Vaccinating with Bacterial Pili

Protection against a number of diseases, including gonorrhea, may be possible by immunizing with the bacterial surface components called pili

An invading bacterium that is trying to infect the human body would seem to have its work cut out for it. Most of the ports of entry are well guarded. Secretions produced all along the digestive and respiratory tracts, for example, wash away most pathogenic bacteria before they do any harm. In addition, these tracts have aids, such as the beating cilia of the cells of the respiratory passages and the normal peristaltic movements of the intestines, that help to propel bacteria along and eventually out of the body.

Despite all these defenses, people obviously do fall ill with any number of bacterial infections. Within the past few years, researchers have learned that in order to infect the respiratory, digestive, and urogenital tracts, pathogenic bacteria must be able to attach themselves to the cells lining those tracts. Says Garth Jones of the University of Michigan, "Many of these pathogens can very successfully colonize parts of the body not invaded by other bacteria. The pathogens must have some special ability and it is turning out to be adhesion." Once the pathogenic bacteria adhere to the cell surfaces they can multiply there and produce their symptoms, often by secreting toxins.

The research on pathogen adhesion, which has gained prominence relatively recently, converges with another, older line of investigation—that concerning the bacterial structures called pili, which are threadlike projections on bacterial cell surfaces. Charles Brinton of the University of Pittsburgh discovered pili some 25 years ago. They are perhaps best known for their role in bacterial mating, during which they transfer genetic material from one cell to another. But several investigators, including J. P. Duguid of Ninewells Hospital in Dundee, Scotland, and Brinton, have evidence for another important function of pili. Some of them serve as the attachment structures by which bacterial pathogens may adhere to target cells.*

Not only is the research on pili and bacterial adhesion helping to explain the nature of pathogenicity—including why some bacteria can cause disease while other, closely related ones can not—it may also provide the basis for developing vaccines against a number of human and animal diseases for which none currently exist. The idea is to elicit production of antibodies against the pili and thus prevent the attachment to cell surfaces of bacteria bearing the projections. Vaccines consisting of pili are now being tested in human volunteers for immunization against gonorrhea and against a bacterial diarrhea. In addition, vaccines to protect newborn calves and pigs against diarrheal diseases are already being marketed in Canada and Europe.

A vaccine for gonorrhea is of particular interest because of the appearance a few years ago of penicillin-resistant strains of the causative bacterium, *Neisseria gonorrhea*. Penicillin has been the mainstay of treatment for gonorrhea, which afflicts some 2 million people in the United States every year. Moreover, the disease is a principal cause of sterility in women, whose fallopian tubes may become permanently blocked as a result of the infection.

Several lines of evidence suggest that the pili of *N. gonorrhea* are needed for the bacteria to be infective. For example, the cultured bacteria can exist in four different colonial forms, only two of which carry pili, and there is a good correlation between the presence of pili and the virulence of gonococcal strains. Moreover, piliated bacteria adhere more readily to cultured human cells than do nonpiliated ones, and isolated pili also attach to a variety of epithelial cell types. (The surfaces of the urogenital tract and the other body cavities are lined with epithelial cells.)

Brinton has developed a vaccine containing gonococcal pili. In preliminary tests of its antigenicity, the vaccine elicited the production of antibodies when it was injected into human volunteers. The antibodies were found both in blood and in the secretions of the urogenital tract, where they may prevent bacterial attachment. According to Edmund Tramont of the Walter Reed Army Medical Center, who has collaborated with Brinton on some of the vaccine work, stimulation of the formation of secretory antibodies is important because they would constitute the first line of defense against local infections of the urogenital tract.

The pili vaccine appears to confer protection on inoculated volunteers who are "challenged" by exposure to *N. gonorrhea*, Brinton says. When they were given a dose of the bacteria that would normally infect about 70 percent of those exposed, 15 of 21 controls came down with gonorrhea, whereas only 9 of 19 immunized males did. When both groups were challenged again at a later date, this time with a bacterial dose that would normally infect 30 percent of those exposed, none of the 16 immunized men still participating in the study developed gonorrhea, but 7 of the 18 remaining controls did.

Tramont is cautious about the significance of this trial, however. "The results are tantalizing and promising," he says, "but they do not answer the question [about the vaccine's effectiveness]." He hopes that a field trial, which will test the vaccine under more natural conditions than those prevailing in the clinic, will provide more definitive answers. The field trial may get under way in about 1 year.

