Vi interacts with host cells through a cell surface associated recognition complex containing prohibitin family of molecules
Introduction

The host-pathogen interactions during infection with *S.typhi*, the causative agent of human typhoid, are not well understood primarily due to non-availability of a suitable animal model. Most of our current understanding of how *S.typhi* interacts with host cells comes largely from studies carried out with *S.typhimurium*, which in mice causes an analogous disease referred to as murine typhoid (Jones and Falkow, 1996). These studies have revealed the presence of a large number of effector molecules secreted by pathogenic *Salmonella* species, which enable them to invade intestinal epithelial cells, survive intracellularly in tissue macrophages and bring about cell death (Galan, 1996; Ginocchio *et al.*, 1992; Lee *et al.*, 1992; Pegues *et al.*, 1995; Monack *et al.*, 2000). The effector molecules belong to a novel protein secretion apparatus called the Type III secretion system (Galan and Collmer, 1999). This system gets activated upon contact of *Salmonella* with host cells and works like a ‘molecular syringe’ in translocating effector molecules inside a host cell. A number of these effector molecules or virulence factors reported in *S.typhimurium* have been shown to be highly conserved in *S.typhi* suggesting that the two microorganisms might utilize similar mechanisms to invade mammalian cells and subsequently to establish themselves within a host (Galan and Curtiss, 1991). However, in spite of this high degree of functional conservation of the effector molecules, recent studies have revealed significant differences in the way these two closely related *Salmonella* serovars interact with host cells. The two differ significantly in their intracellular replication, intracellular survival and cytotoxicity-inducing ability in murine and human mononuclear phagocytes (Alpuche-Aranda *et al.*, 1995; Schwan *et al.*, 2000). *S.typhi* uses Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) for entry into intestinal epithelium and in comparison to *S.typhimurium* induces significantly larger quantities of interleukin-6 in the human intestinal epithelial cells (Pier *et al.*, 1998; Weinstein *et al.*, 1998). These studies, combined with the fact that in humans *S.typhi* produces systemic infection, typhoid, whereas *S.typhimurium* causes enteritis characterized by self-limited fever and diarrhea (Keusch, 1991), suggest that conclusions about *S.typhi*-host cell interaction drawn
from *S. typhimurium* model should be interpreted cautiously. Clearly, molecular entities not conserved between the two species might be playing an important role in host-specific interactions. An important distinction between *S. typhi* and *S. typhimurium* is the presence of a polysaccharide capsule around *S. typhi* (Felix and Pitt, 1934a). The capsule commonly referred to as Vi (for virulence), is a polymer of α (1→4) N-acetyl galacturonic acid with variable O-acetylation at the third carbon. The virulence of *S. typhi* correlates with the expression of this molecule as the majority of *S. typhi* strains isolated from typhoid patients express this antigen (Felix *et al.*, 1935). Studies carried out in mice have shown that Vi+ strains of *S. typhi* had a lower lethal dose 50% (LD50) than Vi− strains. Also, typhoid incidence was higher in human volunteers challenged with Vi+ *S. typhi* than those challenged with Vi− *S. typhi* (Hornick *et al.*, 1970a and 1970b). Furthermore, Vi-encapsulated strains of *S. typhi* have been shown to survive better than Vi negative bacteria in cultured macrophages in vitro (Hirose *et al.*, 1997). The expression of Vi is associated with resistance of *S. typhi* to the action of anti-O antibody, to phagocytosis and to complement mediated killing. The latter two actions can be initiated by antibodies to Vi (Robbins and Robbins, 1984; Looney and Steigbigel, 1986). The potential of this polysaccharide as a vaccine has been extensively studied (Acharya *et al.*, 1987; Klugman *et al.*, 1987; Szu *et al.*, 1991; Kossaczka *et al.*, 1994; Szu *et al.*, 1994) and currently, Vi is one of the vaccines against *S. typhi*. On the other hand, there have been no studies on understanding its role during infection of intestinal epithelial cells with *S. typhi*.

