the art of bacterial warfare

New research reveals how bacteria hijack our body's cells and outwit our immune system—and how we can use their own weapons against them

By B. Brett Finlay

Most bacteria are well-behaved companions. Indeed, if you are ever feeling lonely, remember that the trillions of microbes living in and on the average human body outnumber the human cells by a ratio of 10 to one. Of all the tens of thousands of known bacterial species, only about 100 are renegades that break the rules of peaceful coexistence and make us sick.

Collectively, those pathogens can cause a lot of trouble. Infectious diseases are the second leading cause of death worldwide, and bacteria are well represented among the killers. Tuberculosis alone takes nearly two million lives every year, and *Yersinia pestis,* infamous for causing bubonic plague, killed approximately one third of Europe's population in the 14th century. Investigators have made considerable progress over the past 100 years in taming some species with antibiotics, but the harmful bacteria have also found ways to resist many of those drugs. It is an arms race that humans have been losing of late, in part because we have not understood our enemy very well.

Historically, microbiologists sought to learn how bacteria cause disease by growing them in a nourishing broth, then isolating molecules from the bugs' exterior or extracting their secretions from the medium, and examining the effects of those substances on human cells and animals. Such studies characterized assorted bacterial toxins, but most investigations of the mechanisms of disease virtually ignored the interplay between bacterial pathogens and their hosts. Over the past 20 years, however, a growing body of research has revealed that disease-causing bacteria often

KEY CONCEPTS

■ Bacterial pathogens multiply and make toxins inside human hosts, but how the microbes elude our defenses and deliver their poisons have been poorly understood.

■ Studying host-pathogen inter-actions reveals sophisticated bacterial strategies for co-opting and manipulating host cells to serve a bacterium's needs.

■ A new understanding of bacterial tools and tactics is leading to novel approaches for battling the microbes.

—The Editors

How Bacteria Hijack Host Cells

Disease-causing bacteria actively promote their own survival by co-opting host cells' machinery and communications to do the microbes' bidding and by altering the environment inside and outside cells to suit their needs. In the examples below, microbes invading the intestine wield specialized tools (*inset at far left*) to manipulate a variety of cell types, including epithelial cells, immune cells and harmless bacteria that reside in the gut.

Secretion apparatus

Bacterial outer membrane

Needle

Translocon
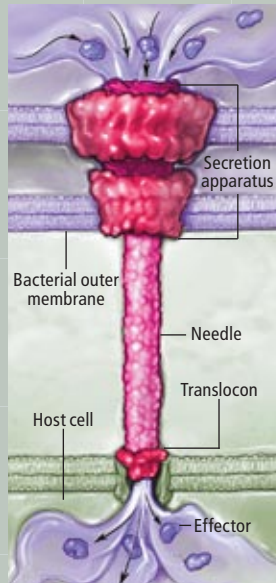
Host cell

Effector

SECRETION SYSTEMS
Specialized devices allow bacteria to inject cell-controlling "effector" molecules directly into host cells. The type 3 secretion system (T3SS) above is typical. The main secretion apparatus in the bacterial membrane lowers the hollow needle to the host cell and delivers a translocon protein to anchor the needle; effector proteins follow.

