member of the immunoglobulin gene superfamily and localizes predominantly to the axon–glial cell junction. Although it was long thought to be essential in the myelination process, mice deficient in MAG myelinate surprisingly well and appear phenotypically normal[9,10]. The peripheral nervous system looks normal in young MAG-deficient mice, but there is evidence of myelin and axonal irregularities, including degeneration, with age[11].

It is unclear whether this axonal loss is due to abnormal interactions with Schwann cells (myelinating cells of the peripheral nervous system) or is secondary to the myelin irregularities that develop in these mice. Nevertheless, there is good evidence to suggest that MAG, through its extracellular domain, has a role in normal axonal function in the peripheral nervous system[12]. Thus, its loss might be expected to contribute to axonal abnormalities. PLP, which is thought to span the lipid bilayer multiple times, is the predominant protein of central nervous system myelin. It is particularly surprising, then, that PLP-null mutants have only subtle myelin defects and appear neurologically normal until relatively old age[13]. The onset of behavioral (motor) abnormalities follows the accumulation of axonal pathology in these mice[14]. It is unclear whether the subtle myelin abnormalities present in the central nervous system of PLP-deficient mice are the direct cause of the axonal abnormalities or whether PLP is supporting axonal function in some other way, perhaps through the mediation of communication between axons and glia[15].

Disconnecting myelin and survival

The current results[1] take the axonal degeneration observed in individuals with CMT1 and multiple sclerosis and in mice lacking MAG and PLP one step further, raising the intriguing and clinically important possibility that a subset of human neurodegenerative conditions might have defects in myelinating cells as their underlying cause, even when there are no apparent myelin abnormalities. Environmental, infectious and genetic factors that primarily affect myelinating cells might be indirectly responsible for a number of other neurodegenerative syndromes, despite the absence of clear myelin abnormalities. The results presented by Lappe-Siefke *et al.*[1] suggest that in our pursuit of the molecular cause of human neurodegenerative disorders, the focus of attention, or at least the scope of inquiry, should include the examination of Schwann cells and oligodendrocytes. Two primary challenges remain: to define the mechanism by which myelinating cells provide axons with sustenance and to determine whether axonal disorders of glial cell origin represent a considerable portion of neurological syndromes. It is clear that myelinating cells are vital for axonal function and survival—not bad for a 'supporting' cell. □

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Intoxicated cells and stomach ulcers

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Helicobacter pylori **induces chronic gastritis in virtually all hosts, yet only a fraction of colonized patients ever develop peptic ulcer disease. A new study shows that binding of the *H. pylori* virulence determinant VacA by a receptor tyrosine phosphatase, Ptprz, modifies the phosphorylation pattern of gastric epithelial cell proteins and leads to cellular detachment. As activation of Ptprz also results in gastric injury and ulceration *in vivo*, these findings help explain why VacA-expressing strains of *H. pylori* augment the risk for peptic ulcer disease**

The human stomach has long been regarded as a biological sanctuary, and although curved bacilli had occasionally been visualized adjacent to the gastric epithelium, their relationship to disease remained obscure until Marshall and Warren's seminal report[1] relating the presence of these organisms, *Helicobacter pylori*, to peptic ulceration. Although *H. pylori*-induced gastritis clearly increases the risk for peptic ulceration, distal gastric adenocarcinoma and gastric mucosal lymphoproliferative disease, only a fraction of colonized persons ever develop clinical sequelae[2]. Pathologic outcomes likely involve choreographed interactions between *H. pylori* virulence determinants and host constituents. On page 375, Akihiro Fujikawa and colleagues[3] describe a novel mechanism through which a specific *H. pylori* disease-related component, VacA, may induce ulcer formation by binding and activating the receptor protein tyrosine phophatase, Ptprz, expressed on gastric epithelial cells. Engagement of Ptprz by VacA leads to cellular detachment, which may heighten the risk for ulcerogenesis by exposing denuded mucosa to noxious gastric contents.

