Fall 1986

# The Surface of a Small Cell: [Dr. Alexander Tomasz]

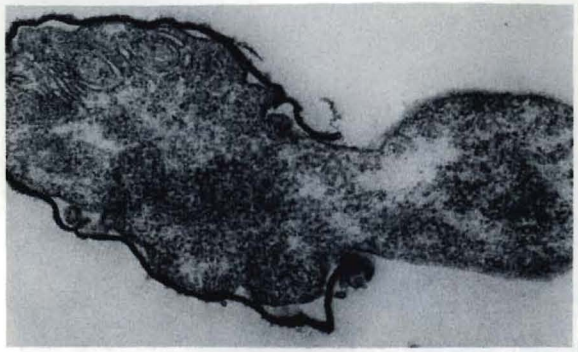
Fulvio Bardossi

Judith N. Schwartz

THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

FALL 1986



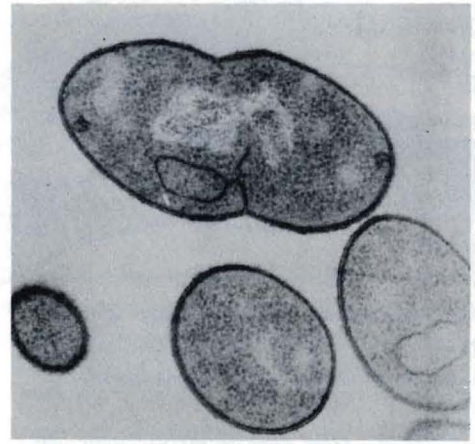
*Bacterial "suicide." A pneumococcal cell explosion triggered by penicillin.*

# The Surface of a Small Cell

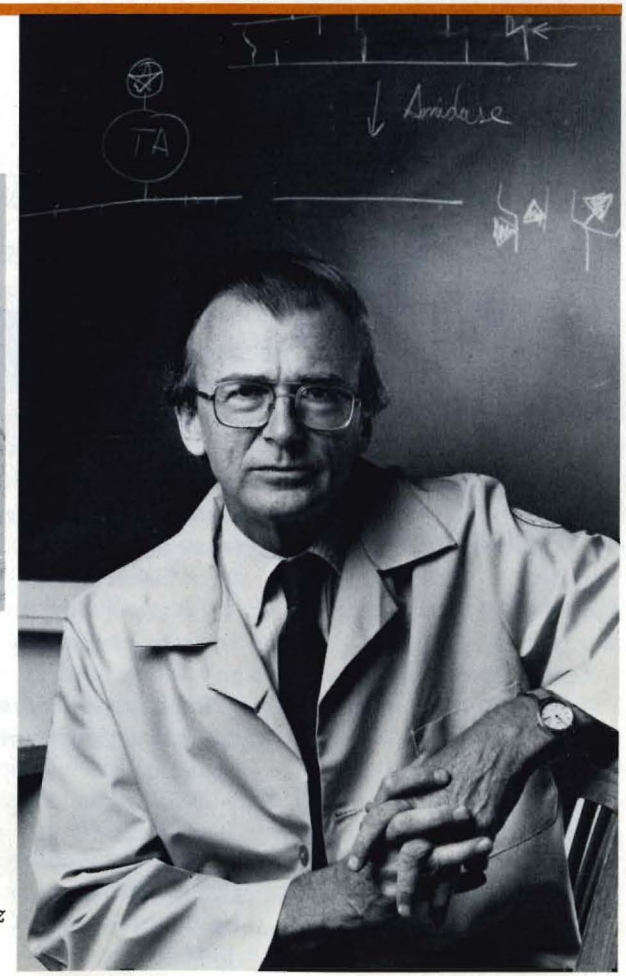
To the naked eye it is a milky liquid in a test tube. But when a droplet of it is seen through the thousandfold magnifying power of the microscope in Alexander Tomasz's laboratory, the field of vision suddenly becomes filled with hundreds of football-like objects, remarkably uniform in shape and size, some of them arranged in pairs, some aligned tip-to-tip like a necklace.

