TRANSPLANTATION REVIEWS

Rapamycins: Antifungal, Antitumor, Antiproliferative, and Immunosuppressive Macrolides

Randall Ellis Morris

What we know is a drop. What we don't know is an ocean.
Isaac Newton

Progress in rapamycin (RPM) research has been rapid and is poised to accelerate even more dramatically. An Investigational New Drug application (IND) for phase I trials of RPM as a treatment for prospective graft recipients was approved less than 2 years after the first published reports[1,2] and public disclosure[3] of the ability of RPM to prolong graft survival in experimental animals. RPM is a macrolide fermentation product that has antifungal and antitumor activity. However, its effects on the immune system have generated the most interest because RPM is structurally similar to another new immunosuppressive macrolide, FK506. RPM is particularly intriguing because it inhibits the activation of immune cells by unique, relatively selective, and extremely potent and highly effective mechanisms. For example, one half microgram of RPM administered daily to mouse recipients of completely mismatched heart allografts prolongs graft survival. When these mice are treated for only 2 weeks with higher doses of RPM, or when a single dose of RPM is administered to rat heart allograft recipients, strain-specific unresponsiveness is induced, and grafts survive indefinitely in both species.

The research on RPM is representative of a significant shift in emphasis in transplantation from the macrocosmic world in which innovative surgical techniques predominated from the 1950s through the 1970s to our current focus on the microcosm of cellular and molecular immunopharmacology. A revolution in the discovery, development, and clinical use of new strategies to control the immune response is clearly upon us: it took more than 35 years to accrue the four imperfect mainstays of immunosuppression for transplantation—steroids, azathioprine, anti–T-cell antibodies, and Cyclosporin A (CsA). In 1992, six new xenobiotic immunosuppressants will be in clinical trials (Fig 1).

This new era in immunosuppression can be traced to the convergence of several lines of research: (1) the discovery and successful clinical use of CsA; (2) an increased understanding of the fundamental biology of immune cells that enables the actions of different immunosuppressants to be better understood and thus lay the foundation for more rational means to discover, develop, and use improved drugs; and (3) organized preclinical research programs designed to identify potentially valuable immunosuppressants and to generate the knowledge needed for these agents to be used intelligently in the clinic. Figure 2 shows the research program used for several years in the Laboratory for Transplantation Immunology at Stanford University that enabled us to identify RPM[5-11] and the morpholinomethyl ester of mycophenolic acid (MPA)[12-16] as immunosuppressants for graft rejection. The mechanisms of action and immunopharmacology of these two compounds, as well as FK506,[17-19] deoxyspergualin (DSG),[20,21] and brequinar sodium (BQR)[22] have also been studied and compared with one another in our laboratory.

Our spectrum of experimental systems begins with in vivo mouse models that are so rapid, quantitative, and inexpensive that we have been able to evaluate hundreds of molecules for suppression of alloimmunity. The vast majority of these drug candidates fail during testing in rodents because they lack efficacy or safety, and they are discarded quickly so that our resources can be concentrated on compounds with the greatest potential. Compounds that show promise are evaluated further in rodent models to identify those with the following ideal characteristics: (1) unique mode of action; (2) high efficacy for the prevention or treatment of acute, accelerated, or chronic rejection; and (3) low toxicity. This Darwinian selection process accomplishes two tasks: first, it insures that only the agents with the greatest poten-

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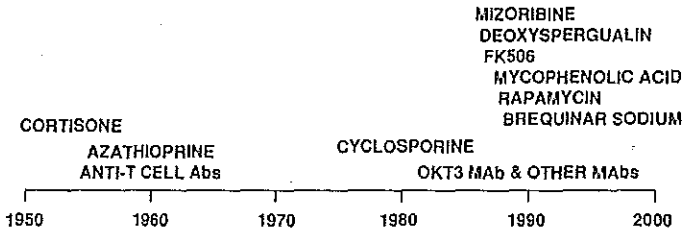

Figure 1. History of the use of drugs used to control graft rejection. All of the following xenobiotics recently discovered to suppress graft rejection in preclinical models have advanced to clinical trials: the antimetabolites such as mizoribine (MZR), MPA in its prodrug form of RS-61443, and BQR; the cyclosporine-like drug FK506, and drugs that define two new classes of immunosuppressants, DSG and RPM.

tial are advanced to the expensive nonhuman primate transplant model; and second, it prepares us to be able to use these compounds intelligently in nonhuman primates. The nonhuman primate model is important because it is highly predictive of the safety and efficacy of a test drug in humans. The sum of all knowledge produced from well-planned preclinical studies is the essential foundation from which successful clinical trials are designed and executed. New drug development is a highly complex, multidisciplinary task, and our contribution to the development and clinical use of new immunosuppressants depends on very close collaboration with scientists and clinicians in the pharmaceutical industry.