Varieties of VacA

H. pylori populations are extremely genetically diverse, which may engender differential host responses that influence clinical outcome. A specific locus of variability is

vacA, which encodes a vacuolating cytotoxin. The gene *vacA* is present in virtually all *H. pylori* isolates[4,5], but strains differ in vacuolating activity owing to variations in *vacA* gene structure. The regions of greatest diversity are localized near the N terminus of VacA (allele families s1a, s1b, s1c or s2) and the mid-region (allele families m1 or m2; ref. 5). Most s1 VacA toxins possess vacuolating activity *in vitro*, whereas s2 VacA proteins possess little if any cytotoxic activity. Strains containing s1 alleles are more commonly isolated from individuals with ulcer disease than from individuals with gastritis alone[5], underscoring the importance of *vacA* as a bacterial locus related to high-grade host responses within the gastric niche.

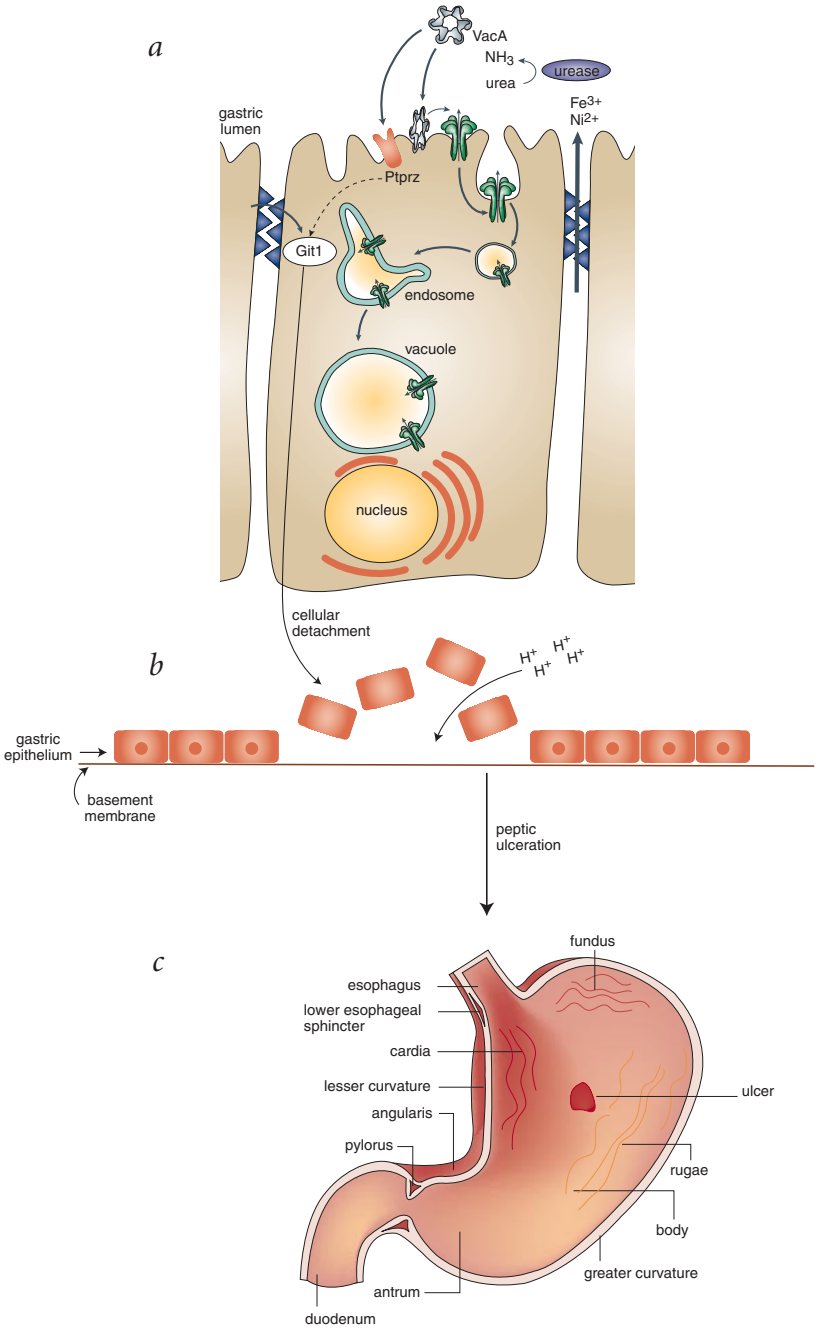
After export, VacA oligomerizes into rosettes that have minimal vacuolating activity[6]. Acid treatment dissociates VacA oligomers into monomers, however, exposing critical hydrophobic regions, which then mediate its insertion into cellular membranes where it forms anion-selective channels (refs. 7,8; see figure). Cytoplasmic internalization of VacA is regulated by active cellular processes[9], and as vacuoles contain membrane markers of late endosomes and lysosomes, vacuole biogenesis seems to occur at the level of late endosomes (see figure).

