New Techniques for Selective Immune Suppression Increase Transplant Odds

Cyclosporin A, antilymphocyte serum, and lymphoid irradiation help transplants survive without exposing the recipient to hazards of infection

The greatest problem in organ transplantation is not suppressing the recipient's immune rejection of the donor organ. Rather, it is suppressing that rejection without destroying the patient's capacity to withstand virulent infections. Most deaths among organ recipients, in fact, arise from pneumonia and similar infections rather than from rejection of the donor organ. Recently, however, at least six different drugs and techniques have begun to show promise of selectively suppressing rejection while allowing the recipient to retain some immune function to fight off infections.

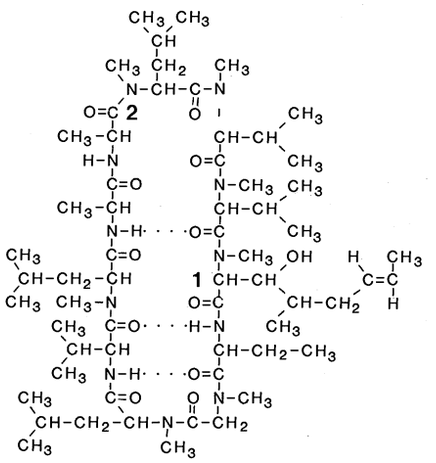
One of the new techniques is transfusion of the recipient with blood prior to kidney transplantation (*Science*, 8 August, p. 673). One involves a new drug, another involves the use of antilymphocyte serum, and a third involves irradiation of the recipient's lymphoid system.

The immune system has two major functional branches, namely cell-mediated immunity and humoral immunity. Cell-mediated immunity is effected by T or thymus-dependent lymphocytes that act directly to incapacitate or destroy tissues bearing foreign antigens or invading microorganisms. T lymphocytes play a part in transplant rejection, immune surveillance, delayed hypersensitivity reactions (as when a protein is injected under the skin), and resistance to viral infections. Humoral immunity results from the production of soluble antibodies by plasma cells, which are derived from B or bone marrow lymphocytes. Antibodies are the first line of defense against many bacterial infections. Other important components of the immune system include macrophages (large, mobile cells that can engulf and destroy foreign matter, including other cells) and a variety of materials secreted by both T and B cells.

Ideally, the transplant surgeon would like to suppress the production of T lymphocytes, especially those whose formation has been stimulated by the foreign tissue, without affecting the humoral system. But conventional methods for suppressing the immune system, such as the administration of steroids, suppress both cell-mediated and humoral immunity. All of the new approaches promise a more selective suppression of the immune reaction, but in each case the precise mechanism is not fully understood.

Possibly the most exciting development for many transplantation immunologists is the use of cyclosporin A, which was discovered by Sandoz Pharmaceuticals of Basel, Switzerland, in 1972. A metabolite of the fungus *Trichoderma polysporum* (Link ex Pers.) Rifai, cyclosporin A is a cyclic polypeptide composed of 11 amino acids. One amino acid (labeled **1** in the diagram) has not been observed in other organisms; one alanine (marked **2**), furthermore, exists in the *dextro-* form rather than the *levo-* form found in most living organisms. The exact mechanism of action of cyclosporin A is not known, but the antibiotic has been shown to be most active against T cells that have been triggered by antigens on the graft. B lymphocytes are almost completely spared.

Sandoz has made the drug available for clinical trials to a small group of transplant surgeons in Britain, France, Canada, and the United States during the past 3 years. Among those who have had the greatest amount of experience with it are Roy Y. Calne of Addenbrooke's Hospital in Cambridge, England, and Thomas E. Starzl of the University of Colorado Health Sciences Center. Calne, using cyclosporin A as the primary tool to suppress rejection, has given 65 cadaver kidneys, livers, and pancreases to 58 patients. Starzl, who has been using it for a somewhat shorter time, has transplanted 65 cadaver kidneys and ten livers. Both say that the antibiotic is the most effective drug they have used. Particularly impressive, Starzl says, is the fact that patients are released from the hospital in less than one-quarter of the time required for conventional therapy.



The structure of cyclosporin A as determined by M. Dreyfus of Sandoz, Ltd.