E. coli

Tir

T3SS

Actin polymer
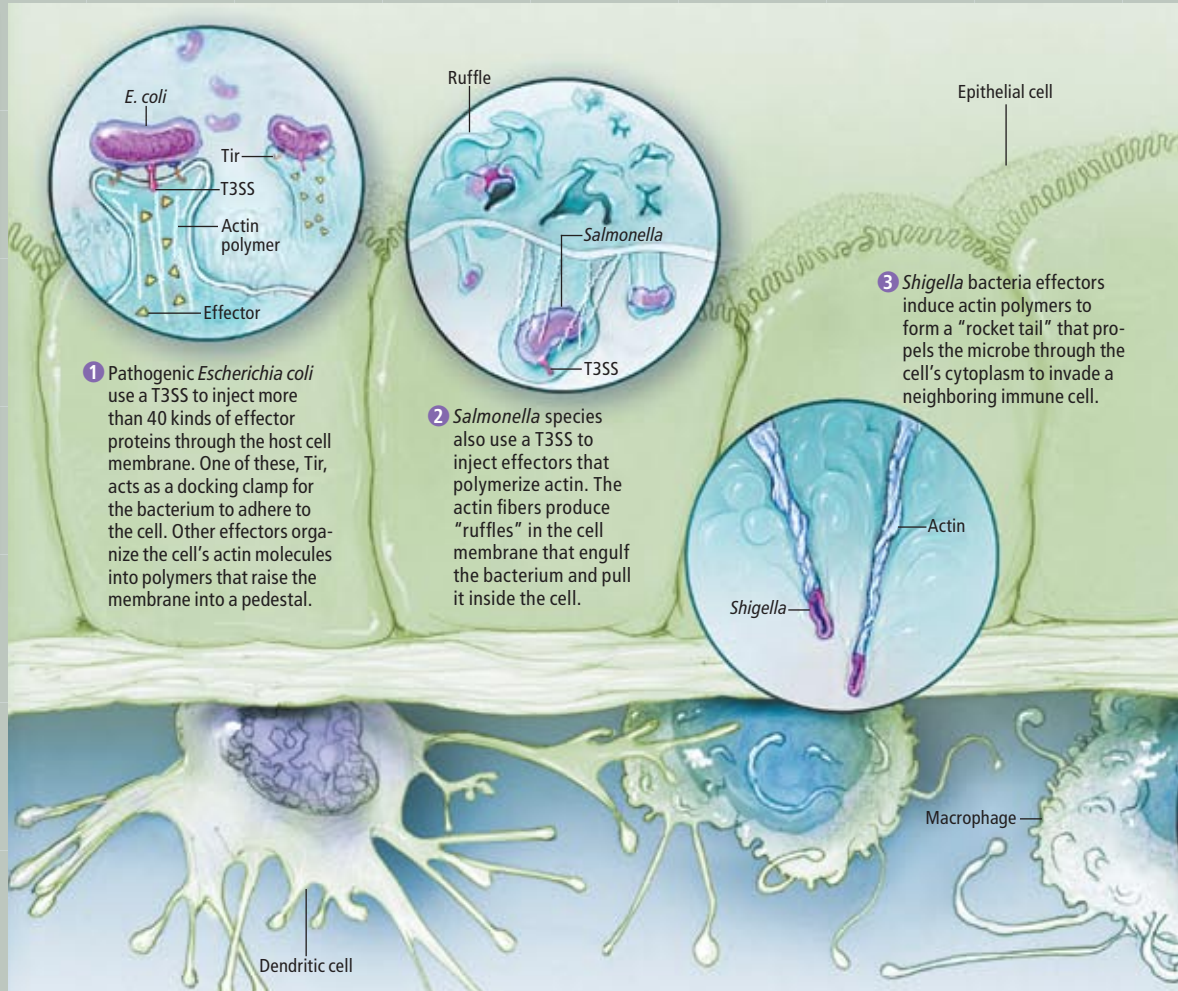
Effector

1 Pathogenic *Escherichia coli* use a T3SS to inject more than 40 kinds of effector proteins through the host cell membrane. One of these, Tir, acts as a docking clamp for the bacterium to adhere to the cell. Other effectors organize the cell's actin molecules into polymers that raise the membrane into a pedestal.

Ruffle

Salmonella

T3SS

2 *Salmonella* species also use a T3SS to inject effectors that polymerize actin. The actin fibers produce "ruffles" in the cell membrane that engulf the bacterium and pull it inside the cell.

Epithelial cell

3 *Shigella* bacteria effectors induce actin polymers to form a "rocket tail" that propels the microbe through the cell's cytoplasm to invade a neighboring immune cell.

Actin

Shigella

Macrophage

Dendritic cell

Breaking and Entering

behave much differently in broth than they do inside a potential host.

To penetrate diverse organs and tissues and to survive and thrive in our bodies, bacteria become skilled subversives, hijacking cells and cellular communication systems, forcing them to behave in ways that serve the bugs' own purposes. Many microbes take control by wielding specialized tools to inject proteins that reprogram the cellular machinery to do the bugs' bidding. A few are also known to employ tactics that rid the body of benign or beneficial bacteria, to better commandeer the environment for themselves. As investigators have identified the aggressive strategies and ingenious weapons used by pathogenic bacteria to invade and outwit their hosts, we have wasted no time in trying to devise therapies that turn the microbes' own weapons against them.

