MEETING AMERICAN SOCIETY FOR MICROBIOLOGY

Microbes Feature as Pathogens And Pals at Gathering

CHICAGO, ILLINOIS—Unlike Leeuwenhoek, who guarded the lens that best magnified the "wee animalcules" that he first saw, today's microbiologists enthusiastically broadcast both their methods and their findings. At the American Society for Microbiology meeting, held here from 30 May to 3 June, about 14,000 of the researchers assembled to discuss findings on topics ranging from a novel protection against *Leishmania* to communication by gut bacteria.

A Bite in Time

Avoiding insects might seem a logical way to avoid insect-borne diseases. But new results suggest that for at least one disease, leishmaniasis, the best protection might be the bite of the very same fly that transmits it. At the meeting, immunologist David Sacks of the National Institute of Allergy and Infectious Diseases (NIAID) reported that mice bitten by sand flies that do not carry *Leishmania*, the tiny protozoan that causes the infection, resist infection later, possibly because something in the flies' saliva revs up the animals' immune response.

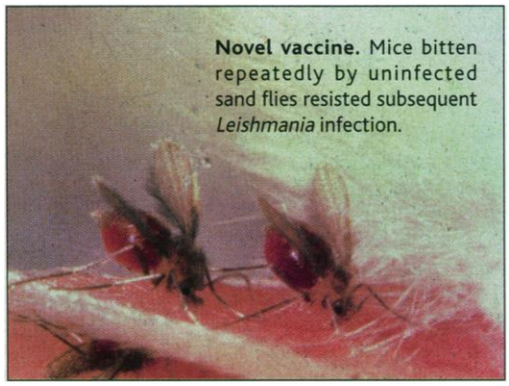
Leishmaniasis afflicts hundreds of thousands of people in tropical and subtropical areas every year, making it the second most common protozoan disease after malaria. Most commonly, the microbe causes a skin infection, but in some cases it infects the internal organs and can be fatal. No one has been able to develop an effective vaccine, so some researchers hope the surprising effect of the sand fly bite may open a chink in *Leishmania*'s armor. "There's a great deal of potential [in this work] in terms of understanding transmission, which boils down to prevention," says Duane Gubler, an epidemiologist at the Centers for Disease Control and Prevention in Fort Collins, Colorado.

Researchers have known for some time that components in sand fly saliva can enhance the infectivity of the parasites they transmit. Among other things, they block blood clotting in the vicinity of the wound, thereby aiding feeding by the insect and also transmission of the parasite. In the current work, Sacks wanted to see if exposure to the saliva of uninfected sand flies might lead to some kind of immune response that would neutralize these enhancing effects.

He and his research team at NIAID and the Walter Reed Army Institute of Research in Washington, D.C., filled small vials with sand flies, clamped the vials against the ears of six mice, and allowed the insects to bite. Infected flies produced lesions on most of the ears within 3 weeks. The lesions healed, but most of them still harbored parasites. In an experiment conducted in parallel, the mice were exposed to uninfected flies twice, at 2-week intervals, before they were bitten by infected flies. Only three of the 12 ears developed lesions, and after they healed, all were parasite-free.

These results suggest that the prior bites not only decrease the incidence of disease, but also reduce the capacity of mice to serve as reservoirs of infection. "Allowing uninfected flies to bite these mice provides as much or more resistance [to *Leishmania*] as the best vaccines known," says Sacks.

But that didn't happen quite the way he expected. Rather than neutralizing the enhancing effects of the saliva, the repeated bites apparently protected the animals by inducing an immune response that would fight the parasite more directly. He found that production of interferon γ increased in the animals. This molecule, one of many cytokines that regulate immune responses, stimulates cell-mediated immunity, a response specialized for thwarting pathogens that reside within host cells, as *Leishmania* does.



Novel vaccine. Mice bitten repeatedly by uninfected sand flies resisted subsequent *Leishmania* infection.

CREDIT: E. ROWTON/WALTER REED ARMY INSTITUTE OF RESEARCH

Because the majority of flies in areas where leishmaniasis is endemic are uninfected, the protective effect of prior bites might explain why the severity of *Leishmania* infections varies from one individual to another, Sacks says. It might also explain anecdotal reports that children and newcomers to parts of the world where the parasites and sand flies live tend to suffer more serious illness than adult natives. He notes that researchers have ascribed such resistance to immunity against the parasite itself gained during earlier infections. Instead, he says, "we're introducing the idea that the history of exposure to vector saliva has a profound effect."

