

Mycophenolate Mofetil: Clinical Update

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Since active clinical transplantation became a reality, physicians have been in constant conflict with the body's immunologic defenses. Steroids and azathioprine were the mainstay of immunosuppressive therapy for many years. During these years, graft survival was modest, with survival rates of 50% or less at one year for cadaver transplants. After the introduction of cyclosporine A in 1983, renal cadaver graft survival rates increased to 60–75%. Since that time, other immunosuppressive agents such as OKT3 and better patient management have increased 1-year graft survival rates well above 80%. Nevertheless, present immunosuppressive regimens remain toxic, nonspecific, and render the patient at increased risk of infection and lymphoproliferative disorders. Presently there exists no “magic bullet” that can render the immune system incapable of rejecting a graft while allowing the patient continued defense against infection. However, a new drug, mycophenolate mofetil (MMF; CellCept[®]; RS-61443) comes surprisingly close to this concept by emphasizing a unique mechanism of action.

PURINE METABOLISM IN LYMPHOCYTES

Mycophenolate mofetil is a fermentation product of several penicillin species. It has been shown to be a selective inhibitor of purine metabolism in lymphocytes. This discovery thrust MMF into the ongoing search for new and more specific immunosuppressive agents. Purine metabolism became the focus for the development of a new immunosuppressive agent when an adenosine deaminase deficiency in children was found to be associated with a decreased number and

function of T- and B-lymphocytes.¹ In contrast, the genetic defect in the major purine salvage pathway catalyzed by hypoxanthine guanine phosphoribosyl transferase (HGPRT) has essentially normal numbers of lymphocytes and the responses.²

Allison³ concluded that human purine salvage in lymphocytes plays a major role compared to other cell types. In resting lymphocytes, purine biosynthesis is low; however, when activated by antigens or mitogens, lymphocytes have greatly increased de novo purine synthesis.

There are two major pathways of purine synthesis⁴ (Fig. 1). In the de novo pathway, the ribophosphate PRPP synthetase and ribonucleotide reductase are allosterically regulated by nucleotides. In livers, PRPP synthetase is inhibited by adenosine nucleotides (AMP and ADP) and activated by guanosine nucleotides (GMP, ADP, and GTP).⁵ Catalytic activity of ribonucleotide reductase is decreased by the binding of dATP and stimulated by the binding of dGTP.⁶ Therefore, an excess of adenosine nucleotides and/or the depletion of guanosine nucleotides can

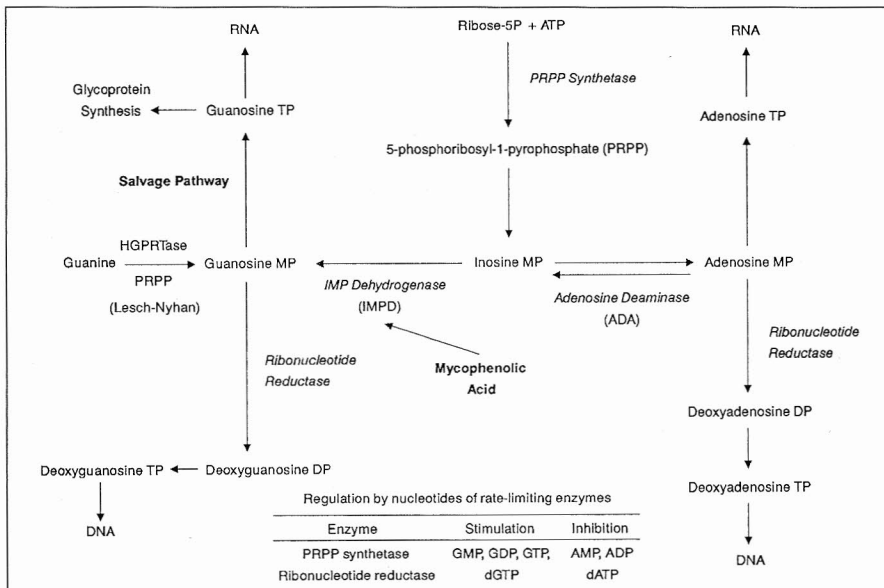


Figure 1. De novo pathways of purine biosynthesis, showing the central position of IMP. MPA inhibits IMP dehydrogenase, thereby depleting GMP, GTP, and dGTP. Two rate-limiting enzymes in lymphocytes are activated by guanosine ribonucleotides and dGTP, but inhibited by AMP, ADP, and dATP.

