other Golgi-associated proteins even if they contain dileucine motifs. Systematic screening of CI-MPR mutants expressed in CI-MPR–negative fibroblasts revealed that GGA2 binds specifically to the MPR dileucine motif. Importantly, Zhu and coworkers also demonstrated that removal of just the dileucine motif from MPRs was sufficient to abolish binding of GGA2.

The Puertollano group provide additional evidence that the interaction between the GGAs and the MPRs drives the production of TGN-derived transport vesicles. Time-lapse confocal imaging of live cells with GGA1 and CD-MPR proteins tagged with different fluorescent dyes revealed that GGA1 and CD-MPRs are localized in the same vesicles budding from the TGN. There is now a wealth of evidence to support the fact that GGAs are sorting proteins that recruit MPRs into vesicles forming at the TGN.

If this is the case, then what does the adaptor protein AP-1 do? Since the first biochemical demonstration that adaptor proteins sort cargo receptors into vesicles (*12*), and more particularly that AP-1 binds to CI-MPRs, it has been assumed that AP-1 sorts MPRs into TGN vesicles destined for the late endosome. Indeed, there are binding sites for AP-1 in the cytoplasmic domains of both CI- and CD-MPRs (*13*). The Zhu and Puertollano experiments appear to rule out the possibility that AP-1 is involved in the TGN–to–late endosome transport of MPRs, a job that clearly belongs to the GGAs. Rather, it now seems more likely that AP-1 binds to MPRs in the endosomal compartment, helping to recycle these cargo receptors back to the TGN. The GGAs have an exquisite specificity built into their VHS domains, which is necessary to ensure the correct sorting of MPRs into TGN vesicles. Future experiments will no doubt seek to understand the exact molecular nature of this specificity.

References

1. R. Puertollano *et al.*, *Science* **292**, 1712 (2001).
2. Y. Zhu *et al.*, *Science* **292**, 1716 (2001).
3. A. L. Boman, C. Zhang, X. Zhu, R. A. Kahn, *Mol. Biol. Cell* **11**, 1241 (2000).
4. E. C. Dell'Angelica *et al.*, *J. Cell Biol.* **149**, 81 (2000).
5. J. Hirst *et al.*, *J. Cell Biol.* **149**, 67 (2000).
6. A. Poussu, O. Lohi, V.-P. Lehto, *J. Biol. Chem.* **275**, 7176 (2000).
7. H. Takatsu, K. Yoshino, K. Nakayama, *Biochem. Biophys. Res. Commun.* **271**, 719 (2000).
8. O. Lohi, V. P. Lehto, *FEBS Lett.* **440**, 255 (1998).
9. R. Puertollano *et al.*, *Cell* **105**, 93 (2001).
10. M. W. Black, H. R. Pelham, *J. Cell Biol.* **151**, 587 (2000).
11. M. S. Nielsen *et al.*, *EMBO J.* **20**, 2180 (2001).
12. J. N. Glickman, E. Conibear, B. M. F. Pearse, *EMBO J.* **8**, 1041 (1989).
13. J. G. Orsel, P. M. Sincock, J. P. Krise, S. R. Pfeffer, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 9047 (2000).

