Fighting Cancer with Designer Cells

Cancer immunotherapist Steven Rosenberg was in the news last month as part of a team conducting the first human gene transfer. For Rosenberg, it was one step in a long journey

IN 1968, STEVEN A. ROSENBERG was a young surgeon in training at Harvard, assigned to the Veterans Administration hospital in Roxbury, when a 63-year-old man checked in to have his gallbladder out. "It seemed to be a fairly routine case," Rosenberg thought at first. Then he read his patient's medical history.

A dozen years earlier, in the height of summer, this same man had been admitted to Roxbury with "epigastric distress." The doctors there found him to have a stomach tumor the size of his fist, with extensive spread to his lymph nodes and liver. As a palliative measure, they cut the tumor out of his stomach, but the situation was deemed ultimately hopeless and the man was sent home to die. Yet here he was, a full 12 years later, lying before surgeon-in-training Rosenberg very much alive and well, except for his gallstones. Rosenberg was flabbergasted: he had just seen his first case of a truly spontaneous remission of cancer.

"Spontaneous remission," Rosenberg said in a recent interview with *Science*, "is extraordinarily rare. In 20 years since, I've only seen two others."

But that first remission marked the beginning of a sustained research effort aimed at proving in dying patients a long-held and intriguing theory—that the immune system can be harnessed to vanquish cancer. Rosenberg, the physician/surgeon, added basic science to his repertoire, conducting experiments first in animals, then in people, and, over the years, his experiments have helped make immunotherapy a reality.

He currently is chief of surgery at the National Cancer Institute in Bethesda, Maryland, and there, after several years of controversial human experiments, he has assembled an impressive but still preliminary body of data. It's not that he's curing cancers in large numbers; but Rosenberg has shown that he can make headway in some cases of patients for whom no traditional therapy has worked. And in some cases—a precious few—a handful of patients with widespread cancer have been literally rescued from the brink of death and have been in complete remission for four or more years.

Whereas once cancer was nearly uniformly fatal, now surgery, radiation, and chemotherapy are effective treatment for some 50% of cancer patients. Cancer Institute officials and others cite this as a great achievement. But, as Rosenberg notes, the other side of this statistic is that current therapy fails in 50% of patients. This, he and his colleagues in oncology hope, is where biological therapy will come into play as a fourth approach to this devastating disease. "But it is in its infancy. In terms of sophistication, we're where chemotherapy was 30 years ago," is the way Rosenberg puts it.

Nonetheless, just last month, Rosenberg and his colleagues R. Michael Blaese of NCI and W. French Anderson of the National Heart, Lung, and Blood Institute, collaborated on a sophisticated and precedent-setting experiment using TIL (tumor-infiltrating lymphocytes) in the nation's first approved human study in gene transfer (*Science*, 26 May, p. 913). Using a retrovirus as a vector or transport vehicle, the researchers inserted a marker gene into a population of TIL that will be infused into ten terminally ill melanoma patients.

The objective is to track TIL cells as they carry out their search-and-destroy mission inside the body. But it is possible that the experiment will pave the way for gene therapy in cancer. Rosenberg, for instance, already is conducting studies in vitro of TIL cells transduced not with a neutral marker gene but with the gene for a known antitumor substance, such as alpha interferon or tumor necrosis factor. Using genes to enhance the potential tumor-fighting capacity of immune cells would create what Rosenberg calls "designer lymphocytes."