Thomas Buchanan of the University of Washington Medical School has also prepared a gonococcal pili vaccine and is testing it in volunteers. So far he has found that it stimulates the production of antibodies in the blood but that it has only a slight, temporary effect on the secretory antibodies of the urogenital tract.

In addition to causing localized infections, the gonococcal bacteria may penetrate into the bloodstream and produce disseminated infection affecting the skin, the joints, and occasionally the heart. Buchanan has found that most—almost 90 percent—of the bacteria isolated from

*The sex pili are different from the attachment ones and some investigators restrict the name pili to those involved in mating and apply the terms fimbriae or fibrillae to the others.

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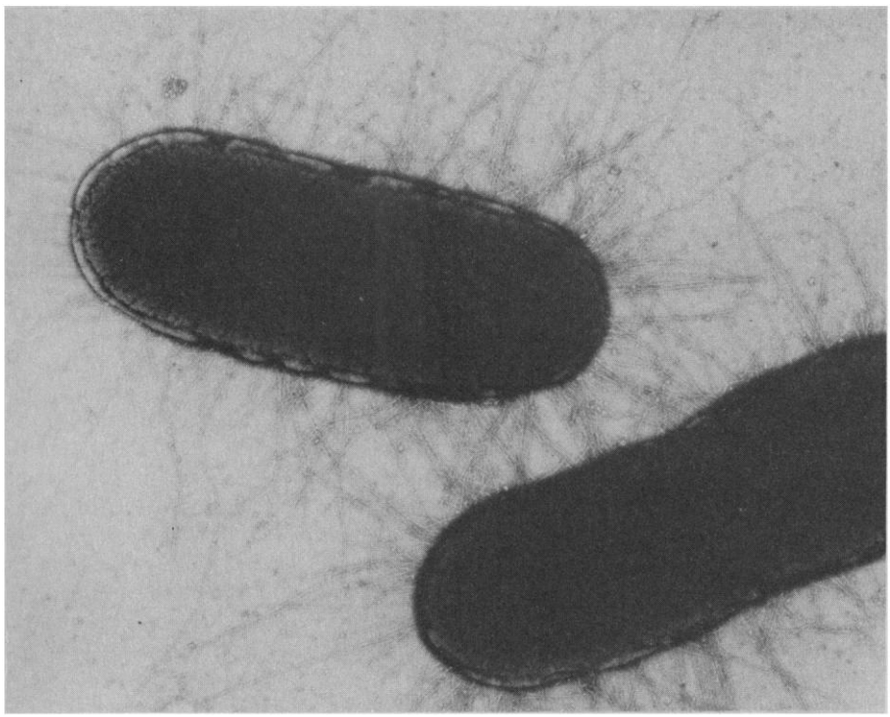

Escherichia coli *bacteria with numerous pili radiating from their surfaces. [Source: Barry Eisenstein and Edwin Beachey, University of Tennessee Medical Center and the Memphis Veterans Administration Hospital]*

women with disseminated gonorrhea contain an unusual form of a membrane protein called POMP (for principal outer membrane protein). The unusual POMP occurs in only about 30 percent of the bacteria isolated from localized infections. He has evidence that this change in POMP structure may contribute to the reduced susceptibility to immune attack that is seen in nearly all strains of *N. gonorrhea* that cause disseminated disease.

Buchanan thinks that it may be possible to develop a POMP-containing vaccine to protect against disseminated gonorrhea, although the results of tests with human volunteers have thus far been disappointing. The first batch of POMP vaccine did not prove to be immunogenic. In theory, however, any bacterial surface component might serve as an antigen in a vaccine.

How many pili variants would have to be included in a gonorrhea vaccine is still unclear. Evidence from a number of investigators suggests that the pili of the different gonococcal strains—and there are about 1000 of them—are antigenically distinct. For example, antibodies against a given piliated strain prevent attachment of the corresponding bacteria to cultured human cells, but do not prevent attachment of other strains. As Tramont describes the current situation, "A pessimist says the pili are all different and antibodies to one won't cross-react with the others. An optimist looks for shared antigens on the different pili in hopes of finding one or a few that will immunize against most of the strains."

