**Abstract.** The impacts of toxins are generally observed as acute intoxications, whereas the environmental health effects of chronic exposure to mostly low levels of toxins are only poorly documented and are an emerging issue. Furthermore, toxins are potentially useful as tools to study the physiological role of particular channels or to determine structural features of channels that are important for their function. Recent studies suggest that vacuolating cytotoxin (VacA) is a novel ligand for receptor protein tyrosine phosphatase (RPTP) β. VacA is a virulence factor of Helicobacter pylori that is involved in gastric ulceration and cancer in experimental animals and humans. VacA may represent the chronic exposure of A-B family of bacterial endotoxins and be a useful probe for RPTPβ functions in the gastrointestinal system.

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**1. Introduction**

*Helicobacter pylori* is a spiral-shaped Gram-negative bacterium endowed with a very powerful urease activity and with polar flagella (1). These features allow the bacterium to survive at acidic pH in the stomach lumen. *H. pylori* is probably most common chronic bacterium infection of humans, present in almost half of the world population. Once acquired, these organisms persist for years, for decades, and possibly for life. The presence of the bacterium in the gastric mucosa is associated with chronic active gastritis and is implicated in more severe gastric diseases, including peptic ulceration, mucosa-associated lymphoid tissue lymphoma, and gastric carcinoma (1,2).

**2. Vacuolating cytotoxin (VacA)**

Many *H. pylori* strains secrete a cytotoxin that collective evidence indicates is an important virulence factor in *H. pylori*-mediated disease (3,4). The toxin was named vacuolating cytotoxin (VacA) because it induces vacuolation of cultured mammalian cells. The VacA gene has a 3864- or 4200-base pair open reading frame, encoding a precursor protein for the mature 87- or 95-kDa VacA. Oral administration of purified VacA to mice induces gastric mucosa degeneration and inflammatory cell recruitment, characteristic of *H. pylori*-mediated diseases (5).

VacA exhibits cellular behavior similar to that of protein toxin with intracellular targets. Most intracellularly acting toxins possess an overall structure corresponding to the A-B family of bacterial endotoxins, which includes diphtheria, cholera, and anthrax toxins (4,6). In general, the B component of an A-B toxin binds to a specific mammalian cell-surface receptor and facilitates the membrane translocation of an enzyme A moiety into the cytosol. The A moiety covalently modifies a specific intracellular target molecule, resulting in the disruption of important cellular functions. VacA has recently been indicated to be an A-B toxin and to bind to specific, high affinity cell surface receptors (7,8).
The formation of the vacuoles is accompanied by a redistribution of lysosomal membrane glycoproteins among endocytic compartments. The membranes of the vacuoles contain the small GTP-binding protein Rab7 (a late endosomal marker) and the membrane glycoprotein Lgp 110 (a lysosomal marker), suggesting that VacA may disrupt normal membrane trafficking at or near the level of late endosomes (9). The function of Rab7 is required for vacuole formation since HeLa cells overexpressing dominant negative mutants of Rab7 do not develop vacuoles upon exposure to VacA (10). Dynamin is a large-molecular weight GTP-binding protein which is required for intracellular vesicle transportation (11-13). A recent study demonstrated that VacA failed to induce vacuolation in HeLa cells transfected with dominant-negative dynamin and that stable transfection of wild-type dynamin augmented VacA-induced vacuolation, indicating a crucial role of dynamin in VacA-induced vacuolation (14). A protein (VIP54) co-localizing with the intermediate filament protein vimentin was recently found to be a possible cytosolic target of VacA (15). The association of intermediate filament proteins with the nuclear membrane and with the plasmalemma is well documented although the role of VacA-VIP54-vimentin in vacuole biogenesis needs to be clarified (15). Vacuole formation is also dependent on the function of a vacuolar-type ATPase protein pump in transfected cells as well as in response to externally added VacA (3,9). Late endosomes may represent an important crossroad involved in the transport of endocytosed ligands and newly synthesized proteins that are destined for lysosomes (9). Any perturbation of cellular function at this level may have important functional consequences. The demonstration that VacA inhibits intracellular degradation of epidermal growth factor (EGF) and inhibits normal maturation of procathespisin D may illustrate two important functional alterations (4,16).