Pioneering studies using LAK (lympho-

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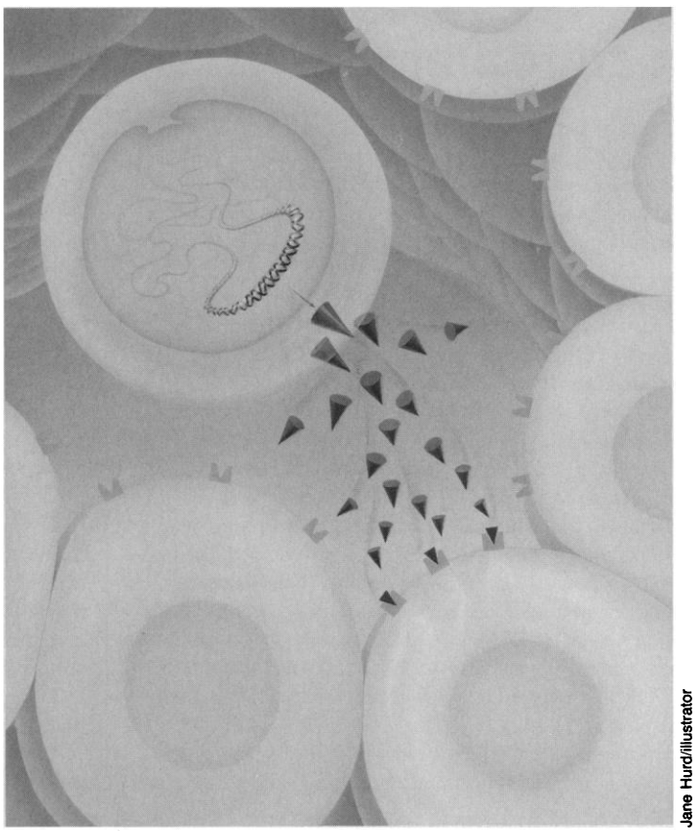
No man is an island. *Steven Rosenberg has drawn on the work of some of the giants of immunology and the talents of many fellows who have contributed to his lab and whose portraits line his wall.*

kine-activated killer) cells and, most recently, potent TIL have shown that sometimes the immune system can be enlisted in the fight against cancer with astonishing success. Advanced melanoma has proved resistant to nearly all forms of treatment. Kidney cancer or renal cell carcinoma is equally intractable. After years of work, Rosenberg now is reporting some stunning success. When what Rosenberg calls "adoptive immunotherapy" works, it works spectacularly.

Many other melanoma and renal cell carcinoma patients have seen their tumors shrink more than 50%. That is not a cure, but in cancers that have been among the most hopeless, it may be a hint of real progress to come.

TIL appear to be unique among immune cells in that they are specific to the tumor from which they came. TIL extracted from a patient's melanoma, grown, and reinfused appear to attack only that patient's melanoma—leaving healthy cells alone. Preliminary evidence suggests that the TIL recognize and home in on similar tumors throughout the body.



Jane Hurd/illustrator

Tumor attack. *Tumor-infiltrating lymphocytes, or TIL, launch an attack on a melanoma from which they originated.*

But when it comes to mechanisms, there is considerable uncertainty. The researchers hope that by tracking the location of infused cells, they will learn something about how they work when they do, and why they fail to work.

"Rosenberg has the first real evidence that immunotherapy works in humans," Alan Rabson, scientific director of the National Cancer Institute, told *Science*. Former NCI director Vincent T. DeVita, Jr., now physician-in-chief at Memorial Sloan-Kettering Cancer Center, adds that "the significance of Steve's work is that he is getting positive results in patients with big tumor masses. We haven't been getting that before."

Complete remission of metastasized cancer is the real goal, which brings us back to the Roxbury VA in 1968 and the man who once had stomach cancer.

Rosenberg saw a clue in his patient's history. A few days after cancer surgery back in 1956, the man developed a severe infection in his stomach, characterized by virulent pus loaded with alpha streptococcus. Rosenberg knew that other researchers had observed an association between shrinkage of stomach cancer and severe abdominal infection. Furthermore, the medical record showed that his patient's stomach cancer had been densely infiltrated by lymphocytes and other immune cells.

Rosenberg was ready for one of his first clinical experiments. Another patient under his care was in the hospital with stomach cancer, a man whose blood type matched that of the man whose tumor had spontaneously vanished. Rosenberg devised a protocol for giving the survivor's blood to the dying cancer victim. One can hear the expectation in his voice even now as Rosenberg relives the early days. The transfusion was given, and with it, perhaps, immune cells that could destroy tumor.

