Just Lookin' for a Home

Many bacteria sneak into cells via entry routes already in place

By MARJORIE CENTOFANTI

Pity the bacteria that find themselves in the human gut. They must adjust to high temperature and acidity, not to mention competition from other bacteria for food and space. Bile salts bathe them in a powerful detergent. The constant downward squeeze of peristalsis threatens to dislodge them.

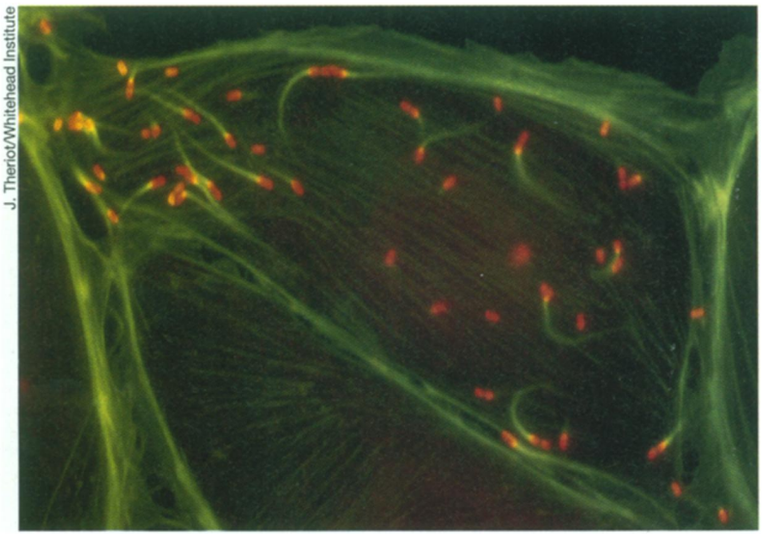
Many bacteria survive these conditions through sheer tenacity. Adhering tightly to the intestinal lining, they feed and reproduce, developing colonies that cling to inner surfaces of their human hosts like bits of wet tissue paper. Other bacteria make use of the adverse conditions to spur their growth.

Some bacteria, such as varieties of the common bacteria *Salmonella* and *Shigella,* however, have broken through the barrier posed by the gut. They've found new homes, either by staying within intestinal wall cells or by slipping through those cells to reach quieter niches in other tissues.

Most bacteria that thrive on the surface of the intestines do no harm to their human hosts. Those that penetrate cells, says Stanford University School of Medicine bacteriologist Stanley Falkow, often become pathogens—microorganisms that cause disease. From the bacterial point of view, Falkow says, pathogenicity is "simply another facet of their extraordinary versatility. It's survival. It's how the bacteria make a living."

From the human point of view, however, this pathogenicity can mean sickness and death. Bacteria often injure cells directly or fan the immune system into a powerful inflammation that does more harm than good. Accordingly, the aim of medical scientists has long centered on wiping out pathogens after a person has become ill. But as more bacteria become resistant to antibiotics, a subtler approach might be better.

"Most of medicine looks at the product of bacterial invasion, at the disease," Falkow says. "But disease may be the last thing we want to study; it comes after all the interesting stuff happens." The interesting stuff, according to Falkow, includes how bacteria attach to and enter the cells and how they survive once inside. The next generation of therapies, he suggests, will arise from solving those problems.



J. Theriot/Whitehead Institute

Long comet tails of the protein actin propel Listeria *bacteria through a kidney epithelial cell.* Listeria, *like* Shigella *bacteria, use these tails to spread from cell to cell.*

"Bacteria adhere everywhere in the environment—rocks, leaves, animals," says infectious disease researcher Elaine I. Tuomanen of Rockefeller University in New York. "The idea that this sticking could relate to human disease grew decades ago from studies on tooth decay," she says. Those bacteria able to stick to teeth, researchers found, produce specific surface molecules—adhesins—that mediate binding.

Since most bacterial infections of host cells, and teeth, begin with some sort of attachment, researchers sought to identify various adhesins. The business part of an adhesin molecule links to sugars or proteins projecting as a fuzz on cell membranes. Because of adhesins, Tuomanen says, bacteria can "cozy up to body cells."