None of the organs transplanted by Calne and only two of those transplanted by Starzl have been rejected. Three of Calne's patients developed malignant lymphoma, a form of cancer that has previously been associated with immunosuppression. Those cases were among his first group of patients, however, and no more cases have been observed since he revised the protocol to lower the dose of cyclosporin A and to use no steroids in conjunction with the drug. Starzl has had no cases of lymphoma among his patients and continues to use steroids (at 10 to 20 percent of the conventional dose) in conjunction with cyclosporin A. Beyond lymphoma, other side effects that all investigators have observed include mild cases of hair growth on the face and limbs, tremors, and toxicity to the kidney.

Other clinicians have had experiences nearly as good. Nicholas L. Tilney of Brigham and Women's Hospital in Boston has used cyclosporin A on nine kidney patients, with good results for six. He withdraws the new drug after 3 months, however, in favor of standard steroid maintenance therapy, partly to reduce the risk of lymphoma. Calvin R. Stiller and Paul A. Keown of University Hospital in London, Ontario, have been successful with five of six kidney patients who have received cyclosporin A.

The only investigator to obtain negative results with cyclosporin A in kidney patients is Paul Sweny of the Royal Free Hospital in London. Of 19 kidney recipients to whom he has given the drug, only eight have retained functioning organs. He has observed frequent and irreversible rejection crises in the patients and severe infections; two young women patients had massive facial hair growth. It is not clear why his results are so different from those of other investigators.

Another area where cyclosporin A has

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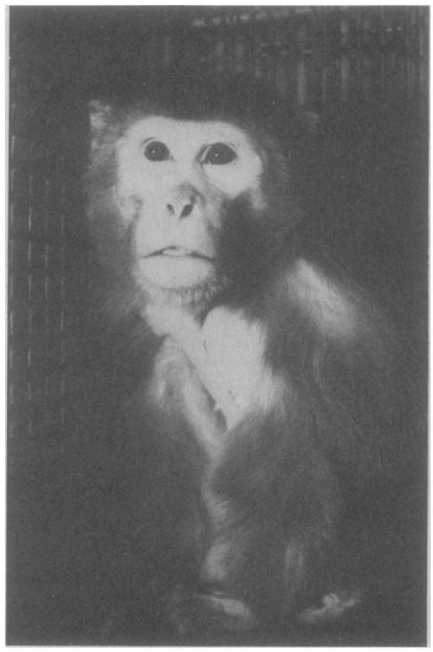
a great potential is heart transplants. Norman E. Shumway, Bruce A. Reitz, and their colleagues at the Stanford University Medical Center have successfully used the drug for heart transplants in rats and, more recently, monkeys. They have performed successful grafts in more than 40 monkeys; eight of the monkeys were later observed to have malignant lymphoma, but these monkeys were among the first transplanted and the surgeons think they now have the problem under control.

The Stanford group has also performed combined heart and lung transplants on five monkeys. This operation had previously been nearly impossible because of the difficulty of transplanting lung tissue and the propensity of sutured tracheas to become infected and not heal. One of the monkeys died of lymphoma after 5 months, but the rest are alive and well; the longest lived has now survived for nearly a year after surgery. Reitz says that cyclosporin A is the best agent for pulmonary transplants that the Stanford group has seen, and it may be the best agent for heart transplants. The group is now planning to begin a clinical trial of cyclosporin A for heart transplants, and possibly for heart and lung transplants, within the next few months.

The final verdict on cyclosporin A is not yet in, however. Investigators feel that at least another 100 kidney transplant patients will need to be studied before its efficacy can be determined precisely. Many heart patients will also need to be studied. It will thus be at least two more years before the true value of cyclosporin A is known for kidney transplants, and probably much longer for heart transplants.

An alternative way to suppress cell-mediated immunity selectively is to produce antibodies against T lymphocytes. These antibodies are known variously as antilymphocyte serums (ALS) or antilymphoblast globulins (ALG). Use of these antibodies when a rejection crisis occurs may remove from the recipient T lymphocytes sensitized against the foreign antigens of the graft and may thus leave the patient free of the effects of such lymphocytes for an extended period of time.

The effects of ALS were probably first noticed in the late 1960's by Sir Peter Medawar, and they have since been studied by a number of investigators. Antibodies against T lymphocytes usually have been produced in rabbits, but horses have been used to produce larger amounts. Among the first in this country to use ALS successfully were Starzl and John S. Najarian of the University of



This monkey at the Stanford Medical Center survived a combined heart and lung transplant. [Source: Bruce A. Reitz]

Minnesota Medical School. Najarian has supplied his preparation to other investigators, and it has been used successfully in more than 1500 kidney transplants. In general, though, it has been difficult to compare results among different investigators because ALS preparations vary with respect to animal source, cell type used for preparation, immunization schedule, and methods to prepare the ALS.

