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.

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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).

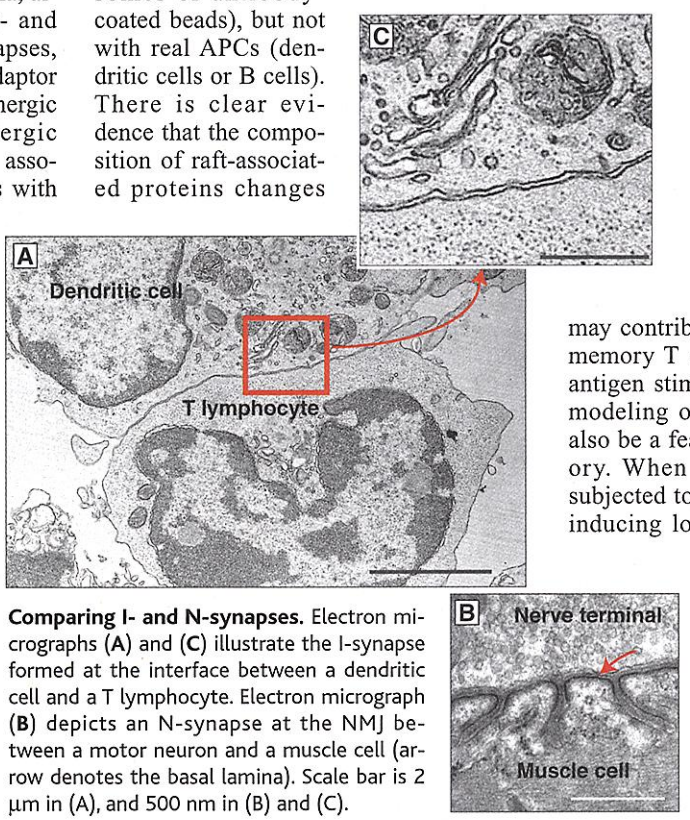
In the middle of the wide synaptic cleft (>40 nm) that separates the presynaptic motor neurons and postsynaptic muscle cells of the vertebrate NMJ is a dense network of collagen called the basal lamina. Trapped in this network are various glycoproteins including agrin, which is secreted by motor nerve 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.

to promote this clustering, in particular with adaptor proteins such as rapsyn, which link AChRs together (*4*). Although agrin is found at synapses in the central nervous system, it is not clear what it does there. Central nervous system synapses have a much narrower synaptic cleft (15 to 30 nm) that is devoid of a basal lamina, allowing direct contact between pre- and postsynaptic neurons. At these synapses, receptor clustering depends upon adaptor proteins—such as gephyrin at glycinergic synapses or PSD-95 at glutamatergic synapses—that allow the homotypic association of neurotransmitter receptors with each other, and the heterotypic association of these receptors with adhesion molecules (*5*).

Khan *et al.* make the intriguing observation that agrin is also found on T lymphocytes. In contrast to neurons, T cells only express the Z^- isoform of agrin. The agrin of resting T lymphocytes ($agrin_{rest}$) appears to be highly glycosylated, whereas that of activated T lymphocytes ($agrin_{act}$) is weakly glycosylated (both forms are recognized by distinct antibodies). $Agrin_{rest}$ is diffusely distributed on the surface of resting T lymphocytes, whereas $agrin_{act}$ colocalizes with clustered TCRs. Picomolar concentrations of agrin purified from activated T lymphocytes (but not $agrin_{rest}$) induce the clustering of TCRs, CD28 costimulatory molecules, and membrane lipid rafts.

Lipid rafts are mobile and dynamic microdomains of the plasma membrane. They are produced by the preferential packing of cholesterol and sphingolipids into platforms that move in more fluid regions of the membrane occupied by unsaturated phospholipids (*6*). They are identified by their low solubility in detergents and by their frequent enrichment with gangliosides such as G_{M1} (which can be detected by the binding of cholera toxin subunit B). Small rafts (<100 nm diameter) can coalesce into superrafts, which in turn can form a cap in one section of the plasma membrane (*7*). A number of groups have proposed that the partition of proteins between rafts and more fluid zones of the plasma membrane influences or perhaps even controls T cell signaling (*8*, *9*). Most of these studies analyzed the protein composition of the detergent-insoluble fraction of the plasma membrane after stimulation by cross-linking antibodies. Given the growing importance attributed to lipid rafts in T cell signaling, Khan *et al.* speculate enthusiastically that the formation of an I-synapse (a prerequisite for T cell signaling) could be driven by agrin-dependent raft aggregation. This may be true, although direct proof is not yet available. The presence of rafts at the I-synapse has been observed with surrogate APCs (submicrometer-sized liposomes or antibody-coated beads), but not with real APCs (dendritic cells or B cells). There is clear evidence that the composition of raft-associated proteins changes after T cell stimulation and that this change is required for signaling through activated TCRs. But there is no evidence that signal transduction at the I-synapse is initiated by raft clustering, whether induced by agrin or some other agent. For now, agrin's job at the I-synapse is still open to debate.



Comparing I- and N-synapses. Electron micrographs (**A**) and (**C**) illustrate the I-synapse formed at the interface between a dendritic cell and a T lymphocyte. Electron micrograph (**B**) depicts an N-synapse at the NMJ between a motor neuron and a muscle cell (arrow denotes the basal lamina). Scale bar is 2 μm in (A), and 500 nm in (B) and (C).

There remain a number of lingering questions, several of which are likely to be answered by the analysis of I-synapses formed between agrin-deficient T cells and APCs (*10*). At the NMJ, agrin secreted by the presynaptic cell acts indirectly to trigger the tyrosine phosphorylation of AChRs. In contrast, at the I-synapse, agrin is secreted by and seems to act directly on T cells (although it could be secreted by other cells in the spleen as well). What is the receptor for agrin on T cells? Is agrin present at the T cell–dendritic cell synapse, which does not have a basal lamina (*11*)? And if so, what is the initial signal responsible for the deglycosylation of agrin, given that only the deglycosylated isoform can induce clustering of TCRs?

Agrin may have additional tasks in the immune system that it does not have at the NMJ. This would not be the first example of a "molecular Lego" game played by evolution resulting in one molecule performing a variety of different tasks depending on its location. For example, it is conceivable that agrin might induce the partial coalescence of rafts in a nonpolarized way, leading to the formation of "signaling clusters," which would increase the sensitivity of T cells to subsequent stimulation (*12*). Such a possibility is consistent with the recent observation that activated T cells have aggregates of TCRs on their surface, a feature that may contribute to the enhanced ability of memory T cells to respond to secondary antigen stimulation (*13*). Intriguingly, remodeling of neuronal membranes could also be a feature of nervous system memory. When hippocampal dendrites are subjected to a tetanic stimulation, thereby inducing long-term potentiation (one of the basic phenomena involved in memory formation), one sees for several tens of minutes enhanced growth of small protrusions that develop into new dendritic spines (*14*, *15*). It is tempting to speculate that, in addition to its involvement in NMJ development and stabilization, agrin also could be required for the reorganization of neuronal and T lymphocyte membranes, which may contribute to the establishment of neuronal and immunological memory, respectively.

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