Vibrio fluvialis Attachs to but Does not Enter HeLa Cell Monolayers

IT Carvalho, V Magalhães, NC Leal, V Melo, M Magalhães

Laboratório Keizo Asami, UFPE, Cidade Universitária, 50670-901 Recife, PE, Brasil *Centro de Pesquisas Aggeu Magalhães-FIOCRUZ, Cidade Universitária, Recife, PE, Brasil

Considering the possibility that invasiveness could be a neglected factor of virulence in Vibrio fluvialis-*linked enteritis, since a dysenteric form of the disease was seen in Bangladesh, we studied 12 Brazilian strains of the organism, six clinical and six environmental, to determine whether they might be able to enter into HeLa cell monolayers or would carry plasmids incidentally involved in invasiveness. Four human and two environmental isolates attached to but did not enter into the cells. Though five strains harbored plasmids, no relationship was found between the carriage of these genetic elements and adhesiveness.*

Key words: *Vibrio fluvialis* - *Vibrio* pathogenicity - diarrhea - HeLa cell adherence

During the late 1970s, Bangladesh experienced an extensive epidemic of diarrhea linked to *Vibrio fluvialis*; more than 500 individuals became ill, most presenting a large number of pus cells and erythrocytes in their stools (Huq et al. 1980). Elicitation of inflammatory reaction is usually considered as a sign of enteric disease caused by invasive organisms, such as, *Shigella, Salmonella,* and *Yersinia enterocolytica* (Dupont & Pickering 1980). Such organisms have the capacity to enter and replicate within cultured epithelial cells (Small et al. 1987), abilities often mediated by plasmid genes (Sansonetti et al. 1981, Jones et al. 1982).

Since the pathogenicity of *V.fluvialis* long has been attributed to enterotoxins and cytolysins (Seidler et al. 1980, Lockwood et al. 1982, Chikahira & Hamada 1988), we decided to investigate whether the bacterium also displays invasive potentialities capable to explain dysentery-like syndromes.

MATERIALS AND METHODS

Bacterial strains - We selected six strains of *V.fluvialis*, isolated as the sole enteropathogen from six patients with diarrhea. For comparison we also studied six environmental cultures recovered from oysters. All strains were confirmed biochemically as *V.fluvialis* by standard procedures (Lee et al. 1981, Brenner et al. 1983; Baumann et al. 1984). Working cultures of each isolate were maintained at -70°C in defibrinated rabbit blood and revived on sheep blood agar when desired.

HeLa cell assays - Adherence to HeLa cells was tested according to the one step 3-hr incubation assay, originally described by Cravioto et al. (1979). An adherent strain of *Escherichia coli* O111:H2 was the positive control.

Strains were tested for invasiveness by using the gentamicin- HeLa cell technique (Vesikari et al. 1982, Small et al. 1987). As positive control, a recent clinical isolate of *Shigella flexneri* was included.

Plasmids - Plasmid DNA extraction was done by the method of Birnboim and Doly (1979) and analyzed by electrophoresis of cell lysates through 0.7% agarose gels. Plasmids were subsequently stained with ethidium bromide and visualized with UV illumination.