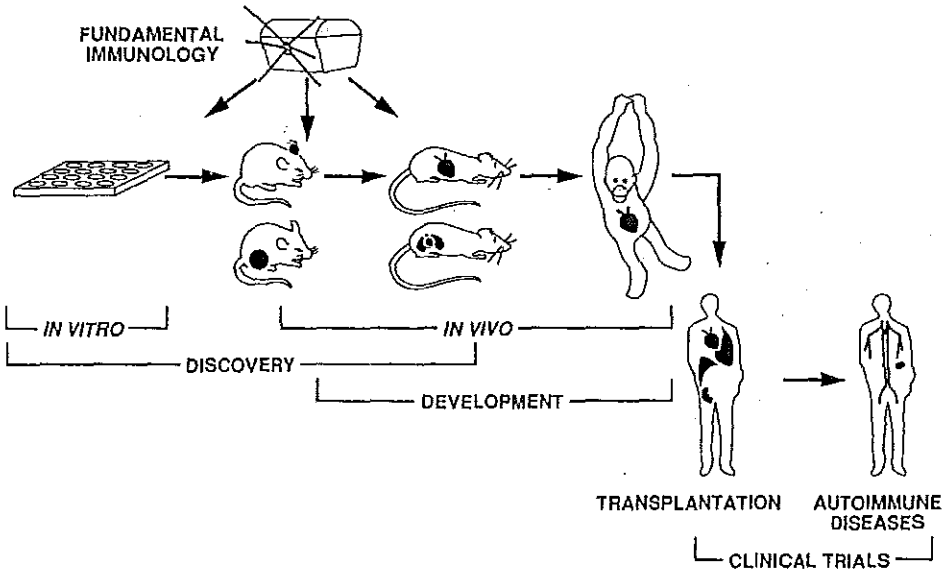

Figure 2. Schematic representation of the program used at the Laboratory of Transplantation Immunology at Stanford University to identify compounds with immunosuppressive activities for transplantation and to develop these compounds for clinical use for the prevention and treatment of rejection. Fundamental knowledge of the immune system coupled with an appreciation of the characteristics of the drug candidate is used to design experiments to profile the activity of the compound and define its mechanisms of action. Heterotopic transplantation of neonatal mouse heart allografts into the ear pinnae of mouse recipients and alloantigenic and mitogenic stimuli of popliteal lymph node hyperplasia are used as rapid and quantitative bioassays before proceeding to the more laborious techniques of primarily vascularized heterotopic (abdominal) and secondarily vascularized heterotopic (subrenal capsule) heart allograft and xenograft transplantion in the rat. Assessment of the efficacy and the safety of the compound in cynomolgus monkey recipients of heterotopic allografts precedes phase I clinical trials in transplant patients and patients with autoimmune diseases.

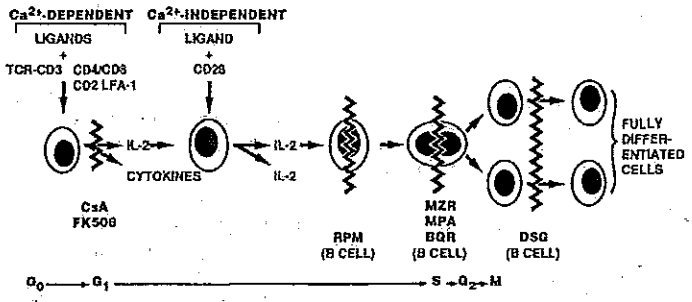

Figure 3. Schematic representation of the possible sites of action of the following immunosuppressants on activated T cells: CsA and FK506 prevent the transcription of early phase cytokine genes; RPM inhibits the signal transduction of IL-2 bound to its receptor and may have other antiproliferative effects unrelated to lymphokine signals; MZR, MPA, and BQR all inhibit purine (MZR, MPA) or pyrimidine (BQR) nucleotide synthesis; DSG seems to inhibit late stages of T-cell maturation. RPM, MZR, MPA, BQR, and DSG also act on activated B cells at the sites shown.

Even more important than the relatively large number of new immunosuppressants that have been discovered is their variety. Each of these new molecules suppresses the immune system by blocking distinctly different biochemical reactions that initiate the activation of immune cells that cause the many forms of graft rejection (Fig 3). Briefly, CsA and FK506 act soon after Ca^{2+}-dependent T-cell activation to prevent the synthesis of cytokines important for the perpetuation and amplification of the immune response.[23-25] RPM acts later to block multiple effects of cytokines on immune cells including the inhibition of interleukin-2–(IL-2–)triggered T-cell proliferation,[26-31] but its antiproliferative effects are not restricted solely to T and B cells. RPM also selectively inhibits the proliferation of growth factor-dependent and growth factor-independent nonimmune cells. Mizoribine (MZR),[32] MPA,[33] and BQR[34] are antimetabolites that inhibit DNA synthesis primarily in lymphocytes. These new antimetabolites are more selective than azathioprine because these compounds block the activity of enzymes restricted only to the de novo purine or pyrimidine biosynthetic pathways. Lymphocytes are more dependent on these pathways for nucleotide synthesis than other cells.

