An Immune Boost to the War on Cancer

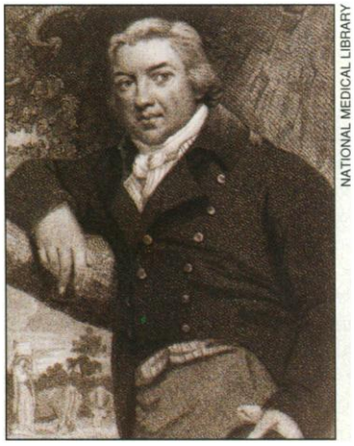
Immunologists hope that what they've learned about the molecular workings of the immune system will lead to effective immunotherapies for cancer, but they have their work cut out for them

To understand the difference between health and disease, researchers need to understand the immune system. As attested to by this special issue, which includes the Editorial on page 13 and five Articles beginning on page 50 as well as this News report, that understanding is emerging in a remarkable outpouring of discoveries about the molecular workings of the immune system. Now, as researchers seek to apply this knowledge in the clinic, *Science* invites all immunologists to air their opinions on what the realistic prospects are for these immunotherapies and when they are likely to happen. You can respond in a questionnaire on *Science*'s World Wide Web site at http://science-mag.aaas.org/science/feature/immuno. We will collate the responses received for a month after publication and post the results in late May. Thank you for your help.

Next month marks the 200th anniversary of one of the world's great experiments: In an effort to protect a child against smallpox—a disease that can leave its victims badly disfigured, if it does not kill them—British doctor Edward Jenner "vaccinated" him with material from a cow infected with cowpox, a related but much milder virus. The experiment worked—the boy remained healthy when subsequently inoculated with smallpox itself—and the door was open to the eventual vanquishing of many of humankind's most serious infectious diseases, including, of course, smallpox itself.

Jenner was able to achieve his success in spite of knowing nothing about how his vaccine elicited immunity to the smallpox virus. Now, 2 centuries later, the molecular biology revolution is bringing immunologists a detailed molecular and cellular explanation of how the immune system operates. And that in turn may yield a valuable payoff as researchers begin applying the information to develop a new generation of vaccines and other treatments based on manipulating the immune system.

"If you compare the vaccination methods used as recently as the 1980s to those now, it's completely different. They were black-box approaches, and there was no real molecular understanding," says tumor immunologist Drew Pardoll at Johns Hopkins University School of Medicine. But, he adds, "it's possible now to consider a whole new range of strategies for vaccines and other immunotherapies to exploit the new knowledge."



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Role model. Current immunologists hope their efforts to develop vaccines will be as successful as Edward Jenner's.

In some cases, the goal of those new strategies is to damp down inappropriate immune responses in an effort to treat rheumatoid arthritis and other autoimmune conditions or combat the rejection of transplanted organs. In others, it's just the opposite, aimed at kick-starting the most appropriate immune responses that can destroy cancer cells or prevent parasitic infections like malaria that aren't eradicated by the natural responses. But even though researchers are hopeful that these efforts will eventually pan out, they also caution that many obstacles, both scientific and regulatory, will have to be overcome. "There are still many things we need to understand," says tumor immunologist Thierry Boon of the Ludwig Institute in Brussels.

Recent work aimed at devising new therapies for cancer shows the promise, but also the potential pitfalls, of these efforts to put basic immunology to work. They have yielded a dizzying array of new approaches in test tube and animal work—but even therapies that have been in the works for a decade and more have been slow to move into clinical trials because what works in experiments often does not translate to patients.

Researchers have long believed that cancers should be subject to immune attack. Indeed, a great deal of experimental evidence from studies in both experimental animals and the test tube has shown that tumor cells are sufficiently different from normal cells to be recognized and destroyed by the immune system. And there are inklings that the same thing happens in humans, at least occasionally. "Some cancers in humans sometimes spontaneously regress with the hallmarks of an immune response," says Pardoll.

All too often, of course, that immune surveillance, as it's called, fails, allowing cancer cells to multiply and form life-threatening tumors. But the knowledge that they can be destroyed by the immune system under some circumstances has spurred many efforts over the years to beef up patients' immune responses, although those efforts met with only mixed results at best. "In 50 years of cancer vaccine trials there have been some sporadic successes but not enough to be really useful," says Boon.

One reason may be that these earlier attempts at cancer immunotherapy were not very specific, relying on crude tumor cell preparations or even general immune stimulants like BCG (Bacillus Calmette-Guérin) in an attempt to boost immune responses to tumors. But the newer strategies take a much more rational approach, says Pardoll.

