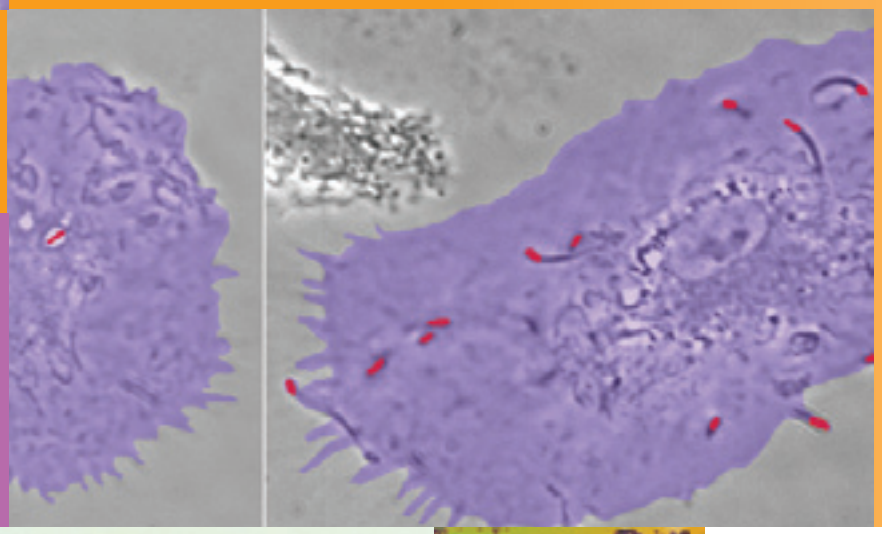
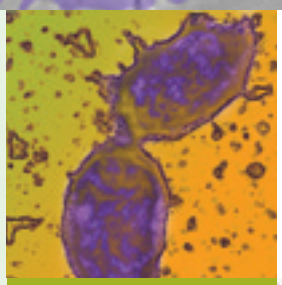
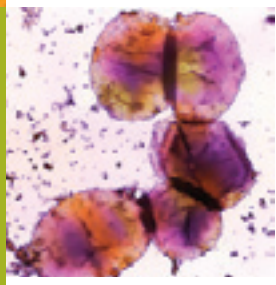
IN THE AGE-OLD STRUGGLE BETWEEN HOST AND PATHOGEN, U-M RESEARCHERS CAUTION THAT TRUCE RATHER THAN VICTORY MAY BE THE ANSWER.



Mouse macrophages infected with *Listeria monocytogenes*



Haemophilus influenzae



Chains of anthrax bacilli killing host macropahges

by Nancy Ross-Flanigan

photos by Martin Vloet

Are the Bugs Winning?

Pick up a newspaper, click on the TV or radio, and you're bound to encounter another scary story. One day it's West Nile virus; the next it's anthrax or antibiotic-resistant *Staphylococcus*. Bad bugs are big news, and even though our battle against them is an ancient one — cases of anthrax were recorded by the Romans — we seem to be losing ground these days. Are the bugs really winning, or are hyperbolic news stories only making it seem that way?

The search for an answer begins with a basic question: Just why are microbes so intent on attacking us? Rest assured, it's nothing personal, infectious disease experts say. You may be the most diligent worker, devoted parent, and decent, upstanding citizen, but to a disease-causing organism, you're just a dwelling. Well, not *just* a dwelling. From the bug's point of view, you're a four-star resort, warm as Cancun in winter and brimming like a buffet table with sugars, vitamins, minerals and other chemical delicacies.

It's no wonder, then, that viruses, bacteria and parasites keep finding ways to outwit the human body's best defenses. In this ongoing evolutionary game of tit-for-tat, the bugs that manage best are the ones that have known us longest, explains Cary Engleberg, M.D., professor of internal medicine and of microbiology and immunology in the Medical School, and chief of the Division of Infectious Diseases.

"The organisms that have the best capacity to survive inside the human host and are able to evade elimination by doing specific things to our immune system are those that are strictly human pathogens," says Engleberg. Over eons of intimacy, these bugs have developed and honed cunning strategies for hiding from or interfering with our immune responses. And though they're constantly trying to thwart our protective measures, these most intimate invaders are the ones that are least likely to kill us outright.

"It's a general tenet of microbial pathogenesis that the successful pathogen doesn't kill its host," Engleberg explains. "So when we see a situation where an organism has developed a way to create a chronic infection — to reproduce itself and maybe cause illness, but not damage the host so badly that it interferes with its own transmission — then we know a significant amount of coevolution has taken place. Organisms that cause very high mortality rates are unlikely to have evolved in human beings."

