Drug Transforms Transplant Medicine

Cyclosporin obviates rejection problems with organ transplants and may also be used to treat autoimmune diseases and parasitic infections

During the past 2 years, the transplantation field has been revolutionized by Cyclosporin A—a new drug that prevents rejections and has surprisingly few side effects. The drug also may be effective in treating autoimmune diseases and—unexpectedly—it seems to kill the schistosomiasis and malaria parasites.

The remarkable effects that Cyclosporin has had on the transplantation field were summarized recently during congressional hearings called by Albert Gore (D–Tenn.), who is chairman of the House Committee on Science and Technology. At the hearings, Thomas Starzl, a surgeon at the University of Pittsburgh School of Medicine, told how Cyclosporin A has changed the outlook for liver transplants. Before the drug became available in 1979, liver transplants were risky at best. "For almost 20 years there seemed no way out of the dilemma. The drugs being used were, on the one hand, unreliable in preventing rejection and, on the other, extremely dangerous." With Cyclosporin, however, the percentage of patients whose liver grafts survive for the crucial first year has increased from about 35 percent to 65 or 70 percent. "These results have been so encouraging that at the University of Pittsburgh liver transplantation is now considered a service as opposed to an experimental procedure," Starzl said.

G. Melville Williams, a surgeon at Johns Hopkins University, testified about kidney transplants, saying, "Four centers in this country have had experience with the new immunosuppressive drug Cyclosporin and all of these investigators agree that many of the practices we have applied [to increase graft survival] may prove to be obsolete when this drug becomes available for general use." Eighty to 90 percent of all cadaver kidney transplants succeed with the use of Cyclosporin whereas without the drug only about 50 percent do. Said Williams, "The field of kidney transplantation is excited and restless awaiting the availability of Cyclosporin."

Heart transplants and heart-lung transplants also are greatly aided by Cyclosporin. Stanford surgeon Norman Shumway testified, "Since we began the use of Cyclosporin in December of 1980, there has not been a single instance of clinically diagnosable rejection of allografted heart."

The hero of the Cyclosporin story is Jean Borel, a soft-spoken Swiss researcher at the Sandoz Corporation in Basel, who discovered the immunosuppressive effects of the drug and doggedly insisted that it be tested and developed. Like many other drug companies, Sandoz requests that when its employees travel abroad they carry back with them a handful of soil to be tested for microorganisms that may make antibiotics. It was Borel's job to screen new compounds isolated from these soil samples to see if they have anticancer or immunoregulatory effects.

In 1970, Sandoz microbiologists set to work on soil from Wisconsin and from Norway and discovered that these samples contained two new strains of fungi. Both the fungi strains produced a water-insoluble substance, now known as Cyclosporin A. When they tested this substance they learned that it seemed to kill a couple of other strains of fungi. Upon further testing, however, the microbiologists realized that it was not a particularly potent antibiotic, but they were intrigued because the substance was surprisingly nontoxic. Since other new substances that were not good antibiotics sometimes had pharmacologically useful properties, Borel decided to do a quick test to see if it suppresses the immune system or kills cancer cells.

> No one yet knows how Cyclosporin works on the molecular level.

To Borel's immense surprise, he learned that the new substance seemed to suppress cell-mediated immunity—the kind involving T cells and the kind that causes graft rejections and certain autoimmune diseases—without killing cells. Not only that, these effects on T cells are reversible. Other immunosuppressive agents work by indiscriminately killing all rapidly dividing cells. Since T cells begin to multiply when they encounter a foreign antigen, these immunosuppressive drugs do prevent cell-mediated immunity but they also kill bone marrow cells, which are the stem cells of all blood cells, and make patients extraordinarily susceptible to infections and at a higher risk for cancer. Still other immunosuppressive drugs—the steroids—kill T cells by unknown mechanisms, but large doses of steroids over long periods of time have serious side effects such as diabetes, high blood pressure, ulcers, and brittle bones.

The effects of Cyclosporin on T cell proliferation intrigued Borel but the management at Sandoz told him they had new goals for their research and that they were planning to drop immunology. Fortunately, he was able to persuade them to allow him to continue to develop the drug, and Sandoz chemists began working on purifying and characterizing it. They learned that Cyclosporin A is a cyclic molecule consisting of 11 amino acids, one of which is in the D form, which is very unusual, and one of which has never before been seen. The new amino acid contains nine carbon atoms and is methylated. Now Sandoz chemists are able to completely synthesize the molecule and to make derivatives of it.

