A Dominant Negative Mutant of *Helicobacter pylori* Vacuolating Toxin (VacA) Inhibits VacA-induced Cell Vacuolation*

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Most *Helicobacter pylori* strains secrete a toxin (VacA) that causes structural and functional alterations in epithelial cells and is thought to play an important role in the pathogenesis of *H. pylori*-associated gastroduodenal diseases. The amino acid sequence, ultrastructural morphology, and cellular effects of VacA are unrelated to those of any other known bacterial protein toxin, and the VacA mechanism of action remains poorly understood. To analyze the functional role of a unique strongly hydrophobic region near the VacA amino terminus, we constructed an *H. pylori* strain that produced a mutant VacA protein (VacA-(Δ6–27)) in which this hydrophobic segment was deleted. VacA-(Δ6–27) was secreted by *H. pylori*, oligomerized properly, and formed two-dimensional lipid-bound crystals with structural features that were indistinguishable from those of wild-type VacA. However, VacA-(Δ6–27) formed ion-conductive channels in planar lipid bilayers significantly more slowly than did wild-type VacA, and the mutant channels were less anion-selective. Mixtures of wild-type VacA and VacA-(Δ6–27) formed membrane channels with properties intermediate between those formed by either isolated species. VacA-(Δ6–27) did not exhibit any detectable defects in binding or uptake by HeLa cells, but this mutant toxin failed to induce cell vacuolation. Moreover, when an equimolar mixture of purified VacA-(Δ6–27) and purified wild-type VacA was added simultaneously to HeLa cells, the mutant toxin exhibited a dominant negative effect, completely inhibiting the vacuolating activity of wild-type VacA. A dominant negative effect also was observed when HeLa cells were co-transfected with plasmids encoding wild-type and mutant toxins. We propose a model in which the dominant negative effects of VacA-(Δ6–27) result from protein-protein interactions between the mutant and wild-type VacA proteins, thereby resulting in the formation of mixed oligomers with defective functional activity.

*Helicobacter pylori* are Gram-negative bacteria that persistently colonize the gastric mucosa of humans (1). Colonization of the gastric mucosa by these bacteria results in mucosal inflammation and is a risk factor for the development of peptic ulcer disease, distal gastric adenocarcinoma, and gastric lymphoma (1–4). Gastric adenocarcinoma is currently one of the most common causes of cancer deaths worldwide and is the only cancer that has been directly linked to a bacterial infection (3).

Most *H. pylori* strains secrete a toxin (VacA) that is unrelated to any other known bacterial protein toxin (5, 6). When VacA is incubated with epithelial cells in vitro, the most prominent effect is the formation of large cytoplasmic vacuoles (5). These vacuoles contain markers for both late endosomes and lysosomes and have an acidic intravacuolar pH (7–9). VacA-induced vacuoles are thought to represent novel intracellular compartments that form as a result of heterotypic fusion events (7–9). In addition to altering the morphology of cells, VacA causes multiple functional changes, including alterations in the intracellular trafficking and processing of procathepsin D and epidermal growth factor (10). When added to polarized epithelial cell monolayers, VacA induces an increase in monolayer permeability for various ions and small uncharged molecules (11). VacA also interferes with the process of antigen presentation, which may be one mechanism by which *H. pylori* resists immune clearance (12).

The *H. pylori* vacA gene is translated as a 140-kDa protoxin, which undergoes amino- and carboxyl-terminal processing to yield a mature secreted toxin of about 87 kDa (13–16). Secretion of VacA probably occurs via a mechanism analogous to that used for secretion of *Neisseria gonorrhoeae* IgA protease (14–15). Mature 87-kDa VacA monomers assemble into complex water-soluble oligomers typically comprised of 12 or 14 subunits (17–18). Upon exposure to acidic pH, these oligomers disassemble into monomeric components (17). Acidification of VacA enhances its cytotoxic activity and permits the toxin to insert into lipid membranes to form anion-conductive channels (19–23).

