IMMUNOTHERAPY

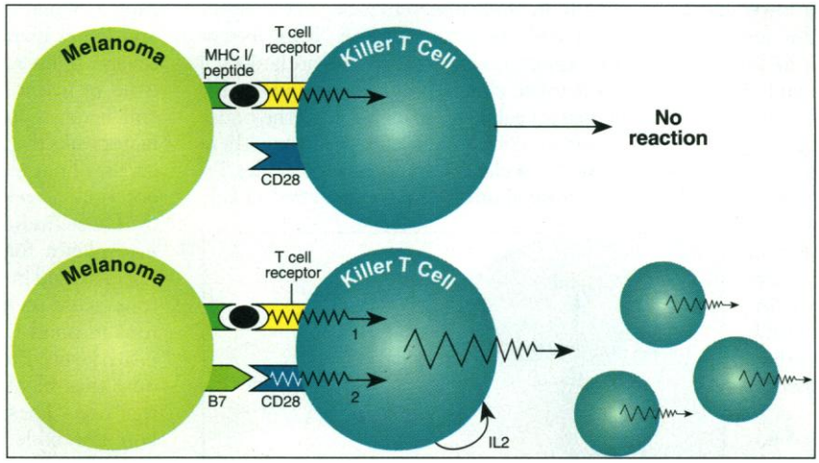
A Stimulating New Approach To Cancer Treatment

In most instances, immune cells are efficient and deadly sentries in our body, quickly identifying and rubbing out invaders such as viruses and bacteria. But when it comes to cancer, the immune system suffers from a frustrating blindspot: Even though many immunologists think cancer cells sport "tumor antigens" that should allow them to be recognized, they somehow escape destruction. Now, aided by recent advances in understanding what it takes to trigger immune cell activity, researchers in at least three independent labs have come up with a novel gene therapy approach that could overcome that frustrating inability of the body's defenses.

The new approach has so far been tested only in mice, but some immunologists are optimistic about its potential. "There's a great deal of promise in this line of therapy. The application could be widespread for most human tumors," says tumor immunologist Larry Kwak of the National Cancer Institute's Biological Response Modifiers Program. But he also notes that the history of cancer immunotherapy is rife with approaches that looked good in animal trials only to prove disappointing in humans. Still, Kwak and others say this new type of immunotherapy looks encouraging enough that clinicians are rushing to try it with human patients.

The three groups working on the new immunotherapy are those of molecular immunologist James Allison and graduate student Sarah Townsend of the University of California, Berkeley, who describe their results on page 368 of this issue of *Science*; Peter Linsley, Lieping Chen, and their colleagues at Bristol-Myers Squibb Pharmaceutical Research Institute in Seattle, who detailed similar work in the 24/31 December issue of *Cell*; and a group led by Laurie Glimcher of the Harvard School of Public Health, whose work is unpublished.

All three teams based their experiments on the understanding that has emerged in the past few years of how the critical immune cells called T cells are called into action. Immunologists had thought T cells are activated by a single signal: the presentation to the T cell of bits of protein known as antigens, on the surface of another cell. But test-tube studies have lately revealed that a second, more general signal from so-called antigen presenting cells is usually also required. Such "costimulation" occurs when a surface molecule, B7, on the presenting cell interacts with a T cell molecule known as CD28. Indeed, a T cell given the first signal but not the second by B7 goes into a state of unresponsiveness. That may be one reason why tumor cells, which don't usually carry the B7 protein, aren't destroyed by immune cells.



Cooperation needed. Melanoma cells engineered to make the B7 surface molecule gain the ability to stimulate the multiplication of killer T cells with antitumor activity.

The three groups who are working on this problem reasoned therefore that if they could somehow add B7 to cancer cells, tumor-specific immune cells could then both recognize the cell through its tumor antigens and also receive the needed second signal from B7. The Seattle group, for instance, introduced the B7 gene (along with a gene for a viral antigen known to provoke a strong immune response) into melanoma cells. The second gene was needed, they felt, to ensure that T cells would receive the first signal—most tumor lines do not induce an immune response. When the researchers injected the doubly-altered cancer cells into mice, tumors began forming, but in every single case the cancers regressed completely within 2 or 3 weeks.