In fact, Tramont and Brinton have evidence for such a common antigen on gonococcal pili. They showed that pili are capable of competitively inhibiting the attachment of antigenically distinct strains of *N. gonorrhea* to human epithelial cells, although the inhibition worked best when the pili and bacteria were antigenically similar. In addition, vaginal secretions from an infected woman, which contained antigonococcal antibodies, inhibited the attachment to the cultured cells of both the infecting gonococcal strain and antigenically different strains. The investigators suggest that the secretory antibodies may be directed against the common pili antigens. Brinton, who is clearly an optimist about a gonorrhea vaccine, says, "We have solved the problem of antigenic heterogenicity." Clinical trials will be needed to determine whether or not the optimism is justified.

Attempts are also under way to develop vaccines, for both human and veterinary use, against diarrhea caused by the bacterium *Escherichia coli*. Many *E. coli* strains are harmless, normal inhabitants of the large intestine, but some produce diarrheal disease that is an important cause of infant mortality in the developing countries. In addition, travelers who drink water contaminated with the bacteria may be afflicted with diarrhea. And the bacterial diarrhea in newborn farm animals, including calves, piglets, and lambs, can result in substantial losses for farmers and ranchers.

The strains of *E. coli* that cause diarrhea all produce toxins that cause extensive loss of fluids from the intestine. Genes for the toxins are carried on plasmids, small circular pieces of DNA that exist independently of the bacterial chromosome and can be transferred from one bacterial strain to another. For a while, bacteriologists thought that any *E. coli* strain that picked up a toxin gene on a plasmid could become pathogenic.

That turned out not to be the case, however. Additional properties, in particular the ability to adhere to the mucosal lining of the small intestine, were required. By the mid-1970's, researchers had identified a half-dozen or so antigenically distinct pili, all of which enable *E. coli* bacteria to stick to the intestinal mucosa.

Development of the veterinary vaccines has been facilitated by the fact that only a few different pili types have been found on the *E. coli* strains that cause the diarrheas in question. In the case of "scours," which occurs in newborn calves, only one pili type, that designated K99, needed to be incorporated into a vaccine that is now being marketed by Connaught Laboratories of Toronto.

This vaccine, which was developed by Stephen Acres and Otto Radostits of the Veterinary Infectious Disease Organization in Saskatoon, Saskatchewan, Canada, is given to pregnant cows about 6 weeks before they are due to calve. The cows produce antibodies against K99 pili, which are secreted into the colostrum and acquired by the newborn calves when they suckle. In this way, the calves take the antibodies directly into their digestive tracts, and there is no need to worry about whether vaccination stimulates the production of secretory antibodies in the intestine.

Protection of newborn piglets against *E. coli*-induced diarrhea will be somewhat more complicated than protection of calves, because at least three pili types will probably have to be included in a vaccine against the swine disease for use in this country. The three pili types—K99 plus K88 and 987—have all been found on the *E. coli* strains affecting North American swine. Antibodies to one do not protect against the others, according to Brinton, who is collaborating on the animal work with Harley Moon of the National Animal Disease Center in Ames, Iowa, and Richard Isaacson, who is now at the University of Michigan.

The investigators immunized pregnant gilts with either K99 or 987 pili. Piglets suckling the gilts were protected against diarrhea caused by *E. coli* bearing the pili used for immunization, but not against diarrhea caused by bacteria carrying the other pili type. The evidence suggests that the antibodies taken in by the suckling pigs worked, as expected, by inhibiting the attachment of the appropriate *E. coli* strain. Even though these results indicate that full protection of newborn piglets against diarrhea caused by *E. coli* will require a vaccine containing all three pili types, such a trivalent vaccine ought to be feasible. In Europe, where only one pili type is required for protection of piglets, Burroughs-Wellcome is already marketing a vaccine for the disease.

There are indications that inhibition of adhesion may be involved in immunity to *E. coli* infections of humans. Myron Levine and his colleagues at the University of Maryland School of Medicine showed that human volunteers who had previously been infected with *E. coli* were protected against diarrhea after reinfection with the same strain. These individuals excreted the *E. coli* strain to the same degree as the controls, who had not been exposed to the bacteria before and who became ill.

According to Levine, this finding means that cell-killing antibodies did not contribute to the immunity he observed. If they had, the individuals who had become immune as a result of the earlier exposure would have excreted fewer bacteria. Instead, he thinks that antibodies inhibiting bacterial adhesion may explain the lower infection rate of those individuals.