"But nothing happened. The patient went on to die."

The road to designer lymphocytes has been long, arduous, and marked by failed hopes.

In 1968, immunology was exploding scientifically as researchers discovered that the immune system is divided into two parts—one dominated by the B cell or antibody-producing arm, the other by T cells which regulate the immune system. A logical extension of this new knowledge was the notion that patients with spontaneous remission were lucky enough to have some rare but powerful immune capacity to wipe out their own tumors.

Although his first attempt at immunotherapy at the Roxbury VA in 1968 did not save his patient, it was in accord with the latest thinking. Rosenberg interrupted his surgical training to get a Ph.D. in biophysics at Harvard, followed by an immunology fellowship at Harvard and another at the National Cancer Institute. In 1974 he made what has been a permanent move when he became chief of surgery at the cancer institute—a position he still holds—and began the series of animal and human experiments that led to the discovery of TIL cells.

In the late 1960s and early 1970s it was not possible to grow immune cells in vitro in any quantity, but they could be harvested from animals. Rosenberg began studying minipigs—small research animals whose immune systems have a certain resemblance to people's. He immunized the pigs with human tumors and in an attempt to transfer antitumor immunity actually treated six patients with minipig lymphocytes. "It didn't work, but there was one important observation. We showed that you could safely give people large numbers of lymphocytes. But no one was much interested and you won't find these studies in the literature. We wrote four papers; they were all rejected."

Then a major breakthrough occurred. In a cancer institute lab not far from Rosenberg's, Robert C. Gallo and his colleagues Doris Morgan and Frank Ruscetti had been trying to grow leukemia cells en masse. In 1976 they reported in *Science* (10 September) the discovery of T cell growth factor or interleukin-2 (IL-2), a substance that makes cells in vitro grow like gangbusters.

Rosenberg was in business. Using IL-2 to mass produce cells, a series of animal studies ensued. He and his colleagues showed, for instance, that infused lymphocytes can destroy tumors in mice. But the problem was finding the right lymphocytes. Some seemed to have antitumor properties. Others did not.

Then, in 1980 Rosenberg and his group discovered LAK cells. "We found that if you incubate just about any lymphocyte in IL-2 for 4 days, it turns into an activated killer. At least, at first we thought we could use any lymphocyte." The trouble with this finding was that the Rosenberg team didn't really want LAK cells—random killers attacking

The Rocky Road to Remission

When press accounts of Steven Rosenberg's experiments with a new form of cancer therapy first appeared 4 years ago, dying patients around the country deluged their physicians with demands for the treatment. But it will be a while before "adoptive immunotherapy" is widely available to patients. The therapy, in fact, provides a case study of the challenges that often face medical researchers going from early trials to widespread use.

To begin with, "adopting" the patient's immune cells—turning them into potent tumor fighters—requires skill and sophisticated facilities. The special attraction of TIL (tumor-infiltrating lymphocytes) cells is their unusual specificity. TIL recognize and infiltrate only the tumors they come from. But to be medically effective they have to be made available in huge numbers—anywhere between 3×10^{10} to 75×10^{10}. And they have to be grown case-by-case for each patient.

A chunk of tumor is surgically excised and the infiltrating lymphocytes extracted from it. These are then mixed with interleukin-2 in 3-liter bags that look like great hot water bottles filled with strawberry juice. It is the IL-2 that make the TIL grow.

The TIL proliferate rapidly, doubling approximately every 2½ days; but even at that rate, it will take 4 to 6 weeks to produce the 150 or so bags of cells that will eventually be spun down and concentrated before being infused in a patient.

Then comes the medical challenge. IL-2 in therapeutic quantities is toxic to patients in and of itself. It takes skilled, experienced physicians and nurses to get cancer victims through the side effects. Virtually all cancer therapy is tough stuff. IL-2 has the reputation of being the toughest among them.