VacA applied extracellularly apparently targets mitochondria (17). Such a translocation could be accomplished in an endosomal compartment, by a mechanism similar to those described for other A-B toxins (18). VacA released by H. pylori is often cleaved into two subunits of 34 kDa (the N-terminal fragment, p34) and 58 kDa (the C-terminal fragment, p58), which remain associated by non-covalent interactions (3,19). The toxin is able to bind a cell receptor through a segment of the p58 domain (7). Upon binding, VacA could either be endocytosed and accumulated in late endosomes where vacuoles originate, or translocated into the cytosol where it induces cytochrome c release and apoptosis (17). VacA p34 is targeted selectively to mitochondria where it induces cytochrome c release and apoptosis (17). Several other bacterial toxins have been shown to induce mitochondrial dysfunction, including clostridium difficile toxin A (20), Escherichia coli lipopolysaccharide (21), and cereulide, an emetic toxin isolated from Bacillus cereus (22). It was also found that the minimal intracellular vacuolating domain of VacA encompasses p34 and <200 residues belonging to the C-terminal moiety of p58 (6).

3. VacA interacts with full-length receptor protein tyrosine phosphatase ß

It was recently reported that VacA is able to interact with target cells by binding to a 250-kDa receptor protein tyrosine phosphatase (RPTP)ß (23,24). VacA is known to cause vacuolation in many cell types, with the notable exception of HeLa-60. Even in this cell, VacA sensitivity clearly paralleled the induction of RPTPß mRNA expression by...
phorbol 12-myristate 13-acetate (PMA) (24). Transfection of RPTPβ gene into the hamster kidney cells that are also insensitive to VacA, resulted in an induction of VacA sensitivity (24). RPTPβ is a receptor-like protein tyrosine phosphatase composed on an extracellular domain, a single transmembrane domain and a cytoplasmic portion that contains two tyrosine phosphatase domains (25,26) (Fig. 1). Three different isoforms of RPTPβ are expressed as a result of alternative splicing: a short and a long form (transmembrane) that differ by the presence of the spacer region of the extracellular domain and a secreted form lacking phosphatase activity. VacA was demonstrated to bind to the full-length 250-kDa isoform (23). Many agents induce cellular responses by regulating the tyrosine phosphorylation of target proteins including the EGF receptor (25,26). It is known that EGF-activated signal transduction pathways are essential for cell proliferation and ulcer healing, and vacuolation induced by VacA is inhibited by an antibody against EGF receptor (8).

4. Novel ligand for RPTPβ

The extracellular region of RPTPβ has a complex structure, with multiple domains that may interact with a variety of ligands. Such ligands include the extracellular matrix protein tenascin, pleiotrophin, and several neuronal cell adhesion molecules (CAMs) from the immunoglobulin superfAMILY, which are important for the establishment of intricate networks of connections in the nervous system (25,26). Recently pleiotrophin was demonstrated to be the first natural ligand for transmembrane forms of RPTPβ as well as for any of the RPTP family (27). Pleiotrophin is a platelet-derived growth factor-inducible, 18-kDa heparin binding cytokine that signals diverse phenotypes in normal and dysregulated cellular growth and differentiation. Pleiotrophin may signal through ligand-dependent receptor inactivation of RPTPβ to increase levels of tyrosine phosphorylation of β-catenin to initiate downstream signaling. It may be important in cell adhesion, tumor invasiveness, and metastasis (27).

RPTPβ isoforms are found in the developing nervous system primarily on radial glia and astrocytes, in patterns suggestive of their involvement in neuronal migration and axonal guidance (25). The isoforms, however, differ in developmental pattern of expression and abundance in different brain regions. It appears that the short form present on the surface of glial cells promotes neurite outgrowth by interacting with certain CAMs, while the long or the secreted form can have either inhibitory or repulsive effects (25,28). The transmembrane forms of RPTPβ can be pharmacologically discriminated from each other by their different sensitivities to VacA. Mapping the receptor binding as well as other functional domains will be important for understanding the mechanism of VacA cytotoxicity, and also useful as a biochemical probe for the long receptor form of RPTPβ.

5. Toxins as tools for channel and receptor functions

Neurons of the mammalian brain express multiple subtypes of sodium channels that are the products of at least six distinct genes (29-33). These channels differ in subcellular localization, developmental pattern of expression, and abundance in different brain regions. These variations are suggestive of functional differences, yet there is no direct information about different physiological roles of particular sodium channel subtypes. Several scorpion toxins have been shown to exert their neurotoxic effects by a direct interaction with voltage-dependent sodium channels (30,31). Recent studies suggest that multiple sodium channel subtypes in mammalian brain can be pharmacologically discriminated by their sensitivity to certain toxins, such as scorpion α-toxins and α-like toxins (32,34). Sodium channel specific scorpion toxins are peptides of 60-76 amino acid residues in length, tightly bound by four disulfide bridge (35). The complete amino acid sequence of 85 distinct peptides are presently known (35). Interestingly, sodium channels have been demonstrated to interact with RPTPβ, which increases sodium currents (36).