In a pilot study with human volunteers, Levine and Brinton have shown

Cornell Evidence for Fifth Quark

The most widely held view among elementary particle physicists is that the fundamental constituents of matter are entities called quarks and leptons and that there are six varieties of each. The existence of four of the quarks is well established, whereas evidence for the fifth is incomplete and no sign of the sixth has yet appeared. At the 20th International Conference on High Energy Physics, held in Madison, Wisconsin, in July, physicists working at Cornell University's electron-positron colliding beam storage ring, CESR, presented evidence that should nail down the case for the fifth quark, provided that additional substantiating data are obtained in the next several months. Physicists give quarks whimsical names. The first four are called up, down, strange, and charm quarks; the fifth is the bottom or b quark, and the sixth, if found, would be the top or t quark.

Physicists believe that quarks never occur as free, isolated particles but only in combination with other quarks. The particle detected at Cornell was therefore not the bottom quark itself but a meson consisting of a bottom quark and a second quark of another variety, probably an up or down quark. (Quarks also occur in combinations of three to make up the heavier subnuclear particles such as the proton and neutron, which are called baryons.) Discovery of the B meson was not at all unexpected and, in fact, high energy physicists generally assumed that it would be found at Cornell because CESR provides collisions of just the right energy to produce the particle. It may be a year or more before experimenters can collect enough data of the type needed for detailed analysis of the properties of the B meson and thereby be certain of their finding.

Cornell's storage ring was completed just over a year ago. At that time, groups working at the Fermi National Accelerator Laboratory near Chicago and the Deutsches Elektronen-Synchrotron Laboratory in Hamburg, West Germany, had already established the existence of the upsilon family of particles which they conjectured to consist of a bottom quark and a bottom antiquark bound together. Confidence in this interpretation was buoyed by the strong similarity between the family of upsilon particles and the exhaustively studied family of psi particles known to consist of charm quark and antiquark pairs. But the bottom character could not be directly verified because its appearances in the quark and antiquark canceled out each other.

In January of this year, two groups working at Cornell (one consisting of researchers from Cornell, Harvard University, Ithaca College, the University of Rochester, Rutgers University, Syracuse University, and Vanderbilt University; and the other consisting of investigators at Columbia University and the State University of New York at Stony Brook) confirmed the existence of the three upsilon particles found earlier. Then in April, the two Cornell groups announced the discovery of a fourth upsilon at a still higher energy. The fourth particle had a much shorter lifetime than the other three, indicating that it was decaying rapidly by a pathway forbidden to the others. By analogy with the charm quark system where the same pattern occurred, the investigators reasoned that the fourth upsilon was energetic enough to decay into two B mesons, one containing the bottom quark and one containing the bottom antiquark. The first three upsilons presumably did not have enough energy to create the extra up or down quarks needed for the creation of the mesons and therefore decayed by another mechanism in which they were transformed into other varieties of lighter particles.

Since April, the investigators have been analyzing their data for evidence to support this proposition. According to Edward Thorndike of Rochester, one telltale sign of bottom quarks was the presence of leptons (electrons and muons) among the decay products. Karl Berkelman of Cornell, who reviewed the results from CESR at the conference, said the mass of the B meson is between 5.18 and 5.28 billion electron volts (GeV) and its lifetime is less than 3×10^{-11} second, in agreement with theoretical predictions.

This is not the first report of finding the B meson. Last summer an international collaboration working at the European Organization for Nuclear Research (CERN) near Geneva said it had evidence for the particle in experiments with CERN's largest accelerator, a proton synchrotron. During the next several months, the group increased its amount of data by a factor of 4, but the signal for the B meson had disappeared. In the Cornell case this is not likely to happen. A real effect has been observed; it is only the interpretation that is not yet ironclad.

—ARTHUR L. ROBINSON

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Vaccines now under development may prevent diarrhea in newborn piglets. The pregnant gilt is vaccinated and the suckling piglets acquire the antibodies from her colostrum. [*Photo: United States Department of Agriculture*]

that a pili vaccine can confer some protection against bacterial diarrhea. Only two of six immunized individuals became ill when challenged with a dose of *E. coli*, whereas all of the controls did. As in the case of *N. gonorrhea*, however, there is still uncertainty about the number of different pili types that would have to be included in a vaccine to achieve adequate protection.