With conventional chemotherapy, a patient's hair falls out. Bone marrow is suppressed, making patients vulnerable to infection. Clotting factor may be affected. Mucosal cells are damaged and nausea and vomiting can be severe, especially when it is the mucosal lining of the stomach that is injured.

But over the years, physicians have become skillful at administering drugs in precise combinations and at minimizing side effects. These days, patients often stop by the hospital for a round of chemotherapy and go home the same day.

Adoptive immunotherapy is different. Patients must be hospitalized for a week or more, sometimes in intensive care. Although TIL themselves appear to be relatively nontoxic, the IL-2 is nasty stuff. It is also essential. Once TIL are administered, subsequent infusions of IL-2 stimulates their continued growth in the body. Without IL-2 neither TIL nor LAK (lymphokine-activated killer) cells will grow in vivo in therapeutic quantities. At least, no one has learned how to do that yet, although researchers imagine one day being able to hook the IL-2 gene onto TIL.

By mechanisms that are only beginning to be understood, IL-2 causes a "capillary leakage" syndrome that makes blood vessels more permeable than they ought to be. Fluid from the body seeps into vessels and is held there. Patients swell up with fluid, sometimes gaining as much as 10 to 20 liquid pounds. IL-2 also causes body temperature to spike dramatically—104 degrees is not uncommon. The heart and kidneys are put under tremendous strain. Patients become confused, sometimes delirious.

Rosenberg believes that even these side effects can be medically managed and go away when the therapy is over. A number of oncologists have serious reservations about the risk. Rosenberg takes the optimistic view. "The real problem with IL-2," he says, "is that the side effects are unlike those most oncologists are used to seeing. That bothers doctors. But I remember that people reacted to the side effects of chemotherapy the same way at first. And, just as with chemotherapy, we're getting better at controlling the side effects of IL-2."

Sometimes the treatment of advanced cancer is as deadly as the disease. Adoptive immunotherapy, toxic as it can be, is not at the top of the list. Indeed, data show that the mortality rate from chemotherapy is somewhere between 2.5% and 5%, depending upon the patient's overall health and the type of treatment. Autologous bone marrow transplantation, in which a patients own marrow is removed, treated, and returned, has a mortality rate of 15% to 20%. In Rosenberg's opinion IL-2 is remarkably safe by comparison. "The mortality rate from the therapy itself," he says, "is only about 1.5%," a risk he thinks is well worth taking—considering the alternative. ■ **B.J.C.**

normal cells and tumors alike.

"We spent a year trying to eliminate LAK cells from our cultures," Rosenberg remembers. "Then, after a while we decided we'd better look at LAK after all."

Research on LAK, which are now in experimental use at some 30 cancer centers, revived an immunological theory that was popular in the 1960s. Immunologists used to hypothesize that we get cancer every day and every day the body's natural system of "immune surveillance" destroyed those wild cells. When the B and T cell arms of the immune system were identified, immune surveillance fell from favor because neither B nor T cells fit the bill as immune surveyors cruising around the blood looking for cancer.

The precursor cells that are transformed into activated killers by IL-2 turn out to be neither B nor T cells. They are "null" cells that make up 5 to 10% of the immune system and "could be part of a primitive surveillance system," Rosenberg notes.

In large numbers, LAK cells can do more than that. By 1984, Rosenberg was using a combination of IL-2 and LAK to eliminate pulmonary tumors in mice. In a paper in the 28 September 1984 issue of *Science*, he and his colleagues showed that the injection of LAK cells and IL-2 into mice with many lung tumors could lead to cancer regression.

In addition, clinical protocols for human tests were being designed, but supply continued to be a problem. It took vats of cells to produce IL-2 in any quantity. Then more vats of cells incubated in IL-2 were required to get sufficient quantities of LAK.