The mechanisms by which VacA causes alterations in cellular morphology and function are not yet well understood. Transfection of HeLa cells with plasmids expressing VacA results in cell vacuolation, which suggests that VacA has an intracellular site of action (24–27). Nearly all bacterial toxins that act intracellularly have an enzymatic activity, but thus far, no enzymatic activity of VacA has been identified. The formation of membrane channels by VacA also may contribute to cytotoxic effects, perhaps analogous to the mechanism by which aerolysin causes vacuolation of cells (28). Structure-function analysis of VacA may be helpful in deci-
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Table I

| H. pylori strain | Site of sacB/kan insertion in vacA |
|-----------------|-----------------------------------|
| VM064           | BglI; 116                         |
| VM022           | BsmFI/EcoNI; 435–890             |
| VM025           | EcoNI/BstXI; 980–1617             |
| VM027           | BstXI/XcmI; 1617–1901             |
| VM028           | XcmI/BglII; 1901–2154             |
| VM002           | BglII/NotI; 2154–2551             |

*With the exception of strain VM064, sacB/kan cassettes replaced the vacA sequences originally present between the indicated restriction sites. Numbers designate nucleotides within the vacA open reading frame.

Characterization of H. pylori vacA Mutants—Mutant H. pylori strains were grown in sulfite-free Brucella broth containing 0.5% charcoal, and proteins in the culture supernatants were concentrated by precipitation with a 50% saturated solution of ammonium sulfate (13). To determine whether mutant strains expressed and secreted VacA, both whole bacterial cells and concentrated broth culture supernatant proteins were immunoblotted with anti-VacA serum (13). Preparations of supernatant proteins also were tested in an antigen-detection ELISA1 (13, 31), which permitted concentrated broth culture supernatant proteins from each mutant strain to be standardized according to VacA concentration. These standardized supernatant protein preparations were tested for vacuolating activity in a HeLa cell assay, and cell vacuolation was assessed by light microscopy (13).

VacA Purification and Quantitation of Vacuolating Activity—VacA was purified from broth culture supernatants as described previously (17). Protein concentrations were determined using a Micro-BCA assay (Pierce). Unless otherwise stated, purified VacA preparations were routinely acid-activated before addition to HeLa cells. Acid activation was accomplished by dropwise addition of 250 mM HCl until a pH of 3 was reached (21). Purified, acid-activated VacA preparations were standardized by protein concentration and added to HeLa cells in minimal essential medium containing 10% fetal bovine serum and 10 mM ammonium chloride at 37 °C for 16 h. The vacuolating activity of purified VacA preparations was quantified using a neutral red uptake assay (32). Neutral red uptake data are presented as OD540 values (mean ± S.D.). Statistical significance was analyzed using Student’s t test.

Atomic Force Microscopy—Purified VacA was added to supported lipid bilayers composed of a total lipid extract from bovine heart (Avanti Polar Lipids, Alabaster, AL), as described previously (19). The protein was injected into a buffer of 1 mM citric acid, pH 2.6, covering the

The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; DEPC, diethyl pyrocarbonate; GFP, green fluorescent protein.
supported bilayer. After incubating for 1 h, the sample was extensively washed and the pH was changed to ~7 to induce crystallization (19). The sample was briefly fixed with 2% glutaraldehyde, prior to imaging by atomic force microscopy (19). Imaging was performed in the contact mode with a NanoScope II AFM (Digital Instruments, Santa Barbara, CA) using “twin tip” Si3N4 cantilevers. The typical scan rate was 7 Hz, and the applied force was minimized to 0.1 nN (19).

Electrophysiologic Analysis of VacA Channel-forming Activity—The planar lipid bilayers, composed of egg phosphatidylcholine/dioleoylphosphatidylserine/cholesterol (55:15:30 mol %) dissolved in n-decane, were prepared, and the membrane currents were measured as described previously (19, 20). The buffer in each experiment was buffer A (5 mM citric acid, pH 4.0, 2 mM EDTA), with the salt composition as described in the figure legends or tables. The potential is indicated relative to the cis-side, defined as the chamber to which the protein was added. Permeability ratios were determined from the Goldman-Hodgkin-Katz equation (33), after measuring the membrane voltage for zero current (reversal potential) in asymmetric salt concentrations. Statistical significance was analyzed using Student’s t test.