That was remarkable, but it was far from the kind of challenge clinicians will face in human beings if this kind of therapy is ever tried. Specifically, the mice had no tumors when the treatment began. In an experiment that more closely parallels the human challenge, the group first injected mice with unaltered melanoma cells so that the animals would have growing tumors at the time of treatment. Four days later, the researchers intravenously introduced the genetically engineered cells into the mice. Again, the results were dramatic: All treated mice lived longer than untreated controls; 40% survived the experiment and appeared tumor-free. The results suggest, Linsley says, that melanoma cells displaying B7 and the viral antigen on their surfaces were able to elicit an immune response to all the melanoma cells.

Allison and Townsend's experiment was very similar to that of the Seattle team, but differed in one important way. The Berkeley workers used the same melanoma cell line, but decided not to cotransfect a gene for a viral antigen. "All we put in was the B7-molecule," says Allison, explaining that they hoped that antigens already on the tumor surface would be sufficient to provide the first signal to the T cell receptor. Their hunch proved correct. Although the mice injected with the B7-enhanced tumor cells developed tumors, they slowly regressed over time, and none of the animals died. That finding is perplexing, because the Seattle group's work had suggested B7 alone would not do the trick. Further work is needed to explain the contradiction.

The Berkeley team has also shown that they can exploit the altered melanoma cells as a sort of cancer vaccine to prevent the growth of melanoma tumors. For example, after first "priming" the immune system by injecting the mice with B7-positive melanoma cells, Allison and Townsend waited more than 2 weeks before challenging the animals by injecting them at a different site with unaltered tumor cells. The "vaccine" protected 89% of their mice for more than 3 months, Allison says. Such a vaccine might prevent metastases in melanoma patients who have had their primary tumors surgically removed.

What apparently happens in the B7 experiments, both groups believe, is that the altered melanoma cells directly costimulate tumor-specific "killer" T cells of the immune system. This costimulation appears to affect gene expression in the cells, increasing the production of interleukin 2 and other growth stimulatory hormones that cause the killer cells to multiply and start an all-out attack on tumors. Ordinarily, killer cells need the extra interleukin 2 produced by another T cell class, the so-called helper cells, to proliferate, but by using antibody to knock out the helper cells in the test animals, each group could show that such assistance wasn't necessary. "We're probably enabling them [the killer cells] to make suffi-

cient interleukin 2 to overcome the need for help," explains Linsley.

Despite the impressive confirmation of costimulation's potential as a therapy by three different groups, gene therapy with B7 may still join the ranks of other strategies that work well on manmade tumors in mice but are not effective in humans. Even Lee Nadler of the Dana-Farber Cancer Institute, who cloned the B7 gene and is eager to try a B7-based therapy on human patients as soon as possible, wonders whether the costimulation strategy can treat naturally occurring cancer. "One should not be waving the flag and saying that this is the answer. I'm excited but very wary," he says. Among his many concerns is that recent work suggests T cells become tolerant of normally developing tumors. If so, costimulation could prove ineffective. Nadler does believe, however, that if cancer immunotherapy does ever come to fruition, costimulation will play a role in it. Drew Pardoll, an oncologist at John Hopkins University who specializes in immunotherapy, agrees: "Cancer won't be cured by the lottery. It'll be beat by understanding what turns on and off the immune system at the molecular level." And for the moment, the on-switch known as B7 is drawing lots of attention.

–John Travis

CHEMISTRY

Catalytic Conversion Could Be a Gas

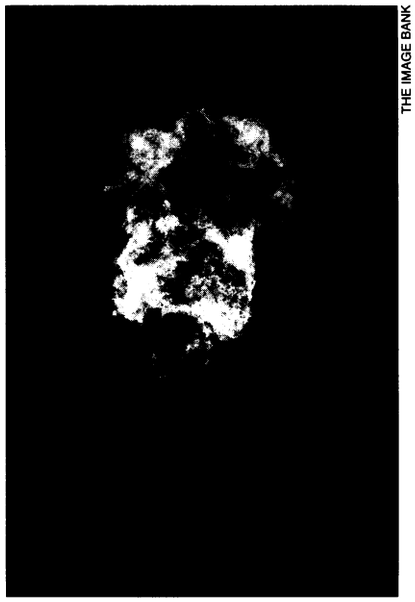
Like lovers, chemists spend time aplenty trying to get the object of their attention to change its ways. In fact, the analogy goes further than that, because generally what lovers want is for the object of their attentions to be more responsive—which is often what chemists desire. And, like lovers, chemists sometimes get their wish. In this issue of *Science*, two groups report using catalytic methods to coax methane (CH4) into being far more reactive than it ordinarily is. The new work might have powerful economic implications: If it proves out, it could render methane (which makes up most natural gas) a rival to petroleum as a fuel and a chemical feedstock.