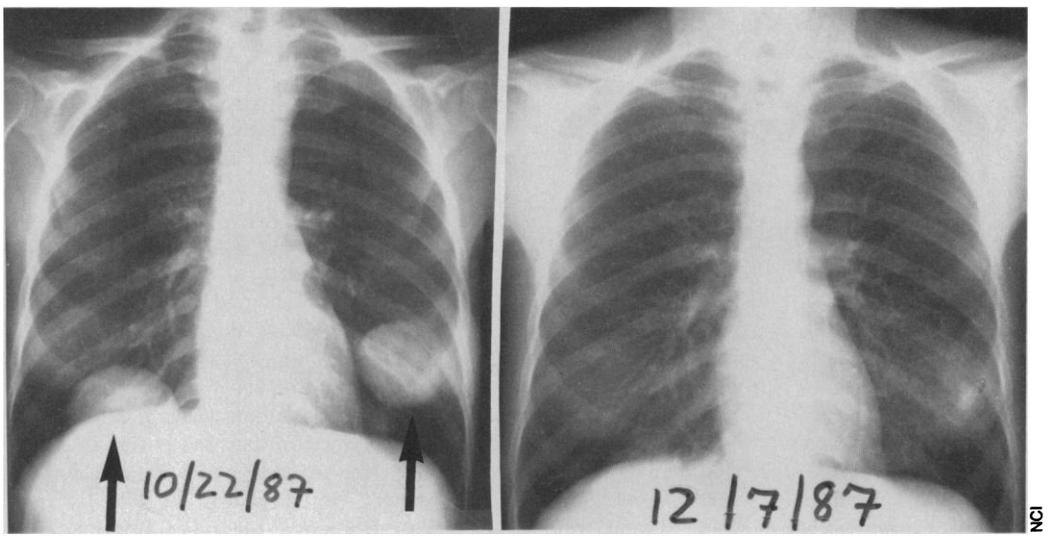
Some clinical experience was already in hand. In 1982, Rosenberg tried augmenting the immune response by giving cells stimulated in culture with phytohemagglutinin. He treated 21 cancer patients. Nothing happened.

In 1984, he began trials giving IL-2 alone. "At the time we were using natural IL-2, made for us by DuPont, but the material was still scarce. We treated 39 patients, using a low dose. There was no toxicity. But the patients did not get better."

He also tried giving LAK cells alone. Six patients were treated. That, too, was "perfectly safe," but had no effect on the patients' tumors.

Altogether, in those early experiments 66 patients were treated and 66 patients went on to die.

Then, just as the Gallo lab's discovery of IL-2 had been a technical breakthrough of major importance, the cloning of the IL-2 gene by Tada Taniguchi and co-workers in Osaka in 1983 was another vital accomplishment along the road to eventual human trials. Cloning the gene meant that IL-2



The tumors vanish. *A patient with melanomas as big as tennis balls in his lungs has a clear x-ray 6 weeks after TIL therapy.*

could be mass-produced by recombinant DNA technology. Rosenberg, working with researchers at Cetus, showed in 1984 that recombinant IL-2 has the same properties as natural IL-2 and soon Cetus began supplying recombinant IL-2 in quantity.

In a repeat of an earlier experiment, some patients were given IL-2 alone, this time in large doses. "We got substantial toxicity but no antitumor effect," Rosenberg reports, in what is beginning to sound like a familiar story.

Then, late in 1984, Rosenberg made his first attempt to treat melanoma with a combination of LAK cells and IL-2. One of his patients was a 29-year-old nurse whose melanoma had metastasized throughout her body.

Finally, success. Within days of treatment, the nurse's cancers began to melt away. Within a month and a half, all tumor was gone. Back home in Florida with her family, she celebrated by holding what she called a "dead tumor" party. Today, she is just fine. Something worked—spectacularly.