"You are looking at pneumococci," explains Dr. Tomasz, "bacteria that are among the most dangerous agents of human disease. You are also looking at the microbe that provided the clues to what many consider the most important discovery of biology in this century: the identification of DNA as the material of the genes."

In the preantibiotic era, up to the 1940s, pneumococcal infection was a leading cause of death, surpassing heart disease and cancer. Even with the introduction of penicillin, the pneumococcus has remained a major killer of children. Not surprisingly, many of the first generation of biologists and chemists at what was originally known as The Rockefeller Institute for Medical Research concentrated on learning more about the pneumococcus. Their efforts were rewarded with some of the most important landmarks of microbiology, including the chemical characterization of the first polysaccharide antigen, the sugary "coat" that enables bacteria to evade a host's immune defenses. The crowning achievement to emerge from

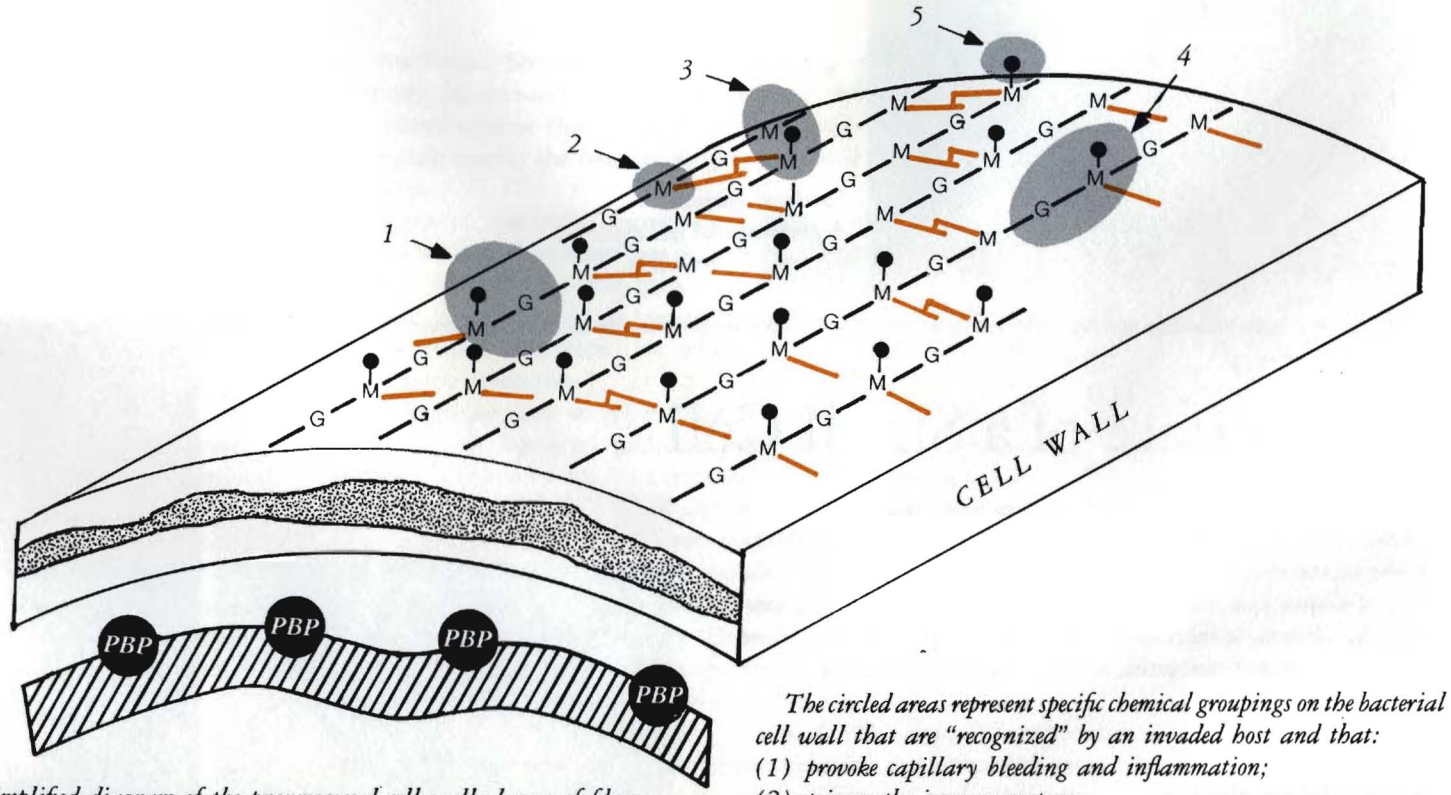


*Pneumococcus surrounded by its protective wall.*




*Alexander Tomasz*

*Elaine Tuomanen reading a computer printout that shows the growth of pneumococcal cultures and their death by penicillin.*




*Simplified diagram of the pneumococcal cell wall, layers of fibers composed of chains of sugars (G and M), interlinked by short peptides (made of amino acids), shown in orange. (G = glucosamine. M = muramic acid.)*

*The small black balls represent other types of sugar chains, an important component of which is choline, essential to normal cell growth.*

*PBPs, penicillin-binding proteins. Responsible for building cell walls, these proteins are the targets of penicillin action.*

*The circled areas represent specific chemical groupings on the bacterial cell wall that are "recognized" by an invaded host and that:*

*(1) provoke capillary bleeding and inflammation;*
*(2) trigger the immune system;*
*(3) serve as an attachment site for proteins of complement, a substance important in host defense;*
*(4) activate complement before attachment;*
*(5) react with the so-called C-reactive protein, which is produced in large amounts in the blood during infection but the function of which is still unclear. This part of the wall also contains choline, the receptor for autolysin, the enzyme which, when activated by penicillin, causes the pneumococcus to explode.*