PERSPECTIVES: MICROBIOLOGY

Cracking *Listeria*'s Password

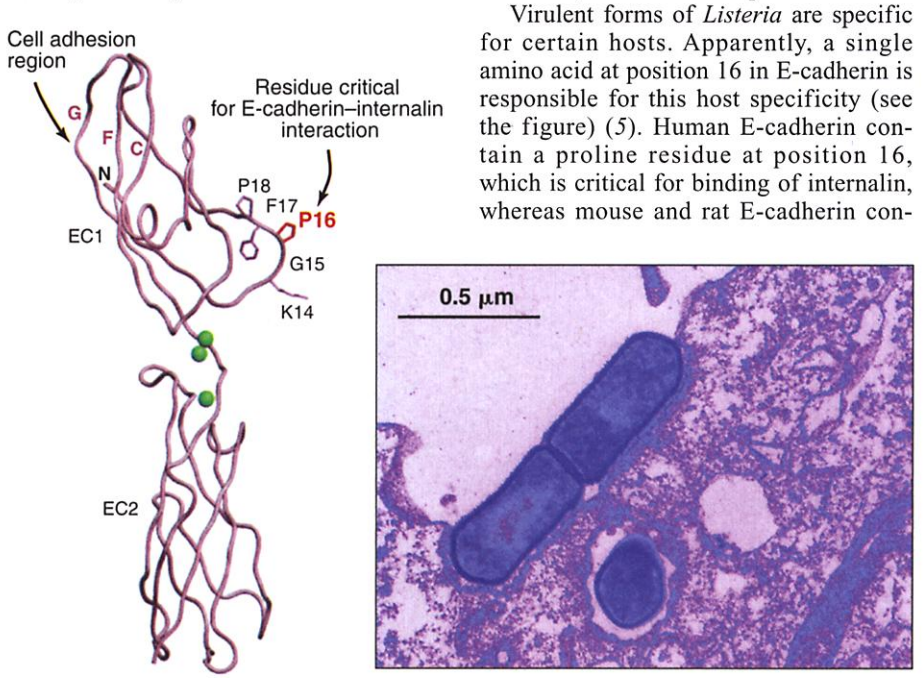
B. Brett Finlay

Listeriosis, a food-borne disease that is particularly severe among infants and the elderly, is caused by the Gram-positive bacterium *Listeria monocytogenes*. Each year in the United States, there are about 2500 cases of listeriosis and 500 deaths (*1*); *Listeria* infection has also been linked to late-term miscarriages in pregnant women. After ingestion of contaminated food, *Listeria* bacteria breach the intestinal barrier, sometimes causing severe gastroenteritis in their human hosts. The bacteria then travel throughout the body, crossing the blood-brain barrier to infect the central nervous system and the placenta of pregnant women to infect the fetus. Although mouse models have been valuable for studying the T cell response to *Listeria* infection (*2*), they cannot reveal how bacteria breach the intestinal barrier because the bacteria are given intravenously rather than orally. This is now set to change with the report by Lecuit *et al.* (*3*) on page 1722 of this issue. These authors have developed an elegant transgenic mouse model and a guinea pig model of listeriosis in which they show that *Listeria* enters gut epithelial cells through binding of its surface protein internalin to an epithelial transmembrane protein called E-cadherin.

The author is in the Biotechnology Laboratory, University of British Columbia, Vancouver, V6T 1Z3, British Columbia, Canada. E-mail: bfinlay@interchange.ubc.ca

Internalin belongs to the invasin family of bacterial surface proteins, all of which contain leucine-rich repeats. Invasins are used by a variety of intracellular bacterial pathogens to invade nonphagocytic host cells such as those of the gut epithelia. The invasin of *Listeria* internalin binds to E-cadherin, which is expressed on the basolateral epithelial cell surface and enables tight junctions to form between epithelial cells (*4*). Noninvasive strains of *Listeria* do not express internalin and cannot invade host epithelial cells, but they become invasive if they are forced to express internalin.

Virulent forms of *Listeria* are specific for certain hosts. Apparently, a single amino acid at position 16 in E-cadherin is responsible for this host specificity (see the figure) (*5*). Human E-cadherin contain a proline residue at position 16, which is critical for binding of internalin, whereas mouse and rat E-cadherin con-



An advantage to being different. (Left) A single amino acid at position 16 in E-cadherin is sufficient to confer host specificity on the bacterial pathogen, *Listeria monocytogenes*. Human and guinea pig gut epithelial cells are susceptible to infection with *Listeria* because the E-cadherin expressed by these cells contains a proline at position 16 to which internalin on the bacterial surface is able to bind. Rat and mouse gut epithelial cells, on the other hand, have a glutamic acid at position 16 and are refractory to infection by all strains of *Listeria* regardless of whether they express internalin. **(Right)** A transmission electron micrograph of *Listeria* invading a cultured human epithelial cell. [Reproduced with permission from (*10*)]

tain a glutamic acid. If the glutamic acid at position 16 is converted to a proline residue, rodent epithelial cells become susceptible to *Listeria* infection.

With their new animal models of listeriosis, Lecuit *et al.* (*3*) confirm that this single amino acid difference in E-cadherin is the reason that *Listeria* readily invades human gut epithelial cells but not rodent cells. This amino acid difference also explains why mice cannot be orally infected with *Listeria* even though oral infection is the normal route in humans. By making a transgenic mouse whose gut epithelial cells overexpress human E-cadherin, the authors have produced a murine model of orally acquired listeriosis. With their transgenic mouse, closer scrutiny of the first steps involved in breaching the host's intestinal barrier is now possible.

Lecuit *et al.* (*3*) examined various E-cadherin amino acid sequences and found that guinea pig E-cadherin, like the human sequence, contains a proline at position 16. They confirmed previous work (*6*) showing that a guinea pig epithelial cell line could be readily infected with *Listeria* in vitro. The authors then demonstrated in vivo that guinea pigs could be infected orally by virulent forms of *Listeria* producing internalin but not by strains lacking internalin.

In the Lecuit *et al.* transgenic mouse, the human E-cadherin gene is under the control of the *iFABP* promoter, which restricts its expression to epithelial cells of the small intestine (*7*). Morphologically, the authors could not detect any abnormalities associated with overexpressing E-cadherin in gut epithelia. However, continued expression of E-cadherin driven by the *iFABP* promoter is known to suppress proliferation of gut epithelial cells, induce apoptosis of cells in the crypts of the gut, and retard the normal movement of epithelial cells from the crypts to the tips of the villi (*7*). Lecuit *et al.* found that orally infecting their transgenic mice with virulent *Listeria* strains resulted in significant mortality, yet similar doses of a bacterial strain lacking internalin did not kill the mice. Virulent bacterial strains were also able to invade cells of the mesenteric lymph nodes, spleen, and liver in these animals. One paradox that the transgenic mouse and guinea pig models do not resolve is how the bacteria in the gut lumen gain access to E-cadherin, which is located on the basolateral surface of epithelial cells.