The potential to alleviate many of the problems associated with variability of the preparations was realized about 8 years ago when the Upjohn Company of Kalamazoo, Michigan, began clinical trials with a commercial ALS known as ATGAM. This material has so far been used in more than 1000 kidney transplant patients. Some results reported this summer at the International Congress of the Transplantation Society are typical of those achieved by other investigators.

A. Benedict Cosimi of Massachusetts General Hospital in Boston used ATGAM to treat patients whose relatives had donated kidneys for transplant. Of 11 patients treated with ATGAM at the first sign of rejection, nine still had functioning kidneys after 2 years. Of 12 patients who received only conventional steroid therapy during the same period, only six had the same long-term success.

Two other groups have used ATGAM to treat rejection crises in patients who received kidneys from cadaver donors. Mark A. Hardy and his colleagues at the Columbia University College of Physicians and Surgeons found that 71 percent of patients who received ATGAM retained their donor kidney after 12 months, whereas only 48 percent of those who did not receive the biological retained the donor kidney. In a similar study by Ronald S. Filo and his associates at the Indiana University Medical Center, 90 percent of 21 patients who received ATGAM withstood their body's first attempt to reject the organ, whereas only 58 percent of the 26 patients who did not receive ATGAM were able to resist the initial rejection episode.

At the end of a full year, 72 percent of Filo's patients who received ATGAM still had their transplants, while only 55 percent of those treated conventionally retained the kidney for that period. Similar results have been achieved with smaller groups of patients by Ronald H. Kerman and his associates at the University of Texas Medical School and by M. H. Butt and his colleagues at the Downstate Medical Center in Brooklyn. In these studies, use of ATGAM was begun at the time of the transplant rather than at a rejection crisis, but the outcomes were much the same.

The side effects of ATGAM and other antilymphocyte serums are modest, according to Barbara E. Loughman of Upjohn, as long as the recipients are not allergic to the animal in which the serum is prepared. The most common effects are fever and chills; these probably result from proteins injected along with the antibodies, but the fever could be caused by endogenous pyrogens (fever inducers) released by destroyed leukocytes. There is also sometimes a modest, transient depression in the concentration of either platelets or white blood cells in the blood stream.

ATGAM has been used primarily for kidney patients, but other investigators have used their own preparations of antilymphocyte serums in experimental transplants of hearts, liver, skin, islets of Langerhans (the part of the pancreas that produces insulin), and bone marrow, and even in the treatment of aplastic anemia. If the present trials of ATGAM continue successfully, Loughman says, Upjohn will probably begin clinical trials in some of these areas as well.

Another technique which can be used to suppress T cell production is total lymphoid irradiation (TLI), or high-dose x-irradiation of the lymph nodes of the neck, chest, and abdomen, the thymus gland, and the spleen. TLI was developed more than 15 years ago by Henry S. Kaplan of Stanford as a technique to treat Hodgkin's disease and other cancers of the lymphoid tissues. Other investigators, such as Samuel Strober of

No Go for Salicylate

One promising approach to immune suppression that has not worked out is the use of sodium salicylate. In January of 1979, Stuart W. Jamieson, Bruce A. Reitz, Nelson A. Burton, and Edward B. Stinson of the Stanford University Medical Center reported that grossly mismatched hearts transplanted in rats could be kept healthy for a minimum of 50 days by giving the rats high doses of sodium salicylate and azathioprine, an immunosuppressive agent commonly used in transplant maintenance. Similar rats receiving azathioprine alone survived only for a mean of 6 days.

Jamieson postulated that the antiplatelet activity of salicylate was responsible for this effect. He had previously suggested that platelets adhere to complexes of host antibodies and donor antigens, releasing substances that break down endothelial cells and create openings through which T lymphocytes can infiltrate the graft. By inhibiting the formation and activity of platelets, he reasoned, salicylate might be able to stave off rejections.

Jamieson's theory might be correct, but its practical application in other animals has not proved successful. Extensive studies in both dogs and monkeys, Reitz says, have shown that the concentrations of salicylate necessary to achieve an immunosuppressive effect are toxic in both species. The Stanford group has thus stopped working with the drug altogether, and Jamieson is searching for another drug that might exhibit stronger antiplatelet activity.—T.H.M.