Exploiting the T cells

One of the most ambitious exploits recent knowledge about how T cells, a key type of immune cell thought to direct the attack on cancer cells, are triggered into action. Immunologists had thought that a T cell is activated by a single signal, a specific antigen delivered by surface molecules on the so-called antigen-presenting cell. But work from several teams during the past few years has shown that T cell activation also requires a second, more general, signal, transmitted by another protein on the antigen-presenting cell surface, known as B7. Without that second signal, in fact, a T cell goes into a state of unresponsiveness instead of being activated by the antigen signal. And that may be one reason why tumor cells aren't destroyed by immune cells. Even though tumors carry antigens that should elicit an immune response, they don't usually carry the B7 protein.

That led several groups to ask whether it would be possible to elicit an immune attack on cancer cells by genetically engineering them to make the B7 protein. The work done so far in animals has given encouraging results, says Pardoll. In those experiments, cells from several different types of tumors were first taken from animals and injected with the B7 gene. Then the cells, which expressed the B7 protein on their surface, were injected into other animals. In

several of the studies, these transfected cells were unable to develop into tumors and were destroyed by the host animals. To Pardoll, results like these point up the importance of basic work like that leading to the discovery of costimulation. "Cancer won't be cured by the lottery," he says. "It'll be beaten by understanding what turns on and off the immune system at the molecular level."

In fact, the animal results were sufficiently encouraging that Mario Sznol, at the National Cancer Institute, has begun the first clinical trial of B7-transfected tumor cells in 12 patients with advanced melanoma. In these initial studies, the goal is to see whether the treatment is safe and shows any sign of inducing an immune response to the patient's tumor cells, but researchers hope that in the future the modified cells might be used to prevent recurrence of melanoma by boosting a patient's immunity.

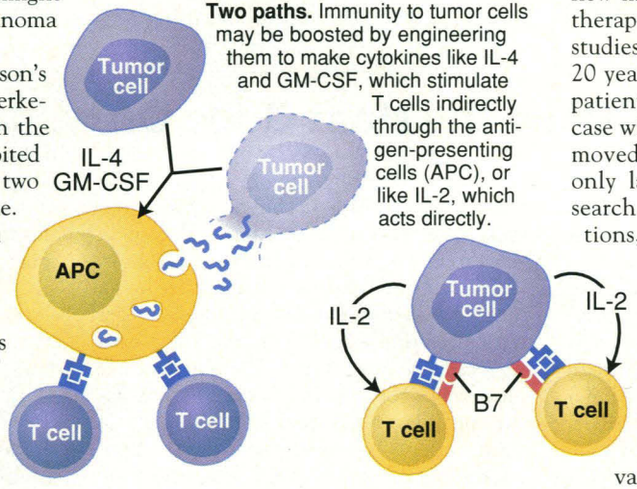
Other recent work by James Allison's team at the University of California, Berkeley, suggests a different way in which the costimulatory pathway might be exploited to boost tumor immunity. T cells carry two receptors that can bind the B7 molecule. When the protein binds the one called CD28, it activates the T cells, provided that they have also received the specific antigen signal. But B7 binding to the other receptor, known as CTLA-4, puts a powerful brake on T cell activation, limiting the immune response. So in work described in the 22 March issue of *Science* (pp. 1691 and 1734), Allison's team injected antibodies against CTLA-4 into mice with tumors. They found that the antibodies, which themselves bind to CTLA-4 and block it from interacting with B7, enhance the animals' anti-tumor response by removing the inhibitory signal. Allison and his colleagues suggest that combining antibody treatment with B7 transfection might be a means to boost responses even further.

Putting cytokines to work

Besides responding directly to the antigen-presenting cells that carry these costimulatory molecules, T cells also respond to messages released from cells, in the form of molecules called cytokines. They, too, offer an opportunity to boost the anti-tumor response, by turning up the cytokine signal. Many researchers have found that if they introduce appropriate cytokine genes into tumor cells from experimental animals, the cells will secrete the cytokines. And when transplanted back into the animals, the engineered cells may provoke an immune response.

Clinical studies based on these promising results are already under way. In one approach, genes for cytokines that stimulate T cells directly, such as interleukin-2 and interferon γ, are introduced into tumor cells; in another, the transplanted genes code for cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4, that act indirectly by stimulating the antigen-presenting cells. "The application could be appropriate for a range of human tumors," says tumor immunologist Larry Kwak of the National Cancer Institute, who is working on immunotherapies to treat cancers of blood cells.

While the gene therapy experiments are aimed at boosting the signals that tell the immune system to respond to tumor antigens, another strategy focuses on the antigens themselves. Like traditional vaccination, it aims to trigger a response by deliberately exposing the immune system to a dose of antigen. In the past, researchers pursuing this approach administered complex cocktails of tumor cell material—and had little success. "For effective immunization against tumors you need defined tumor antigens," says Kwak. But now, says Boon of the Ludwig Institute, "we have plausible and defined antigens on tumor cells that we didn't have 5 years ago."