pneumococcal research at The Rockefeller—the demonstration that DNA was the molecular carrier of heredity—was reported by Oswald Avery, Colin MacLeod, and Maclyn McCarty in 1944.

"Ironically," Dr. Tomasz remarks, "the original question addressed by the early studies, namely, how do as few as a hundred of these tiny bacteria kill a child, has remained unanswered." For the past several years, Dr. Tomasz's laboratory has been

readdressing this question. "I'm convinced," he says, "that the answers lie in a better understanding of the chemistry of the pneumococcal surface, specifically, the outermost layer called the cell wall.

"What fascinates me about the cell wall," he says, "is the improbable nature and number of tasks it has to perform. It has to be rigid enough to maintain the integrity of the cell. It has to reproduce itself, in perfect replicas, in each cell division. And it has to regulate the opening and closing of its many gates, doors, and windows to allow the orderly traffic of thousands of nutrient molecules in and out of the cell every second. It also serves as the cell's communication center, receiving and transmitting signals from and to the outside world."

A slender band measuring about a thousandth of a millimeter and visible only through the electron microscope, the cell wall is, in fact, an enormous molecular envelope, conforming to the size and shape of the cell and surrounding the entire cell with an uninterrupted protective network of chemical bonds. Bacterial walls represent the largest molecules in nature. Bacteria can reproduce these "miniature architectural masterpieces," as Dr. Tomasz calls them, with speed and precision in each cell division. He notes: "I often watch and admire the logistics that go into the construction of a building in New York. Imagine a house already inhabited and yet under continuous expansion with such miraculous efficiency that a new, identical twin structure arises every half hour without inconveniencing the tenants."

## BACTERIA TALKING TO ONE ANOTHER

Hungarian-born Alexander Tomasz briefly considered a career in music before deciding to study biology and chemistry at the University of Budapest. He fled to Austria after the Soviet suppression of the Hungarian uprising in 1956, crossing the border hidden in a canvas-covered farm truck. Shortly after, he came to New York.