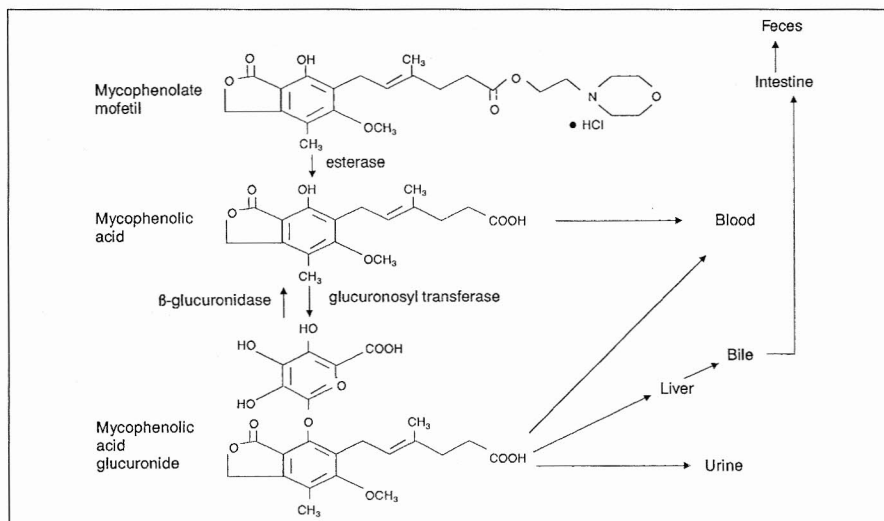


Figure 2. Structure of the morpholinoethyl ester of MPA (MM), MPA and its glucuronide, and sites of interconversion and excretion.

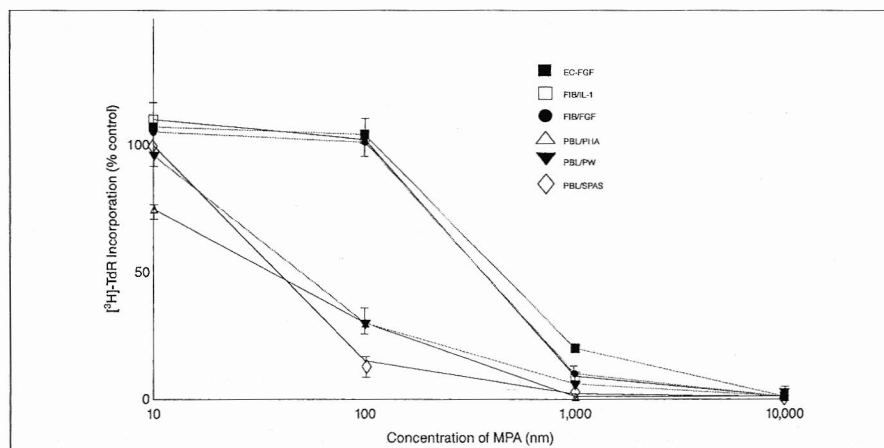


Figure 3. Potent inhibition by MPA of the proliferation of human peripheral blood lymphocytes (PBL) responding to stimulation by various mitogens. Higher concentrations of MPA are required to inhibit the proliferation of human dermal fibroblasts (FIB) and human umbilical vein endothelial cells (EC).

decrease the pool of PRPP, and thereby decrease the pool of substrates for DNA polymerase activity. Observations by Giblett⁷ and Allison^{8,9} in children with various purine salvage pathway deficiencies led to Allison's conclusion that de novo purine synthesis is essential for the proliferation of human T- and B-lymphocytes to mitogens.

In Figure 1, it can be seen that IMPDH plays a crucial role. By inhibiting IMPDH, a subsequent depletion of guanosine nucleotides and nucleosides occurs. MMF accomplishes this task.

MYCOPHENOLATE MOFETIL (RS-61443)

Mycophenolic Acid (MPA)

Mycophenolic acid (MPA; Fig. 2) selectively inhibits inosine monophosphate dehydrogenase (IMPDH) in a noncompetitive, reversible manner.^{10,11} MPA does not require phosphorylation to inhibit IMPDH, which unlike other nucleotide analogs, does not inhibit DNA repair enzymes or produce chromosomal breaks.

MPA has been shown to inhibit humoral and cell-mediated murine responses in mice.¹² Also, MPA has been found to be effective against psoriasis and rheumatoid arthritis.¹³⁻¹⁵

In 1982, Allison and Eugui at Syntex¹⁶ found MPA strongly inhibited responses of human lymphocytes to mitogenic stimulation and in mixed lymphocyte responses. Prior to this, MPA and its analogs had been studied for anti-tumor effects by Japanese investigators.^{17,18} They noted immunosuppressive activity, but these effects were not pursued.

