"The" Magic in Magic Bullets

Researchers are using their knowledge of how substances get into cells to make toxins that kill unwanted cells

In March a group of researchers at the University of Minnesota tried a daring new treatment for leukemia. Frequently a patient with leukemia who has had several relapses after being treated with chemotherapy is offered a bone marrow transplant. But the donor bone marrow may contain cells that can cause the serious complication of graft versus host disease in the leukemic patient. These scientists took donor marrow and pretreated it with an immunotoxin—an extremely potent poison hooked up to a monoclonal antibody that recognizes only those cells in the donor marrow that can cause graft versus host disease. The experiment, says John Kersey of the University of Minnesota, "marks what may be the start of a new era in using more selective agents to get rid of unwanted cells."

Although the use of immunotoxins in medicine is new, the idea of these poisons, often called "magic bullets," has been around for at least 75 years. Link a potent toxin to an antibody directed against a certain type of cell that you want to kill, such as a cancer cell or a cell that can cause graft versus host disease. Then the antibody should attach itself directly to those cells and destroy them, while at the same time sparing other cells that do not have these antigens on their surfaces. But medical researchers have only recently begun to make effective immunotoxins as they finally begin to learn how toxins and other substances get into cells and how to use this knowledge to make immunotoxins that do not get blocked at the cell surface or get destroyed by enzymes inside the cell.

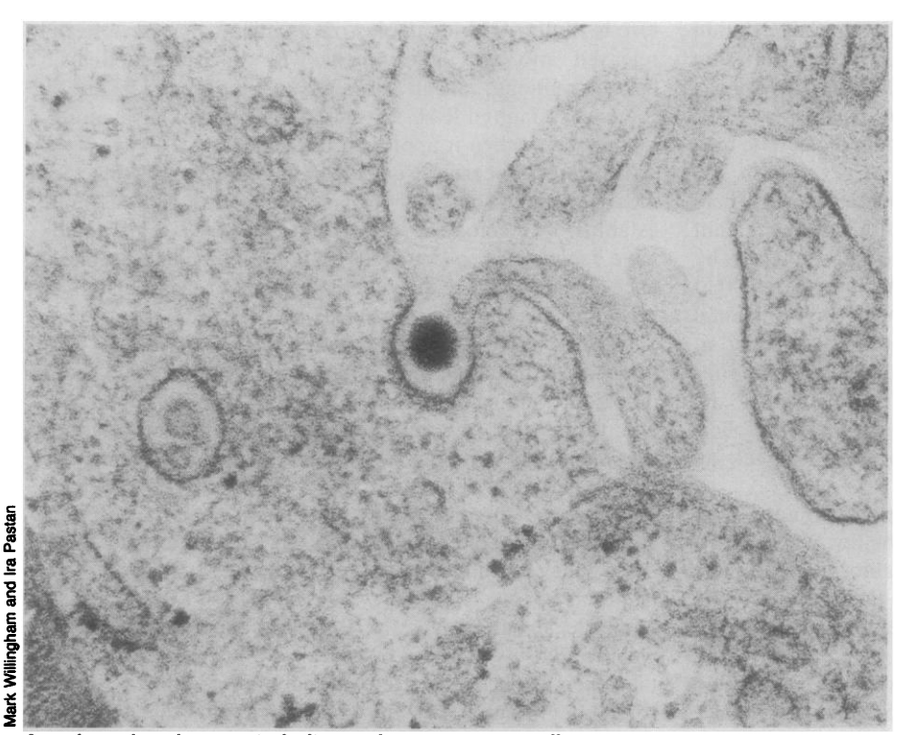
Scientists first began to understand how substances get into cells about 8 years ago, and since that time an intriguing picture has emerged. Eight years ago, it was recognized that hormones such as insulin, nutritional substances such as low density lipoproteins (LDL), viruses such as adenovirus, and toxins such as diptheria toxin, bind to specific receptors on the surfaces of cells. But no one thought that those receptors actually entered the cells. Then, Michael Brown, Joseph Goldstein, and Richard G. W. Anderson of the University of Texas Health Science Center at Dallas found that LDL, which carries cholesterol to cells, is taken up by cells together with its receptor. At around the same time, Stanley Cohen of Vanderbilt University discovered that epidermal growth factor enters cells along with its receptor; and Donald Steiner and Susan Perris of the University of Chicago learned that insulin receptors and insulin enter cells together.

These findings were hard to believe. Steiner says that when he saw the results of his experiments showing that insulin receptors get into cells, he wondered if he had made a mistake. His colleagues were incredulous. "Quite frankly, many in the field didn't believe it," he says.