In this study, the interaction of Vi with intestinal epithelial cells and mononuclear phagocytes, the two main cell types that *S. typhi* is believed to infect during typhoid, was investigated.

**Results**

Vi capsular polysaccharide of *S. typhi* binds to human intestinal epithelial cells and mononuclear phagocytes
The binding of Vi with a model human intestinal epithelial cell line, Caco-2, was analysed by flow cytometry using anti-Vi monoclonal antibody as a probe. The polysaccharide showed a dose-dependent binding with these cells (Figure 1a). The binding was not seen if cells were incubated with LPS instead of Vi, or if an isotype matched antibody was used in place of anti-Vi MoAb. Importantly, binding was also seen with culture supernatant derived from Vi positive S.typhi but not with culture supernatant from Vi negative S.typhi (Figure 1b). The degree of binding obtained with the culture supernatant suggested that Vi positive S.typhi released Vi in abundance during its in vitro growth in LB medium.

Caco-2 cells stained with Vi were also observed on a Nikon TE-2000 fluorescence microscope. As can be seen in Figure 2a, cell surface associated fluorescence obtained with the polysaccharide was not uniform but showed a punctate pattern. In fact, when cells stained with Vi at 4°C were transferred to 37°C, the fluorescent foci became bigger with time suggesting that Vi-binding molecules might undergo aggregation upon interaction of cells with Vi at 37°C (Figure 2b). No detectable staining was observed in the absence of Vi.

To analyze the role of acetyl groups in the interaction of Vi with cells, its binding to Caco-2 cells was investigated in the presence of polygalacturonic acid (PGUA). The latter differs from Vi in the absence of N-acetyl and O-acetyl groups at positions C-2 and C-3 respectively. The results showed that binding of Vi to cells could not be blocked by excess PGUA (Figure 3), indicating that acetyl groups might be involved in the interaction of Vi with cells. The specificity of Vi-Caco-2 cell interaction was also demonstrated by the inability of LPS derived from S.typhi to inhibit binding of Vi to cells (Figure 3).

The interaction of Vi with host cells was not restricted to intestinal epithelial cells. The polysaccharide also bound to a human promyelomonocytic cell line, U937, in a dose-dependent manner (Figure 4).

**Vi is internalized after binding to cells**

To investigate if Vi could get internalized in Caco-2 cells, surface staining of Vi was analyzed in cells which had been incubated with the polysaccharide at 4°C
Figure 1. Binding of Vi to Caco-2 cells analysed by flow cytometry. Cells were incubated with varying concentrations of Vi or culture supernatants derived from S.typhi, at 4°C followed after washing with anti-Vi MoAb and FITC-anti-mouse Ig antibody. Control cells (shaded histogram) were incubated only with anti-Vi MoAb and FITC-anti-mouse Ig antibody. (a) binding of Caco-2 cells with Vi. (b) binding with culture supernatants derived from Vi (Vi+ CS) and Vi S.typhi (Vi CS).
Figure 2. Immunofluorescence showing binding of Vi to Caco-2 cells. (a) Cells were incubated with Vi (10μg/ml) for 1h at 4°C, followed by incubation with anti-Vi MoAb and FITC-anti mouse Ig antibodies. Cells were observed on a TE-2000 microscope (Nikon) fitted with a digital camera. Images were captured using ACT program and were transferred to Adobe photoshop for printing (magnification 30x). a' shows a magnified section of (a). (b) a representative image showing staining after cells (stained at 4°C as in a) were transferred to 37°C for 2h (magnification 60x).
Figure 3. Interaction of Vi with Caco-2 cells is not blocked by PGUA or LPS. Cells were preincubated with PGUA or LPS (both 100 μg/ml) for 1h at 4°C after which these cells were washed and incubated with Vi (10 μg/ml) for 1h at 4°C. Staining for Vi was done using anti-Vi MoAb followed by FITC- anti-mouse Ig antibodies. Control cells (shaded histogram) were incubated only with anti-Vi MoAb and FITC-anti-mouse Ig antibodies.
Figure 4. Vi binds to a human promyelomonocytic cell line, U937. Cells were incubated with varying concentrations of Vi at 4°C followed after washing with anti-Vi MoAb and FITC-anti-mouse Ig antibodies, and analyzed by flow cytometry.
and then transferred to 37°C for various time periods. There was a gradual decrease in the cell surface associated fluorescence when cells were shifted from 4°C to 37°C, suggesting a time-dependent internalization of the polysaccharide (Figure 5a). Internalization was confirmed by intracellular staining for Vi in permeabilized cells. As expected, Caco-2 cells, which were transferred to 37°C after binding with Vi at 4°C, showed significant reduction in cell surface staining (Figure 5b). However, when these two sets i.e. cells incubated with Vi at 4°C and those transferred to 37°C after incubation with Vi at 4°C were permeabilised, there was no significant difference in total cell staining (Figure 5c). This clearly showed that loss of cell surface associated Vi during transfer of cells from lower to higher temperature was due to internalization of Vi in cells at 37°C and not due to shedding off of the polysaccharide from cells.