Toxins released by bacteria are only one source of the illnesses they produce. Some of the symptoms of bacterial infections arise directly from the bugs' tactics for staying alive. Because many pathogens produce a similar array of symptoms—diarrhea, fever, and so forth—it may seem logical to think that they cause disease in similar ways, too. Although many pathogens do act on some of the same fundamental elements of cellular machinery, such as certain proteins that make up the cell's internal skeleton, the microbes use surprisingly diverse and complex methods to attack.

The first step in any bacterial assault, for instance, is attachment to the host's cells. A disease-causing strain of *Escherichia coli*, known as enterohemorrhagic *E. coli* O157, has perhaps
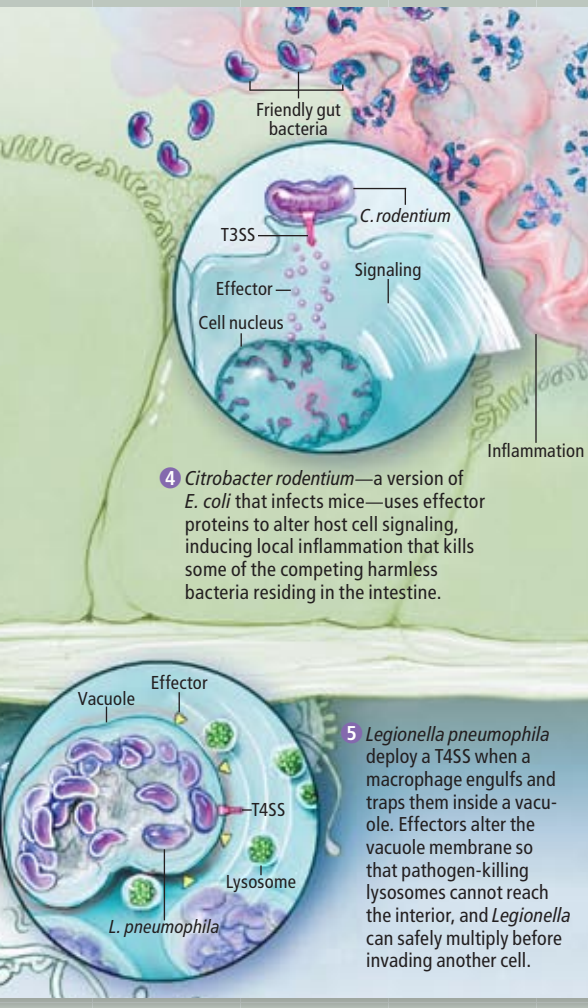
④ *Citrobacter rodentium*—a version of *E. coli* that infects mice—uses effector proteins to alter host cell signaling, inducing local inflammation that kills some of the competing harmless bacteria residing in the intestine.

⑤ *Legionella pneumophila* deploy a T4SS when a macrophage engulfs and traps them inside a vacuole. Effectors alter the vacuole membrane so that pathogen-killing lysosomes cannot reach the interior, and *Legionella* can safely multiply before invading another cell.

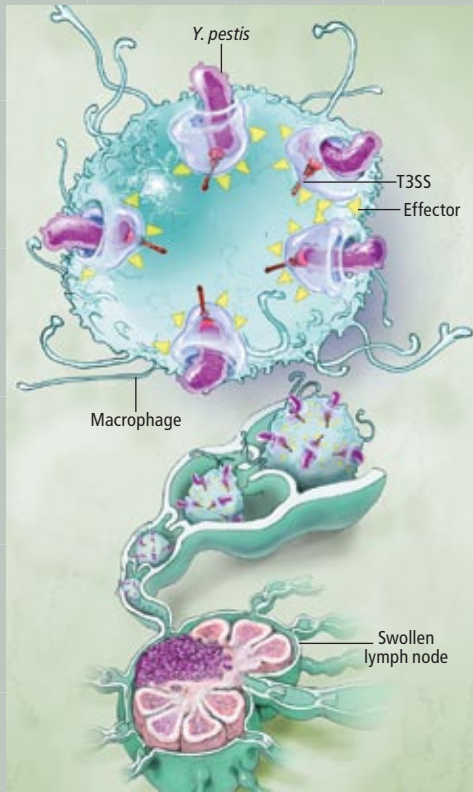ANDREW SWIFT (*bacterial hijacking illustrations*)

the most remarkable method of locking itself onto a host cell. People typically pick up this pathogen by eating tainted food; once inside the gastrointestinal tract, O157 attaches to the intestinal wall and produces a toxin that induces bloody diarrhea. At one time, scientists thought that this virulent form of *E. coli,* like all other adherent pathogens, latched onto a receptor molecule already present on the host's intestinal cells. More recent work has shown, though, that O157 actually makes its own receptor and delivers it into the cell through a specialized device known as a type 3 secretion system, or T3SS for short. (Secretion systems have historically been named based on the order of their discovery.)

