Interaction of *Vibrio cholerae* O139 with an intestinal parasite, *Entamoeba histolytica*

*Vibrio cholerae* is the causative agent of cholera, a form of diarrhoea, which continues to rage and remains a major public health problem in the developing world. The organism has the capacity to survive in diverse estuarine environments, as well as in the human host. Recent studies have suggested that interaction with a freshwater amoeba, *Acanthamoeba castellanii*, could be one possible mode of survival in the aquatic environment (Abd et al., 2005; Thom et al., 1992). It was also shown that *V. cholerae* could replicate intracellularly in *A. castellanii*. This prompted us to study its interaction with a parasitic amoeba, *Entamoeba histolytica*.

*E. histolytica*, the causative agent of amoebic colitis and amoebic liver abscess, is the second most common cause of death from parasitic disease worldwide (Stanley, 2003). In their natural environment, trophozoites of *E. histolytica* live in the colon of the human intestine together with the resident microflora, which under normal conditions is usually composed of a complex mixture of mainly anaerobic or microaerophilic bacteria (Mirelman, 1987). In order to examine the interaction of *V. cholerae* with the trophozoites of *E. histolytica*, a gentamicin assay was employed, as described previously (Venkataraman et al., 1997). In this assay, *E. histolytica* HM-1:IMSS trophozoites (a kind gift from Professor Sudha Bhattacharyya, JawaharLal Nehru University, New Delhi, India) were suspended in serum-free TYI-S-33 medium at a concentration of $10^5$ amoebae ml$^{-1}$. Trophozoites were incubated in triplicate in 24-well tissue culture plates (Falcon).

Subsequently, *V. cholerae* O139, strain SG24 (a kind gift from Dr Ranjan Nandy, National Institute of Cholera and Enteric Research, Calcutta, India), was added to a final concentration of $10^7$ cells ml$^{-1}$. The samples were incubated at 36°C for 1 h, followed by the addition of 200 µg gentamicin ml$^{-1}$ for 2 h to kill the extracellular bacteria. After gentamicin treatment, the trophozoites were washed three times with PBS by centrifuging at 280 g for 7 min. After washing, trophozoites were counted with trypan blue to check viability and lysed by syringe passage in the presence of 0.01 % Triton X-100. Dilutions were plated on Luria–Bertani agar plates for colony enumeration, which was found to be $5.53 \pm 0.18 \times 10^3$. The data suggested the presence of intracellular bacteria within the trophozoites of *E. histolytica*, with the trophozoites providing a protective barrier for *V. cholerae* against the effect of gentamicin.

To investigate the intracellular localization of the bacteria further, we carried out

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**Fig. 1.** (a) Electron micrograph of a thin section of *E. histolytica* trophozoites incubated for 1 h at 36°C with *V. cholerae* SG24. (b) Vacuole magnification. Thin sections were prepared after epoxy-resin embedding. Bacteria were found inside the vacuoles (arrow). Bars, 500 nm (a) and 200 nm (b).
transmission electron microscopy of infected trophozoites. Microscopic pictures of trophozoites 1 h after infection revealed that *V. cholerae* O139 cells were localized intracellularly in the vacuoles (Fig. 1).

Taken together, these findings suggest that *V. cholerae* O139 cells are recognized by the trophozoites of *E. histolytica* and are localized primarily in the vacuoles. Previously, various surface molecules of *V. cholerae* have been shown to be involved in diverse functions. For example, the extensively studied type 4 pilus, the toxin-coregulated pilus, is necessary for colonization of the mammalian intestine (Thelin & Taylor, 1996). Similarly, a mannose-sensitive haemagglutinin (MSHA) was found to promote the adherence of *V. cholerae* to zooplankton (Chiavelli et al., 2001). Besides promoting adherence to biotic surfaces, MSHA is also important for biofilm formation on abiotic (borosilicate glass) surfaces (Watnick et al., 1999). Currently, the cell-surface molecules of *V. cholerae* that mediate its interaction with the trophozoites of *E. histolytica* are not known. Additional studies are necessary to determine this. Furthermore, association of *V. cholerae* with free-living as well as parasitic amoebae may serve as a model for further exploration of the biology of the bacterium. This is the first report describing the interaction of *V. cholerae* with the human intestinal parasitic protozoan *E. histolytica*.

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