Consider, for example, the difference between the bugs that cause gonorrhea and West Nile fever. *Neisseria gonorrhea,* the bacterium responsible for the sexually transmitted disease, lives only in humans, "and as far as we know exists nowhere else in nature," says Engleberg. Its many methods of eluding the immune system — from disabling antibodies to disguising itself in ever-changing costumes of surface proteins➤

— are evidence of its long, close relationship with us. West Nile virus, on the other hand, isn't accustomed to living in humans, Engleberg says. "It really is adapted to insects, and it also survives in birds. The human being is an accidental host, but if humans didn't exist, the virus would still be here; it'd be happy." Since it hasn't discovered how to hang out in humans without wreaking havoc, West Nile — like other encephalitis viruses — is a killer. And as international travel and commerce increasingly blur geographic boundaries, our chances of encountering such dangerous, unfamiliar pathogens are growing.

Plots and Counterplots

Understanding the strategies successful pathogens use is the first step in plotting a counterattack. That's why researchers like Joel Swanson, Ph.D., professor of microbiology and immunology; Philip Hanna, Ph.D., assistant professor of microbiology and immunology; and Brian Akerley, Ph.D., assistant professor of microbiology and immunology, focus on specific processes and players in the host-pathogen interaction.

Swanson's lab concentrates on the biology of the macrophage, an amoeba-like white blood cell that engulfs invaders by the process of phagocytosis and takes in other tidbits through endocytosis. "We try to understand how those processes work in the normal macrophage — that is, a macrophage doing its job successfully," says Swanson. "Secondarily, we study how various pathogens perturb the normal process of phagocytosis." To that end, Swanson's research team builds microscopes and develops techniques for observing and analyzing the chemistry inside living macrophages.

Currently, they're exploring what happens when a macrophage swallows up *Listeria monocytogenes*, a bacterium that is implicated in food poisoning and can cause fever, meningitis and encephalitis. Within a half hour of being taken up and sequestered inside a special compartment in the macrophage, *Listeria* performs a Houdini act. The wily bug escapes its prison by secreting a protein that dissolves the compartment's lining. Unless, that is, the macrophage is on the alert, tipped off by signaling molecules sent from other immune system cells. In this activated state, the macrophage somehow prevents the captive *Listeria* from pulling off its escape act.

Joel Swanson

"We're trying to understand what chemistries are involved in that escape process, and, conversely, what chemistry the macrophage uses to stop it," says Swanson. "Researchers have a kind of outline picture of what those chemistries are — they know that reactive oxygen and nitrogen species may be involved, but how they work together to accomplish these things is unknown. That's what we'd like to understand."

West Nile virus hasn't discovered how to hang out in humans without wreaking havoc; it, like other encephalitis viruses, is a killer. And as international travel and commerce increasingly blur geographic boundaries, our chances of encountering such dangerous, unfamiliar pathogens are growing.

A technique called fluorescence resonance energy transfer (FRET) is giving Swanson's team a clearer view of what goes on inside a macrophage. "FRET allows you to see if two proteins inside a cell are actually interacting with each other, which then allows you to ask quantitative questions about the chemistry," says Swanson. "FRET technology has a lot of promise for figuring out what kinds of signals are generated inside the cells and how those signals may be modified by pathogens."

In other work, Swanson and collaborators at Harvard University are trying to unravel the process by which anthrax toxin kills macrophages. Anthrax toxin comes in two forms, lethal toxin and edema toxin. Lethal toxin works mainly on macrophages and is made up of two components: lethal factor, which does the dirty work, and protective antigen, which acts as a "landing craft" for lethal factor.

"We would eventually like to know how lethal factor kills macrophages, but at this point we're studying how it's delivered into macrophages," says Swanson. What the researchers know so far is that the two toxin components — each harmless by itself — are processed into a functional toxin on the surface of the macrophage. Then, the toxin molecule is taken into the macrophage by endocytosis, ending up in a vesicle inside the macrophage. "The acidic pH inside the vesicle causes the toxin to insert in the vesicle membrane, creating a pore that

Cary Engleberg

Philip Hanna

allows the delivery of lethal factor across the membrane into the cytoplasm, where it does its thing to kill the macrophage," Swanson explains. "If you can prevent lethal factor from being delivered across the membrane, nothing will happen — the cell won't die — so that's why we're interested in the basic mechanism of delivery."

Anthrax is also the subject of Hanna's research, which focuses on the first few hours of infection. Under certain conditions, *Bacillus anthracis,* the bacterium that causes anthrax, forms spores that resist drying, heat, sunlight and many disinfectants. These hardy spores can remain dormant in the soil for decades — perhaps even centuries or millennia. But once they get inside the body — by being inhaled or swallowed or entering through a cut or scratch in the skin — they quickly germinate and start causing problems.