Analysis of VacA Binding and Uptake by HeLa Cells—Purified VacA was iodinated using the IODO-GEN method (Pierce). IODO-GEN (2 μg) in chloroform was plated onto the wall of a microcentrifuge tube, and the chloroform was evaporated under a stream of N2. To the IODO-GEN-containing tube, 1 μCi of [125I]iodide in 50 mM sodium phosphate buffer, pH 7.2, and 50 μg of purified VacA were added in a final volume of 100 μl and incubated for 10 min at 25 °C. The liquid phase of the reaction was then removed, added to 10 mM non-radioactive iodide, and the free [125I] was removed by gel filtration on a 10-ml G-25 Sephadex column equilibrated with 10 mM EDTA and 25 μl per ml bovine serum albumin. This procedure resulted in effective radioiodination of VacA without a detectable loss of vacuolating activity.

HeLa cells were grown to confluency on 35-mm dishes. Acid-activated [125I-VacA (500 ng/dish)] was added to the cells for 3 h at 4 °C in Heps-buffered saline (50 mM Hepes and 100 mM NaCl, pH 7.2) containing 1 mM CaCl2, 1 mM MgSO4, and 100 μg/ml bovine serum albumin. Cells were then washed three times to remove unbound VacA and were incubated for 4 h at 37 °C in Eagle’s medium containing 10% fetal bovine serum and 10 mM ammonium chloride. In selected experiments, cells were treated with proteinase K (250 μg/ml for 30 min at 4 °C) to remove or digest surface-bound [125I]-VacA, and pelleted cells then were immediately lysed by boiling in SDS-polyacrylamide gel electrophoresis sample buffer. Proteins in cell lysates were separated by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

Inactivation of VacA by Treatment with DEPC—Diethyl pyrocarbonate (DEPC, Sigma) and purified VacA were mixed at pH 8.0 in a ratio of 100 DEPC molecules per VacA histidine residue (34). After incubation on ice for 1 h, the chemically modified VacA sample was mixed with an equal volume of minimal essential medium containing 10% fetal bovine serum. DEPC-treated samples were tested in HeLa cell assays within 1 h of preparation.

Transfection of HeLa Cells—HeLa cells were plated (200 μl) at a density of 5.0 × 104 cells per ml in 96-well tissue culture plates (Corning; Cambridge, MA) in Dulbecco’s modified Eagle’s medium supplemented with 2.5% fetal calf serum and 100 units penicillin/ml and 100 mg of streptomycin/ml. HeLa cells were first infected with recombinant vaccinia virus (vT7) bearing the T7 RNA polymerase gene (26). Vaccinia virus stock was titered at 37 °C for 30 min and added to HeLa cells (26). After infection for 30 min, virus stock was removed, and the HeLa cells were transfected using the calcium phosphate method (26). Plasmids used for transfection included pET-20b containing an insert encoding residues 1–953 of VacA fused to GFP (26), pET-20b expressing the same VacA-GFP protein but with a 22-amino-acid deletion (36–27), or pET-20b encoding GFP only. Co-transfections were done by transfecting cells with a mixture of two different plasmid preparations in a 1:1 ratio. Mock-transfected cells were infected with vT7 and treated with transfection reagent only. Following the transfection procedure, the cells were incubated in Dulbecco’s modified Eagle’s medium plus 5 mM ammonium chloride at 37 °C for 20 h prior to analysis.

RESULTS

Expression of Mutant VacA Proteins—In an effort to construct an H. pylori VacA mutant protein that had altered functional properties but no gross alterations in structure, we introduced 11 different in-frame deletion mutations into the chromosomal vacA gene of H. pylori 60190, as described under “Materials and Methods.” Each mutant strain was tested by immunoblot analysis (13) for the capacity to express and secrete VacA. Seven of these mutant strains expressed and secreted truncated vacA products of the expected size, but no vacA products were detected in either bacterial cells or supernatants from four mutant strains (Fig. 2). Concentrated culture supernatants from the seven VacA-expressing mutant strains were adjusted to a uniform VacA concentration based on the results of a VacA antigen-detection ELISA (13, 31), and these preparations then were tested for activity in a HeLa cell assay.