Interaction of Vi with cells is mediated through a cell-surface associated receptor complex containing prohibitin family of molecules

After establishing that Vi capsular polysaccharide could interact with cells, it was pertinent to investigate if this interaction was mediated through specific recognition molecules on host cells. This analysis was carried out by immunoprecipitation with Vi using surface biotinylated Caco-2 cells. Vi was able to bind to a molecular complex containing two major proteins of about 30kDa and 35kDa and a minor protein of about 68kDa (Figure 6, lane 1). Similar profile was obtained when culture supernatant from Vi positive S.typhi was used as a source of Vi (Figure 6, lane 2). The bands were not seen when culture supernatant from Vi negative S.typhi was used in immunoprecipitation. (Figure 6, lane 3). Vi pulled down a similar complex from a human monocytic cell line, U937 (Figure 7).

To determine the identity of Vi-binding proteins, these molecules were isolated by bulk immunoprecipitation with cell lysate prepared from 10⁹-10¹⁰ Caco-2 cells. The immunoprecipitated complex was run in a SDS-PAG and bands were stained with Coomassie Brilliant Blue. The bands corresponding to 30kDa and 35kDa were excised from the gel and subjected to mass spectrometric analysis. The results showed that these two proteins contained many peptide sequences
Figure 5. Internalization of Vi in Caco-2 cells. (a) Caco-2 cells were incubated with Vi (10μg/ml) for 1h at 4°C, washed and transferred to 37°C for 5, 15 and 60 minutes. Subsequently, cells were incubated with anti-Vi MoAb and FITC anti-mouse Ig antibodies, and analyzed by flow cytometry. (b) Cells were incubated with Vi for 1h at 4°C, washed and transferred to 37°C. After 1h, cells were stained for Vi as outlined under (a). In (c), cells were treated as described under (b), permeabilized with saponin and stained for Vi. C1 (shaded histogram) and C2 represent control cells incubated with PBS instead of Vi at 4°C and 37°C respectively.
Figure 6. **Vi interacts with a cell surface associated molecular complex in Caco-2 cells.** Cells (15x10⁶) were surface biotinylated using NHS-biotin, lysed with TKM buffer containing 1% Triton-X-100 lysis buffer and centrifuged at 15,000xg. The supernatant (filtered through 0.22μ membrane) was incubated with protein-G-Sepharose beads preloaded with anti-Vi MoAb and Vi. The beads were washed, boiled with Laemmli sample buffer (non-reducing) and electrophoresed in a 10% SDS-PAG. Proteins were transferred to a nitrocellulose sheet and incubated with HRP-labeled Extravidin. The blot was developed using ECL. Lane 1- lysate incubated with Protein G-Sepharose beads preloaded with anti-Vi MoAb and Vi, lane 2- as in lane 1 except that culture supernatant from Vi⁺ *S.typhi* was used in place of purified Vi, and lane 3- as in lane 1 except that culture supernatant from Vi⁺ *S.typhi* was used in place of purified Vi.
Figure 7. Interaction of Vi with a molecular complex derived from a human monocytic cell line, U937. Cells (170x10^6) were surface biotinylated, lysed and centrifuged at 15,000xg. The supernatant was passed through a 0.22μm membrane and precleared using protein-G-Sepharose beads incubated with anti Vi MoAb. The precleared supernatant was incubated with protein G-Sepharose beads incubated with anti-Vi MoAb and Vi (Vi positive culture supernatant was used as the source of Vi) as described under Figure 6. Lane 1- Caco-2 supernatant incubated with Protein-G beads preloaded with anti-Vi MoAb and LB, Lane 2 as in Lane 1 except that Vi positive culture supernatant was used in place of LB.
corresponding to a recently identified tumor suppressor protein, prohibitin, and its closely related higher molecular weight homolog, BAP37 (B cell receptor associated protein; Figure 8). The mass spectrometric data was confirmed by immunoblot with anti-prohibitin antibodies. These antibodies reacted strongly with the 30kDa prohibitin band immunoprecipitated with Vi and also recognized the band representing BAP-37. The antibodies also reacted, though weakly with the 68kDa molecule present in the immunoprecipitated complex (Figure 9), suggesting that this molecule might also share some similarity with prohibitin family of molecules. Though the exact identity of this protein is yet to be established, a polyubiquitinated prohibitin of about 65KDa has been recently reported in mammalian sperm (Thompson et al., 2003). It is therefore possible that the 68kDa Vi-binding protein represents a similar molecule. Vi did not bind to prohibitin and BAP37 after these molecules were electrophoresed and transferred to nitrocellulose suggesting that the interaction of the polysaccharide with the proteins in the complex may be indirect or the molecules need to be in their native form and associated with each other in order to interact with this polysaccharide.

