic allograft rejection, can be inhibited by MMF. Clinically relevant concentrations of MMF have demonstrated the ability to inhibit human arterial smooth muscle proliferation in vitro and in animal models of chronic rejection. In cynomolgus monkey recipients of aortic allografts, MMF treatment significantly suppressed graft vascular disease.

In systemic lupus erythematosus (SLE), accelerated atherosclerosis is a known complication facilitated by the oxidation of apolipoprotein-A1 (ApoA1) in high-density lipoprotein (HDL). ApoA1 normally helps prevent atherosclerosis by inhibiting LDL oxidation and facilitating the removal of cholesterol from macrophages. However, when HDL is oxidized, these protective functions are compromised. Mycophenolate mofetil (MMF) is expected to slow down the progression of atherosclerosis in SLE patients by inhibiting these oxidative processes, whereas other immunosuppressive agents may exacerbate it.

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