"Like so many other refugees," he says, "I came with only the clothes I wore. After I had found a job as a technician at Sloan-Kettering Institute and a small flat, I invested my first paycheck in some clothes, a record player, and two favorite recordings—the Bach double concerto for violins and the Mass in B Minor. Furniture came later."

Resuming his scientific education, he earned a Ph.D. in biochemistry at Columbia University. "My mind was only vaguely set as to what I wanted to do next. I was interested in two subjects, molecular genetics and the surfaces of cells."

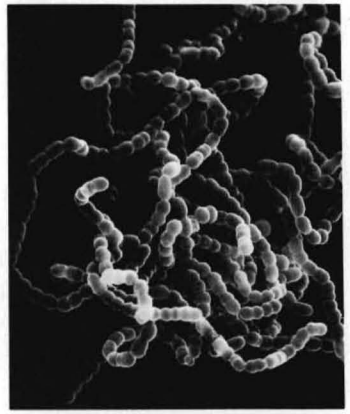
The pneumococcus has the capacity, rare among bacteria, to take up free-floating strands of DNA from its environment. This capacity was dubbed "competence" by the investigators who took advantage of it in studying the mechanisms by which nonvirulent pneumococci are transformed by absorbing DNA molecules from virulent neighbor pneumococci (which led to the recognition of DNA as the genetic bearer).

Through his reading, Dr. Tomasz came upon the perfect problem to engage both his interests: how pneumococci capture DNA molecules during the process of genetic transformation. It was clear that the place to approach it was The Rockefeller University laboratory of Rollin Hotchkiss, whose group had made enormous advances in refining the discovery of Avery, MacLeod, and McCarty.

"The reputation of the place was formidable," says Dr. Tomasz. "I still remember my interview with Hotchkiss. At the end of it I was so excited that I inadvertantly put on his somewhat oversized galoshes instead of my own. Hotchkiss observed with quiet amusement and then asked, 'How does it feel to be in my shoes?'"

The work on competence started out with "a lot of frustration," Dr. Tomasz recalls. "Most people in the field were interested in it simply as a technique to assess the quality of the DNA molecule. I was at heart a cell biologist. I was interested in the competent cell itself. I wanted to know what special properties of the pneumococcus enable it to recognize bare DNA floating by. Are there special entry points, special doors on the cell surface? Could one see them?"

Soon after his arrival, he consulted with Walther Stoeckenius, now a professor of biophysics at the University of California, San Francisco, who showed him how to mix pneumococci with



*Scanning electron micrograph of pneumococcal cells stuck together in long chains. The inhibition of normal cell division is one of the consequences when the bacteria are grown in a medium containing the abnormal compound ethanolamine in place of choline. Another consequence is penicillin tolerance.*

DNA molecules and, by means of a special technique new at the time, see both cells and molecules in the electron microscope.

"I will never forget my excitement and amazement when, after searching and searching in vain, I suddenly saw the contour of a long, slender fiber of DNA attached to the side of a pneumococcus. I knew that I was fortunate to have caught a cell at the moment of uptake. I ran into the hall and called everyone in to look." (The micrograph has since appeared in several microbiology textbooks.)

Despite his thrill and "aesthetic satisfaction" he still hadn't found the "door" he was looking for. "As it turned out," he says, "I was soon to find the 'key' for the door, but the nature of the door itself has remained pretty much an enigma."

It had been known for some time that in pneumococcal cultures passing through a typical growth cycle the ability to take up DNA emerged in a peculiar manner. At low cell concentrations competence was undetectable. Then, as the cells multiplied, it appeared abruptly and spread through the culture until virtually every cell was catching DNA from the medium. Dr. Tomasz wondered whether the sudden and synchronous sweep of competence could possibly result from the expression of a physiological state, some kind of temporary "differentiation" enforced by some factor produced by one cell and transmitted to another. Is it possible that these bacteria "talk" to each other?

