Intimin and the intimate attachment of bacteria to human cells.

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Editorial

Escherichia coli was first implicated as a cause of diarrhea in humans by epidemiological studies conducted more than 40 years ago. These strains, which now comprise the enteropathogenic *E. coli* (EPEC) biotype, primarily infect the small intestine, where they cause a protracted diarrheal illness of infants and children living in developing countries (1). In contrast, the more recently discovered enterohemorrhagic *E. coli* (EHEC) biotype causes both sporadic illnesses and epidemic outbreaks of hemorrhagic colitis, including a recent rash of cases in the northwestern United States (2). While the epidemiology, clinical manifestations, and site of intestinal involvement of EPEC and EHEC infections are strikingly different, the interaction of these organisms with cultured epithelial cells is remarkably alike. When incubated with Hep-2 cell monolayers, both EPEC and EHEC form tightly adherent microcolonies on the cell surfaces, and beneath these colonies, f-actin condensation can be demonstrated (3). Ultrastructural studies of EPEC-infected tissue culture cells and small bowel biopsies from EPEC-infected children show close juxtaposition of the outer membrane of the bacteria and the underlying plasma membrane, localized elevation and invagination of the plasma membrane that partially envelops the bacteria, and effacement of microvillae near the adherent bacterial colony. Identical changes are evident in the colonic epithelium of EHEC-infected newborn piglets (3). These findings indicate that both EPEC and EHEC cause a profound, but localized rearrangement of the cytoskeleton. This combination of histologic features constitutes the "attaching and effacing" phenotype of these strains, and because the attachment is associated with underlying changes of the cytoskeleton, it has been termed "intimate" attachment. Now in this issue of *The Journal,* Donnenberg et al. (3, 4) show in two separate studies that "intimin," a protein expressed by both EPEC and EHEC strains, not only is required for the production of the attaching and effacing phenotype in vitro, but is expressed in vivo as a biologically active molecule.

Intimin is structurally similar to the invasin protein of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica:* they exhibit 51% amino acid sequence similarity; are of approximately the same molecular weight (ranging between 835 and 939 residues); are both deployed on the outer membrane of their respective species where they interact with eucaryotic cell plasma membranes; and both are chromosomally encoded (5). *Y. pseudotuberculosis* and *Y. enterocolitica* are facultative intracellular pathogens that invade epithelial cells, and through this process they may enter the underlying lymphoid elements of the intestine. When the invasin-encoding *inv* gene from *Y. enterocolitica* is cloned into a normally noninvasive *E. coli,* the recombinant strain can adhere to and enter cultured epithelial cells through a process that in the following manner resembles the interaction of EPEC and EHEC with cells: reorganization of the cytoskeleton is evident and this is associated with the accumulation of polymerized actin around attached and entering bacteria (6, 7).

It is useful to consider the biology of intimin in the context of what has been learned about the cellular receptor and functional domains of invasin since its discovery in 1985 (8). Invasin interacts with members of the integrin superfamily of eukaryotic cell adhesion molecules and in particular with integrins that contain the beta1 chain (9). Integrins are membrane-spanning, cell surface heterodimeric molecules that have a large extracellular ligand-binding domain and a small intracellular domain. Present evidence indicates that they are involved in a variety of cell adhesion processes, including the attachment of cells to the extracellular matrix. During the internalization of invasin-bearing bacteria, the organism establishes multiple points of contact between its outer membrane and the plasma membrane of the cell; soon thereafter the bacteria is internalized within a membrane-bound vesicle. During this process, in addition to polymerized actin, the actin-associated proteins filamen, talin, and the beta1 integrin subunit accumulate around the entering bacteria (7). These observations are compatible with a model that specifies a chain of interacting proteins (including talin, vinculin, and alpha actinin) that are thought to couple microfilaments to an integrin cytoplasmic domain. Through their membrane-spanning and ligand-binding properties, integrins may in turn provide a linkage between the extracellular environment and the cytoskeleton. According to this model, the invasin-mediated internalization process is initiated when invasin binds to an extracellular domain of a beta1-integrin, followed by an interaction between the cytoplasmic domain of the integrin and proteins that comprise the cytoskeleton (7).