The findings suggest that sand fly saliva might be a useful component of an anti-leishmaniasis vaccine. And they point to an irony, says Sacks: "One reason the Army has been keeping [the flies used in the experiment] is to figure out how to keep them off soldiers. Maybe what they should be doing instead is allowing their soldiers to come into contact with uninfected flies before they encounter the infected ones."

How Yeast Mitochondria May Meander

Like good parents, normal cells equip their offspring properly before sending them off into the world. That means, among other things, ensuring they each receive the organelles called mitochondria. Thought to be the descendants of symbiotic bacteria, mitochondria provide cells with energy, and a dividing cell employs active mechanisms to ensure that each offspring gets its fair share. About 5 years ago, for example, researchers found that in dividing yeast, mitochondria seem to amble to the emerging bud along cables of the protein actin. Results described at the meeting by cell biologist Liza Pon of Columbia University may now indicate what propels them.

Pon's work suggests that mitochondria move with the aid of a seven-protein complex called Arp2/3, which helps propel the movements of crawling cells by initiating the addition of actin subunits at the interface between the cell membrane and actin polymers. "If it's true, it's very interesting and novel because everyone has focused on the Arp complex in cell motility, not organellar movement," says David Drubin, a cell biologist at the University of California, Berkeley. The findings also highlight the link between mitochondria and their proposed evolutionary predecessors, the *Rickettsia* bacteria, because these microbes also use Arp2/3 to propel themselves, building a tail of actin to push themselves from cell to cell.

Pon originally set out to test the idea that mitochondria move with the aid of the motor protein myosin, which is known to run along actin filaments and is what powers muscle contraction. But she found that mitochondria could still zip along actin cables in yeast strains whose myosin genes were defective.

"We hit the wall looking at myosin," Pon said. That spurred her to look for other molecules that might yoke mitochondria to actin.

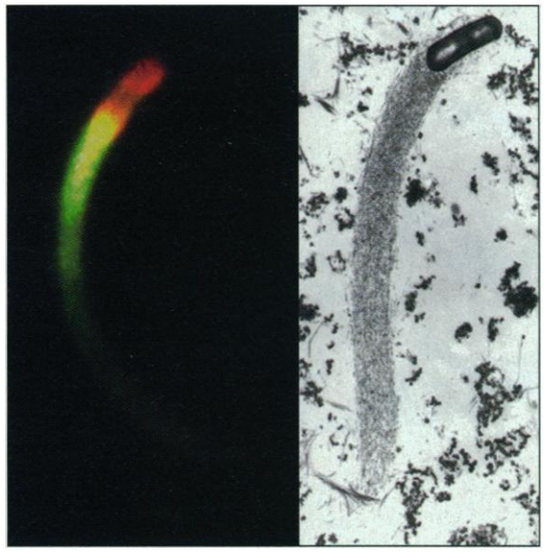
She eventually identified six proteins that might fit the bill. Sequence analysis revealed that one of them was Arc15p, a member of the Arp2/3 complex. With fluorescently labeled antibodies that bind to Arc15p, she confirmed that the protein lies on the mitochondrial surface along with another subunit of the Arp2/3 complex, Arp2p.

To determine whether these proteins are in fact needed for the mitochondria to move, Pon examined a yeast strain with a mutant *arp2* gene that contains a functional protein at 25°C, but not at 39°C. Although actin organization appeared normal, the mitochondria ceased to move when she raised the temperature to 39°C. An *arc15* mutant gave similar results. Finally, Pon found that a drug that interferes with actin polymerization in a mutant strain that contains an abnormally high number of cables decreased the number of moving mitochondria and rendered those that did move more sluggish.

Based on these results, Pon suggested that yeast mitochondria push themselves ahead by polymerizing actin behind them, in effect building an actin tail like those of their supposed bacterial ancestors. Unlike the bacteria, however, the mitochondria move along actin tracks. This mechanism may be special to budding yeast. Other cells use another type of cable, the microtubule, to move their mitochondria, but yeast microtubules are mainly used to pull the nucleus apart during cell division.

Pon and others caution that more work will be required to confirm the finding. "It's an intriguing hypothesis, and it bears closer examination, but there are some published experimental results that aren't consistent with a direct role for actin in mitochondrial inheritance in yeast," says Michael Yaffe, a cell biologist at the University of California, San Diego. For example, he says, certain mutant strains of yeast have no detectable actin cables but still seem to be able to divvy up their mitochondria properly.