The Lecuit *et al.* work is noteworthy for several reasons. Whereas earlier virulence studies with systemic murine models of listeriosis called into question the importance of internalin as a virulence factor, the new data argue strongly that internalin is indeed crucial for initial penetration of the intestinal epithelial barrier. Furthermore, their transgenic mice provide the first rodent model of orally acquired listeriosis. In several transgenic mouse models of viral diseases such as polio, mouse tissues express human receptors for the virus (*8*). Lecuit *et al.*, however, are the first to make a transgenic mouse expressing a human receptor for a bacterial pathogen. Despite their success, the guinea pig and transgenic mouse models still do not provide a completely accurate picture of human listeriosis. Further refinements such as driving expression of human E-cadherin in cells of the mouse blood-brain barrier or placenta should enable features of listerial meningitis and neonatal disease to be reproduced more accurately.

Molecules involved in pathogenesis are being elucidated for other bacterial pathogens that live inside cells (*9*). Once these molecules are discovered, transgenic technology should enable development of relevant animal models. Although the pathogenesis of bacterial diseases is extremely complex, uncovering one key virulence factor is often sufficient to provide new opportunities for their study and treatment.

References

1. P. S. Mead *et al.*, *Emerg. Infect. Dis.* **5**, 841 (1999).
2. D. H. Busch, K. Kerksiek, E. G. Pamer, *Immunol. Rev.* **172**, 163 (1999).
3. M. Lecuit *et al.*, *Science* **292**, 1722 (2001).
4. J. Mengaud *et al.*, *Cell* **84**, 923 (1996).
5. M. Lecuit *et al.*, *EMBO J.* **18**, 3956 (1999).
6. P. Racz, K. Tenner, E. Mero, *Lab. Invest.* **26**, 694 (1972).
7. M. L. Hermiston, M. H. Wong, J. I. Gordon, *Genes Dev.* **10**, 985 (1996).
8. R. B. Ren *et al.*, *Cell* **63**, 353 (1990).
9. B. B. Finlay, *Cell* **96**, 315 (1999).
10. P. Cossart, M. Lecuit, *EMBO J.* **17**, 3797 (1998).

PERSPECTIVES: IMMUNOLOGY

Agrin—A Bridge Between the Nervous and Immune Systems

Alain Trautmann and Eric Vivier

The immune system and the nervous system share a number of unique features. They are both composed of complex networks of primary and accessory cells that are in constant communication with each other. In both systems, a record of a primary cell's encounter with a stimulus is preserved, such that upon reencountering the stimulus, the system remembers it and responds. Transmembrane signaling through surface receptors of both neurons and lymphocytes is regulated by the clustering of these receptors with each other and with other molecules. In both systems, information is transferred at points of contact between cells called synapses (see the figure). Although synapses in the nervous and immune systems (N- and I-synapses, respectively) are clearly different, they have in common a number of molecules that are required for synapse formation and operation. For example, major histocompatibility complex (MHC) proteins, which are key players in the immune system, are also involved in the formation of the nervous system (*1*). On page 1681 of this week's *Science*, Khan and colleagues (*2*) report that agrin—a well-characterized glycoprotein in neuromuscular junctions (NMJs), which are specialized synapses between motor neurons and muscle cells—is also present in the immune system. They propose that immune cell agrin may participate in the clustering of antigen-specific T cell receptors (TCRs) and accessory costimulatory molecules at the I-synapse between T lymphocytes and antigen-presenting cells (APCs).

In the middle of the wide synaptic cleft (>40 nm) that separates the presynaptic motor neurons and postsynaptic muscle cells of the vertebrate NMJ is a dense network of collagen called the basal lamina. Trapped in this network are various glycoproteins including agrin, which is secreted by motor nerve terminals and is important for formation of NMJs during development. The alternatively spliced product of the agrin gene Z^+ (but not the Z^- isoform) induces clustering of acetylcholine receptors (AChRs) and other postsynaptic proteins in the NMJ muscle cell membrane. It does this by activating a muscle-specific receptor tyrosine kinase, which phosphorylates AChRs, thereby controlling their association with cytoskeletal components of the NMJ (*3*). There are additional ways

A. Trautmann is in the Laboratoire d'Immuno-Pharmacologie, CNRS UPR 415, ICGM, Paris, France; e-mail: trautmann@cochin.inserm.fr. E. Vivier is at the Institut Universitaire de France and Centre d'Immunologie de Marseille–Luminy, CNRS-INSERM–Université Medicale, Campus de Luminy, Marseille, France; e-mail: vivier@ciml.univ-mrs.fr.