Attempts at developing a stable synthetic derivative of MPA with good bioavailability were unsuccessful. Then, the morpholinoethyl ester of MPA (Fig. 3) was found to have improved bioavailability in primates compared to MPA.¹⁹ The ester is rapidly hydrolyzed to yield MPA both in human peripheral blood mononuclear cell cultures and in vivo. The ester of MPA is designated "RS-61443."

In vitro Effects of Mycophenolic Acid

MPA is a potent inhibitor of proliferative responses of human leukocytes.²⁰⁻²² Concentrates of MPA as low as 100 nM almost completely inhibit B-cell proliferative responses to pokeweed mitogen, a T-dependent B-cell mitogen (PWM), and abrogate antibody production.^{21,23} Both MPA and mycophenolate mofetil strongly inhibit proliferation of all human T- and B-lymphocyte cell lines tested.²³

Mycophenolate mofetil or MPA at 1 μ M can be achieved clinically and completely inhibits lymphocyte proliferation, but has no antiproliferative effects on fibroblasts or endothelial cells²⁰ (Fig. 4). In addition, similar concentrations (1–10 μ M) inhibit the proliferation of arterial smooth muscle cells in culture (Fig. 5), which may be clinically beneficial in the treatment of chronic rejection. Moreover, MPA in concentrations up to 10 mM/L do not demonstrably effect phagocytosis and killing bacteria by human neutrophils.²⁴

Another interesting aspect of MPA is its effect on adhesion molecules, which are important for cell-to-cell interactions. It has been established that nucleotide intermediates are necessary for the glycosylation of proteins and lipids. Glucose, galactose, and their amines are transferred to dolichol phosphates and then to protein through uridine-diphospho intermediates, whereas sucrose and mannose are transferred through guanosine-diphospho intermediates.²⁵ With MPA-mediated depletion of GTP, mannose and fucose transfer to glycoproteins is decreased. Some of these are adhesion molecules.

Studies by Muller et al.²⁶ demonstrated that one of the lymphocyte glycoproteins effected in VLA-4, the ligand for VCAM-1 on activated endothelial cells.²⁷ Also, treatment of either T-cells or IL-1 activated endothelial cells with MPA concentrations of 1–10 mM decreased lymphocyte attachment. In vivo, this effect may decrease recruitment of leukocytes into sites of ongoing rejection, even if clonal expansion has occurred.

In vivo Effects

As stated previously, the morpholinoethyl ester of MPA is rapidly hydrolyzed to yield MPA in vivo, the principle hepatic metabolite being the glucuronide, most of which is excreted in the urine. Eugui²⁸ demonstrated that intestinal beta-glucuronidase activity in the mouse hydrolyzes the glucuronide to MPA. Recycling of these two products via the enterohepatic circulation has been demonstrated.

Moreover, stimulated lymphocytes and macrophages have increased beta-glucuronidase activity, which implies MPA glucuronide may be hydrolyzed by these cells to release MPA at sites of inflammation and graft rejection.²⁹ Eugui¹² also showed that the antiproliferative effects of MPA are lymphocyte-selective in vivo. MPA-treated mice exposed to allogeneic tumor cells intraperitoneally had a dose-dependent reduction in generation of