VacA also exerts other biological effects that may influence clinical outcome. Inoculation of mice with either purified VacA or broth filtrates containing VacA leads to epithelial cell injury[10,11], and *in vitro*, VacA induces gastric epithelial cell apoptosis[12]. VacA functions as a transmembrane pore, permeabilizing host cells to urea[13], which may allow *H. pylori* to manipulate gastric pH by generating ammonia (see figure). When added to polarized monolayers, VacA increases paracellular permeability to organic molecules, iron, and nickel (ref. 14; see figure). Collectively, these data indicate that VacA can induce multiple physiologic consequences that may contribute to pathogenesis.

Targeting a phosphatase

VacA has been reported to bind to a variety of high-affinity cell-surface receptors, including a receptor-type protein tyrosine phosphatase, Ptprz (also known as PTPζ and RPTPβ; ref. 15). Protein tyrosine phosphatases constitute a diverse family of cytoplasmic and transmembrane receptor–like enzymes that regulate cellular proliferation, differentiation and adhesion. Specific ligands for Ptprz have been well characterized, and binding of one of these, pleiotrophin, mediates cellular attachment and migration.

Git1 (G protein–coupled receptor kinase-interactor 1, also called Cat-1, Cool-associated, tyrosine-phosphorylated 1) has recently been identified as a potential substrate of Ptprz, and stimulation of neuroblastoma cells with pleiotrophin paradoxically increases phosphorylation of Git1. As pleiotrophin also increases phosphorylation of β-catenin, Ptprz substrates are probably under constitutive



Gut check. Working model of cellular alterations induced by the *H. pylori* VacA toxin that lead to ulcer formation. ***a***, VacA alters cellular tight junctions and enhances paracellular permeability to iron, nickel and other organic molecules, which may provide essential nutrients required for *H. pylori* growth in the gastric niche. VacA also inserts into the plasma membrane, where it forms an anion-selective channel that can transport urea from the cell cytosol, which, in turn, acts as a substrate for the generation of ammonia by *H. pylori* urease. VacA channels are then endocytosed and incorporated into endosomes, and then they form vacuoles. In a separate pathway, VacA binds to a specific receptor-type protein tyrosine phosphatase, Ptprz, and activates Git1 by an as yet unidentified mechanism. Git1 integrates multiple intracellular signals that regulate membrane trafficking, organelle structure, actin cytoskeletal changes and cellular adhesion. ***b***, Engagement of Ptprz by VacA leads to epithelial cell detachment from the underlying basement membrane, thereby rendering the lamina propria vulnerable to the damaging effects of acid in the stomach. ***c***, Prolonged exposure of denuded and inflamed gastric mucosa to acid ultimately leads to peptic ulceration.

negative regulation, and specific ligands may reduce the catalytic activity of Ptprz. Git1 regulates ADP-ribosylation factor GTPases that mediate membrane trafficking, actin cytoskeletal changes and the organization of focal adhesion complexes. As Ptprz regulates cellular phenotypes (for example, adhesion) that may contribute to mucosal damage and may represent a receptor for VacA, Fujikawa *et al.*[3] investigated the role of VacA–Ptprz interactions in gastric injury using complementary *in vivo* and *ex vivo* genetic models of Ptprz deficiency.