Culture supernatant from H. pylori AV320 (containing VacA-(Δ517–536)) induced cell vacuolation, whereas each of the other mutant VacA proteins lacked detectable vacuolating activity. To determine whether these mutant VacA proteins could form water-soluble oligomeric structures, culture supernatants from each mutant strain were fractionated by gel filtration chromatography, and high molecular mass fractions were immunoblotted with anti-VacA serum. VacA-(Δ6–27) and VacA-(Δ517–536) were detected in the same high molecular mass (900 kDa) fractions as in wild-type VacA is typically found (13, 17), which indicated that these mutant proteins could form water-soluble oligomeric structures in a manner similar to wild-type VacA. In contrast, no high molecular mass oligomeric forms of the remaining 5 mutant VacA proteins were detected. Thus, only one mutant VacA protein (VacA-(Δ6–27)) was identified which seemed to be structurally intact, yet lacked vacuolating cytotoxic activity. This mutant VacA protein was selected for further detailed characterization.
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Structural Characterization and Lipid Interactions of VacA-(Δ6–27)—The 22-amino acid deletion in VacA-(Δ6–27) is located within a unique region of strong predicted hydrophobicity near the amino terminus of VacA (Fig. 3). To examine structural properties of this mutant VacA protein, purified VacA-(Δ6–27) was incubated with supported lipid bilayers, and the bilayers then were imaged by atomic force microscopy (19, 35, 36). At pH values below 5, a high density of oligomeric mutant VacA associated with anionic lipid membranes (data not shown), in a manner similar to that observed for wild-type toxin (19). Adsorption of VacA-(Δ6–27) to the membrane at pH <5, followed by raising the pH to 7, resulted in the formation of two-dimensional crystal patches that could be imaged to a high degree of resolution (Fig. 4). All features of these crystals, including the lattice parameters, the inner diameter of the central rings, and the height by which the oligomers protrude from the bilayer, were identical to those described previously for wild-type VacA (19). These results indicate that the amino-terminal hydrophobic region of VacA is not required for oligomerization, association with lipids, or two-dimensional crystal formation within a unique region of strong predicted hydrophobicity near the amino terminus of VacA (19).

Electrophysiologic Properties of Channels Formed by Mixtures of VacA-(Δ6–27) and Wild-type VacA—Acidification of wild-type VacA results in the disassembly of VacA oligomers into monomeric components (17, 22) and reassembly of monomers into oligomers can occur when VacA-containing solutions are shifted from acid to neutral pH (17). Therefore, we hypothesized that wild-type and mutant VacA monomers might assemble into hetero-oligomeric channels under the conditions of the planar lipid bilayer assay. To test this hypothesis, the two VacA species (each 30 nM) were mixed together at neutral pH, and the mixture then was acidified to pH 3 and maintained at this pH for 1 h before being added to planar lipid bilayers. The time required for the mixture to produce a current of 100 pA was significantly longer than that observed for wild-type VacA alone, regardless whether at 60 or 30 nM concentrations (p < 0.01), but was much shorter than that detected for 60 nM VacA-(Δ6–27) alone (p = 0.005). (Fig. 5). This latter observation indicates that within the period required for the mixture to generate 100 pA, few, if any, homo-oligomeric VacA-(Δ6–27) channels could form in the bilayer. Therefore, the macroscopic current produced by the mixture could arise via two possible mechanisms: (i) formation of hetero-oligomeric channels, or (ii) formation of primarily homo-oligomeric channels of wild-type VacA, with a delay caused by blockage of binding sites in the bilayer by the VacA-(Δ6–27) proteins. To discriminate between these alternatives, we determined the ion selectivity by measuring the reverse potential in asymmetric salt solutions. The channels formed by this mixture of VacA proteins exhibited a permeability ratio markedly different from that measured for homo-oligomeric channels of wild-type VacA (p = 0.01) (Table II). Taken together, these data suggest that the mixture of wild-type and mutant VacA proteins forms hetero-oligomeric channels.