**Vi-binding molecules are enriched in lipid rafts**

To gain insight into the localization of Vi-binding molecules in the membrane, detergent insoluble membrane domains or lipid rafts prepared from Caco-2 cells were probed with anti-prohibitin antibodies. As can be seen from Figure 10a, both prohibitin and BAP-37 were enriched in lipid rafts represented by fractions 3-5. Our attempts to identify a lipid raft marker ganglioside GM1 or caveolin-1 in these membrane microdomains were not successful. This might be due to low level expression of these raft markers in Caco-2 cells as reported by Orlandi et al. (1998). However, lipid rafts prepared from canine kidney cell line, MDCK, or murine fibroblast cell line, NIH3T3 under identical conditions showed expression of caveolin-1 (Figure 10b). Significantly, when lipid rafts were prepared from Caco-2 cells which had been incubated with Vi at 4°C, the polysaccharide was found to be associated with these membrane microdomains (Figure 11). The rafts
Figure 8. Vi-binding proteins belong to prohibitin family of molecules. Vi-binding proteins were isolated from Caco-2 cells by bulk immunoprecipitation. The proteins were run on a SDS-PAG and bands corresponding to 30kDa and 35kDa were subjected to mass spectrometry. The 30kDa protein contained sequences corresponding to Prohibitin while the 35kDa had peptide sequences corresponding to a higher molecular weight homolog of prohibitin, BAP37 (B-cell associated protein). Amino acid sequences shown in red are the sequences which were identified in the two Vi-binding proteins. The amino acid sequences of Prohibitin and BAP-37 were from NCBI database.
Figure 9. Immunoblot with anti-prohibitin antibodies. Caco-2 cells (14x10⁶) were lysed with TKM buffer containing 1% Triton X-100. The lysate was incubated with Protein G-Sepharose beads pre-loaded with anti-Vi MoAb and culture supernatant from Vi⁺ S.typhi. The beads were washed, boiled with sample buffer (non-reducing) and samples were electrophoresed in SDS-PAG. Proteins were transferred to a NC membrane and blotted with rabbit anti-prohibitin antibodies followed by HRP-labeled anti-rabbit Ig antibodies. The blot was developed using ECL. Lane 1- Caco-2 lysate was incubated with Protein G-Sepharose beads preloaded with anti-Vi MoAb and LB. Lane 2- same as in lane 1, except that Vi⁺ CS was used in place of LB. Lane 3- Protein G-Sepharose beads incubated with anti-Vi MoAb followed by LB. Lane 4- same as in lane 3, except that Vi⁺ CS was used in place of LB. Lane 5- Protein G-Sepharose beads incubated with Vi⁺ CS. Lane 6- Protein G-Sepharose beads incubated with Caco-2 cell lysate. Lanes 7-9, lysates derived from 0.35, 0.7 and 1.4x10⁵ Caco-2 cells, respectively.