The bacterium's T3SS injects a molecule called Tir, along with 40 or more other "effector" proteins directly into the membrane of the host cell

and then locks one of its own surface molecules onto Tir. But that is only its first step in taking over the cell. Tir and some of the other injected effectors also induce the host cell's internal skeleton to behave abnormally. A key cytoskeletal building block, actin, interacts with the bacterial proteins and begins forming polymers that push on the cell membrane from the inside until it forms a pedestal. The *E. coli* remains outside the cell, securely anchored to its new throne, while the effectors and toxins it has injected into the cell do their dirty work. The exact function of these striking pedestals remains unknown, but investigators have demonstrated that they are central to the bacterium's ability to cause disease.

Another potentially lethal pathogen, *Helicobacter pylori,* attaches itself to the epithelial cells lining the stomach, then begins customizing its environment to promote its own survival. *H. pylori* releases an enzyme called urease that locally counters the stomach's high acidity, which normally kills most bacteria. Not all strains cause disease, but those that do can generate gastric ulcers and even stomach cancer—making it the only bacterium known to cause cancer. The pathogenic strains produce a type 4 secretion system that injects an effector protein called CagA. The protein's exact purpose is unclear, but recent work suggests that it can induce stomach epithelial cells to display more of the receptors to which *H. pylori* attaches. The effector may also directly alter the stomach cells' internal signaling in a way that makes them elongate, scatter and ultimately die, contributing to ulcer formation.

E. coli O157 and *H. pylori* bacteria do not need to enter cells to cause disease, but *Salmonella* species, which are closely related to *E. coli* and cause diarrhea in more than a billion people worldwide every year, do penetrate cell walls. Indeed, to thrive, *Salmonella* bacteria have to pass into and through epithelial cells that line the intestine. This invasion begins when the bacteria use a T3SS variant known as *Salmonella* pathogenicity island 1 (SPI-1) to inject epithelial cells with effectors that reorganize actin polymerization in a way that produces "ruffles" in the cell membrane—similar to *E. coli*'s pedestal. The ruffle structures reach up and around a bacterium attached to the outside of the cell membrane, causing the cell to literally drag the microbe inside. Molecules injected through SPI-1 also induce the diarrhea characteristic of these infections, but the *Salmonella* bacteria do not stop there.

Macrophages and other cells belonging to

HUMAN MICROBIOTA

HUMAN BODY COMPOSITION

10 TRILLION
human cells

100 TRILLION
bacterial cells

BACTERIAL SPECIES LIVING IN THE HUMAN …

5,000–35,000
… intestine

300–500
… mouth

120
… skin

BACTERIAL SPECIES PATHOGENIC TO HUMANS

100

PERCENTAGE OF HUMANS COLONIZED (WITH OR WITHOUT ILLNESS) BY COMMON PATHOGENS

33%
Mycobacterium tuberculosis

50%
Helicobacter pylori

50%
Staphylococcus aureus

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Outwitting the Guards

Immune cells and the antibodies they manufacture are supposed to neutralize invaders, but bacterial pathogens can evade those host defenses with diverse tools and tactics, such as depicted in the examples below.