The LAK–IL-2 combination was tried on a series of 25 patients. First, LAK cells are grown from the patient's blood in vitro in IL-2. Following infusion of LAK cells, IL-2 is infused to stimulate continued LAK cell production in vivo. Some patients received up to 90 doses of IL-2.

The nurse was the only patient whose cancer went into complete remission, but some 50% of tumor was destroyed in ten others, resulting in a partial remission. Fourteen other patients did not get better at all.

But progress had been seen at last and it generated considerable enthusiasm. In fact, one could argue, the enthusiasm was way out of proportion to reality. Rosenberg reported his data in *The New England Journal of Medicine* on 5 December 1985. At least some of the press went wild. *Fortune* heralded a "cancer breakthrough" on the cover of its 25 November issue and phones at the National Cancer Institute rang off the hook with calls from desperate patients who were only going to be disappointed.

But experimentation went on, as the Rosenberg team continued to try out various doses and combinations in its attempt to save the terminally ill. A year after the *New England Journal* paper, Rosenberg reported on a study of high-dose IL-2 alone in ten patients, this time writing in the 12 December 1986 issue of the *Journal of the American Medical Association*. He found that in only three patients did tumor regress. None had a complete remission. But the study did show for the first time that IL-2 alone could shrink tumors.

By now, the enthusiasm for adoptive immunotherapy was high. But some researchers thought that its potential had been blown out of all proportion. In an editorial in the same issue of *JAMA*, Charles Moertel of the Mayo Clinic said the work should be discontinued because the treatment produced too much toxicity and too little promise. Again, IL-2 hit the news (*Science*, 7 January 1987, p. 154).

Rosenberg continued to plug away. "Desperate diseases demand desperate responses," he says.

While human experiments with LAK–IL-2 were taking place in the hospital, efforts to find something better went on at the bench. Harking back to the very early observation that lymphocytes seem to inflitrate tumors in patients who show some recovery. Rosenberg and his colleagues discovered TIL cells. They reported in the 19 September 1986 issue of *Science* that in mice tumor infiltrating lymphocytes are 50 to 100 times more potent than LAK cells.

Furthermore, TIL cells appeared to be the long sought holy grail of oncology—something that is highly specific to the tumor from which it comes and utterly harmless to the normal cells and tissues around it.

But even those mouse TIL studies showed the frustrating mix of failure and success that has characterized Rosenberg's experiments. "In preliminary experiments, we found no impact of TIL on large established metastases," he reported. Using TIL in combination with IL-2 also "had no impact on survival of mice."

Undaunted, the investigators added a third ingredient to their brew—the immunosuppressant cyclophosphamide. Bingo. "The combination of Cy given on day 8 along with TIL and IL-2 resulted in long-term cure of all mice. . . ."

More important, Rosenberg found that TIL can be isolated from human tumors and grown in IL-2 to fantastic numbers.

It sounds simple. One surgically removes a chunk of tumor that is then minced up in a grinder. Add IL-2 and the TIL grow and grow, doubling every couple of days. However, the process hinges on technical nuances that are not always fully understood.

Now, Rosenberg's attention is fixed on TIL therapy and its possible future modifications. Already, at NCI and at a couple of other centers, TIL are being given in combination with alpha interferon and tumor necrosis factor. The data are not in.

Overall, experience with adoptive immunotherapy has been mixed. Just this April, oncologists collaborating at six cancer centers reported confirmation of Rosenberg's LAK–IL-2 regimen and wrote in the *Journal of Clinical Oncology* that their data support "the concept of adoptive immunotherapy as an important new treatment" for metastatic melanoma.

At the same time, Rosenberg's colleagues in oncology are cautious. Many have decided that the time is not yet ripe for more widespread use of adoptive immunotherapy.

When all the numbers are added up, they reveal a few stunning successes, a fair number of partial remissions, and many failures. More than 650 patients have been treated with one form of adoptive immunotherapy or another; some 20% have responded. There is still a long way to go but Rosenberg is a patient man.

■ **Barbara J. Culliton**