What triggers germination? That's what Hanna and colleagues are trying to find out. "We want to know what signals the body contributes and how the bug senses them and then very quickly changes from an inert particle to a rapidly growing, toxic bacterium," he says. In a study published in the March 2002 *Journal of Bacteriology,* Hanna and John A.W. Ireland, Ph.D., a research fellow in the Department of Molecular, Cellular, and Developmental Biology, showed that germination depends upon the coordinated activity of several genes, receptor proteins and amino acids in at least two separate signaling pathways. Apparently, the process starts when ring-shaped structures found on certain amino acids and ribonucleosides bind to receptor proteins on the spore's membrane.

Hanna began studying anthrax a decade ago, long before anthrax-laced letters set off a nationwide panic in 2001. Though his work seems especially relevant in light of recent events, it would be valuable even without the threat of bioterrorism.

"The reason we began to study anthrax in the first place is that we have no real clue — for any bacterium — of what goes on in the first few hours of infection," Hanna explains. "Anthrax is a great model for studying this problem because it comes into the host as a dormant spore; then it germinates quickly. It's a rapid, synchronous process, so we can study each step in each stage of the disease, looking at the expression profiles of its genes inside the vacuole of the macrophage, inside the cytoplasm of the macrophage, and once it leaves the macrophage and enters the bloodstream. The hope is that we will learn about how our body's main immune system responds to a whole class of bacteria, and that the work will transcend just anthrax."

Like Hanna, Brian Akerley is studying a specific pathogen — *Haemophilus influenzae* — for insights that he hopes will apply more broadly. *H. influenzae,* a bacterium, is one of those bugs that have made a habit of living in humans. Some 75 percent of healthy children and adults harbor it in their upper respiratory tracts, but it has never been detected in any other animal species. *Haemophilus influenzae* is often the culprit in otitis media, the ear infection that plagues young children, and it can also cause respiratory tract infections and pneumonia in infants, children and adults.

But it wasn't the bug's prevalence or its disease-causing capabilities that caught Akerley's interest when he was beginning his postdoctoral fellowship in 1995. *Haemophilus* had another claim to fame as the first free-living organism to have its entire genome sequenced, and that's what appealed to Akerley.

"At that time, I was studying *Bordatella pertussis,* the respiratory pathogen that causes whooping cough, but I decided it would be advantageous to work on a sequenced organism," he says. However, as Akerley and many other researchers soon realized, just knowing an organism's genome sequence wasn't terribly informative.

"There wasn't really a direct pathway for going from the genome sequence to ➤

the function of the genes in that genome," Akerley recalls. Applying single-gene approaches to an entire genome was laborious and required large groups of researchers. "Just beginning my postdoc, I didn't really want to start a consortium," he laughs. So he came up with a new approach that ultimately allowed him to pinpoint the bug's weak spots.

Akerley knew that *Haemophilus* is a whiz at taking up DNA from its surroundings and incorporating it into its chromosome. He also knew that other researchers were developing test-tube methods employing transposons — pieces of DNA that can move at random, jumping into genes and causing mutations. Combining those two bits of knowledge, he devised a way of mixing *Haemophilus* DNA in a test tube with the enzymes that mediate transposon hopping, creating recombinant *Haemophilus* DNA. Then he fed the recombinant DNA molecules back to *Haemophilus*, which took them up into its chromosome and recombined them to generate mutations.

"I was able to create an extremely efficient and rapid mutagenesis system for *Haemophilus*, an organism that previously was intractable to transposon mutagenesis," says Akerley. The system also allowed Akerley to do something that hadn't been possible before: decide where in the *Haemophilus* chromosome he wanted a transposon to land and then target it directly to that location. By doing that to every section of the chromosome, he could induce mutations at virtually every possible insertion site.

"Then, using a technique called genetic footprinting, we can locate where those mutations have landed, and that tells us which genes have been mutated," Akerley explains. "It should be all the genes in the region except for one category — the genes that are essential for growth under the conditions that we use to select the mutants."

Using his system, Akerley identified a large number of genes that are necessary for the bug's growth or survival — exactly the genes that should be of interest to pharmaceutical companies looking for drug targets. Comparing his findings to a database of known genes in other bacterial pathogens, he found that some of the essential genes in *Haemophilus* are also present in other bacteria, such as *Mycobacterium tuberculosis*, which causes tuberculosis. While that doesn't prove that the same genes are essential in other bacteria, it does suggest good candidates to explore as potential therapeutic targets.

Putting the Pieces Together

While Swanson, Hanna and Akerley concentrate on specific pathogens and processes, Denise Kirschner, Ph.D., associate professor of microbiology and immunology, uses mathematical models to pull disparate pieces together into a cohesive picture.

