Triple Killer

Physicians create new cells to stave off common viruses that threaten transplant patients. **BY AL STAROPOLI**

After seeing many of her bone marrow transplant patients become seriously ill, physician Catherine Bollard decided to fashion a new immune cell to fight common viruses.

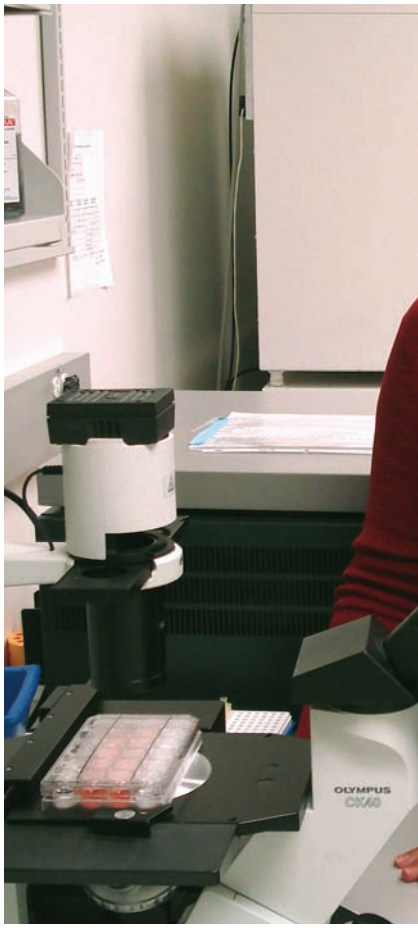
"We see viral infection in about 70 percent of our patients after transplant," says Bollard, a pediatric hematologist at the Texas Children's Cancer Center. The viruses, which are generally benign to healthy patients, can be life-threatening to transplant patients and others with compromised immune systems.

Transplant patients have traditionally been treated with antiviral drugs to prevent infections, but these drugs are expensive, have many toxic side effects, and need to be administered intravenously every day for approximately four months. In addition, when these medicines are stopped, patients are still prone to viral infections.

Instead of using antiviral drugs, Bollard decided to try a different strategy—one that employs the body's own cells to fight off infection. To accomplish this, she and her colleagues developed killer T cells that, when infused into patients, could protect against three of the most common causes of post-transplant infection: Epstein-Barr virus (EBV), cytomegalovirus (CMV), and adenovirus.

Epstein-Barr virus, which causes mononucleosis, and CMV are commonplace among adults. By age 40, up to 95 percent of adults have been infected with EBV. In most people, EBV causes mild, flu-like symptoms. Thereafter, EBV becomes dormant but can re-emerge in transplant patients, causing serious illness or death. CMV similarly infects and becomes dormant in many people, retaining the potential to cause serious infections—usually affecting the lungs and causing severe pneumonia—in patients with weakened immune systems.

To give patients a better chance for recovery, Bollard partnered with Malcom Brenner, who directs the National Gene Vector Laboratory (NGVL) at Baylor College of Medicine. Brenner used the NCRR-funded laboratory to engineer an adenovirus—a common virus that can infect many different types of cells—to produce proteins from CMV, in addition to adenovirus proteins. This hybrid virus was then used to infect immune cells, called B cells, that were already harboring EBV. When infected, B cells are known to stimulate the growth of killer T cells in tissue culture, which can in turn destroy virus-containing cells.



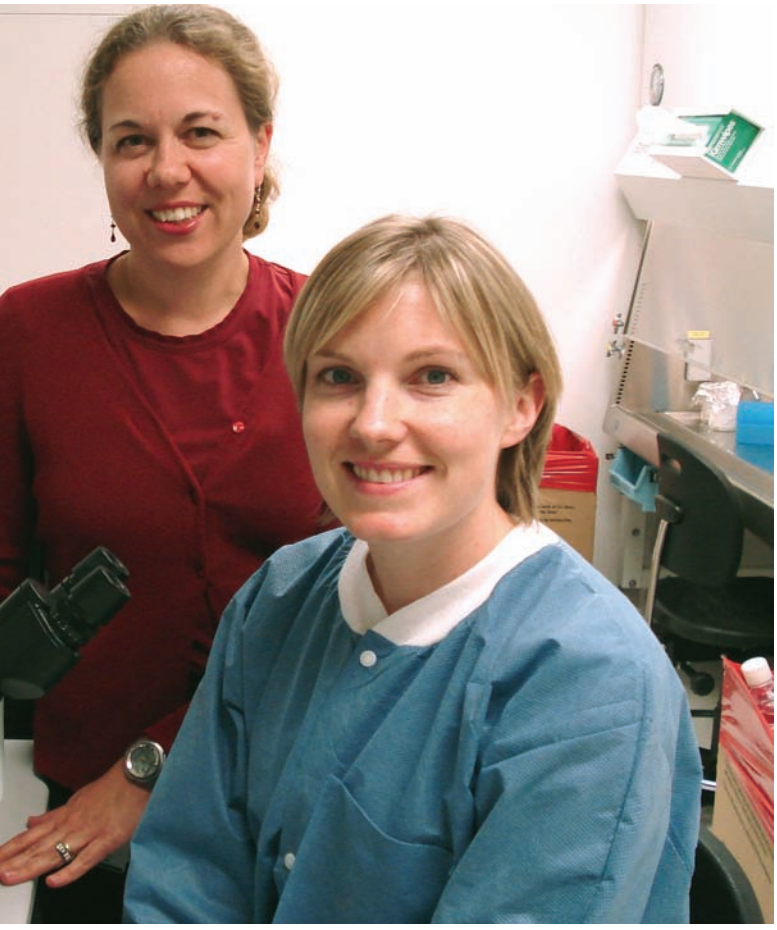
Catherine Bollard (back) and colleague Ann Leen developed a new type of killer T cell that can fight viral infections in bone marrow transplant patients.