His experiments confirmed his hunch. He discovered a highly specific factor released by competent cells—a protein he called "activator"—which could then induce competence in other cells that were not yet competent. "Therefore, the communication on the genetic level, that is, the uptake of DNA molecules," he says, "appears to be preceded by another type of physiological 'communication' alerting and preparing cells to receive and allow the entry of DNA molecules. Interactions between groups of cells, via hormone chemical messengers, is common among multicellular organisms, but this type of mechanism had not been seen before in bacteria."

Dr. Tomasz remembers that just at that time Dr. Hotchkiss had published a historical paper on Avery. "He took out his pen and wrote on a reprint of it, 'To Alex Tomasz, Avery's grandson.' When I reminded him that Avery had never married, he promptly corrected the dedication, 'To Alex Tomasz, Avery's illegitimate grandson.' "

With activator in hand, Dr. Tomasz started a series of experiments to understand, in biochemical terms, what this substance actually does to cells. It was clear that activator was some sort of a key that opened a door on the cell wall through which DNA molecules could now move inward and attach to receptors located somewhere deeper under the cell surface.

## SUICIDAL ENZYMES

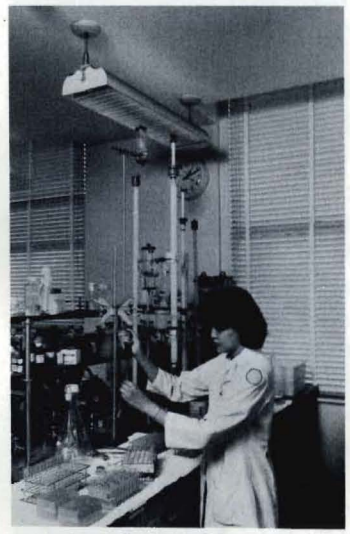
While searching for the DNA receptor, Dr. Tomasz came upon a chemical compound, called choline, common in higher organisms, but which had not been seen before in bacterial cell walls. He further discovered that it was essential to the pneumococcus. Without choline in the culture medium the bacteria did not grow. When he tried to feed the cells some different but related compounds, the results were "astonishing."

Ethanolamine was one of the compounds the cells accepted. As the wall was built, the ethanolamine took up the position normal to choline, but the cells in which they did so lost the ability to perform many activities. Daughter cells were unable to separate at the end of division, the cells could no longer take up DNA, even when treated with activator, and most interestingly, they did not blow apart when treated with penicillin. Dr. Tomasz knew he had "struck gold."

The internal pressure of a bacterial cell is almost always greater than that of its surrounding milieu. It would therefore instantly explode were it not for the strong, supportive fibers of the wall that encase it with an uninterrupted network of chemical bonds. If the continuity of this network is broken, as seems to happen in penicillin-treated cells, the contents of the cell bursts out, or lyses.

Several years of study in the laboratory revealed that choline serves as the receptor to which an enzyme called autolysin attaches when it receives the "go" signal to start breaking chemical bonds in the cell-wall network. Dr. Tomasz and his co-workers think that the command is normally released at the

*Dr. Tomasz, laboratory assistant Jill Schwartz, and Dr. Tuomanen studying fluorograms of penicillin-binding proteins from South African strains of penicillin-resistant pneumococci. The bacteria were given penicillin tagged with radioactive tritium, which reacts with the penicillin-binding proteins to form a complex that can be visualized on X ray film.*



*Marilyn Chung, who is assisting with biochemical analyses of pneumococcal cell walls.*

end of cell division so the autolysin can split fibers to complete the separation of daughter cells. When the choline receptors were missing, the autolysin could not obey the go command and thus daughter cells remained linked to one another forming infinitely long chains.

"Most exciting," says Dr. Tomasz, "was the evidence that the destructive secret of penicillin lies in its ability to fool the bacteria into releasing the signal at the wrong time, on the wrong scale, and at the wrong places along the cell surface, thereby causing bacerial suicide. When pneumococci with inhibited autolysis (in which ethanolamine replaced choline) were treated with penicillin, the cells didn't explode, but just stopped growing and died more slowly."