Recent reviews[35-38] discuss these and other immunosuppressants. RPM has recently been the subject of four brief reviews,[39-41] a long review,[42] and has been included in reviews that have primarily focused on FK506.[43-45] This review provides a complete profile of RPM from work published through the end of August 1991. Despite the progress made in understanding RPM since the first publication on this compound in 1975,[46] the description of its ability to suppress graft rejection has stimulated renewed interest by a wide variety of investigators whose work has not yet been published. As a result, research on macrolide immunosuppressants has become fluid and extremely fast-paced. Because unpublished data generally are not available for evaluation, I have not referred to unpublished work or personal communications. However, I have relied on many studies of RPM from the Laboratory of Transplantation Immunology at Stanford University that have yet to be published in full. In most of these cases, I have supplied the data from which conclusions in the text are drawn.

Because this review is being written relatively early in the research life of RPM, and because the majority of the work on this complex molecule has yet to be published, the material subsequently presented should be regarded more as a preview rather than as a review. At the very least, this article will provide a logical framework that other investigators can use to organize and to evaluate new information on RPM as it is published. For many investigators with highly specialized interests, only selected sections will be of use. For others, it is essential to understand all that is known about a new and unique molecule such as RPM. Without an understanding of RPM that is both deep and broad, it will be difficult to meet the challenging tasks of using RPM as a tool to learn more about the immune system, maximizing its therapeutic potential, and discovering new and improved members of this class of immunosuppressant. If we strive to understand thoroughly the little that is now known about RPM, we will make more efficient and rapid progress toward our goal of understanding all of the important biological effects of this molecule.

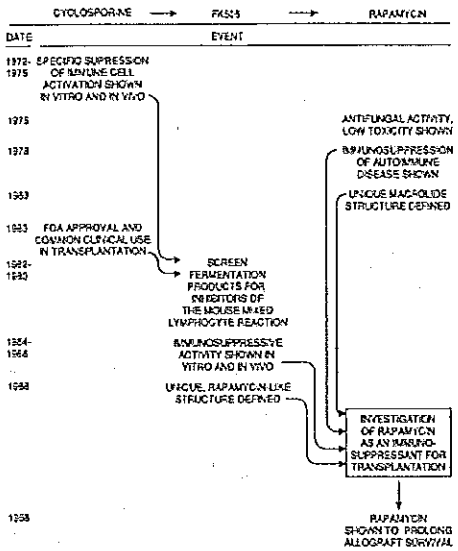

Figure 4. Evolutionary path of RPM as an immunosuppressant for transplantation.

In addition to reviewing the information on RPM, this article warns of the danger of inductive reasoning in which, in an adolescent field like immunology, arguing from highly specific cases to general laws often promotes the illusion of knowledge rather than its true acquisition. However, by interrelating information concerning the structure, the molecular mechanisms, and the actions of RPM on defined cell types in vitro, its effects in vivo, as well as its disposition in the body and its toxicity, new and important insights into the actions of RPM can be gained. In general, the conceptual tools used in this review to analyze the data from experiments on RPM can be applied to the study of many other immunosuppressants, especially other xenobiotics.

Before dissecting and examining every aspect of RPM in detail, it is worth reviewing the events that led to the attention RPM is now receiving. Figure 4 shows the relationship of the evolution of RPM as an immunosuppressant to the development of CsA and FK506 as immunosuppressants. Table 1 provides a more detailed outline of the sequence of the main events that have defined progress in RPM research in its first 15 years.[14-27,29,30-32] The ancestors of RPM are CsA and FK506. As shown in Fig 4, CsA stimulated the organization of a rational screening program designed to discover other fermentation products with mechanisms of immunosuppressive action identical to CsA. The discovery of FK506 was the product of this program,[33] and when the structure of FK506 was defined, its similarity to the structure of RPM was immediately recognized.[34] Years before, the structure of RPM had been determined as a conse-

Table 1. History of RPM Drug Development: The First 15 Years

Discovery	*Year*	*References*
Isolation from Easter Island (Rapa Nui) soil sample and characterization of antimicrobial activity	1975	Vezina, Kudelski, and Sehgal[16] Sehgal, Baker, and Vezina[17]
In vivo use: Toxicity Pharmacokinetics Bioavailability Antifungal activity	1978	Baker, Sidorowicz, Sehgal, et al[18]
Immunosuppression of autoimmune disease	1977	Martel, Klicius, and Galet[19]
Elucidation of structure	1980	Findlay and Radics[20]
Antitumor activity described	1981	Douros and Suffness[21]
Immunosuppression of allograft rejection		
RPM alone	1989	Morris and Meiser[1] Calne, Collier, Lim, et al[2]
RPM in combination with CsA	1990	Meiser, Wang, and Morris[3]
Differentiation of effects of RPM and FK506 on immune cells in vitro	1989	Tocci, Matkovich, Collier, et al[27]
	1990	Metcalfe and Richards[28] Dumont, Staruch, Koprak, et al[29]
Differentiation of effects of RPM and FK506 on immune system in vivo	1990	Morris, Wu, and Shorthouse[3]
Demonstration of binding of RPM to FK506 binding protein	1989	Harding, Galat, Uehling, et al[32]

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