Structure-function analysis of invasin has localized the integrin receptor binding domain to the COOH-terminal 192 amino acids, a region that lacks the Arg-Gly-Asp motif common to many other extracellular, integrin-binding matrix proteins (10). Comparison of the *Y. pseudotuberculosis* and *Y. enterocolitica* invasin sequences with the EPEC and EHEC intimin sequences shows that the region with the largest number of identical amino acids corresponds to residues 145–392 of the *Y. pseudotuberculosis* invasin sequence, a region that appears to be required for the outer membrane localization of the protein. In contrast, relatively less homology is evident in the ligand-binding, COOH-terminal region, suggesting that intimin may bind a different receptor than invasin (5).

In spite of all that has been learned about the domain structure and cell biology of invasin, little is known about its pathogenic role in animals or humans; other virulence determinants of these two *Yersinia* species have been identified, including *ail* (an attachment invasion locus), which also confers the invasion phenotype. Paradoxically, while considerably less is known about the structure-function relationships and cellular receptor of intimin, the studies reported here by Donnenberg et al. (3, 4) demonstrate unequivocally that $intimin_{EPEC}$ is expressed in vivo and is required for the full virulence of EPEC in experimentally infected humans, and that $intimin_{EHEC}$ is required for the production of the attaching/effacing lesion in EHEC-infected newborn piglets. However, attempts to identify the mechanism by which intimin exerts these effects using in vitro experiments have not led to as clear an explanation as analogous experiments conducted with the invasin proteins. Specifically, a "knock-out" mutation of eae_{EPEC}, the chromo-

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somal locus that codes for intimin$_{EPEC}$, causes loss of the intimate attachment phenotype. However, unlike *inv*, the *eae*$_{EPEC}$ gene does not confer either the invasion or the attaching/effacing phenotype when cloned into a normally noninvasive and nonattaching *E. coli*. Moreover, the *eae*$_{EPEC}$ mutant retains its capacity to effect subtle changes in the organization of the cytoskeleton of the cell to which it is attached possibly through its capacity to induce host cell tyrosine kinase activity (11). These findings suggest that another, quite separate genetic locus encodes an activator of host cell tyrosine kinase and that the attaching/effacing phenotype of EPEC may require two separate gene products, one that codes for intimin and for close attachment per se, and another that is responsible, at least in part, for changes in the cytoskeleton and for the increased levels of intracellular calcium (11). Thus, at this time, it is not clear whether intimin only confers the intimate attachment phenotype and therefore mainly acts as a bacterial adhesin or whether, like the structurally related invasin protein, it also directly effects changes in the cytoskeleton, perhaps in concert with other virulence determinants of the organism. As the authors point out, the occurrence of diarrhea in 4 of the 11 volunteers receiving the *eae*$_{EPEC}$ mutant strain may have been due to the expression of one or more of these other determinants (3).

These studies exemplify an experimental strategy of considerable power: the combined use of molecular genetics to create specific mutations in putative virulence genes and the study of these mutants in experimentally infected human volunteers or in relevant animal models of the disease. For EPEC, it should now be possible to extend these studies to clearly define the role not only of intimin, but of the putative tyrosine kinase activator and of the recently discovered bundle-forming pilus of the organism (12). In addition to their capacity to reveal the pathogenic mechanisms of EPEC-induced diarrhea, studies of this kind could lead to the development of an EPEC vaccine. However, because the porcine model used here for the study of EHEC does not exhibit the hemorrhagic colitis syndrome and because human volunteer studies with EHEC cannot be conducted owing to the severity of the illness, progress in the understanding of this disease may be slower. Here, public health measures to prevent infection with this organism will be the major means of control.

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