

Understanding *Listeria*

This pathogen endangers pregnant women, newborns, and those with weak immune systems. No wonder the U.S. Food and Drug Administration calls it a "bad bug."

Boutique *fromage* shops in Paris elevate raw-milk cheese to an art form, but pregnant women are advised to avoid those delights. *Listeria monocytogenes* is the reason—it's a pathogen found in uncooked meat, raw vegetables, and foods like cold cuts and soft cheeses that are tainted after processing. Antibiotics clear up the infection, but because a pregnant mother's symptoms can be mild, the pathogen often goes undetected, killing the developing fetus or leading to meningitis or other serious brain diseases.

What makes *Listeria* particularly troublesome is its unique ability to penetrate three barriers in humans: the intestinal barrier, the placental barrier, and the blood-brain barrier.

"When we started in 1986 we knew very little," says HHMI international research scholar Pascale Cossart, who is also head of the Unit of Bacteria-Cell Interactions, INSERM U604, at the Pasteur Institute in Paris. "We sequenced and published the first gene of *Listeria*, and now the whole genome is finished."

With colleague Philippe Glaser, Cossart headed a consortium of 10 laboratories that published the full genome sequences of *L. monocytogenes* and its non-disease-causing relative *Listeria innocua* in 2001. The information generated by that project is now helping to uncover new virulence factors. But it was Cossart's experiments in classical genetics that paved the way to begin to uncover the molecular tricks by which *L. monocytogenes* crosses the three barriers and wreaks its destruction.

In a finding published earlier this year in the *Proceedings of the National Academy of Sciences*, Cossart and Marc Lecuit—the doctor on the Pasteur team—describe how the bacterium crosses the placenta to infect the fetus. It's the first time anyone has explained how a pathogen crosses the so-called mater-

nofetal barrier, and their work could now provide clues for other groups studying toxoplasmosis or cytomegalovirus, which share that capacity and hence also pose a threat to pregnant women and their babies.

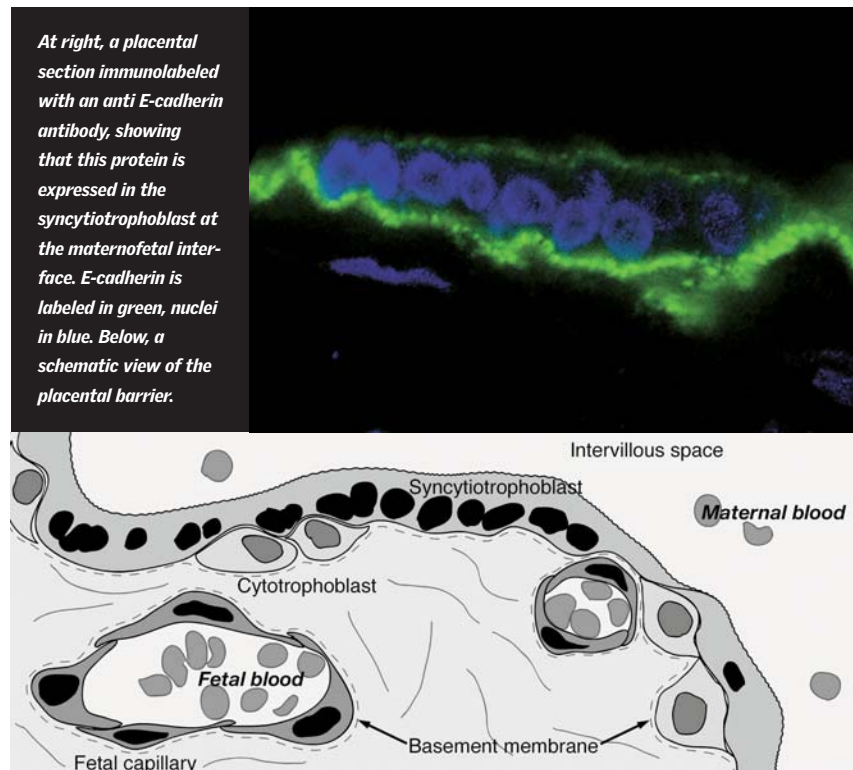
The mechanism they describe relies on a single protein, internalin, which exists on the surface of the bacterium and interacts with a receptor on the surface of mammalian cells called E-cadherin. The "E" stands for epithelial, because E-cadherin is the molecule that causes cells to stick together in the epithelia of various tissues of the body, such as the intestine, liver, and brain. Cossart had earlier identified internalin by focusing on those *L. monocytogenes* mutants unable to enter cultured

human epithelial cells. She found that only those lacking internalin could not do so.