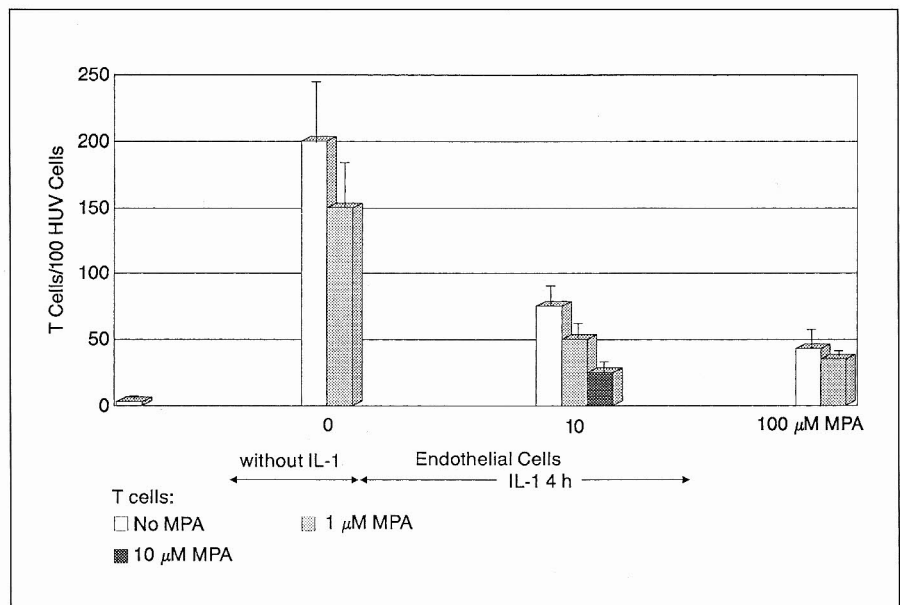


Figure 4. Graph showing that treatment of either T cells or IL-1 activated endothelial cells with MPA in therapeutically attainable concentrations (1–10 μ M) decreased lymphocyte attachment to the endothelial cells. HUV=human umbilical vein.

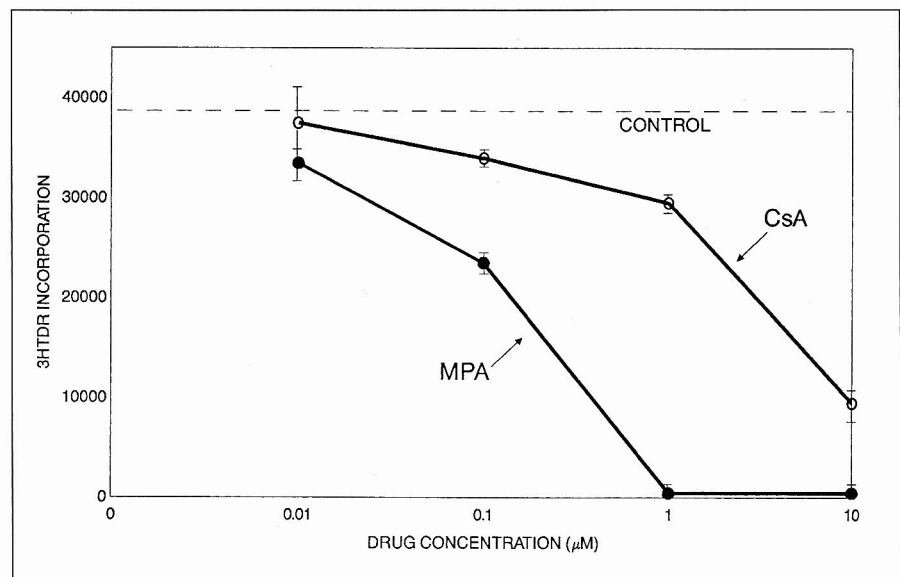


Figure 5. Effect of MPA and CSA on the proliferation of human arterial smooth muscle cells in culture.

cytotoxic T-lymphocytes. Viable tumor cells were recovered from the peritoneum during treatment, while untreated mice eliminated the tumor cells rapidly. Studies by Moss et al.^{30,31} in rat cardiac allograft models demonstrated tolerance with prompt rejection of third-party tissue in the same treated mice. Knechtle et al.³² investigated the possibility that MPA might be efficacious in inhibiting antibody formation.³³ He used male ACI rats as skin graft donors to Lewis rats. Three successful skin grafts were placed on Lewis recipients at 13- to 14-day intervals, which resulted in higher titers of complement fixing antibodies as measured by ⁵¹Cr re-

lease. Heterotopic rat cadaver allografts were then performed in sensitized animals. Study groups consisted of MPA at an oral dose of 20 mcg/kg/day, CSA at 10 mcg/kg/day, and a combination of the two.

Rejection in control rats occurred between 1 and 4 days postoperatively, whereas MMF increased median survival to 7 days. This was not significant; however, MMF in combination with CSA increased survival to 14 days. These experiments showed that (1) cytotoxic antibody titers of individual rats did not correlate with graft survival in any group; (2) possible mechanisms of effect include the

inhibition of recruitment of effector cells and alteration of the effector membranes of antibody-mediated rejection; and (3) rejection by sensitized lymphocytes may be more difficult to prevent than rejection by anti-donor antibody.

Platz et al.³⁴ showed MMF (20 mcg/kg/day) plus CsA (5 mcg/kg/day) and methylprednisolone (0.1 mcg/kg/day) were effective in prolonging canine renal allografts. No nephrotoxicity, hepatotoxicity, or bone marrow suppression was observed. In subsequent experiments, MMF reversed acute renal allograft rejection in some dogs.³⁵

Chronic rejection, unlike acute rejection, is a formidable problem for long-term graft survival.^{36,37} Chronic rejection is characterized by proliferation of smooth muscle cells and fibroblasts. This process begins in small- and medium-sized arteries, and later throughout the entire arterial system of heart, kidneys, and liver.³⁸ Lesions are characterized by concentric intimal thickening, whereas atherosclerotic lesions tend to be focal and asymmetric.