DISABLING MACHINERY
When a macrophage tries to engulf *Yersinia pestis,* the bacterium uses its T3SS to inject effectors that paralyze the immune cell's uptake machinery. *Yersinia* then ride the cell into lymph nodes, where the microbes multiply, causing the swollen nodes called bubos that are characteristic of bubonic plague.
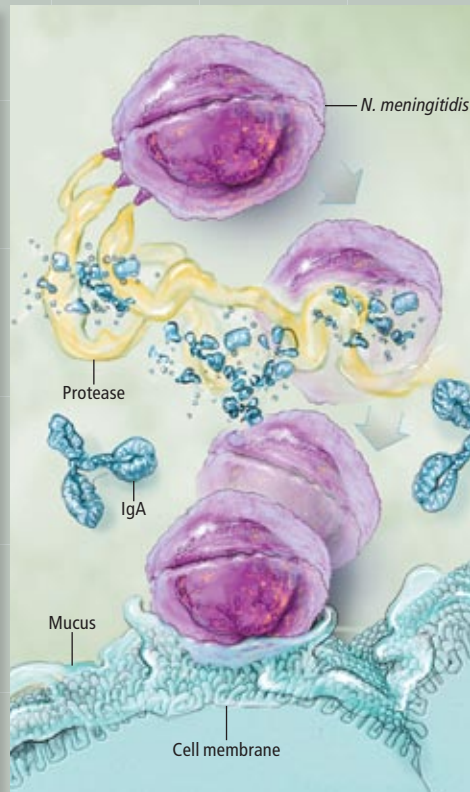
DESTROYING ANTIBODIES
Immunoglobulin A (IgA) antibodies block bacteria from adhering to epithelial cells that line nasal passages and similar mucosal surfaces in the body. But *Neisseria meningitidis,* a cause of meningitis, can colonize such cells by first releasing a protease (enzyme) that degrades the antibodies.
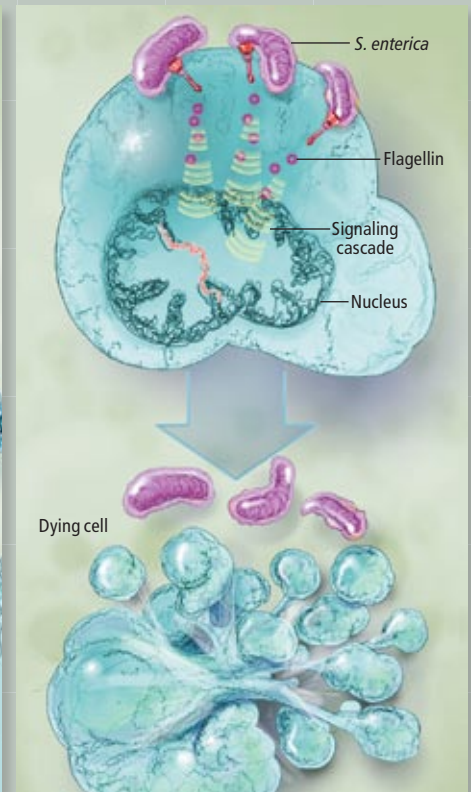
TRIGGERING SELF-DESTRUCTION
Certain host cells such as macrophages train other immune cells to recognize a pathogen, but *Salmonella enterica* prevents that from happening. The bacterium uses its T3SS to inject the cell with flagellin, a protein that sets off signaling cascades that ultimately trigger a cell suicide mechanism.

what is called the innate arm of the immune system, such as neutrophils and dendritic cells, normally ingest and destroy ("phagocytose") any invaders. These phagocytes engulf bacteria and sequester them in membrane-bound vacuoles where killing molecules destroy the captives. But *Salmonella* species penetrate the intestinal lining by passing from epithelial cells to immune cells waiting on the other side. Once inside the phagocytic vacuole, the bacteria deploy a second T3SS, called SPI-2, which releases effector proteins that convert the vacuole into a safe haven where *Salmonella* can multiply. The proteins cause this switch from death chamber to sanctuary by altering the vacuole membrane so that the killing molecules cannot get in.

The SPI-2 system is critical to the success of *Salmonella typhi*, the strain that causes typhoid fever. By allowing the microbes to survive inside the phagocytic cells, which travel within the body via the bloodstream and lymphatic system, SPI-2 enables the organisms to reach and replicate in tissues far beyond the intestine, such as the liver and spleen.

An ability to live long term inside a host's cells is a trait common to many bacterial pathogens that cause serious disease, including those responsible for tuberculosis and Legionnaires' disease. Indeed, *Legionella pneumophila* is particularly intriguing in that it injects at least 80 different effectors into phagocytic cells through its T4SS. Although the function of only a handful of these proteins is known, at least some of them serve to convert the phagocytic vacuole into a safe haven.