Having previously shown that Ptprz is primarily expressed in the brain, the authors first sought to establish its presence in gastric tissue. Virtually all glands in the gastric corpus of wild-type mice contained detectable Ptprz and expression was localized to the glandular basal region, whereas no staining was present in gastric tissue harvested from Ptprz-deficient mice. The ability of VacA to bind to gastric tissue was then determined by passing gastric mucosal homogenates across a VacA-laden chip. The binding capacity of extracts from Ptprz-deficient mice was 30% less than that from wild-type mice, and these differences resolved in the presence of antagonistic Ptprz antibodies. The ability of VacA to associate with Ptprz was also shown by immunoprecipitation experiments in which Ptprz-containing beads were co-incubated with purified VacA. Resolution with antibodies against Ptprz and against VacA revealed immunoreactive bands, indicating that VacA binds to Ptprz *in vitro* and *in vivo* but that Ptprz only represents a fraction of the total VacA-binding sites in gastric epithelium.

How to cause an ulcer

Fueled by these results and previous data showing the ability of VacA *per se* to induce mucosal injury[10,11], Fujikawa *et al.*[3] next delivered purified VacA by gavage to wild-type and Ptprz-deficient mice. VacA was detected in the cytoplasm of a variety of epithelial cells from both genotypes and there were no differences in VacA distribution, supporting the conclusion that multiple VacA receptors are present in gastric tissue. In contrast, VacA induced a dose-dependent increase in gastric injury only in wild-type mice, with most developing severe gastric hemorrhage and ulcers when challenged with the highest dose of VacA (500 μg per kg of body weight).

To investigate the role of Ptprz in VacA-induced injury at a molecular level, primary gastric epithelial cells from wild-type and Ptprz-deficient mice were co-incubated with VacA *in vitro*. VacA was incorporated equally into wild-type and Ptprz-deficient cells, which mirrored the *in vivo* pattern of VacA distribution. When primary cells grown on a reconstituted basement membrane were exposed to VacA, however, only wild-type cells detached. The functional consequences of VacA–Ptprz binding were further explored by transfecting BHK-21 cells that lack Ptprz but contain its substrate Git1 with Ptprz or a phosphatase-inactive Ptprz construct. VacA induced phosphorylation of Git1 in cells transfected with functional Ptprz but not in cells transfected with defective Ptprz or with vector, recapitulating events that occur after treatment with pleiotrophin.

Fujikawa *et al.*[3] then returned to their *in vivo* model and treated mice with oral doses of pleiotrophin. Wild-type, but not Ptprz-deficient, mice developed severe gastritis and ulcers after treatment with pleiotrophin, and there was no evidence of vacuolation. The authors then extended these findings by showing that Ptprz is expressed in human gastric tissue in a pattern similar to that observed in mice, which strengthens the biological relevance of this model to *H. pylori*-induced ulcerogenesis in humans.

Models and mechanisms

The results from this study invoke a model in which VacA binds specifically to Ptprz, leading to cellular detachment through modification of the phosphorylation patterns of cellular proteins, a pathway that is distinct from vacuole biogenesis (see figure). Although these experiments have provided fresh insights regarding determinants that may influence ulcer formation, there are new questions and hypotheses to be explored. As vacuolation and cellular responses induced by the binding of Ptprz seem to be independent events, do non- or minimally-toxigenic VacA molecules bind Ptprz or induce cellular detachment? What specific region(s) of VacA are required for Ptprz binding and activation? Do additional *H. pylori* constituents contribute to ulcer formation?

Despite these questions, mechanistic studies such as this are extremely valuable, not only because of the significance of *H. pylori* as a human pathogen, but also because they may facilitate translation of developments from the laboratory to the clinical setting. For example, understanding the role of specific *H. pylori* virulence determinants in the development of peptic ulceration could contribute to vaccine development. Understanding the mechanisms through which specific bacterial factors interact with host pathways may allow identification of infected individuals at high risk for clinical disease. Finally, understanding the pathogenesis of one well defined cause of microbially induced disease could lead to its use as a model system for other forms of inflammation and injury that develop within the context of bacterial infections. □

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