Not everybody in the field is willing to buy that claim immediately—but they're intrigued. "The researcher in me says these are pretty interesting" findings, says Peter Barone of the Gas Research Institute in Chicago. "The engineer in me says that [researchers] need to take a strong look at the economics" of the processes for converting methane into more widely usable products before the real promise of the processes can be assessed.

These two groups are hardly the first to be intrigued by methane's potential. The 9000 trillion cubic feet of natural gas in the world's known and projected reserves harbors an amount of energy comparable to the world's 1.5 trillion barrels of oil, note Roy Periana and his coauthors from Catalytica Inc., in Mountain View, California (see page 340). But several factors have conspired to relegate natural gas to the minor leagues compared to oil. The world's largest reserves—in places like Southeast Asia and Canada's northern reaches—are vast distances or oceans away from the centers of consumption, note the authors of the second paper, chemical engineers Lanny D. Schmidt of the University of Minnesota and Dan Hickman of Dow Chemical Company (see page 341). Transporting it as a rarefied gas is expensive, yet compressing or liquefying it to make transport easier raises the risk of fiery or explosive catastrophe.

That's the reason most natural gas is "flared" (burned off) as it vents upward when oil companies pump the world's primary energy lifeline, oil, which is found in the same fields. Natural gas is often "cheaper to waste than to transport," says George Lester, who works at Allied Signal Research, where he helped develop the catalytic converter technology used in automobiles—the technology that forms the basis of Hickman's and Schmidt's process.

Yet natural gas is an excellent source of energy that would find more extensive use if it could be made safer and cheaper to transport. That potential has inspired plenty of research directed at converting methane into something else, such as liquid methanol (CH_3OH), an easily transportable fuel, or synthesis gas ("syngas"), a combination of hydrogen and carbon monoxide that can serve both as a chemical feedstock and as a precursor to methanol. In fact, researchers have been pulling these and other conversions off for decades for specialized purposes, using mostly energy intensive and high-temperature processes, but they haven't been able to do so in a way that keeps the bottom line happy in large-scale use.



THE IMAGE BANK

Flareup. A fiery "flare" marks natural gas being burned off in an oil field.

To do that means grappling with a real chemical challenge: the four strong bonds between methane's central carbon atom and its surrounding quartet of hydrogen atoms. "It's a very inert molecule," Schmidt says. "Once you find conditions extreme enough to attack it, it goes all the way to carbon dioxide and water," rather than stopping at the intermediate—and economically useful—stages of syngas or methanol.

Hickman and Schmidt tackle the task by flowing room-temperature methane and oxygen (or air) through a heated, sponge-like ceramic disk whose surfaces have been coated with platinum or rhodium. Rather than oxidizing to water and carbon monoxide, the methane molecules form hydrogen and carbon monoxide, yielding a hot syngas. Using gas chromatographic analyses of the products, Hickman and Schmidt conclude that 90% of the methane going through this catalytic gauntlet is converted to syngas, with the reaction occurring in about a thousandth of a second. At that rate, Schmidt projects, industry could build house-sized methane conversion plants at the remote sites of natural gas reserves capable of producing syngas at the same clip as a billion-dollar syngas plant bigger than a city block that uses current technology. Concedes Lester: "This could be a contender."

The Catalytica team found another way to partially oxidize methane, this time into methanol. The combo of sulfuric acid and a catalytic amount of metal ions such as mercury 2+, which readily accepts electrons, forces methane to replace one of its C-H bonds with a $COSO_3H$ bond and become methyl bisulfate (CH_3OSO_3H). That compound serves as a "protected form of methanol," Periana says. It doesn't oxidize further the way methane does, and in water it readily converts to methanol while regenerating the sulfuric acid. The Catalytica team claims the yield of methanol from this process is about 40%, compared to yields of less than 5% obtained so far in other approaches using direct oxidation of methane.

"Petroleum is civilization's feedstock for energy and carbon-based materials," Periana says. But, he cautions, "it will last maybe 50 to 75 years." Methane conversion schemes such as those proposed by Periana, Hickman, and Schmidt could help to fill the gap after that, allows Lester of Allied Signal. But not, he adds, until "industry takes a close look"and then only if the new methods genuinely prove superior to classical ones.

–Ivan Amato