**Interactions of VacA-(Δ6–27) with HeLa Cells**—To compare the cell-vacuolating activities of wild-type VacA and VacA-(Δ6–27), purified acid-activated proteins of each type were incubated with HeLa cells. Purified wild-type VacA caused the formation of large intracellular vacuoles, whereas purified VacA-(Δ6–27) lacked any detectable vacuolating activity for HeLa cells (Fig. 6). One possible explanation for the failure of

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**Fig. 3.** Hydrophilicity plot of the mature secreted VacA toxin from *H. pylori* 60190, generated by Kyte-Doolittle analysis. The amino-terminal sequence of mature, secreted VacA is shown in capital letters. The underlined amino acids are located within a strongly hydrophobic region and were deleted in VacA-(Δ6–27).

**Fig. 4.** Structural analysis of VacA-(Δ6–27). Purified VacA-(Δ6–27) was added to supported lipid bilayers composed of total lipid extract from bovine heart and incubated for 1 h at pH 2.6. After washing the membrane, the pH was raised to 7 to induce crystallization, and the crystals were analyzed by atomic force microscopy. The two-dimensional crystals produced by VacA-(Δ6–27) were indistinguishable from those of wild-type VacA (19). Scale bar, 50 nm.

**Fig. 5.** Kinetics of channel formation by wild-type VacA and VacA-(Δ6–27). Mutant or wild-type VacA preparations (30 or 60 nM concentrations, as indicated) were added to planar lipid bilayers composed of egg phosphatidylcholine/dioleoylphosphatidylserine/cholesterol (55:15:30 mol %) in buffer A with 100 mM NaCl. The time required to produce a current of 100 pA at −50 mV was then determined. In addition, results are shown for a 1:1 mixture of wild-type VacA and VacA-(Δ6–27) (each 30 nM). Results represent the mean ± S.D. from at least three independent determinations for each sample tested.
Purified acid-activated wild-type VacA (Δ6–27) to induce cell vacuolation might be that HeLa cells fail to bind or internalize this mutant toxin. To test this hypothesis, we examined and compared interactions of purified radiolabeled wild-type VacA and VacA-(Δ6–27) with HeLa cells. Both forms of VacA bound to cells at 4 °C, and the surface-exposed ~87-kDa VacA proteins bound at this temperature were susceptible to digestion with protease K (Fig. 7, lanes a and b). After incubation of VacA proteins with cells at 37 °C for 4 h, both wild-type and mutant forms of the ~87-kDa toxin became resistant to protease K digestion (Fig. 7, lanes c–f). This inaccessibility to protease digestion provides strong evidence that both the wild-type and mutant forms of VacA are internalized by HeLa cells (27, 37). In the presence of a 100-fold excess of unlabeled acid-activated wild-type VacA during the 4 °C binding step inhibited the cellular uptake of radiolabeled wild-type and mutant VacA-(Δ6–27) wild-type VacA and wild-type VacA-(Δ6–27) (1:1) were incubated with HeLa cells for 16 h at 37 °C. Vacuolating activity was quantified using a neutral red uptake assay, whereas intracellular expression of VacA-(Δ6–27) was detected (data not shown). Thus, VacA-(Δ6–27) exerted a dominant negative effect, whereas DEPC-treated VacA lacked this property.

Acid-activation of wild-type VacA results in markedly enhanced vacuolating activity (17, 21). Therefore, we compared the capacity of acid-activated and untreated VacA-(Δ6–27) to inhibit wild-type VacA activity. Acid-activated VacA-(Δ6–27) completely inhibited the activity of the wild-type toxin, whereas non-acid-activated VacA-(Δ6–27) had minimal inhibitory effects (Fig. 9). This suggests that the mutant toxin must undergo an acid-induced structural change before it can exert its dominant negative effect.