Unfortunately, however, no one yet knows exactly how Cyclosporin works on the molecular level nor what portions of the molecule are necessary for its effects. Cyclosporin seems to allow T cells to be primed to proliferate but it blocks them from actually dividing. Once Cyclosporin is removed, the T cells start to multiply. On the other hand, researchers find that they can take some species of animals off the drug after they have had organ transplants and the animals remain tolerant—which is a totally baffling effect. David Winter, director of research for Sandoz, remarks, "There's a lot of argument as to what is really going on. The story changes from week to week." The only accurate statement that Winter feels comfortable with is admittedly vague: Cyclosporin appears to inactivate subsets of T cells.

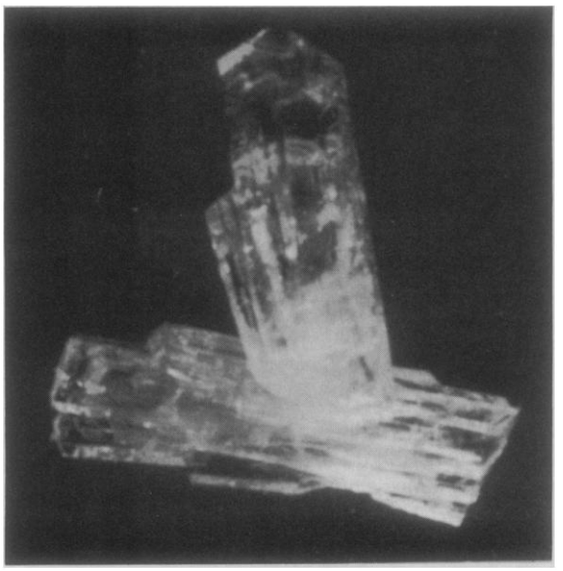
Asked whether the unusual amino acid and the D amino acid in Cyclosporin are necessary for its immunological activity, Winter says that they are but, he adds, "a very great proportion of the molecule is necessary. It may be that all of the molecule is necessary. The chemists right now are trying to tease it apart but it takes a couple of years to characterize each change in the molecule to see if [the derivative] has exactly the same activity as [the original]. It will take forever."

But the clinical uses of Cyclosporin

did not wait for its biological and chemical characterization. In 1977, David White, of Cambridge University, and Roy Y. Calne, of Addenbrook's Hospital in Cambridge, decided to try using the drug with heart transplants in rats. They were extraordinarily successful. The drug seemed to prevent the transplanted hearts from being rejected and, says White, the most important observation was that "a short course of Cyclosporin A produces a very prolonged graft survival." White, Calne, and others then tried the drug in other animals and with other kinds of transplants, including kidney, liver, skin, pancreas, small bowel, and muscle. The results were impressive.

In the meantime, Sandoz researchers were looking for adverse side effects of the drug in rats and rhesus monkeys and were learning that Cyclosporin is indeed specific for T cells and that it does not kill bone marrow cells. It was decided that the drug should be tried in humans. But a new problem arose. When volunteers swallowed gelatin capsules containing Cyclosporin, the drug did not get into their blood. Borel insisted that the problem was with the mode of delivery of the drug and, to prove his point, he mixed up what he calls "a distasteful drink," consisting of Cyclosporin A, pure ethanol, some water, and the solvent Tween-80. He got, he says, "a little tipsy," but the experiment worked. The drug got into his bloodstream in pharmacologically active quantities. Subsequently, the Sandoz researchers learned that a better way to take Cyclosporin is to dissolve it in olive oil. Patients now drink the drug in this form.

In the past few years the Food and Drug Administration (FDA) has allowed Cyclosporin to be used in a limited number of hospitals to prevent graft rejections. FDA approval of the drug, which would make it generally available, is expected soon. As more and more researchers have used Cyclosporin, they have learned that the drug is more powerful than they thought. Yet it is surprisingly safe. At first, physicians would combine Cyclosporin with other immunosuppressive drugs. But, says Winter, "we've learned the hard way that if we use Cyclosporin together with other immunosuppressive agents, the two together turn out to be so severe, the immune system so depressed, that lymphomas may occur. We learned that this drug is more powerful than we originally thought." Now, Cyclosporin is given alone or with low doses of steroids and, Winter remarks, "cancer is less of a concern."



Crystals of Cyclosporin

The miracle substance made by fungi has been purified and crystallized.

The major adverse side effect of Cyclosporin is that it seems to affect the kidneys in about 10 to 15 percent of patients. These patients have elevated levels of creatinine in their blood, which is an indirect indication of abnormal kidney functioning. But no one knows yet what part of the kidney might be affected or how. At a recent international meeting on Cyclosporin, says Berry Kahan of the University of Texas at Houston, "four or five pathologists stood up one after the other and all described different things that might be wrong with the kidney. There is no consensus." Some patients in England have been taking Cyclosporin for as long as 5 years, says Winter, and their serum creatinine levels have remained constantly elevated, yet their kidneys seem to be working fine.