Figure 10. Vi-binding proteins, Prohibitin and BAP-37, are enriched in lipid rafts. Lipid rafts were prepared from Caco-2, NIH3T3 and MDCK cells. Cells (20x10⁶) were lysed with 1ml TKM buffer containing 1% Triton X-100 for 30 minutes at 4°C. The suspension was centrifuged at 500xg to remove unlysed cells and the cell debris. The supernatant was placed at the bottom of a centrifuge tube and mixed with an equal volume of 85% sucrose solution. The mixture was overlaid with 35% sucrose (6ml) followed by 5% sucrose (4ml) and centrifuged at 200,000xg for 16h. Twelve 1ml fractions were collected, run in a SDS-PAG, transferred to a NC membrane and probed with antibodies. (a) reactivity with anti-prohibitin antibodies with fractions obtained from Caco-2 cells. Fractions 3-5 represent detergent insoluble membrane domains. (b) reactivity of detergent insoluble membrane domains obtained from Caco-2, NIH3T3 and MDCK with anti-caveolin-1 antibodies. Blots were developed using ECL.
Figure 11. Vi associates with lipid rafts in Caco-2 cells Caco-2 cells (20x10⁶) were incubated with 50μg Vi (A) or PBS (B) for 1h at 4°C. Cells were washed to remove unbound Vi and lipid rafts were prepared from these cells as described under Figure 10. 5μl of each fraction were dotted onto a NC membrane. The membrane was blocked with non-fat milk protein (1% in PBS), incubated with anti-Vi MoAb followed by HRP-labeled anti-mouse Ig antibodies and developed with ECL.
prepared from control cells as expected, did not show any staining for Vi; low level binding seen with fractions 10-12 derived from these cells was non-specific reactivity. Importantly, immunofluorescence analysis using Confocal microscopy showed that membrane associated prohibitin co-localized with Vi in Caco-2 cells which had been incubated with Vi (Figure 12).

**S.typhi employs Vi to adhere to and gain entry into Caco-2 cells**

To assess the role of Vi in adherence of *S.typhi* to cells, binding of Vi positive *S.typhi* to Caco-2 cells was carried out in the presence of soluble Vi. Figure 13 shows that Vi inhibited binding of Vi+ *S.typhi* to Caco-2 cells in a dose-dependent fashion. The inhibition mediated by Vi was specific, as binding of Vi positive *S.typhi* was not significantly affected when cells were preincubated with LPS derived from this pathogen. Furthermore, Vi did not affect binding of Vi+ *S.typhi* to Caco-2 cells (Figure 14). Further analysis revealed that *S.typhi* could employ Vi not only to adhere to Caco-2 cells but also to gain access into cells. The uptake of Vi-positive *S.typhi* was significantly inhibited when infection of these cells with *S.typhi* was carried out in the presence of anti-Vi MoAb (Figure 15). On the other hand, anti-*S.typhi* flagellin monoclonal antibodies did not have a significant effect on the uptake of Vi positive *S.typhi* by Caco-2 cells.