A few years later, Brown, Goldstein, and Anderson discovered how the LDL receptor and LDL get into cells. The receptor moves to places on the cell surface called coated pits where it binds LDL. Then, says Brown, the receptor and its LDL are "gobbled up by the cell." The Texas group also reported that these coated pits are essential for the entry of LDL into cells. They had as a patient a child whose cells could not take up LDL. The problem was that the LDL receptors, although they could bind LDL, were not recognized by the coated pits. "The functional consequence was the same as not having LDL receptors," says Brown.

Soon afterwards, Ari Helinius of Yale University reported that a number of viruses get into cells by picking out normal cell surface receptors that have the ability to go to coated pits and piggybacking on these receptors. It also was discovered that the potent plant toxin ricin enters cells through coated pits as do the bacterial toxins produced by diphtheria and *Pseudomonas*. Now at least 12 large molecules, including viruses and hormones, are known to get into cells through coated pits.

Several researchers including Helinius and Ira Pastan and Mark Willingham of the National Cancer Institute then asked, What happens to the receptors and the ligands that are bound to them



Mark Willingham and Ira Pastan

An adenovirus in a coated pit on a human cancer cell

This virus particle has bound to its receptor on the surface of the cell and is on its way to the cell interior.

after they enter the cell? They discovered that the receptors and ligands accumulate in sacs inside the cells which have been called "endosomes" or "receptosomes." There the receptors and ligands have an acid bath and the ligand separates from its receptor. Then, in many cases, the ligand goes to the lysosome where it is digested and the receptor goes back to the cell surface.

At the same time as cell biologists were studying how receptors get into endosomes, David Neville and his associates at the National Institute of Mental Health (NIMH) began to explore how proteins get into the cytosol, which is the main body of the cell and the place where proteins are made. Certain poisons, such as ricin and diphtheria toxin, kill cells by stopping protein synthesis and these poisons are so powerful that only one molecule of them is lethal to a cell. Neville's idea was to link the A chain of diphtheria—which is the part that kills cells—to human placental lactogen, a polypeptide hormone.

It took Neville and his associates Tamin Chang and Alice Dazord 4 years to make a hormone-toxin molecule, but when they tried it out in 1977, they found that it did not get into the cytosol of cells. This raised the possibility that either the diphtheria receptor or the diphtheria B chain is necessary for the toxin to get in.

By this time, the stage was set for researchers to try to develop immunotoxins. They were able to make monoclonal antibodies that specifically recognize molecules on cell surfaces and they were beginning to realize how toxins get into cells.

Among the researchers who have used this new knowledge are Neville and Richard Youle of NIMH. Reasoning that the A chain of ricin, like the A chain of diphtheria, may not by itself get into cells to kill them, they attached the entire ricin molecule to monoclonal antibodies to T cells. These immunotoxins get into T cells and kill them. But since they use the entire ricin molecule, the ricin itself can recognize its receptors on cell surfaces and so can get into other kinds of cells. In order for this immunotoxin to be specific for T cells, it is necessary to block the ricin receptors on other cell types. This is easy to do in vitro, the NIMH scientists report, because, as Youle and Gary Murray discovered, the sugar lactose blocks the ricin receptors. All they have to do is bathe the cells in lactose.

There is an immediate clinical use for these ricin immunotoxins in bone marrow transplants. Patients with leukemia

Neptune Ring Fades Again

The wave of new planetary ring discoveries that seemed to be sweeping the solar system has again petered out short of Neptune, the outermost major planet and the last of the four Jovian planets still lacking a known ring system. A group of astronomers suggested last year that they may have detected a ring of Neptune in 1968 without recognizing it at the time, but a thorough search this June as a faint star passed behind the planet has eliminated the possibility of any but the faintest Neptunian rings. An absence of rings would make the odd Neptune system odder still.

As Neptune crept slowly toward a faint star on 15 June, astronomers positioned from Tasmania to Taiwan strained to detect the slightest flicker in the brightness of the star that might signal its passage behind a ring. They found nothing. They are still refining their analysis and reducing the maximum opacity of any undetected rings, but they are now sure of several things. James Elliot of the Massachusetts Institute of Technology, whose team observed June's stellar occultation from the Kuiper Airborne Observatory flying at 12,000 meters over the western Pacific, says that they could have detected a ring four times more opaque than the least opaque of Uranus's narrow rings. Further analysis should half this upper limit. Even flying above much of Earth's atmosphere, Elliot could not have detected rings as wispy as Jupiter's, which is about 1000 times less opaque than Uranus's most diffuse ring.