Intracellular Expression of VacA-(Δ6–27)—As shown in Fig. 7, VacA-(Δ6–27) did not exhibit any obvious defects in binding or entry into HeLa cells, which suggests that this mutant toxin is defective in intracellular activity. To test this hypothesis, HeLa cells that previously had been infected with vT7 were transfected with either pET20b harboring a gene encoding wild-type VacA fused to GFP (26) or pET20b harboring a gene encoding VacA-(Δ6–27)-GFP. Transfected cells were analyzed after 18 h for both vacuolating activity and GFP fluorescence. Fluorescence microscopy revealed that the wild-type and mutant proteins each were expressed within target cells. Intracellular expression of wild-type VacA resulted in cell vacuolation (detected by both light microscopy and neutral red uptake assay), whereas intracellular expression of VacA-(Δ6–27) produced no detectable morphologic changes (Fig. 10, p < 0.001).

We next tested the possibility that VacA-(Δ6–27) could inhibit the function of wild-type VacA when co-expressed within the same target cell. HeLa cells were co-transfected with plasmids encoding both VacA-(Δ6–27)-GFP and wild-type VacA, and the extent of vacuolation was determined by light micros-

| VacA sample     | $P_{NC}/P_{C1}$ | Single channel conductance (pS) a |
|-----------------|-----------------|----------------------------------|
| Wild-type VacA  | 0.19 ± 0.02 (n = 6) | 159 ± 3 (n = 4)                  |
| VacA-(Δ6–27)    | 0.57 ± 0.05 (n = 8) | 154 ± 31 (n = 4)                |
| Wild-type VacA +| 0.38 ± 0.14 (n = 7) | ND                               |

a Macroscopic current was measured in buffer A with 100–150 mM NaCl using 30 nM wild-type VacA, 60 nM VacA-(Δ6–27), or a mixture of the two samples (each 30 nM). The number of experiments is designated (n). Results represent mean ± S.D. from triplicate determinations.

b Current at −100 mV in buffer A with 1.5 mM NaBr using 3 nM wild-type VacA or 45 nM VacA-(Δ6–27).

ND, not determined.
protein toxin. Transfection of mammalian cells with plasmids encoding the amino-terminal 422 amino acids of VacA is sufficient to induce cell vacuolation (25, 26), and antibodies reactive with the carboxyl-terminal portion of mature secreted VacA (amino acids 509–836) inhibit VacA binding to cells (27). These results indicate that the amino-terminal portion of VacA corresponds to an intracellularly active domain, and the carboxyl-terminal portion may correspond to a cell-binding domain. Additional efforts to analyze VacA structure-function relationships have involved the construction of VacA mutant proteins. VacA has not been expressed successfully as a functional recombinant protein in E. coli (29), and therefore, the construction and expression of VacA mutant proteins has been accomplished by manipulating the vacA gene in H. pylori. In previous studies, mutagenesis of two histidine residues (30) and mutagenesis of a surface-exposed domain corresponding to VacA amino acids 327–372 (38) have failed to alter VacA activity. The only inactive VacA mutant constructed thus far has contained a large deletion (corresponding to amino acids 91–330) in the amino terminus of the toxin (39). This mutant VacA protein was secreted by H. pylori but formed dimers rather than typical six or seven-sided oligomers (39). Five of the mutant VacA proteins described in this study, each containing deletions in the region between amino acids 27 and 294, also failed to form typical oligomeric structures. The mechanistic basis for failure of these mutants to oligomerize properly is not clear, but we speculate that sequences located between amino acids 27 and 294 may directly mediate contact between adjacent monomers. Alternatively, it is possible that deletions in this region result in drastic alterations in VacA folding, thereby precluding proper oligomerization. Future studies involving site-directed mutagenesis may be helpful in clarifying whether residues 27–294 comprise an oligomerization domain. Notably, all of the VacA mutants that fail to oligomerize properly lack vacuolating cytotoxic activity (Ref. 39 and this study), which raises the possibility that VacA oligomerization may be essential for cytotoxic activity.