Two paths. Immunity to tumor cells may be boosted by engineering them to make cytokines like IL-4 and GM-CSF, which stimulate T cells indirectly through the antigen-presenting cells (APC), or like IL-2, which acts directly.

Several of them have already moved into clinical trials in Europe and the United States. About 6 years ago, for example, Boon and his colleagues identified the first of a family of human genes encoding antigens found primarily on melanoma cells, and occasionally on other tumor cell types, but not on normal cells. After experiments in mice suggested that one of these genes, designated MAGE-1 (for melanoma antigen 1), could be an effective anti-melanoma vaccine, researchers at the Ludwig Institute began clinical trials to see whether peptide fragments of MAGE-1 and MAGE-3 can evoke an immune response and shrink tumors in advanced melanoma patients. In addition, Steven Rosenberg's team at the National Cancer Institute has discovered several other melanoma-related antigen genes and is currently coordinating a trial testing two of them, known as MART (for melanoma antigen responding to T cells) and GP100, in 70 patients with metastatic melanoma who have failed conventional melanoma therapies.

In some cases, the antigens being tested as experimental cancer vaccines are themselves antibodies. Kwak, for example, is testing whether antibodies, which are the tumor antigens of malignant antibody-producing cells in diseases such as myeloma and lymphoma, can be used to boost an immune response against these cells. In clinical trials, he has shown that those antibodies, called idiotypes, did induce such a response.

A cautionary tale

But the idiotype approach also delivers a cautionary lesson that applies to all these new immunological approaches to cancer therapy. Kwak points out that the idiotype studies first began in animal experiments 20 years ago and only moved into human patients about 7 years ago. "As is often the case with basic research, the original team moved on to other issues and the work was only later revisited by several other research groups thinking about applications," says Kwak.

The long path to get idiotypes into the clinic strikes a familiar chord with immunologist Ellen Vitetta of the University of Texas Southwestern Medical Center in Dallas, who has been pursuing another antibody-based approach to cancer therapy since the early 1980s. Rather than use vaccines, which aim to boost the recipients' own immune responses, this strategy provides a direct weapon in the form of toxins coupled with antibodies targeted to cancer cells. In theory, the antibodies should direct the toxin to the tumor without damaging normal cells. It's taken 15 years, however, to move some of the antibody-toxin molecules from animal experiments to the late stage of dosage tests in humans.

Most kinds of gene therapy and immunotherapy face the same hurdles her team has seen, Vitetta says: "There's a significant reality check needed to make the move from mouse to man. An enormous amount of patience is needed for trials."

One of the key problems in moving from animals to humans, she says, is the greater variability of the results seen in patients, each of whom comes with a different history. Another scientific obstacle that may hinder application of the new immunotherapies for cancer is that humans may respond poorly to them, no matter how cleverly they tweak the immune system, because the disease often weakens the immune system. "Some won't make an immune response to anything," Kwak says.

And beyond these scientific issues, a host of regulatory and financial concerns needs

to be addressed. Until recently, the regulatory procedure was geared to drugs, usually relatively simple chemicals, that could be mass-produced and given to anyone suffering from a given condition. But some of the new immunotherapies need to be individually designed. That can be the case, for example, for the gene therapies, in which each patient's own, appropriately modified, tumor cells have to be used.

Still, David Glover, vice president of medical development at Cambridge Antibody Technology, believes the general attitude of the regulatory authorities is becoming more helpful in the development of antibodies and other biological therapies. "Everybody is interested in the new technologies, and getting agreement on new medicines is not so difficult as it was 4 to 5 years ago," he says. The fact that some of the therapies need to be individually tailored may also make them less appealing to drug companies, who tend to shy away from products for which demand is likely to be small.

And even after a clinical trial has received regulatory approval, frustrations can arise. One cited by Vitetta is difficulty in changing course. An example comes from her own team, which wanted to change the antibody used in one of their trials to one that is more "humanized" and therefore less likely to generate an immune attack on the therapeutic molecules themselves. But that, Vitetta says, "would mean the start of a whole new trial"—and a whole new round of regulatory approvals.

But despite the formidable difficulties, the advances in understanding how the immune system works and the resulting new armory of experimental immunotherapies are enough to keep many researchers optimistic. "I'm sure cancer vaccines are ultimately going to be an exciting example of the translation of laboratory success into human treatments," says Kwak. If he's right, even Jenner might be impressed by the vigor of his legacy 200 years later.

–Nigel Williams

Additional Reading

J. P. Allison and M. F. Krummel, "The Yin and Yang of T Cell Costimulation," *Science* **270**, p. 932 (1995).

A. Van Pel *et al.*, "Genes Coding for Tumor Antigens Recognized by Cytolytic T Lymphocytes," *Immunological Reviews*, No. 14, p. 229 (1995).