Observers are also confident that they probed close enough to Neptune to rule out any ring like the one tentatively proposed last year by Edward Guinan and his colleagues at Villanova University. They reported a recently recognized dip in brightness recorded just after a 1968 Neptune occultation (*Science*, 9 July 1982, p. 143). If a ring caused the dip, it would extend from 4,800 to 11,100 kilometers above the planet. No observed occultation had probed that close to the planet until last June. Elliot is confident that in June they probed within 1000 kilometers of the top of the atmosphere in the plane of any equatorial rings. And they could not have missed any dip in brightness as large as the apparent 1968 dip. Guinan now presumes that a thin passing cloud produced their brightness dip; other possible explanations are highly unlikely.

If Neptune had no rings at all, it would be strange indeed. It would be the only ringless planet of the four gas giants, although it and Uranus are nearly identical in size and mass. The Neptune system already includes the solar system's most elongated satellite orbit—Nereid's steeply inclined orbit carries it 10 times farther out than its closest approach to Neptune. It also has the only large inner satellite, Triton, that orbits in the direction opposite to the rotation of its planet or in a plane steeply inclined to the planet's equator. This unique satellite system has prompted speculation that some intruder, perhaps a tenth planet, wandered through the Neptune system, disrupted the motions of its satellites, and even tossed a third satellite out of the system that became the planet Pluto. Dynamicists have raised various objections to the intruder theory, but whatever produced this irregular satellite system, the reasoning goes, may have denied Neptune its rings.

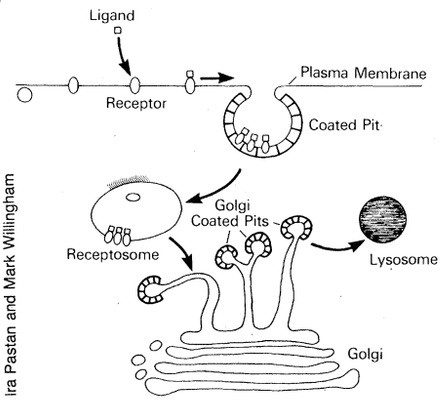
There is still one tantalizing hint of possible Neptunian rings. On 24 May 1981, Harold Reitsema and his colleagues at the University of Arizona detected a dip in the combined brightness of a star and Neptune as the two passed each other in the sky. Apparently, something near Neptune, presumably a satellite, totally blocked the light of the star. If it was a third satellite and it orbits in the planet's equatorial plane, it is at least 180 kilometers across and was 50,000 kilometers above the planet. The chances of catching a lone satellite in front of the star were slim, perhaps 1000 to 1. That has prompted speculation that there are many more small, inner satellites. Around all three of the ringed planets, such moons shape and even rejuvenate rings. Is Neptune the exception? The next decade may tell. Although there are no predictions yet, Neptune could occult a much brighter star, allowing detection of fainter rings. Space Telescope will also make more sensitive searches after its launch in 1986. And Voyager 2, with luck, will encounter Neptune in 1989.—**RICHARD A. KERR**

or certain diseases of the immune system can, in theory, be treated with bone marrow transplants. First they are given high doses of radiation to kill their own bone marrow cells. Then they are given bone marrow from a donor who is immunologically compatible. The problem, however, is in finding a compatible donor. If the marrow is from an identical twin, there is, of course, no problem. But if the marrow is not from an identical twin, the T cells of the donor marrow may see the marrow recipient as a foreign object and attack it. The result is graft versus host disease, a "terribly debilitating disease," says Kersey in which patients die because the grafted cells attack their bodies and never work very well as an immune system.

The major antigens responsible for graft versus host disease are the HLA antigens. People inherit one set of these antigens from each parent and so siblings have one chance in four of having the same HLA antigens. If the HLA antigens are mismatched in a bone marrow transplant, there is an 85 percent chance that the marrow recipient will develop graft versus host disease. That chance is greatly reduced if the HLA antigens are matched.