In contrast to the VacA deletion mutants discussed above, VacA(Δ6–27) forms oligomeric structures similar to those of wild-type VacA. Moreover, VacA(Δ6–27) binds to lipids and forms two-dimensional crystals with a structure indistinguishable from that of wild-type VacA. Collectively, these data indicate that the overall folding of VacA(Δ6–27) remains intact despite the presence of a 22-amino acid deletion. VacA(Δ6–27) does not exhibit any obvious defects in binding or uptake by HeLa cells but fails to induce vacuole formation. The inability of VacA(Δ6–27) to induce vacuolation when expressed intracellularly suggests that this mutant toxin is primarily defective in intracellular function.

Several previous studies have proposed that formation of intracellular membrane channels is important for the morphogenesis of VacA-induced cell vacuoles (19, 20, 23). The loss of vacuolating activity and alteration of channel-forming activity that both result from deleting the VacA amino-terminal hydrophobic segment provide evidence in support of this hypothesis. One unresolved issue relates to understanding the role of channel formation in VacA cellular intoxication concerns the role of acidic pH in VacA activation. Specifically, VacA channel formation in planar lipid bilayers requires exposure of the toxin to acidic pH (19, 20, 23). In contrast, VacA expressed intracellularly in mammalian cells effectively forms vacuoles without any apparent exposure of the protein to acidic pH. This apparent discrepancy could be related in part to the fact that VacA is purified from H. pylori supernatants in an oligomeric form (13, 17, 18), whereas VacA may exist predominantly in a monomeric form when expressed within mammalian cells. We spec-
ulate that the formation of VacA membrane channels may involve oligomerization of membrane-bound monomers (43), and therefore, the role of acidic pH in VacA channel formation in vitro might simply be to disrupt VacA oligomers into monomeric components (17).

A remarkable property of VacA-(Δ6–27) is its capacity to inhibit the cytotoxic activity of the wild-type toxin. One possible explanation for this phenomenon is that VacA-(Δ6–27) might competitively inhibit the binding of wild-type toxin to a putative VacA receptor on the surface of cells (40–42). However, inhibition of wild-type toxin activity was detectable when the ratio of wild-type toxin to mutant VacA was 5:1. Typically, a substantial excess of mutant protein is required to inhibit binding of an active ligand to cell-surface receptors. Moreover, the strongest evidence against competitive inhibition at a cellular surface site is that intracellular expression of a VacA protein containing the Δ6–27 deletion inhibited the vacuolating activity of wild-type VacA.

A more likely explanation for the dominant negative phenotype exerted by VacA-(Δ6–27) is the formation of dysfunctional mixed oligomers, comprised of both mutant and wild-type VacA monomeric components. This model is consistent with the capacity of purified mutant VacA to inhibit wild-type VacA activity when the ratio of wild-type to mutant VacA is 5:1 (i.e. a dominant negative effect). Indeed, the capacity of VacA-(Δ6–27) to alter dramatically the channel-forming activity of wild-type VacA provides evidence that these two species can interact to form dysfunctional hetero-oligomeric structures. Further insight comes from the observation that VacA-(Δ6–27) can exert a dominant negative phenotype when co-expressed with wild-type VacA from within target cells. This raises the possibility that an intracellular oligomeric form of VacA might be required for vacuolating cytotoxic activity. Intracellular interactions between VacA molecules potentially could be critical for the formation of membrane channels or for establishing a quaternary structure with unique binding or enzymatic properties. However, at this time we cannot rule out alternate interpretations of the data. For example, VacA-(Δ6–27) might form oligomers more readily than wild-type VacA, and formation of intracellular hetero-oligomers might deplete the intracellular pool of active monomeric wild-type VacA. Alternatively, compared with wild-type VacA, VacA-(Δ6–27) might exhibit increased avidity for an intracellular target molecule. Further construction and analysis of VacA mutants should be helpful in clarifying the intracellular mechanisms by which VacA alters cellular function.

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