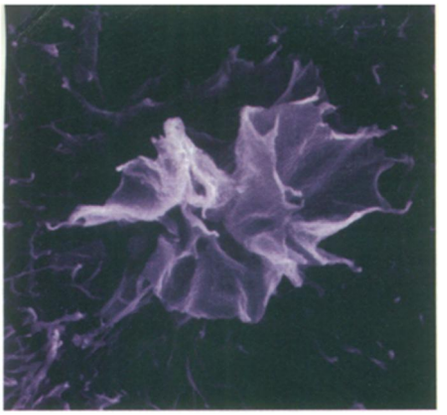
Yet more interesting, she adds, is that some bacterial adhesins resemble animal cell lectins—molecules whose role is to arrange temporary liaisons between different types of cells.

Lectins help eggs and sperm get together, for example, and allow certain blood vessel cells to snag passing white blood cells as a requisite for the white cells' entry into nearby tissues. As Tuomanen's group reported in the July 29, 1990 CELL, *Bordatella pertussis*, the persistent agent of whooping cough, makes an adhesin that's such an effective mimic that the white cells attach to the bacteria instead of binding to the blood vessel.

"In the last year or so, though," Tuomanen says, "the field's changed dramatically." Researchers have found novel bacterial molecules that target animal cells at more specific sites than adhesins do. By means of these molecules, bacteria appear to exploit common routines cells use to maintain themselves.

Like a thief intent on breaking in, a bacterium gradually evolves to take advantage of a cell's routines, adapting one or more of them to gain entry. It can ease into food intake pathways, into between-cell transport systems, and into the mechanisms that recycle cellular molecules.

With *Salmonella*, the cause of food poisoning and life-threatening infections such as typhoid fever, cell entry is flamboyant. A bacterium and


Sansonetti

Shigella *begins its push into a neighboring cell.*

potential host cell first chat; then the host cell ruffles and allows the bacterium entrance.

Getting into the body from contaminated food or water, the bug often enters the elongated cells that line the intestine. There it sets up housekeeping or, depending on its species, moves elsewhere, most often to the liver and spleen.

To get to distant tissues, researchers speculate, *Salmonella* may hitch a ride in the very immune cells sent to destroy it. To do this, "the bacteria may intercept a pathway the immune cells use to recycle materials," says Falkow, and may "gimmick" their way inside, avoiding the digestion that would normally destroy them. Once at its target site, the *Salmonella* species that causes typhoid apparently stays there—unlike the food poisoning species, which is quickly cleared. Humans, Typhoid Mary being the obvious example, can become carriers.

The flamboyance comes when *Salmonella* bacteria first approach host cells. "The hosts are there; the bacteria know it. There's evidence that bacterial chemistry changes," says Jorge E. Galán of the State University of New York at Stony Brook. "*Salmonella* participate in a remarkable phenomenon: a biological cross talk, a molecular conversation with the host cells."

Galán and his coworkers have found at least 10 *Salmonella* genes that code for proteins involved in the cross-talk, he explains in the October 1995 INFECTION AND IMMUNITY. Some of the proteins end up in the host cell, where they trigger cascades of reactions. One such cascade, Galán found, resembles a reaction sequence that a common cellular growth factor initiates, just before it triggers cell division.

After a minute of chatting, the host cell membrane nearest the bacterium ruffles like a flamenco dancer's skirt and engulfs any *Salmonella* present. Then wide channels—the sort cells use to take in fluids—form in the host cell membrane, allowing the bugs to slip inside.

Shigella similarly triggers ruffles outside, then it flourishes within host cells. It sparks widespread damage to the gut—largely from the immune system—which brings on a trademark dysentery. The damage, according to work by Philippe Sansonetti of the Pasteur Institute in Paris, gives the microbe greater access to host cells.

Once inside a host cell, however, *Shigella* taps into between-cell transport systems. It seems to harness actin, a key mobility molecule, to propel itself. Researchers watching videos of the microbe find that it moves smoothly within intestinal cells, pushed by its newly acquired actin tail. Each *Shigella* soon thuds against the cell's membrane, distorting the membrane and indenting an adjacent cell.