"Much of science is reductionist, aimed at understanding one gene that's causing the host or the microbe to do something. What I try to do is synthesize the whole story from all the parts," she explains. "Our chairman, Michael Savageau, who has been a leader in the field of mathematical modeling of biological processes for 30 years, coined the term 'reconstructionist' to explain what we do. While you need the reductionist approach to figure out what the pieces are, you also need the reconstructionist approach to put the pieces together, because as yet there is no experimental tool with which to integrate all the parts."

The underlying question in much of Kirschner's research is why a chronic infection, such as tuberculosis or HIV, makes some people very sick, while others go years without showing signs of active disease. In some people infected with the bacteria that cause tuberculosis, for example, the infection remains latent for their entire lives. The bacteria stay alive, but they do not cause disease. In other people, especially those with weak immune systems, the bacteria become active and multiply, resulting in the reactive form of the disease.

In work reported in the *Journal of Immunology* last year, Kirschner and colleagues showed, via a mathematical model, that interleukin-10 (IL-10) may play a more crucial role in tuberculosis than previously thought. Experiments on mice had suggested that the response to infection with Mycobacterium tuber-

Denise Kirschner

Brian Akerley

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Carol Chenoweth

culosis is the same, whether or not the mouse is able to make IL-10. But in their virtual model of human TB infection, Kirschner and colleagues found that depleting IL-10 set up an oscillation, throwing the system out of equilibrium. "What that tells us is that IL-10 acts as a stabilizer, helping to maintain latency," says Kirschner. "This suggests that, without IL-10, you have a greater chance of developing reactive disease." Collaborators at the University of Pittsburgh and Albert Einstein School of Medicine in Bronx, New York, have experiments underway to find out if the model's predictions hold true in the lab.

Battling on the Front Lines

While researchers devise strategies for battling bugs, clinicians fight on the front lines every day. Their most formidable foe is not a particular pathogen, but the problem of antibiotic resistance.

"It is *the* problem in infectious diseases," says Cary Engleberg. "Nobody has figured out a way to design an antimicrobial agent that an organism cannot become resistant to. Every single one that is on the market had a spectrum of activity in the microbial world when it was first launched, and in every case, resistant organisms appeared very quickly after it started being used. It may take a year or five years or 10 years or 20 years before the drug has to be replaced, but it eventually always happens."

In a sense, antibiotic resistance is changing the whole microbe vs. mortal game. While developing ways to interfere with the host immune response usually involves complicated evolutionary mechanisms, played out over years, acquiring antimicrobial resistance is a snap. It's not a matter of magic; it's just the result of natural selection. When bacteria are exposed to antibiotics, the drug-sensitive bugs die. But in any population there are always variants with unusual traits — in this case, the ability to survive in the presence of one or more antibiotics. Killing off the susceptible bacteria clears the way for resistant ones to thrive and multiply. These indomitable bugs can also transfer their resistance genes to other bacteria that never have been exposed to those antibiotics.

Though antibiotic resistance occurs by a natural process, human habits and practices add to the problem. Over-prescribing of antibiotics and their use in livestock have contributed, says Carol Chenoweth (M.D. 1984, Residency 1991), clinical associate professor of internal medicine and assistant professor of epidemiology. In addition, more people are contracting and spreading infections, requiring increased antibiotic use.

"Hospitals are having a harder time with this now, because the patients we have are much sicker, and we're performing so many procedures on them that we didn't do 20 or 30 years ago," says Chenoweth. "We didn't do liver transplants; we didn't do lung-heart transplants; patients who came in with severe trauma died, whereas now we have better ways of keeping them alive. With changes in healthcare, we're also sending patients home much sooner, so those we're left with are patients who are immuno-compromised or have been exposed to invasive procedures and are at extremely high risk of getting infections."

The U-M Health System takes a multi-pronged approach to preventing antibiotic resistance, carefully controlling antibiotic use and following infection control procedures recommended by the Centers for Disease Control and Prevention. But vigilance can go only so far. In the end, clinicians look to researchers to keep coming up with better ways of deterring pathogens.

"I don't want to sound pessimistic and say that we're losing — I don't think that's necessarily true — but I do think maybe we have to change our ways and look for new methods of treating and preventing infections," says Chenoweth. "And I believe we'll find them — there are a lot of smart people working on the problem."

One of those smart people, Hanna, is optimistic, too. But he's pinning his hopes more on a truce than on complete victory.

"I don't think any infectious disease researchers or physicians would say that we'll ever become infectious disease-free — the bugs multiply and adapt to environments far more rapidly than people do," says Hanna. "The trick will be to continue to develop new tools and to keep effective the tools that we already have."