There is also evidence that adherence by bacterial pili is an important step in infection of the upper respiratory tract by streptococcal bacteria and of the bladder and kidney by *E. coli* and *Proteus mirabilis*. For example, Edwin Beachey, of the University of Tennessee Medical Center and the Veterans Administration Hospital in Memphis, and Itzhak Ofek, of Tel Aviv University in Israel, have shown that *Streptococcus pyogenes*, the cause of strep throat and rheumatic fever, sticks to the epithelial cells of the throat by a nonprotein component of the bacterial pili. And tooth decay can be considered an infection caused by the bacterium *Streptococcus mutans*, which binds to tooth surfaces and secretes acids that break down dental enamel.

Much of the information about the distribution of *S. mutans* and the other oral bacteria comes from the laboratory of Ronald Gibbons at the Forsyth Dental Center in Boston. Beginning in the early 1970's, the Forsyth work helped to draw attention to the highly specific nature of the attachments formed by these bacteria. For example, *S. mutans* is present in large numbers on tooth surfaces, but few are found elsewhere in the mouth. Many of the other investigators who are studying bacterial adhesion credit Gibbons' research with sparking the current explosion of interest in the subject. Before that, bacterial adhesion had been relatively neglected, even though the need for viruses to attach to their target cells had long been recognized.

Even though pili appear necessary for the attachment of many kinds of pathogenic bacteria to mucosal surfaces, and thus for the initiation of infection by those bacteria, there are indications that pili may be a handicap for pathogens that have penetrated into the bloodstream. In the case of kidney infections by *P. mirabilis*, for example, Fredric Silverblatt of the Sepulveda (California) Veterans Administration Hospital and Ofek found that pili favor the development of infections localized to the mucosa of the renal pelvis (the funnel-shaped cavity of the kidney into which the urine is discharged), which are apparently caused by bacteria entering the kidney from the urinary tract.

The bacteria producing these localized infections may push through the mucosa to infect other parts of the kidney, however. In that event, pili may be disadvantageous, possibly because the bacteria now come into contact with certain white blood cells that participate in the immune response.

According to Silverblatt and Ofek, *P. mirabilis* bacteria that are heavily piliated produce fewer kidney abscesses when they are injected into the bloodstream than do lightly piliated bacteria. They have evidence suggesting that this may be the result of the pili making the bacteria more susceptible to attack by phagocytic white blood cells. Silverblatt and Ofek postulate that in order to infect all regions of the kidney, *P. mirabilis* must undergo an adaptive transition during which the bacterial cells lose their pili, thus allowing the bacteria to spread from the mucosa, where the infection began, to other parts of the kidney.

Antibodies against pili can apparently decrease bacterial infectivity by preventing attachment to cells, and so can some antibiotics, including penicillin and streptomycin. In high doses, these two antibiotics act by directly killing bacteria, but there are now indications that at lower concentrations they may also have more subtle effects.

Ofek and Beachey have shown that *E. coli* or *S. pyogenes* bacteria grown with sublethal doses of penicillin or streptomycin may either fail to develop their normal adhesive properties or lose the adhesive molecules they had already acquired. Most of the effects on adhesiveness may reflect the known activities of the antibiotics on cell walls (penicillin) or on protein synthesis (streptomycin). According to Beachey, the antibiotic effects should prove to be a useful tool in dissecting the biochemistry of pathogen adhesion.

Adhesion for some pathogens may be more complicated than a simple attachment of bacterial pili to receptors on mucosal cells, however. Rolf Freter of the University of Michigan points out that many of the laboratory tests used to study interactions between bacteria and cells do not completely reflect the situation in the living animal. For example, the epithelial cells of the intestinal lining are covered with a layer of complex glycolipids and glycoproteins (sometimes called the mucous gel), a barrier which bacteria have to penetrate before they can reach the cells.

Other investigators have shown that only motile *Vibrio cholerae* bacteria are capable of penetrating the mucous gel. And Freter's work with the bacteria suggests that to be able to penetrate the gel and colonize the intestine, they must also be attracted by, and follow the gradient of, an as yet unidentified chemical. In Freter's view, then, "bacterial adhesion to epithelial cell surfaces represents only the final step of several leading to association with the mucosa." Consequently, there may be a number of points at which it is possible to interfere with the early stages of infection. All in all, the research on pili and bacterial adhesion has provided some promising new leads for prevention of a wide range of important bacterial diseases.—JEAN L. MARX