At the distortion, the bacteria make contact with membrane junctions that form part of a cell-to-cell transport network. The bacteria use a molecule called cadherin, which bridges these junctions, to move from cell to cell, Sansonetti reported in the March 11,1994 CELL. The effect is a bit like having a party that spreads from one train car to the next through the connecting doors.

What's fascinating, says Sansonetti, is that *Shigella* can spread without leaving the interior of cells. This allows *Shigella* to evade the immune system, adding to the microbe's virulence.

Some bacteria have evolved mechanisms that take advantage of molecule recycling in the membranes. In her studies on a microbe that causes pneumonia and meningitis Tuomanen noted that 40 percent of people who carry *Streptococcus pneumoniae* don't get sick. In those who do, she speculates in the Oct. 5, 1995 NATURE, microbes hitch a ride into the lung cells, or through the lungs into the blood, by attaching to a receptor that comes to the cell surface during inflammation. Blocking the receptor with a compound that prevents the bacteria from hooking up to it, Tuomanen found, causes the bacteria to lose the ability to invade.

"You can't block receptors, of course, if they're for some vital function in the body, but you could, perhaps, block some for a time, early in infection," she adds. "You'd stop the disease at the benign stage and allow your body to recover. Then you'd also have immunity."

Although many bacteria use sneak attacks, some seem simply to ring the doorbell. At Tufts University in Boston, Ralph Isberg focuses on the intestinal bacterium, *Yersinia,* which typically infect wildlife such as birds with little effect. In children, however, *Yersinia* causes a severe diarrhea; in adults it can trigger arthritis.

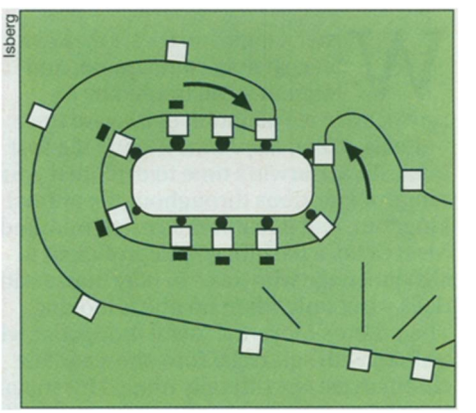
To pinpoint why *Yersinia* is such "an excellent invader," Isberg cloned a variety of *Yersinia* genes—a shotgun approach—and inserted them one at a time into normally benign *Escherichia coli* bacteria. Could any of these altered *E.coli* begin slipping into cells? If so, Isberg would know he had an invader gene.

In the March 5, 1995 TRENDS IN CELL BIOLOGY, Isberg and his colleague Tran Van Nhieu summarized their studies of a gene they had turned up using this technique. The gene codes for a protein, called invasin, that in effect rings the doorbell to enter intestinal cells.

Invasin binds tightly to specific receptor molecules in cell membranes. Normally, these receptors form part of a structural network of proteins that extends outside, inside, and between cells, keeping tissues intact.

By binding to those bacterial surface receptors, Isberg's team found, invasin switches on reactions in host cells that probably rearrange the network, in effect opening the door. The physical result, he suggests, is that a cell's membrane wraps around the bacterium, enabling it to enter the cell.

By seeing how the bacteria pervert normal cell routines, researchers move closer to finding out exactly what makes bacteria harmful—for their unobtrusive entry often forms a basis for virulence. Cell biologists may also benefit from the research.


Isberg

Like a friendly arm, a host cell engulfs a Yersinia *bacterium in this possible scenario. Receptors on the host cell mass in the vicinity of the bacterium, then link with its proteins. Once enough receptors surround the bacterium, mobile cell molecules (arrows) push shut the open ends.*

"I almost think of bacteria now as bioprobes that can clarify various aspects of cell biology," Tuomanen says. "You follow where the bacteria go and what takes them in. Study how actin works? A short list of bacteria deal with that. Cadherins? There's that too."

The surprise, Falkow adds, may be that in finding out about the bacteria, "we learn as much or more about the biology of the humans they invade." □