

## STRUCTURE-FUNCTION RELATIONSHIP OF IMMUNOSUPPRESSIVE DRUGS: A CAUTIONARY TALE

Bierer BE, Somers PK, Wandless TJ, Burakoff SJ, Schreiber SL. Probing immunosuppressant action with a nonnatural immunophilin ligand. *Science* 1990;250:556-559.

### ABSTRACT

The immunosuppressants FK506 and rapamycin bind to the same immunophilin, FK506 binding protein (FKBP), and inhibit distinct signal transduction pathways in T lymphocytes. A nonnatural immunophilin ligand, 506BD, which contains only the common structural elements of FK506 and rapamycin, was synthesized and found to be a high-affinity ligand of FKBP and a potent inhibitor of FKBP rotamase activity. Whereas 506BD does not interfere with T cell activation, it does block the immunosuppressive effects of both FK506 and rapamycin. Thus, the common immunophilin binding element of these immunosuppressants, which is responsible for rotamase inhibition, is fused to different effector elements, resulting in the inhibition of different signaling pathways. Inhibition of rotamase activity is an insufficient requirement for mediating these effects.

### COMMENTS

Organ transplantation, despite numerous technical advances in surgical procedure, remains limited by graft rejection. The basis of rejection of foreign tissue or organ grafts arises from the immunological incompatibility of the donor and the recipient. This immunological incompatibility is determined by a system of tissue antigens termed the major histocompatibility complex (MHC), which is expressed on virtually all cells (1). The human MHC system is termed HLA and contains at least six expressed genetic regions that belong to one of two major classes: class I, which includes the HLA-A, HLA-B and HLA-C regions, and class II, which includes HLA-DR, HLA-DQ and HLA-DP. Class I genes are expressed on the surface of all nucleated cells, whereas class II genes are normally restricted in their distribution to antigen-presenting cells such as macrophages. Each locus is extremely polymorphic. From 6 to 30 distinct alleles have been discovered at any one locus, and thus two randomly chosen individuals are unlikely to be "HLA-identical." T cells in the transplant recipient recognize and react to the foreign HLA antigens present on transplanted tissues. These target antigens are readily

available to T cells because class I MHC antigens are constitutively expressed on the surface of all nucleated cells. Furthermore, activation of many cell types (by inflammatory lymphokines or even by the "trauma" of transplantation) increases class I expression and can also induce the *de novo* expression of class II MHC genes, processes that may increase the immunogenicity of the transplanted organ (2).

The immunological response to foreign HLA antigens is orchestrated by T cells through the production of lymphokines. When activated, T cells secrete a number of lymphokines including interleukin 2 (IL-2). IL-2, in turn, supports the initial clonal expansion of lymphokine-secreting helper T cells and cytotoxic T cells. Together, IL-2 and other lymphokines, including tumor necrosis factor, interferon- $\gamma$  and IL-4, promote the activation and proliferation of mature effector cells such as monocytes and cytotoxic T cells and also induce B cells to generate specific opsonizing and complement-fixing antibodies. In the absence of immunosuppression, these events proceed vigorously and rapidly, leading to destruction of the transplanted tissue within days to weeks.

For organ transplantation to be successful, some form of suppression of the host immune response is necessary. Initial studies performed with organic solvents and nitrogen mustards showed that these substances were effective at prolonging allograft survival, but they were too toxic for routine use. The development of 6-mercaptopurine (of which azathioprine is a derivative) and its successful use in 1959 ushered in the modern era of immunosuppression. From then until the early 1980s, the combination of azathioprine and prednisone was the mainstay of pharmacological immunosuppression. However, both agents are relatively nonspecific, and the subsequent discovery and development of cyclosporine represented a significant advance in both specificity and efficacy.