He named this phenomenon "antibiotic tolerance." Since his initial description, penicillin-tolerant mutants have been observed among many different kinds of pathogenic bacteria isolated from patients. "These mutants may be causing complications in the treatment of patients with defective immune systems, or in heart-valve infection or meningitis, body sites that are poorly accessible to the immune system."

## ARMAMENTS RACE

In 1977, South African scientists described an epidemic caused by a new and frightening breed of pneumococcus that had become over a thousand times more resistant to penicillin than any pneumococci before. Many of the strains had also acquired resistance to other antibiotics, making traditional therapies useless in diseases such as pneumococcal meningitis.

Specimens of the South African strains were sent to Dr. Tomasz. In the course of studying them, Sonia Zighelboim of his laboratory group discovered a new bacterial ploy. It was not the familiar trick of producing an enzyme that can destroy penicillin, which had been seen, for example, in staphyloccoccal infections. These pneumococci had managed an even more herculean feat. They had rebuilt the target proteins of the antibiotic.

"The normal job of such proteins, which are enzymes, is to take the 'bricks' constantly emerging from inside the cell and bolt them to the growing wall," Dr. Tomasz explains. "They work in a kind of assembly line. If you confuse or inhibit even one of them, by genetic manipulation or by drugs, the result is a bacteria with a totally different shape. Footballs become long spaghetti or round potatoes."

Penicillin's devastating effect on bacteria is due to the fact that it is mistaken by the wall-assembly proteins for the bricks. The antibiotic molecules thus gain entry into the labyrinths of these proteins, reaching the active center of the enzyme and inactivating it by the formation of complexes called penicillin-binding proteins.

A thorough study of the penicillin-binding proteins of the South African pneumococci revealed that they had undergone subtle alterations, or mutations, in their DNA blueprints. As a result, the cavities in the wall-assembly proteins were so deformed they impeded inward passage of the penicillin molecule. Dr. Tomasz's laboratory is now working with a group of chemists who are trying to remodel the shape of antibiotic molecules in order to refit them into the remodeled bacterial enzymes.

The appearance of penicillin resistance among pneumococci and other pathogens represented a new stage in the armaments race between bacteria and science. "One of the important lessons bacteria have taught us," says Dr. Tomasz, "is that they are capable of many ingenious methods of counteracting the weapons deployed against them."

An extremely interesting puzzle posed by resistance is currently being studied in Dr. Tomasz's laboratory by José Garcia-Bustos. Reasoning that if the antibacterial activity of penicillin is based on the structural similarity between parts of the antibiotic molecule and parts of the cell-wall building block—so much so that the wall-building enzymes mistake one for the other—then one may expect that resistant cells with abnormal penicillin-binding proteins may have to pay a price for their resistance, for example, by having to build slightly different walls. Dr. Garcia-Bustos is currently comparing walls of resistant and sensitive cells, applying highly sophisticated biochemical analysis in the hope of developing a high resolution map of the chemistry of the pneumococcal cell wall. "What he learns," says Dr. Tomasz, "will benefit all our studies in this

area, since all the miraculous properties of the pneumococcal cell wall ultimately depend on the chemical fine structure and topography of the pneumococcal surface."

## BACTERIA TALKING TO HUMAN CELLS

Before the development of antibiotics, almost half of all pneumococcal lung infections and nearly all cases of meningitis were fatal. With the introduction of penicillin, the percentages dropped dramatically, but the pneumococcus is still a serious threat. Mortality from pneumococcal meningitis remains greater than thirty percent.

Elaine Tuomanen, a medical graduate of McGill University and a member of Dr. Tomasz's laboratory since 1981, arrived with clinical experience in pediatric infections and a desire to study the body's reactions to bacterial invaders using animal models of disease.