Legionella's behavior also offers a window

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into the likely origin of bacterial secretion systems, which apparently evolved not to sicken humans but to protect the bugs from attack by single-celled organisms in the soil. *Legionella* normally uses its T4SS to survive when ingested by soil amoebas, which are remarkably similar in many of their mechanisms to human phagocytic cells. This association with amoebas even gave the microbe its name. At an American Legion meeting in Philadelphia in 1976, amoebas containing *Legionella* bacteria were blown through air-conditioning ducts into the hotel and delivered deep into the conventioneers' lungs. Macrophages in the humans' alveoli ingested the *Legionella*, much as an amoeba would. Thirty-four people died of the resulting respiratory illness, and Legionnaires' disease was born.

Dodging the Sentries

The ability of bacteria to set up housekeeping inside immune cells—the very cells meant to kill them—attests to the versatility of the microbes' tool kit for co-opting cellular machinery. The similarity between human immune cells and bacterial predators outside human hosts may explain the origin of other bacterial survival tactics as well. Some of the most sophisticated mechanisms that bacteria are known to deploy are devoted to evading host defenses and even enlisting immune cells to help the microbes thrive.

Y. pestis, for instance, is transmitted from rats to humans by flea bites that deliver the microbe directly into the blood. When circulating phagocytic cells attempt to engulf and kill the pathogen, *Yersinia*'s T3SS injects a set of at least four effectors that collectively paralyze the phagocytic machinery before the immune cells can swallow their prey. The circulating phagocytes, with *Yersinia* bacteria adhering to their surface, then filter into the lymph nodes, where the microbes start multiplying, causing the painful swellings, or bubos, that give bubonic plague its name.

Many pathogens have evolved secretion/injection systems able to selectively reprogram cell signaling and immune responses. *Shigella dysenteriae,* the causative agent of dysentery, exemplifies the range of tactics that a single species of bacteria can sometimes employ in the course of infection. Although *Shigella* bacteria are extremely similar genetically to harmless strains of *E. coli*, *Shigella* possess a T3SS that injects 25 to 30 effectors, which cause host cells to take up the microbes, much as *Salmonella* are drawn in. *Shigella* then co-opt the cytoskeletal

machinery to travel through the cell and penetrate a neighboring cell, thus avoiding any encounters with immune cells and antibody molecules that might wait outside the cells.

Shigella's other immune evasion and reprogramming mechanisms are not completely understood, but several of its effectors are known to interact directly with the internal signaling systems in host cells in ways that neutralize some of the distress calls an infected cell would normally send out. Not all host cell signals are silenced, however. The microbe also counts on a certain amount of signaling to draw dendritic cells to the site of infection. It then penetrates those phagocytic cells, using them as a Trojan horse to carry them through the intestinal wall—a journey that disrupts the wall and causes the severe diarrhea characteristic of dysentery.

It is not only the innate immune system that bacteria dupe. Some have learned to avoid the "acquired" immune response, which consists of T cells and antibody-producing B cells that are trained by innate immune cells to recognize a specific pathogen by its surface features (antigens). Microbes may dodge these defenses, either by constantly changing surface proteins to evade antibodies or by secreting enzymes that degrade antibodies. *Shigella* is one of several pathogens able to block antibodies from ever being made, by preventing phagocytic cells from presenting antigens to the cells of the acquired immune system. *Salmonella* is also able to trigger an internal signaling cascade that induces phagocytic cells to commit suicide before they can interact with cells of the acquired immune system.

A Competitive Community

To thrive in a body, pathogens need to do more than manipulate cell signaling and outwit immune defenses. They also have to outcompete the body's hordes of normal, friendly bacteria—players that were virtually ignored by most microbiologists and immunologists until recently. All the surfaces of the body exposed to the environment, including the lining of the gastrointestinal tract, contain an enormous population of these "commensal" microbes. Each gram of the large intestine's contents, for example, contains approximately 60 billion bacteria—10 times the number of people on the planet.

One of the most obvious ways to eliminate competition is to cause diarrhea and thereby flush one's opponents out of the body, at least temporarily. My colleagues and I have shown that a mouse version of pathogenic *E. coli,*

Secretion systems evolved not to sicken humans but to protect the bugs from attack by single-celled organisms in the soil.