Cyclosporine, a fungal polypeptide, acts as an immunosuppressant by inhibiting helper T cells from producing IL-2 and other lymphokines (3). This prevents graft rejection by depriving immune effector cells of lymphokines required for their clonal expansion and/or activation. Whereas cyclosporine represents a significant advance in therapy, its ability to suppress the immune system is incomplete. In addition, cyclosporine therapy has been associated with a number of side effects, particularly nephrotoxicity, that can limit its use. Thus the search for new immunosuppressants continues, focusing on agents with nonoverlapping toxicities and on those with distinct mechanisms of action. In particular, the latter might be useful in

designing combination regimens that block distinct phases of the immune response. To this end, much research is directed toward determining the mechanism of action of these drugs, and these studies can also lead to important insights into the physiological processes of the immune system. For example, in 1984, Handschumaker et al. (4) described a cyclosporine-binding protein present in the cell cytosol termed cyclophilin. Cyclophilin, or its closely related isoforms, is found in virtually all mammalian cell types and in fungi, yeast and plants. Subsequent studies demonstrated that cyclophilin was a cis-trans peptidyl-prolyl isomerase, an enzyme that catalyzes the interconversion of the cis-rotamers and trans-rotamers of peptidyl-prolyl amide bonds, thus assisting in protein folding (5, 6). Cyclosporine blocks the isomerase activity of cyclophilin, an effect that is necessary but not sufficient for its immunosuppressive activity. How this leads to the inhibition of IL-2 gene transcription is not yet known.

Recently, a number of studies have been published that investigate the mechanism of action of two relatively new agents, FK 506 and rapamycin. Together, they present a cautionary tale. Although structurally dissimilar, FK 506 appears to act in a very similar fashion as cyclosporine, blocking IL-2 gene transcription after T-cell activation (7). Furthermore, FK 506 binds to a cytoplasmic enzyme (FK 506-binding protein or FKBP) distinct from cyclophilin but that also acts as a cis-trans peptidyl-prolyl isomerase (7). Like cyclosporine, FK 506 blocks the rotamase activity of its binding protein, and neither drug affects the activity of the other rotamase. On the basis of this data, one might predict that cyclosporine and FK 506 would have additive effects *in vivo*, and this has indeed been shown in preliminary studies of murine skin and canine renal allografts (8, 9).

The discovery of FK 506 renewed interest in rapamycin, which, like FK 506, is a macrolide antibiotic with immunosuppressant properties. However, despite this structural similarity rapamycin has a distinct site of action: blockade of the response to IL-2 without interfering with IL-2 production (7). The fact that rapamycin acted at a phase of the immune response distinct from that of cyclosporine or FK 506 suggested that it might have synergistic effects when used *in vivo* in combination with either of these agents. This is probably true for cyclosporine; however, the combination of FK 506 and rapamycin appears to be more complicated. Despite their distinct modes of action, FK 506 and rapamycin can act as reciprocal antagonists *in vitro* (10). This finding, coupled with their common chemical structure, led to the suggestion that FK 506 and rapamycin might share a common intracellular binding protein, thereby accounting for their reciprocal antagonism, and this has now been confirmed.

In this article, Bierer et al. have built on these observations by synthesizing an analog of FK 506 termed 506BD. In synthesizing 506BD, the authors attempted to retain the rotamase-binding domain of FK

506 but eliminate additional structural elements. Like FK 506, 506BD also bound to FK 506-binding protein and blocked its rotamase activity; however, 506BD did not interfere with T-cell activation *in vitro* or the production of IL-2. Previous work by this group demonstrated that rapamycin also blocks FKBP rotamase activity, even though rapamycin does not affect IL-2 gene transcription (11). These findings demonstrate that inhibition of the rotamase activity of FK 506-binding protein is by itself insufficient to mediate the biological activity of FK 506.

Bierer et al. next investigated whether their inactive compound, 506BD, could block the activity of FK 506 or rapamycin. When added at a 100-fold excess, 506BD was able to block the immunosuppressive action of FK 506, presumably by displacing FK 506 from its binding protein. As expected, because they bind to different rotamases, 506BD had no effect on the ability of cyclosporine to inhibit the same responses (T-cell proliferation, IL-2 production). Importantly, 506BD also blocked the immunosuppressive effects of rapamycin, although again a 100-fold excess of 506BD was required. These data suggest a model whereby FK 506 and rapamycin each bind to FK 506-binding protein at the same site using similar structural motifs but that the resulting drug-protein complexes use additional and functionally distinct domains to exert their effects on different target molecules. These target molecules would regulate different signal transduction pathways, one initiated on T-cell activation that induces the transcription of lymphokine genes such as IL-2 and one that results in cell proliferation as a result of IL-2 binding to its cell surface receptor.