Many symptoms of bacterial disease can be traced to molecular events and interactions between the surface of the bacterial pathogen and the host. Just as pneumococci can recognize DNA molecules and "talk" to each other with the signals of activator, they can also recognize a spectrum of important host molecules and send signals to human cells, which respond with the alarm "sirens and bells" of an invaded host. This molecular-level dialogue constitutes "disease."

Dr. Tuomanen is also exploring the added dimensions of complexity the environment of a living being adds to the mode of action of antibiotics; what happens, for example, when the explosion of a bacteria, triggered by penicillin, occurs not in a test tube but in the brain of a patient with meningitis. "We've long been puzzled," she says, "by the fact that meningitis patients sometimes die even after penicillin has destroyed all the invading pneumococci."

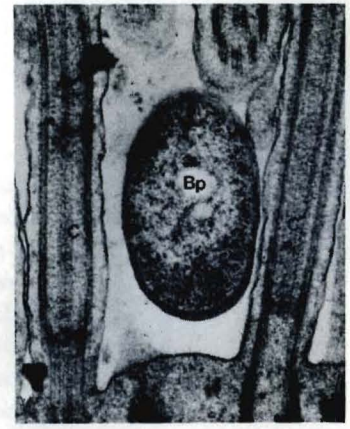
Working with rabbits, Dr. Tuomanen has found evidence that when the bacteria blow themselves apart what was one invader is now sensed by the immune system as thousands. Each fragment of debris, mostly pieces of cell wall, attracts white cells in enormous numbers. The inflammation this causes in the limited cerebrospinal fluid space puts dangerous pressure on the brain. She has been experimenting with the use of nonsteroidal anti-inflammatory drugs combined with penicillin to protect against this overreaction. Her hope is that the encouraging results she has had with rabbits will hold true with children.

"Some of the most intriguing events of infection," Dr. Tuomanen says, "are those that occur *before* the immune response begins. How do bacteria gain a foothold in the host? How does the host recognize an invader's presence? If we could intervene in these first steps, we might be able to develop new therapeutic strategies."

A model for such an approach is emerging from her studies with another bacterial pathogen, *Bordetella pertussis,* which causes whooping cough, or pertussis, a highly contagious and potentially fatal disease. The widely administered DTP vaccine (for diptheria, tetanus, and pertussis) has a high rate of side effects, and some parents have become wary of having their children innocoulated. As a result, whooping cough is on the rise in England, Japan, and the United States.

In contrast to the pneumococcus, which invades compartments of the body, *Bordetella* does its damage while clinging to mucosal surfaces of the airways leading to the lungs. These passages are covered with cilia, brushlike structures that normally sweep debris away from the lungs. The surface of *Bordetella* bristles with proteins, called adhesins, that attach to sugar molecules at the base of the cilia. Dr. Tuomanen is working on biochemical means for preventing this attachment. She has been able to block the attachment in the test tube by using antibodies that cover the anchoring sites on human cells. Alternately, bacteria treated with sugars that look like the anchoring site bind the sugar instead of the cell. Both approaches are now being tested in animals.

"People often think of a laboratory as a remote, unreal place," Dr. Tomasz reflects. "But for us the opposite seems true. The story of pneumococcus is about the closeness of the world of test tubes and research and the 'real' world of clinics and medicine. For all the interesting insights into basic biology that pneumococcus has given us, I think that the most exciting ones are yet to come as we try to understand disease in molecular terms." □




Bordetella pertussis *(Bp), the bacterium responsible for whooping cough, attaching to the base of a cilium (C) of an epithelial cell from the lining of the airways to the lungs.*

RESEARCH PROFILES is published four times a year by The Rockefeller University. This is issue Number 26, Fall 1986. Inquiries should be addressed to the University's Public Information Office, 1230 York Avenue, New York 10021; or phone (212) 570-8967. Photographs, by Ingbet Grüttner, technical illustrations by Dennis Stillwell. Design by Angelica Design Group, Ltd. 