Billingham³⁹ postulated that this process is mediated by T-lymphocytes, while others believe antibodies against donor antigens are involved.³⁶ In all likelihood, both humoral and cellular membranes are involved. MMF may be effective in curbing chronic rejection by its inhibition of T-lymphocyte responses and inhibition of antibody formation. Morris et al.³¹ used rat heterotopic heart allografts as a model for chronic rejection. Neither moderate doses of CSA or tacrolimus were able to prevent graft coronary disease. When treated with MMF, long-term recipients had a low incidence and severity of proliferative arteriopathy. These findings were corroborated by Steele et al.⁴⁰

CLINICAL OBSERVATIONS

Phase I clinical trials were performed with escalating doses of MMF from 100 to 3,500 mg/day orally in combination with CSA and prednisone in patients receiving primary cadaver kidney transplants.^{20,41} Follow-up ranged from 2 to 9 months. Treatment was well tolerated, with GI complaints being the most prominent (mild ileus, gastritis, occasional nausea and vomiting). The fewest episodes of rejection occurred at doses of 2,000 mg/day or more.

A multicenter pilot rescue study⁴² for refractory kidney transplant rejection enrolled 75 patients who had failed

high-dose steroid or OKT3 therapy. Dosing was 1,000–1,500 mg p.o. b.i.d. Fifty-two of 75 (69%) patients were rescued. Those with an entering creatinine of 4 mg/dL or lower had better results (79% vs. 52% for those with a creatinine >4 mg/dL). Klintmalm⁴³ reported a multicenter liver trial for refractory rejection and showed an excellent response rate as well. Deierhoi et al.⁴⁴ reported results from a single center involved in a larger multicenter trial. Twenty-one patients were enrolled in the open-label, dose-ranging trial in primary cadaver transplants. Mean follow-up was 26 months. Patient and graft survival at 2 years was 100% and 95%, respectively. A second phase of the study assessed MMF's efficacy in reversing refractory renal allograft rejection. All patients had biopsy-proven rejection and had at least one course of high-dose steroid or OKT3 therapy. Of 26 patients enrolled, one (4%) was withdrawn for side effects. There were two deaths. Mean follow-up of 20 months showed patient and graft survival of 91% and 54%.

MMF's efficacy has also been shown in human cardiac cadaver grafts. Kirklin⁴⁵ showed a decrease in rejection episodes from 0.67 rejection episodes/patient/month before MMF to 0.27 rejection episodes/patient/month after MMF ($p < 0.0001$). There was no difference in the frequency of infection.

Sollinger et al.⁴⁶ conducted a randomized, double-blind multicenter study which enrolled 499 patients who received primary cadaver renal allografts. Patients were randomized into three groups: Group 1 (MMF 1.0 g b.i.d.); Group 2 (MMF 1.5 g b.i.d.); or Group 3 (azathioprine 1–2 mg/kg/day). Cyclosporine, corticosteroids, and antithymocyte globulin (ATGAM) were administered as part of a quadruple induction protocol. 47.6% of patients in Group 3 had biopsy-proven rejection episodes, compared to 31.1% in Group 1 ($p = 0.0015$) and 31.3% in Group 2 ($p = 0.002$). In addition, Group 3 patients received a greater number of full courses of antirejection treatment as compared to Group 1 and Group 2. Moreover, antilymphocyte agents were used more frequently in Group 3. The incidence and types of adverse events were similar in all groups, but there was a higher incidence of diarrhea in the MMF groups. Of note, the MMF groups had a higher incidence of lymphoma-lymphoproliferative disorder (3 patients). Sollinger concluded that MMF at doses of 2–3 g/day in

combination with cyclosporine and corticosteroids was more effective than present regimens.

CONCLUSION

MMF is a new immunosuppressant that acts by a unique mechanism. IMPDH is essential in the de novo pathway of purine synthesis. By inhibiting this enzyme, MMF inhibits both T and B cells. Experimental and clinical trials have conclusively proven MMF's efficacy in treating acute rejection and the possibility that it may be effective in chronic rejection. There has been no evidence of nephrotoxicity, hepatotoxicity, or bone marrow suppression. There was also no increased incidence in infectious complications.

MMF promises to reduce the incidence of rejection episodes and well may be a major drug in the battle against chronic rejection. **STI**

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