There is a way, however, to transplant mismatched bone marrow cells. Daniel Vallera of the University of Minnesota and others showed that if the mature T cells in the donor marrow can be selectively killed, the immature cells remaining will grow up to recognize the transplant recipient as "self" and will not cause graft versus host disease. This is where the immunotoxin comes in. About 1 year ago, Vallera and Kersey of the University of Minnesota working with Youle and Neville of the NIMH showed that they could take bone marrow cells from mice and transplant them into other mice that have different HLA antigens if they first kill off the mature T cells in the donor marrow with the immunotoxin. Less than 15 percent of the recipient mice got graft versus host disease whereas without the immunotoxin treatment all of them would have succumbed. Then Vallera and his colleagues showed that the immunotoxin can kill mature T cells in human bone marrow. Now they are beginning clinical studies to see if they can use their immunotoxin to prevent graft versus host disease in humans. The results so far, in the three patients that they have treated, are "encouraging," they say.

Investigators would, of course, like to make immunotoxins that work in vivo as well as in vitro. If they attach the whole ricin molecule to a monoclonal antibody,



Ira Pastan and Mark Willingham

How molecules get into cells

A ligand, which may be a hormone, virus, or toxin, for example, binds to a receptor on the surface of cells which transports it to a coated pit. The coated pit engulfs the receptor and its ligand and takes it to a receptosome or endosome. From there, it enters the cell interior or goes to a lysosome where it is destroyed.

they are faced with the problem of somehow blocking the ricin from binding to its own receptors on cells throughout the body. Moreover, says Neville, "The window between toxicity to target cells and nontarget cells is not great. The ratio of nontarget to target cells is between one thousand and one million. There are so many more nontarget cells that they're going to sop up this stuff."

One possible way out of this dilemma is to avoid hooking the whole ricin molecule to an antibody. But, as Neville found, the ricin toxin does not kill cells very efficiently when only the A chain is used. However, Youle and Neville have found that if they hook the A chain of ricin to an antibody and add the B chain to cells separately, three to five times as much toxin gets into the cell cytosol and far more cells are killed.

Now Ellen Vitetta, William Cushley, and Jonathan Uhr at the University of Texas Health Science Center at Dallas have further refined this solution. What they did was to separate the ricin molecule into A and B chains and then hook each chain up separately to an antibody. "The A and B chains bind to the surface of the cell and we guess that they end up engulfed in the same vesicle. Then the A and B chains may be released from the antibodies and form the native poison," Uhr says. The Texas researchers report that this system results in a "marked potentiation" of ricin's toxicity as compared to immunotoxins that deliver the A chain alone and the independent delivery of the two ricin chains by the antibodies eliminates the nonspecificity of ricin binding. Uhr speculates that the B chain is used to get the ricin poison out of the endosome and into the interior of the cell. But, he remarks, "There is still a great deal to learn. It is not as though we fully understand the intracellular events."

Pastan and Willingham, working with Ian Trowbridge and David Fitzgerald at the Salk Institute also are studying ways to get toxins out of endosomes and into cells and they, too, have found a potentially useful method. They hooked *Pseudomonas* toxin to antibodies to the transferrin receptor—which is the receptor for the iron carrying protein. This immunotoxin by itself is not very potent, presumably because the toxin does not easily escape from the endosome. But Pastan and his associates had found previously that adenovirus gets into cells from endosomes by punching a hole in these vesicles. When they added adenovirus to the immunotoxin, they found that it was 100 to 300 times more effective in killing cells.

Pastan does not believe that this use of adenovirus will be of immediate clinical use, but he does think it may be useful in the long term. "If we could understand the mechanism by which adenovirus lyses [the endosome], we could isolate the viral protein and make a conjugate with it," he says. In other words, Pastan envisions linking together the toxin, a monoclonal antibody, and an adenovirus protein that will burst the endosome. Another possibility, Pastan remarks, is to exploit the mechanism diphtheria toxin uses to get out of the endosome. This toxin, apparently, slips through the endosome membrane. It may be possible, according to Pastan, to engineer a molecule that slips through the endosome. In any event, it is clear that research on how molecules get into cells is a key to making effective immunotoxins. "We knew when we made immunotoxins that they were not as active as we wanted them to be, but it was not clear why. Now we know that one of the important problems is getting across the membrane," Pastan remarks.

For the time being, then, immunotoxins will likely be of limited clinical use—although their use in bone marrow transplants is by no means unimportant. But investigators are optimistic that these toxins will eventually become extremely useful. "From the point of view of basic research it's an interesting problem. And it is well worth pursuing from a practical point of view. But it's going to take awhile," says Trowbridge.

—GINA KOLATA

Additional Readings

1. M. S. Brown, R. G. W. Anderson, J. L. Goldstein, *Cell* **32**, 663, (1983).
2. E. S. Vitetta, K. A. Krolick, M. Miyama-Inaba, W. Cushley, J. W. Uhr, *Science*, **219**, 644 (1983).