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> **Many pathogens have evolved from harmless microbes by acquiring genes that confer new properties.**

known as *Citrobacter rodentium,* intentionally triggers inflammation of the intestine, an influx of innate immune cells that kills off a particular subset of the animal's normal gut microbiota. Without these rivals for resources, the pathogens multiply rapidly, and their dominance lasts until the acquired immune system becomes activated against them. The immune cells ultimately clear the pathogens, and then normal flora repopulate the gut, returning to approximately their original composition and numbers.

Similarly, a mouse version of *Salmonella* adapts its behavior to the makeup of the host's microbiota. The bacterium usually causes a systemic typhoidlike disease in mice; however, if the normal mouse microbiota are altered in advance by pretreating the mice with high doses of antibiotics, the pathogen produces a disease that is limited to the gastrointestinal tract. Competition from the resident gut microbes seemingly drives the *Salmonella* to invade the body and cause systemic illness, but when the resident flora are changed, *Salmonella* are content to remain in the gut.

Interactions among microbes, both pathogenic and benign, inside a host's body also provide opportunities for pathogens to gain and exchange weapons. Indeed, many pathogens have evolved from harmless microbes by acquiring genes that confer new properties. In this sense, the gut can be considered a great microbial genetic Internet, allowing the sharing of genes encoding "virulence factors"—the tools and tricks that enhance bacterial virulence, such as secretion systems or effector proteins.

Acquiring new pathogenicity islands can give the microbe an advantage by allowing it to colonize a new host or to become more aggressive. The deadly *E. coli* O157, for example, is thought to have appeared for the first time in the late 1970s, when a relatively benign *E. coli* acquired a pathogenicity island encoding a new T3SS and gained the gene for making Shiga toxin—properties that together produce severe diarrhea and kidney disease in O157 infections.

Building New Weapons

Discovery of injection systems and other tools that help pathogens to survive and thrive inside a host is suggesting ideas for therapy that go beyond the classic antibiotic strategy of directly damaging bacterial cells. My research group, for instance, has taken advantage of our knowledge of secretion systems to devise a novel vaccine against *E. coli* O157.

The vaccine contains pieces of the pathogen's T3SS as well as several of its effectors, so the acquired immune system can learn to immediately recognize and neutralize the proteins, preventing the bacterium from deploying them. This particular vaccine protects people from afar: it is for cows. *E. coli* O157 resides harmlessly in about half of domesticated cattle, but cow fecal matter can spread it to human food and water supplies, which is why this pathogen most often causes illness through tainted meat or produce. By eliminating O157 at its source, the vaccine—which is now used in Canadian cattle and is undergoing approval in the U.S.—can help keep O157 from ever finding its way to a human host.

Many investigators are exploring other creative strategies for disabling pathogens. Once a bacterium's virulence factors are known, one might develop therapies that render the microbe harmless by shutting off the genes that give rise to those factors. A related approach is creating molecules that block a bacterium's cell adhesion molecules—preventing it from gaining a foothold in the host. Such an antiadhesin targeted against pathogenic *E. coli* has already completed human efficacy trials, and similar drugs are in earlier stages of development.

Interfering with organisms' ability to communicate with one another is also an intriguing possibility. Bacteria such as *E. coli* gauge their location in the gut by "listening" to chemical signals from normal microbiota and host cells, and that information plays a part in their decision to attack. Another pathogen, *Pseudomonas aeruginosa,* forms colonies called biofilms in the lungs, and investigators at the University of Copenhagen recently showed that constituents of the biofilm send out signals to warn of approaching immune cells, which causes the other bacteria to secrete an immune cell–killing peptide.

One of the advantages of targeting bacterial factors that help make us sick is that those molecules are not usually essential to the microbe's ability to survive outside our bodies. In contrast to traditional antibiotics, which attempt to kill pathogens outright, newer treatments blocking communication and other virulence mechanisms would leave organisms harmless but alive, and so resistance to the treatments would probably arise more slowly, if at all.

Even more indirect methods of subverting pathogens focus on making the environment unfriendly for them. The prospect that host microbiota could be altered to compete with pathogens is being hotly pursued by many investiga-

VITAL VACCINE: Cattle can carry *E. coli* O157:H7 without becoming sick, but the bacteria can cause lethal kidney failure in humans. Econiche, a cattle vaccine against the pathogen, is licensed in Canada and awaiting approval in the U.S. It protects people by keeping the pathogen out of the food supply.