C. B. Kobrin and L. W. Kwak, "The Current Status of Cancer Vaccines," *Expert Opinion in Investigational Drugs* **3**, p. 1241 (1994).

MATERIALS SCIENCE

Ceramic Shrinks When the Heat Goes On

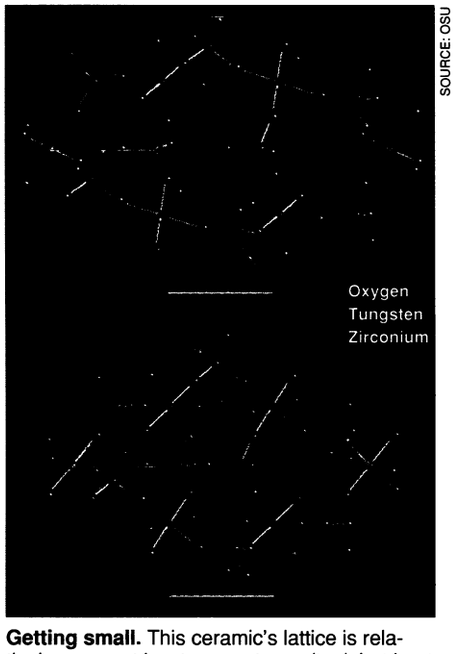
It's hard to break the laws of physics, but sometimes one can bend the rules. Take the well-founded principle that more heat means more size: Added energy—heat—usually causes atoms to swing around more violently, taking up more space and causing materials to expand. But on page 90 of this issue, researchers at Oregon State University in Corvallis and at Brookhaven National Laboratory in Upton, New York, describe a ceramic that's found a huge loophole in the rule: It steadily shrinks as the temperature rises over 1000 degrees.

The shrinking ceramic is a blend of tungsten, oxygen, and zirconium atoms known as zirconium tungstate. Its slimming secret, the researchers have found, is the behavior of its oxygens, which vibrate in a restricted manner that actually pulls the lattice closer together as the heat goes on. "This is really a very unusual material," says Mas Subramanian, a solid-state chemist at DuPont Central Research and Development in Wilmington, Delaware.

A few other materials also slim slightly as they warm; cordierite, for instance, shrinks as its temperature rises from roughly 20 to 500 degrees Celsius. But this ceramic shrinks in just one direction, while it still expands in the other two. The zirconium tungstate's performance is much more dramatic, and it contracts on all sides. That might make the ceramic useful in the construction of electronic circuit boards, where it could counter the heat-driven swelling that can break apart components.

The ceramic's own construction—its atomic lattice—is a complex web of oxygen atoms with tungsten and zirconium atoms scattered among them. Each tungsten atom binds to four surrounding oxygens, while each zirconium atom binds to six oxygens. The many bonds, pulling in different directions, make it difficult for a tungsten or zirconium atom to move around when excited by heat.

Most of the oxygens, on the other hand, bind just two other atoms, hanging between a tungsten and a zirconium like a flag on a rope used in a tug of war. The oxygen can't move much toward either side, all forces being equal, but it can shake up and down or side to side. When heat is added to the crystal, this is exactly what the oxygens do. And the result mirrors what would happen if someone pushed the center of a tug-of-war rope in a direction perpendicular to the tugs. The two people—or in this case, atoms—on either end would be drawn closer together. "The distance between the zirconium or tungsten and oxygen doesn't change," says chemist Art Sleight of Oregon State, who led the research effort. "But the distance between the tungsten and zirconium decreases" as the material warms from 0 to 1050 kelvin, says Sleight.



SOURCE: OSU

Getting small. This ceramic's lattice is relatively roomy at low temperatures *(top)*, but heat causes oxygen atoms to shake, tugging their zirconium and tungsten partners closer *(bottom)* and contracting the material.

Because these "ropes" extend in all directions, the decrease causes the overall material to shrink evenly by 0.75%, an effect the group measured with an atom position tracking technique known as neutron diffraction. The vibrating oxygens don't force the material to bulge, either, because the lattice has large enough voids for them to move without forcing neighboring atoms outward. The overall effect is so strong, Sleight says, that the shrinking stops only when the temperature climbs high enough to destroy the zirconium tungstate material itself.

By blending such shrinking ceramics with the polymers now used in circuit boards, engineers might be able to compensate for the polymers' tendency to expand as they are heated, disrupting circuits. But the effort could run into some minor resistance. Circuit boards must allow electric currents to flow freely through the embedded wires, and polymers, although not as robust as engineers might like, perform this task quite well. The zirconium tungstate, on the other hand, is slightly more electron hungry and would swipe electrons from embedded circuits. Still, Subramanian believes that researchers "may be able to work a compromise" by tailoring the composition of a polymer-ceramic blend so that the final material isn't too electron hungry. If so, shrinking ceramics could be in growing demand.

–Robert F. Service