Stanford, observed that the technique causes a profound and prolonged depression of lymphocyte production, perhaps for 10 years or more. Yet despite this suppression of immunity, less than 1 percent of the Hodgkin's patients treated at Stanford in this manner developed severe infections requiring hospitalization, and none developed secondary cancers associated with the irradiation.

Working with mice and rats, Strober and his colleagues found that TLI permitted them to transplant bone marrow, skin, and hearts from unrelated donors without rejection, even when the animals received no other form of immunosuppression. Skin and heart grafts were taken from the same donor as the bone marrow, suggesting that the donor lymphoid tissue in the marrow somehow paralyzes the host's immune system, allowing it to accept what otherwise would have been considered foreign skin and heart tissue. Moreover, the recipients of the graft do not exhibit graft versus host disease, in which white blood cells produced by the transplanted marrow attack the host's body.

The mechanism of this phenomenon is unclear, but Donna King of Stanford has found that TLI results in the development of a specific population of lymphocytes, called suppressor cells, that prevent both the donor and the host lymphocytes from attacking each other, engendering a sort of "mutual tolerance." The technique works for organs other than bone marrow only if bone marrow is transplanted first, though, and both the bone marrow and any other organs must be from the same donor.

Strober and his colleagues are now working with Shumway's cardiac transplantation team to test the effect of TLI on heart transplants in dogs and monkeys; the preliminary results look promising. They are also working with Oscar Salvatierra and his colleagues at the University of California Medical School in San Francisco in preparation for clinical trials on humans receiving kidney transplants. Initially, the group will use azathioprine in conjunction with TLI, but they hope to eliminate the steroids entirely.

Meanwhile, Najarian and his colleagues at Minnesota have already used TLI in conjunction with steroids on 17 kidney transplant patients. All 17 subjects were high-risk patients who had previously rejected at least one kidney and thus were highly likely to reject another. The first transplant involving TLI was nearly 2 years ago, and all but two of the patients are still alive with functioning grafts: one of these two died of lymphoma, but another committed suicide for reasons not directly related to the transplant. Najarian thinks that the immunosuppression in most of these cases has been greater than necessary, and he is cutting back on the amount of radiation the patients receive.

Unlike Strober, though, Najarian does not think TLI will find great application for first kidney transplants because it is too complicated. The treatment regimen at Minnesota requires about 6 weeks of preparation before the actual transplant is performed and, ideally, the transplant should be carried out as soon as possible upon its completion. Unless the donor organ comes from a relative, however, compatible organs do not arrive on such schedules. In some cases—in which cytotoxic antibodies developed after a first graft, making it hard to find an acceptable donor—Najarian has had to wait several weeks after completion of the preliminaries before a suitable kidney became available. He keeps such patients prepared with small, intermittent doses of radiation, but he does not think this is the optimum way to proceed. For the time being, then, he will use the procedure only for high-risk patients. Strober, in contrast, is confident that his group will find cadaver donors for 90 percent of their first kidney patients within 6 weeks after preparations are completed.

In essence, TLI is a technique of conditioning the recipient by depleting him of lymphocytes. Lymphoid depletion can also be accomplished by the mechanical removal of lymphocytes from lymph collected from the thoracic duct. This procedure, known as thoracic duct drainage, was first performed in clinical transplantation by Curt Franksson of Stockholm in 1963, and it was given a number of trials with conflicting results 10 to 15 years ago.

Recently, however, Starzl and Keith Johnson of the Vanderbilt University School of Medicine have shown that many of the early investigators were not treating the patients long enough with thoracic duct drainage, and that optimal use of the technique requires that the treatment begin at least 28 days before transplantation. With adequate advance treatment of patients, who also were given azathioprine and steroids for maintenance immunosuppression, the incidence of early rejection was reduced to less than 10 percent in recipients of first cadaver kidneys, and kidney survival after 1 year was 80 percent. Unfortunately, as is the case with TLI, the inconvenience and expense of thoracic duct drainage will probably impede widespread use of the technique.

All of the techniques discussed so far act in the organ recipient to reduce his reaction to the foreign tissues of the graft. Other scientists are working with the donor organ itself to find some way to reduce its immunogenicity. Such studies will be the subject of a subsequent article.—THOMAS H. MAUGH II