The identification of cyclophilin as a protein with cis-trans peptidyl-prolyl rotamase activity seemed curious at the time because no role for this activity in lymphokine gene induction was known. It was similarly surprising that both FK 506 and rapamycin bound to a protein with similar activity, particularly when they had distinct effects. The demonstration by Bierer et al. that inhibition of rotamase activity is apparently by itself insufficient for the action of these agents solves one problem but raises another. That is, how does the drug-rotamase complex interfere with T-cell responses?

Even at high concentrations of rapamycin or FK 506, most FKBP remains unbound by the drug, which is consistent with the finding that inhibition of rotamase activity is not itself the target action of rapamycin or FK 506 but rather that the drug-FKBP complex is responsible for the observed effects. Bierer, et al. also present evidence that whereas high concentrations of FK 506 and rapamycin are antagonistic of each others actions (blockade of IL-2 production or IL-2-dependent T-cell proliferation, respectively), at low concentrations FK 506 and rapamycin can have an additive effect on T-cell activation. The fact that FK 506 and rapamycin exert their effects at different points in the T-cell activation cascade (blockade of IL-2 production or IL-2-dependent T-cell proliferation, respectively), suggests that the FK 506-FKBP complex and the rapamycin-FKBP complex

have distinct intracellular targets. A recent report (Goebel MG, Cell 1991; 64:1051-1052, Correspondence), tentatively identifying FKBP as a previously described 12 kD endogenous inhibitor of protein kinase C, raises the possibility that FK 506 exerts its effect by blocking activation of protein kinase C, a key step in antigen-induced T-cell activation. One possible model integrating this report with the work of Bierer et al. would be that FK 506 must bind to FKBP to elicit the protein kinase C inhibitory capacity of FKBP. FK 506 might activate FKBP by a conformational change or by directing FKBP to the proper subcellular compartment. In either case, only the FK 506-FKBP complex is effective. Presumably, the rapamycin-FKBP complex acts in a different fashion or at a distinct intracellular site.

These studies highlight how much has been learned in recent years of the molecular events initiated by T-cell activation. They also underscore the complexity of the immune response and the risks of predicting drug effects based solely on chemical similarities. Furthermore, the difficulty in determining whether drugs with apparently distinct effects *in vitro* will be useful in combination should serve to emphasize that there is no substitute for rigorous *in vivo* testing. Ultimately, the goal of transplantation research is to induce a state of tolerance, or immunological unresponsiveness, to the graft. Although this remains an elusive goal, studies such as these advance us one step further by dissecting intracellular events that occur during the immune response.

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#### SPLIT-LIVER TRANSPLANTATION: ONE PLUS ONE DOESN'T ALWAYS EQUAL TWO

*Emond JC, Whittington PF, Thistlewaite JR, Cherqui D, Alonso EA, Woodle IS, Vogelbach P, et al.* Transplantation of two patients with one liver: Analysis of a preliminary experience with "split-liver" grafting. Ann Surg 1990;212:14-22.

#### ABSTRACT

**Surgical reduction of donor livers to treat small children has been performed successfully in several centers. While this procedure improves the allocation of livers, it does not increase the organ supply. We have extended reduced-size orthotopic liver transplantation (OLT) to treat 18 patients with 9 livers, accounting for 26% of our transplants during a 10-month period and have evaluated the results. In 18 split liver OLTs, patient survival was 67% and graft survival was 50%. In comparison, for 34 patients treated with full-size OLT during the same period, patient survival was 84% (p = 0.298) and graft survival was 76% (p = 0.126). Biliary complications were significantly more frequent in split grafts, occurring in 27%, as compared to 4% in full-sized grafts (p = 0.017). Primary nonfunction (4% versus 5.5%) and arterial thrombosis (6% versus 9%) occurred with similar frequency in split and full-size OLT (p = not significant). These results demonstrated that split-liver OLT is feasible and could have a substantial impact in transplant practice. We believe that biliary complications can be prevented by technical improvements and that split-liver OLT will improve transplant therapy by making more livers available.**

*Broelsch CE, Emond JC, Whittington PF, Thistlethwaite JR, Baker AL, Lichtor JL.* Application of reduced-size liver transplants as split grafts, auxiliary orthotopic grafts, and living related segmental transplants. Ann Surg 1990;212:368-375.

#### ABSTRACT

**The University of Chicago program in pediatric liver transplantation continues actively to seek innovative surgical solutions to problems related to the management of children with end-stage liver disease. Among the most important problems facing these children is a shortage of donor organs, which results from three factors in addition to the actual supply of**