"The surprise came when we started to look at the interaction between internalin and E-cadherin in different cells," she says. "We can precisely study this interaction by using latex beads coated with internalin." Whereas mouse cells seemed to be resistant to "infection" by internalin-bearing beads, human cells were not. What was going on? Mouse E-cadherin and human E-cadherin were previously thought to be identical and function similarly, but they are only 86 percent similar. One amino acid in particular, the 16th in the sequence, turned out to be critical for the ability to bind internalin, and hence to the difference in infectivity. "If you mutate that amino acid in humans, you lose that interaction; mutate it in mice, and you gain it," explains Cossart.

Together, these results explain why the wild-type bacteria and the internalin mutants were equally bad at infecting the mouse. And they paved the way for Cossart's group, in collaboration with Charles Babinet, also at the Pasteur Institute, to create a transgenic mouse model for listeriosis—a mouse that expressed human E-cadherin in its intestine but nowhere else. Then they were able to explore how the binding of

At right, a placental section immunolabeled with an anti E-cadherin antibody, showing that this protein is expressed in the syncytiotrophoblast at the maternofetal interface. E-cadherin is labeled in green, nuclei in blue. Below, a schematic view of the placental barrier.



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internalin to E-cadherin triggers the swallowing up of the bacterium by enterocytes, the host cells that represent the first step in spreading the bacteria through the cells of the intestinal epithelium.

Cossart was delighted when a group at the German Research Center for Biotechnology in Braunschweig, Germany, led by Dirk W. Heinz, published the crystal structure of the internalin/human E-cadherin complex in *Cell* in 2002. “It clearly showed how amino acid 16 sits in a loop and interacts with one of the repeated units in internalin,” she says.

She and others also described most of the other steps in this infection cycle, including the mechanism by which *Listeria* and other pathogens form actin tails to propel them through the host cytoplasm. Her discovery of the ActA protein was a breakthrough in that respect. ActA is a surface protein of *L. monocytogenes* that recruits the Arp2/3 protein complex from the host cell to induce actin polymerization. When she looked at another bacterium, *Rickettsia*

conorii—which is spread by tick bites and causes Mediterranean spotted fever—she found to her surprise that it also exploits Arp2/3. But it does so by using its own surface protein, RickA (a finding also published this year by Cossart, Edith Gouin, and colleagues, in *Nature*). Cossart thinks these structural differences between the two bacteria—the tails of *Listeria* consist of short, highly branched actin filaments, while those of *Rickettsia* are unbranched, more flowing, and hair-like—must spring from different properties of the bacterial proteins.

While she worked on the details, her mind remained on the bigger picture. Lecuit and Cossart wondered if the interaction between internalin and E-cadherin could also be responsible for the bacterium breaching other epithelia in the body—the maternofetal barrier, for instance. The answer turned out to be yes.



At the Pasteur Institute, Pascale Cossart studies bacterial molecular tricks.

For the first time, researchers explained how a pathogen crosses the maternofetal barrier. The work could advance the study of other threats to pregnant women.

Working with placental tissue extracts from women who had been infected with *L. monocytogenes*, Lecuit (in collaboration with D. Michael Nelson at the Washington University School of Medicine in St. Louis) noticed that the bacteria accumulated in a layer of cells called syncytiotrophoblasts, which lie on the placental surface that bathes in the mother’s blood. These cells also express E-cadherin. When he took extracts of the same cells from the placental tissue of healthy women and exposed them in a dish to *L. monocytogenes* that either did or did not express internalin, he found that only those bacteria expressing internalin were able to infect the cells efficiently.

“With one single protein interaction, a pathogen can specifically target and cross two very different barriers,” says Cossart, who is now busy creating a population of transgenic mice

that express human E-cadherin throughout their bodies, to see if the findings hold in vivo in the mouse model. And because E-cadherin is also known to be present at places in the blood-brain barrier, she has a hunch that a similar interaction may be happening there.

One final piece fits into the *Listeria* jigsaw. Cossart noticed that internalin can mutate, and that the presence of this mutation determined the effectiveness of the internalin/E-cadherin interaction. If the internalin gene mutates, the protein is truncated and is secreted by the bacterium rather than remaining attached to it. In other words, it loses its crucial binding capacity and the bacterium becomes unable to infect human cells.

This finding led Lecuit and Cossart, in collaboration with the Pasteur Institute’s Christine Jacquet, to survey the strains of *L. monocytogenes* isolated from infected women and from food in a single year in France, which they published this year in the *Journal of Infectious Diseases*. “We found that 100 percent of the strains responsible for fetal placental infections had full-length internalin, and 35 percent of the food strains had the truncated internalin which was secreted,” says Cossart. “That means there are many, many strains in food which will never infect humans.”

Mothers-to-be are not the only ones vulnerable to listeriosis, but their risk is 20 times higher than that of other healthy adults because of the natural suppression of their immune systems that allows their bodies to tolerate the growing fetus. Because their symptoms are so hard to detect, the emphasis, from a public health standpoint, has been firmly on prevention. Cossart thinks the findings of their survey could make the job of prevention a little easier by providing the basis of a test for distinguishing safe foods from dangerous ones.

—LAURA SPINNEY