In the laboratory, Bollard's team developed 15 lines of killer T cells from bone marrow donors. Of these lines, 14 responded to EBV, CMV, and adenoviruses. The newly fashioned T cells were tested in a Phase I clinical trial conducted in Houston at the Methodist Hospital and the Texas Children's Hospital. The T cells were administered intravenously to 11 patients who had received bone marrow transplants.

Each patient in the Phase I clinical trial received an initial cell infusion after their bone marrow transplantation. Following the infusion, immunosuppression was discontinued in about half of the patients and conventional antiviral therapy was discontinued in all of the patients. None of the patients developed graft-versus-host-disease—a condition in which the newly transplanted cells attack the patient—or other toxicities in over three months of safety monitoring after infusion.

Other teams of investigators also have evaluated similar types of T cell therapies in the past, but their cells were not "stimulated" to fight as many viruses as those used by Bollard and her colleagues. The other therapies showed some evidence of controlling antiviral activity, but they sometimes led to an increase of graft-versus-host disease.

Although Bollard's Phase I trial was designed to evaluate safety



of the T cell therapy, the researchers also found evidence of virus-fighting potential. Patients who had active infections with any of the three viruses at the start of therapy showed rapid improvements in viral titer and disease symptoms, and none developed new viral infections after the killer T cells were infused.

"We treated three patients who had a lot of problems with CMV reactivation prior to infusion. They had to be on antiviral agents for weeks after the transplantation. But once the T cells were given, none of them had to receive antiviral agents," says Bollard.

"None of the patients developed subsequent adenovirus infections," adds Brenner. "This is the first time we've described a cell culture that can protect against adenoviruses, which is a major problem in immunocompromised patients," he adds.

While the frequency of T cells specific to EBV and CMV rose, an increase in adenovirus-specific T cells was seen only in patients with a previous adenoviral infection. Only five individuals tested positive for adenovirus infection before infusion, and it was exclusively in these individuals that a maximal increase in adenovirus-specific T cells was seen. In all of these patients, the adenoviral infections eventually subsided.

"One of our marrow transplant patients had adenovirus pneumonia and was also on a ventilator. This condition has an incredibly high mortality rate, and most people did not think he was going to pull through. But this patient had a dramatic clinical response after receiving the therapy," says Bollard.

The Phase I trial was supported in part by the NCRR-funded General Clinical Research Center, which provided the needed medical care and follow up for patients. The NGVL, also funded by NCRR, was critical in the development of the cells. "We helped Dr. Bollard by finding the vector that would work best for her application. We also developed the vector and tested it. It was a smooth process," says Brenner.

The NGVL also helped Bollard to develop the preclinical information needed for the application to the Food and Drug Administration. Since 1995, the NGVL network has helped to create new therapies by supporting the development of new vectors at no cost to researchers. These vectors can be designed to infect and specifically modify other cells.

Ongoing clinical trials are evaluating the potential of Bollard's therapy, but Brenner sees future possibilities. "There's no reason for limiting this therapy to only three viruses. We could protect against other problematic viruses and perhaps even fungi," he says. The new killer T cells proved effective and safe in all 11 patients studied. Unlike drugs, which control only viruses, the cellular infusions addressed the underlying problem of creating a stronger immune system, without generating toxic effects.

The research described in this article was funded in part by NCRR and the National Heart, Lung, and Blood Institute. For more information on the GCRCs and NGVLs, visit www.ncrr.nih.gov/scientific_rsrcs.asp.

ADDITIONAL READING: Leen, A. M., Myers, G. D., Sili, U., et al., Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. *Nature Medicine* 12:1160-1166, 2006.

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