Targeting Bacterial Weapons

With a better understanding of the tools bacteria use to subvert host cells and defenses, scientists are developing a variety of approaches to counter the bugs' attack. A few of the examples below are in early (phase 1 or phase 2) human testing, but most are in preclinical (laboratory) stages of development.

TARGET	SUBSTANCE (HOW IT WORKS)	TESTING STAGE
Adhesion to human cells	**Immunoglobulin** (blocks operation of bacterial adhesion proteins)	Phase 2*
	Glycodendrimers (act as decoys for bacterial adhesion proteins)	Preclinical
	Pilicides (impede manufacture of adhesion proteins)	Preclinical
Type 3 secretion systems	**Salicylidene acylhydrazides** (block assembly of secretion system)	Preclinical
Virulence genes	**Virstatin** (blocks manufacture of toxin and adhesion molecules)	Preclinical
	Inhibitory autoinducing peptides (block manufacture of communication molecules)	Preclinical
Communication	**Azithromycin** (interferes with multiple aspects of bacterial communication)	Preclinical*
Host immune cells	**IMX942** (modifies signaling and inflammation)	Phase 1 (Canada)
	Sodium butyrate (induces production of antimicrobial peptides)	Phase 2

*Already FDA-approved for other uses.

tors. The principle of introducing probiotics (harmless bacteria such as *Lactobacillus*) and prebiotics (sugars to enhance growth of beneficial bacteria) to protect against disease is widely known, and many people have used substances such as yogurt to try to enhance their commensal populations. But those strategies have not yet been tested rigorously enough to determine which friendly bacteria would be most beneficial, nor has anyone identified specific microbes that would be powerful enough to combat an established infection.

Work is somewhat further along in finding ways to boost the ability of human immune cells to fight off pathogens, however. Many immune-stimulating substances are already widely used in minute amounts as additives to vaccines, without harmful side effects. And several biotechnology companies are currently in early stages of research or in early clinical trials with new substances designed to enhance or refine natural immune responses. This approach could be used to augment other therapies and possibly to prevent or even treat active infections.

Perhaps the greatest hurdle in the effort to develop new drugs for this purpose is uncoupling the beneficial aspects of inflammation—its normal role is to rally needed immune cells to battle the invader—from harmful levels of inflammation that can hurt the host. Evidence gathered so far suggests that obstacle can be overcome, though. One example is a drug based on my group's research with our University of British Columbia colleague Robert Hancock into host

defense peptides: small proteins produced by innate immune cells in response to pathogens. Some of these directly penetrate microbial cell membranes to kill the intruder; others act as signaling molecules to call for immune cell reinforcements. A peptide we discovered, called IDR-1, is in the latter group. It induces dendritic cells to emit chemical signals calling for macrophages to battle pathogens but does not induce the dendritic cells to send out certain types of signals—substances such as tumor necrosis factor-alpha—that can cause a cascade of runaway inflammation. In fact, in animal trials, the molecule reduced inflammation while increasing the response of desirable immune cells to the site of infection.

Turnabout is fair play, and if microbes can learn to manipulate human immune cells' signaling, certainly people can do the same. As scientists' knowledge about how bacteria cause disease has grown exponentially over the past two decades, the sophistication of microbial virulence mechanisms has become increasingly apparent. Pathogens have evolved along with their hosts, fine-tuning their tool kits to an extraordinary degree. But just as the microbes have an impressive array of tricks up their proverbial sleeves, so do we. Studying the remarkable methods that bacteria use to invade and outwit hosts has improved understanding of immunity and disease processes as well. This growing understanding of host-pathogen-microbiota interactions is already allowing scientists to design new ways to prevent and treat bacterial infections—alternatives that cannot come too soon. ∎

➡ **MORE TO EXPLORE**

An Anti-infective Peptide That Selectively Modulates the Innate Immune Response. Monisha G. Scott et al. in *Nature Biotechnology,* Vol. 25, No. 4, pages 465–472; published online, March 25, 2007.

Manipulation of Host-Cell Pathways by Bacterial Pathogens. Amit P. Bhavsar, Julian A. Guttman and B. Brett Finlay in *Nature,* Vol. 449, pages 827–834; October 18, 2007.

Molecular Mechanisms of *Escherichia coli* Pathogenicity. Matthew A. Croxen and B. Brett Finlay in *Nature Reviews Microbiology;* published online, December 7, 2009.

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