Calcium channels play key roles in numerous physiological processes including neurotransmitter release, hormone secretion, neurite outgrowth, and gene expression (37,38). Neurons express several distinct types of voltage-gated calcium channels, which fall into two main categories: high voltage-activated and low voltage-activated channels. High voltage-activated channels are classified as L-, N-, P/Q-, and R-types, based primarily on pharmacological criteria (39,40). The N-type channels are uniquely sensitive to spider toxin ω-conotoxin GVIA and M VIIA, and P-type channels to low concentrations of ω-agatoxin-IVA (37,38,41). Another spider toxin ω-agatoxin IIIA may define a unique, high affinity binding sites on N-, P/Q-, and R-type calcium channels (41).

Potassium channels represent a large and diverse collection of membrane proteins whose common property is that they allow the permeation of potassium with high selectivity (42). Potassium channels participate in a large number of important cellular functions such as control of cell electrical excitability and excitation/response coupling. Various inhibitors of potassium channels were obtained from a variety of sources (42-45). Peptides from scorpions were found to block either of two major classes of K+ channels, voltage-gated (Kv-type) and high conductance Ca2+ -activated (BK-type) K+ channels (43). Other examples include the muscarinic and nicotinic acetylcholine receptors. Muscarinic acetylcholine receptors exist as five subtypes that are widely distributed throughout the body. They are involved in the regulation of fundamental physiological processes, like the modulation of the heart rate, control of motor system and modulation of learning and memory (46). Conventional pharmacological agents were not highly selective for particular subtypes, making investigations on the functional significance of the subtypes difficult (46,47). The venom of the green mamba was found to contain the most specific antagonists known for M1 and M4 receptors (m1-toxin and m4-toxin). The m2-toxin has recently been isolated which binds with high selectivity to M2 receptors (48). A number of mamba venoms have also curaremimetic α-neurotoxins (homologous to the well-known α-bungarotoxin) that bind tightly to nicotinic acetylcholine receptors (47). Nicotinic acetylcholine receptors appear to be important for a number of neurophysiological processes including cognition, learning and memory. α-conotoxins are family of Cys-enriched peptides found in several marine snails (49). They behave pharmacologically as competitive antagonists of several
nicotinic acetylcholine receptors. The anatoxin-A, an algae
toxin, has also been reported to interact in acute subtoxic and
naturally occurring concentrations with human
4ß2-nicotinic acetylcholine receptors (50). These are only some examples
that have been found to be informative molecular probes. It
is expected that many more such toxins, with unexpected
specifications, will be described in the coming years, since
many scorpion and other venoms remain investigated.

6. Concluding remarks

It is understood that the pathogenetic bacterium \textit{H. pylori} is the major risk factor for gastroduodenal diseases including
cancer. Infection has also been implicated in the aetiology of
ischemic heart disease and hypertension, as well as chronic
urticaria, rosacea, autoimmune thrombocytopenic purpura and
diminished growth in children (51). How chronic exposure of
VacA and other virulent factors leads to such health problem
is a promising field of investigation.

Cytotoxin-associated gene A (CagA) is another virulent
factor, which is translocated from the bacteria into gastric
epithelial cells. A recent study indicates that CagA can perturb
mammalian signal transduction machineries and modify
cellular functions by physically interacting with a host cell
protein SHP-2, SRC homology 2 domain-containing tyrosine
phosphatase (52). Dysregulation of SHP-2 by CagA may
induce abnormal proliferation and movement of gastric
epithelial cells, promoting the acquisition of a transformed
phenotype (52).

Genetically well defined and manipulatable animal models
are needed to dissect the molecular mechanisms that underlie
a postulated sequence of \textit{H. pylori}-associated changes in
infected hosts (3.53). Transgenic mice that lack parietal
cells by the use of A-fragment of diphtheria toxin has been
successfully used to examine the outcome of the colonization,
since a determinant of the outcome is the ability of the
bacterium to attach to the gastric epithelium by expressing a
variety of adhesins that recognize a number of epithelial
receptors (3.54). Recently the mutation was introduced in the
RPTPß gene, which abolished expression of all the three
isofoms of RPTPß (55). The RPTPß-deficient mice appeared
to be normal in their gross general behavior and with respect
to fertility, body weight, and life span. Such mutant mice
should help to determine not only how the cytotoxins are
important for \textit{H. pylori} pathogenesis in the stomach and
potentially in other sites, but also how tyrosine phosphatases
are important for the control of gastrointestinal functions.

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