**Discussion**

Vi capsular polysaccharide of *S.typhi* is a polymer of galacturonic acid with N-acetyl and O-acetyl at positions C-2 and C-3 respectively. Vi was first identified as a 'virulence antigen' of *S.typhi* (Felix *et al.*, 1934), the causative agent of typhoid fever in humans. It protects *S.typhi* against many host defense mechanisms (Looney and Steigbigel, 1986). However, its role during infection of intestinal epithelium with *S.typhi* is incompletely understood. The results described in this chapter demonstrate that Vi can bind to human intestinal epithelial cells and mononuclear phagocytic cells, the two important cell types that are infected by *S.typhi* during typhoid fever. The binding of Vi to Caco-2 cells required presence of acetyl groups in the polysaccharide. It is possible that
Figure 12. **Vi and prohibitins co-localize in Caco-2 cell membrane.** Cells grown on coverslips in a 24-well tissue culture plate were incubated with Vi, followed by biotinylated anti-Vi MoAb and PE-labeled strepavidin. Subsequently, cells were permeabilised at 4°C for 5 minutes with chilled methanol containing 0.01% TritonX-100, washed and incubated with rabbit anti-prohibitin Ab, followed by FITC-anti-rabbit Ig antibodies. Cells were washed and the coverslips were placed on glass slides in the presence of antifade reagent. Cells were observed under a Zeiss LSM 510 confocal microscope. **A**-staining for Vi, **B**-staining for prohibitin and **C**- **A** and **B** merged. (magnification 100x).
Figure 13. *S.typhi* engages Vi to bind to Caco-2 cells. Caco-2 cells were incubated with PBS, Vi, or LPS for 1h followed by incubation with Vi* S.typhi* at moi of 1 (A) or 10 (B) for 1h at 4°C. Cells were washed to remove unbound bacteria, lysed and the lysate plated on SS agar plates. Colony forming units were determined after overnight incubation at 37°C. Percent of control adherence was calculated as (% adherence in the presence of inhibitor/ % adherence in the absence of any inhibitor) x 100; binding in the absence of any inhibitor (preincubation of cells with PBS) was taken as 100% control.
Figure 14. Vi does not affect binding of Vi− S.typhi to Caco-2 cells. Caco-2 cells were incubated with PBS or Vi (12.5μg/ml) for 1h at 4°C followed by incubation with Vi negative S.typhi (10 moi). After 1h incubation at 4°C, cells were washed to remove unbound bacteria, lysed and plated on SS agar plate. The plates were incubated overnight at 37°C and the number of colony forming units was determined.
Figure 15. Anti-Vi MoAb inhibits uptake of *S.typhi* by Caco-2 cells. Cells were infected with Vi⁺ *S.typhi* (moi 10) for 20 minutes at 37°C in presence of varying concentrations of anti-Vi or anti-*S.typhi* flagellin MoAbs. Cells were washed to remove unbound bacteria and incubated for 2h in RPMI-1640 supplemented with FCS (10%) and gentamycin (100μg/ml). Thereafter, cells were washed, lysed with TKM buffer containing 1% Triton X-100. The number of cell associated and intracellular bacteria was determined by a sandwich ELISA using anti-flagellin MoAb as a capture antibody and HRP-labeled anti-Vi MoAb as the detection antibody.
these groups are involved in hydrophobic interactions with recognition molecules on host cells. Indeed, studies carried out on physiochemical characteristics of Vi and various derivatives have suggested that N-or O-acetyl groups in Vi could interact with alanine and/or lysine on cellular receptors (Szu and Bystricky, 2003).