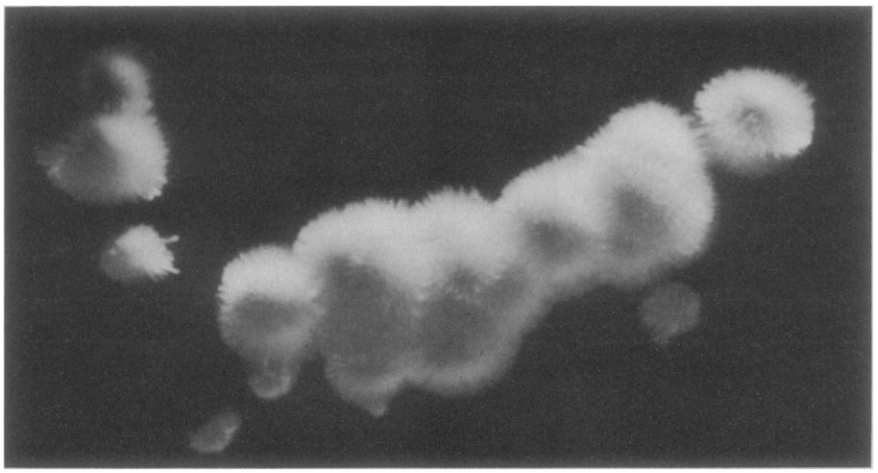
More minor side effects of Cyclosporin are increased hair growth, overgrowth of the gums, transient fatigue, a tingling sensation about the lips, and increased sensitivity to hot and cold. Yet, Winter emphasizes, "It is not a trivial drug. It is not like taking aspirin." Although Sandoz was willing to move quickly to get Cyclosporin used for transplant patients—who often are facing life-or-death situations—the company is far more cautious in using the drug for autoimmune diseases or parasitic infections, where it also may prove useful but where patients may not be better off taking it rather than more conventional treatments. But studies of these other uses for the drug are now going ahead.

Robert Nussenblatt of the National Eye Institute has recently completed a very encouraging pilot study using Cyclosporin to treat the autoimmune disease uveitis and is now recruiting patients for a full-scale controlled clinical trial. He had no trouble convincing patients to try the drug. "Patients generally have been willing to take the drug," he says, "but these are patients with their backs against the wall."

Uveitis refers to an inflammation anywhere in the eye, but often it is caused by an autoimmune reaction. Mild forms of uveitis can be treated with steroid-containing eye drops but more severe forms, which lead to blindness within 3 years if not treated, respond only to more drastic therapy. Patients with autoimmune uveitis are given immunosuppressive drugs to try to stem the inevitable deterioration in their vision.

In the past few years, Walden Wacker, of the University of Louisville in Kentucky, Nussenblatt, and their colleagues found that they could produce severe uveitis in guinea pigs, rats, and monkeys by immunizing them with an antigen, called the S antigen, found in the retina. Then they learned that not only the animals but also some people with severe uveitis have T lymphocytes that divide in response to the S antigen, indicating that the uveitis may be caused by a T lymphocyte reaction.

About 2 years ago, Nussenblatt decided to see if Cyclosporin could ameliorate uveitis in the rat. "I didn't know what the effect would be. I expected to modulate the disease. It turned out that we could totally prevent it," he says. After immunizing rats with high doses of S antigen, he could give them Cyclosporin and none of the rats would get the disease whereas ordinarily all would. Even if he waited until the disease had begun to develop and the rats' eyes were being destroyed, he could stop the disease with Cyclosporin. "There was talk early on that Cyclosporin is effective in preventing the beginning of the immune response. Here we're saying that we could



Crystals of a new amino acid

This never-before-seen amino acid seems to be necessary for Cyclosporin's actions.

have an immune response and turn it off."

At this point, Nussenblatt began thinking about a pilot study of Cyclosporin in humans with severe uveitis. He accepted only patients who could not take or were unresponsive to conventional immunosuppressive drugs so that they really had no other treatment available. Of the 31 patients who have taken the drug, all seem to be doing well, Nussenblatt reports. The inflammation in their eyes improved and their vision either stopped deteriorating or actually got better. Now Nussenblatt and his associates are recruiting 200 to 300 patients with severe uveitis whom they will randomly assign to steroids or Cyclosporin in a controlled clinical trial.