Assuming Pon's results hold up, they might say more about the facility with which subcellular particles can co-opt actin than about the evolutionary roots of mitochondrial movement, says Julie Theriot, a cell biologist at Stanford University School of Medicine. Pon's observations might simply suggest that "it's easy for an organelle—or a microbe—to pick up this kind of motility." Indeed, several labs have shown that other organelles—endosomal vesicles that transport proteins within cells, for example—can apparently move in a similar way, says Daniel Portnoy, a microbiologist at the University of California, Berkeley. "This may be just the tip of the iceberg of actin-based mechanisms in organelle movement."



A mitochondrial cousin? Yeast mitochondria, like these *Listeria* bacteria, may be pushed through cells by a growing actin tail.

CREDIT: J. THERIOT AND M. COUGHLIN, STANFORD UNIVERSITY SCHOOL OF MEDICINE

Communication in the Gut

Disease-causing bacteria give microbes a bad name, but the fact is, humans have lots of microbial pals. Among them are the bacteria that dwell within our intestines and protect us from disease, help digest food, make vitamins, and even help shape the immune system. But although such indigenous bacteria are clearly important, scientists don't know much about how they operate. "The [human gut] epithelial lining is about an inch [2.5 cm] thick with bacteria, and we have no idea what they're doing," says Stuart Levy of Tufts University School of Medicine in Boston and ASM president. New results reported by Jeffrey Gordon, a molecular biologist at Washington University School of Medicine in St. Louis, provide the first detailed insights into how a gut bacterium communicates with its mammalian host, presumably to the benefit of both.

Gordon described how he and Lora Hooper, a postdoc in his lab, are dissecting the molecular signaling system by which *Bacteroides thetaiotaomicron*, a common bacterium in both the mouse and human intestine, induces the intestinal lining to make a carbohydrate that the bacterium uses for food. Their results suggest that the microbe can control the signal so that it communicates the need for the carbohydrate only when the sugar runs low.

What the mouse gets in return is not yet clear. The bacterium's success undoubtedly makes it more difficult for pathogens to invade, and its alteration of the host environment probably helps build a healthy microbial community, suggests Gordon. "This is the first clear molecular definition of how a commensal bacterium plays a role in the development of a mammalian cell environment," says Levy. It could ultimately point to ways for keeping the gut microflora healthy when it is under assault, for example, in patients taking broad-spectrum antibiotics.

The current finding is an outgrowth of studies the Gordon team has been performing for several years on mice raised in a germ-free environment. Normally the intestine begins producing particular carbohydrates that contain the sugar fucose shortly after birth. But in the germ-free animals, the researchers found, production of this compound wanes as animals are weaned. By introducing various bacterial species individually into the animals, Gordon's group found that *B. thetaiotaomicron* was the missing factor, somehow signaling gut cells to make the fucosylated carbohydrate (*Science*, 6 September 1996, p. 1380).

Since then, Hooper has identified some of the molecular switches that control this signaling. Using a variety of genetic and biochemical techniques, she showed that the bacteria make a protein called FucR, which regulates both how the bacteria metabolize fucose and signal the host cells to make more of it. When the bacteria have ample fucose, the sugar binds to FucR. This binding both relieves the inhibition the protein would otherwise exert on the genes needed for fucose breakdown and turns off the signal to the intestinal cells.

Hooper found, for example, that a *B. thetaiotaomicron* strain carrying a mutation that eliminates FucR, and thus prevents it from responding to fucose, induces production of the sugar. The same mechanism would explain why a different mutant strain has the opposite behavior: It never tells the host to make the sugar. The researchers found that because of an enzyme deficiency, this mutant can't break down fucose, so the sugar builds up. As a result, Gordon and Hooper hypothesize, the sugar is always bound to FucR, which keeps the signal permanently "off."

Gordon says the ability of bacteria such as *B. thetaiotaomicron* to modify the intestinal ecosystem probably plays an important role in assembling and shaping its microbial communities. "At birth, the intestine is an unoccupied wilderness," he says. "How is this mass society of microbes assembled and maintained along the length of the gut? We're trying to lay the groundwork for understanding that by unraveling the schemes hatched by microbes for surviving and prospering in this competitive ecosystem."

–EVELYN STRAUSS