The interaction with host cells was mediated through binding of Vi to a specific cell surface associated complex containing prohibitin and its closely related homolog, BAP37 (B-cell associated protein) as major components. Prohibitin is a putative tumor suppressor molecule that has been linked to human cancers (Spurdle et al., 2002). The prohibitin gene was originally cloned based on its anti-proliferative activity causing cell cycle arrest at G1/S (McLung et al., 1989). This protein interacts with E2F family of transcription factors that play an important role in cell cycle progression and suppresses their activity (Wang et al., 1999a; 1999b). Proteins of the prohibitin family are ubiquitous, abundant and highly conserved across species. Prohibitin and BAP37 are remarkable similar in the middle regions and contain a series of repeated leucine residues. In contrast the amino and the carboxy termini of the two molecules are different. The high degree of homology between the two proteins suggests that their functions might be related. Western blot analysis of murine tissue extracts has shown that the levels of prohibitin and BAP37 vary in different organs but their ratio remains constant (Coates et al., 1997).

Prohibitin and its related homologs are also involved in the stabilization of mitochondrial proteins (Nijtmans et al., 2000). In fact, prohibitin family of molecules are most abundant in mitochondria (Ikonen et al., 1994). However, localizations in the cell membrane and the nucleus have also been reported. In the cell membrane, prohibitin, BAP-37 and other closely related homologs have been shown to interact with the transmembrane domain of IgM and IgD in cells of B-lineage (Terashima et al., 1994; Kim et al., 1994). Prohibitin co-localizes with E2F and p53 in the nucleus and is exported to perinuclear regions upon apoptotic stimuli (Fusaro et al., 2003). In yeast, deletion of PHB1 and PHB2 (homologs of prohibitin and BAP37 in yeast) which are present as a heterodimer in the
mitochondrial membrane, leads to replicative senescence (Coates et al., 1997). In Drosophila, disruption of a homolog of prohibitin produces a lethal phenotype (Eveleth and Marsh, 1986). All these studies suggest that prohibitin family of molecules play an important role in many cellular processes.

In our analysis, both prohibitin and BAP37 were found concentrated in lipid rafts. More importantly, Vi readily associated with these membrane microdomains and co-localized with prohibitin in the membrane. Lipid rafts are specialized membrane microdomains that are enriched in cholesterol and sphingolipids and are believed to be centers where many intracellular signaling events are initiated and modulated (Simons and Toomre, 2000). These microdomains can change their size and composition during cellular activation. This is associated with specific protein-protein interactions in a microenvironment where the phosphorylation state of a protein could be modified by local kinases and phosphatases that results in downstream signaling (Simons et al., 2000). Recent studies have shown that many pathogenic microorganisms might target these domains to manipulate cellular signaling in order to establish themselves within a host. Vacuolating toxin (VacA) of Helicobacter pylori has been shown to associate with lipid rafts (Schraw et al., 2002) and this association is important for its cytotoxicity. Disruption of lipid rafts significantly decreased internalization of Shiga toxin 1 B-subunit of Enterohemorrhagic Escherichia coli that interacts with a glycolipid receptor, Gb3 present on epithelial cells (Kovbasnjuk et al., 2001). Host defence against Pseudomonas aeruginosa requires ceramide-rich membrane rafts (Grassme et al., 2003) and initial steps of Shigella infection depend on the cholesterol/sphingolipid raft mediated CD44-IpaB interaction (Lafont et al., 2002). Other pathogens including Mycobacterium bovis, Campylobacter jejuni, the SV40, measles, sindbis and influenza viruses, Plasmodium falciparum and even prions have been shown to use rafts or raft like structures during infection of mammalian cells (Grassme et al., 2003).

Taken together, the results described in this chapter suggest that Vi can target lipid rafts in intestinal epithelial cells and through interaction with prohibitin family of molecules that are concentrated in these membrane
microdomains, might modulate intracellular signaling and consequently various cellular functions. The significance of this interaction will be discussed in the next two chapters.