At University Hospital in London, Ontario, a group of researchers including Calvin Stiller, Andreas Laupacis, John Dupre, Morrison Jenner, Paul Keown, Wilson Rodger, and Bernard Wolfe are ready to start a controlled clinical trial of Cyclosporin in newly diagnosed juvenile diabetics. This form of diabetes is suspected of being an autoimmune disease. They already have shown that Cyclosporin prevents diabetes in the BB rat—a strain that otherwise gets the disease. And they have conducted a pilot study in humans with encouraging results.

"The reason we did the study in humans is that we know there is a reversible component [in juvenile diabetes]," Stiller says. He and his associates have now given Cyclosporin to more than 20 diabetics. Of those treated within the first 6 weeks after their diagnosis, all were able to reduce their insulin dose by at least half and three were able to stop taking insulin entirely. But Stiller is cautious in interpreting his results. About 3 percent of newly diagnosed diabetics spontaneously go into remissions lasting from 2 weeks to 1 year, although all do eventually became diabetic again. So it is not yet proved that Cyclosporin actually caused the remissions. Moreover, says Stiller, "Even if we had a 100 percent cure, my feeling would be that without a controlled trial we never would know the risk-benefit ratio."

Stiller and his associates are starting pilot studies of Cyclosporin for other autoimmune diseases including multiple sclerosis, sarcoidosis, inflammatory bowel disease, and glomerulonephritis. Although he stresses the need to be cautious and deliberate, he remarks, "The rationale for the use of Cyclosporin in autoimmune disease is *compelling*."

Winter of Sandoz agrees with Stiller but says his fear is that once the FDA approves Cyclosporin for transplants (the drug is getting an expedited review) it will be generally available and "people can theoretically use it for anything they want. These are such emotionally charged areas. Our real concern is to keep this thing under control." Sandoz plans to sponsor clinical trials of Cyclosporin as a treatment for multiple sclerosis and possibly for lupus in addition to sponsoring the trials involving diabetes and uveitis.

The most surprising potential use for Cyclosporin is in the treatment of parasitic diseases. Ernest Bueding of Johns Hopkins University School of Medicine, for example, learned to his amazement that the drug kills schistosomes—the worms that cause schistosomiasis. He and his associates originally thought that perhaps the drug might ameliorate the symptoms of the disease. Eggs of the parasite travel to the liver where they cause a pronounced immune reaction and liver damage. "We thought that if we inhibited the T cells we might inhibit the host reaction to the eggs. We tried this [in mice] and it looked very good. There were no eggs. Then we looked at the worms and the worms were very much damaged. Purely by accident we found an effect on the worms."

Bueding and his associates now have learned that Cyclosporin acts by inhibiting an enzyme in the worm that degrades hemoglobin. The drug is effective against all three species of schistosomes and the species that is most resistant to conventional drugs is most sensitive to Cyclosporin. Moreover, when Bueding tested six Cyclosporin derivatives that were not immunosuppressive, he found that two of the six were effective against the schistosomes, indicating that it may be possible to treat schistosomiasis without suppressing the immune system.

The other parasite that Cyclosporin seems to inhibit is malaria. "It's sort of a weird idea," says Leonard Scheibel of the Uniformed Services University of the Health Sciences. "[Gerald] Cole [of Johns Hopkins University] and I thought that since T cells seem to play some role in protection from malaria, possibly Cyclosporin would make malaria worse in mice." So Scheibel, Cole, and S. P. Nickell of Johns Hopkins gave the drug to mice with malaria. "Much to our surprise, the animals with malaria were cured. I said, 'We must have done the experiment wrong,' so we went back and did it several times," Scheibel recalls. The drug seemed to work. Cole then found that it also works in owl monkeys, which are the only primates that get malaria, but he had to use rather high doses of the drug to see an effect.

At that time Scheibel was at Rockefeller University where he and William Trager were growing malaria parasites in vitro. He decided to see if Cyclosporin would kill the malaria parasites in vitro, reasoning that if it did, it would eliminate factors of host immunity. "I put Cyclosporin in the petri dishes. Lo and behold, it killed them," Scheibel recalls. The drug seems just as effective against chloroquine-resistant malaria strains as against those that are sensitive to chloroquine. Although no one knows how the Cyclosporin works, Scheibel suspects that it may inhibit an enzyme that the malaria parasites use to break down hemoglobin. Just as with the schistosomiasis parasites, Cyclosporin derivatives that have no effect on the immune system still kill the malaria parasites.

The biggest problem with Cyclosporin research now, says Winter, is to keep things under control. The drug seems so amazing and its possibilities are so great that many researchers can hardly wait to get their hands on it.—**GINA KOLATA**