RESULTS

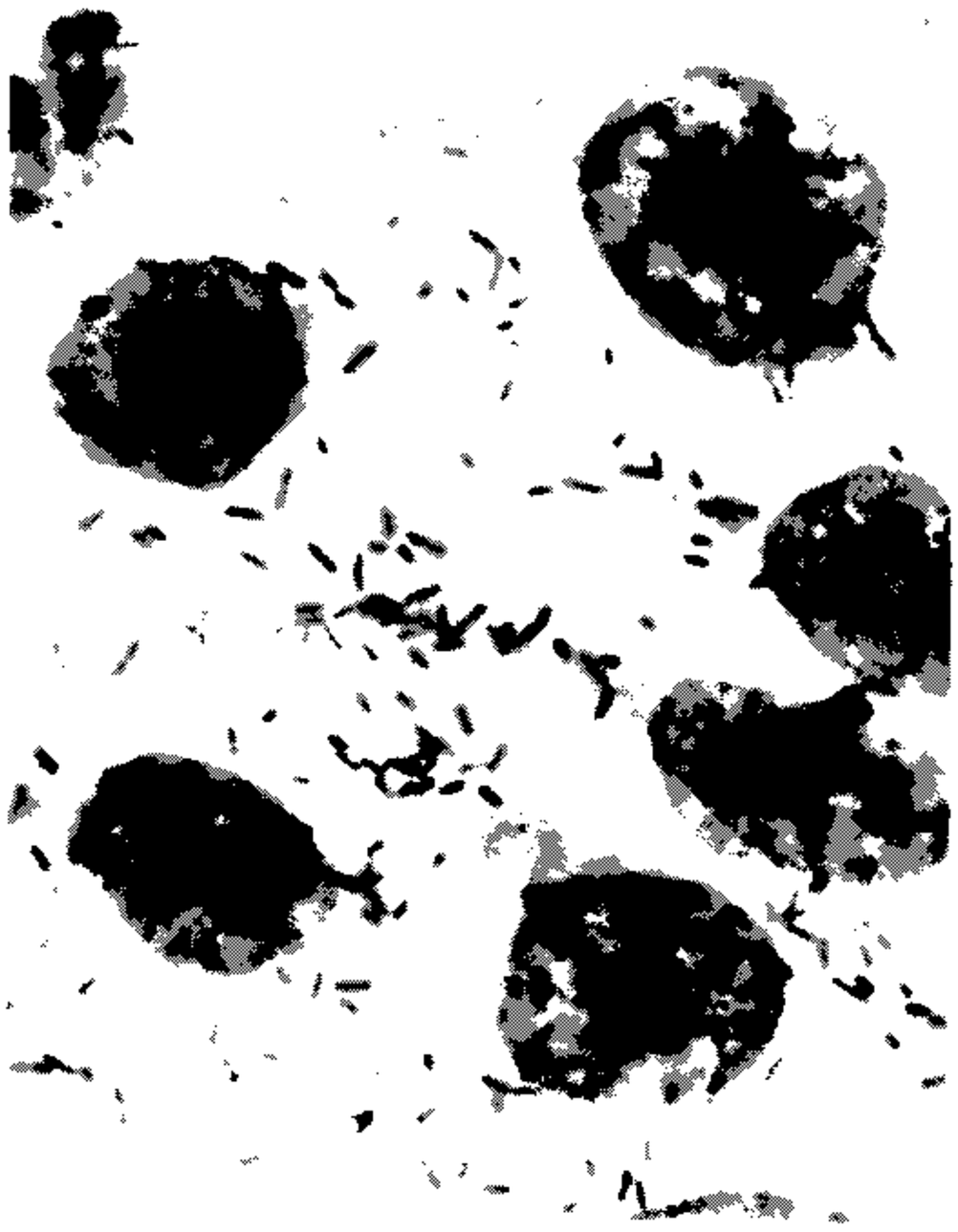
Of 12 strains tested, only five (42%) presented mannose-resistant adherence to HeLa cells (Table). Microscopic examination of Giemsa-stained cell monolayers showed a pattern of adherence similar to that displayed by diffuse adhering *E.coli* strains (Fig.). In the gentamicin-HeLa cell assay, unlike *S.flexneri*, none of the strains of *V.fluvialis* were able to penetrate into the cell cytoplasm.

Three human isolates and two environmental strains of *V.fluvialis* harbored plasmids, but no relationship between adhesiveness and the carriage of these genetic elements could be found (Table).

DISCUSSION

Most bacterial pathogens initiate infectious diseases by adhering to host cells (Isberg 1991). Adherence represents the first step in the process of invasiveness, and it is a universal factor of

Supported by Japan International Cooperation Agency.
Received 5 November 1993
Accepted 20 December 1993



Difuse adhesion of *Vibrio fluvialis* to the HeLa cell monolayer. The incubation of HeLa cells with 10^6 bacteria was done at 37°C for 3 hr in cell line medium and 1% D-mannose. Giemsa stain. Magnification: X900.

virulence in many pathogenic genera of bacteria, including *Vibrio*. In this vein, binding to human fetal intestinal cells was used as a criterium for differentiating virulent and avirulent strains of *V.parahaemolyticus* (Hackney et al. 1980).

In enterobacteriology, the ability to enter into epithelial cells or the production of toxins decides the outcome of the clinical picture of the enteritis: dysentery or watery diarrhea. Most studies on the mechanism of pathogenicity of *V.fluvialis* point out enterotoxins and cytolysins as their major virulence factors (Seidler et al. 1980, Lockwood et al. 1982, Wall et al. 1984, Chikahira & Hamada 1988). Indeed, our patients infected with *V.fluvialis* show a self limited disease, always characterized by profuse watery diarrhea without fecal leukocytes or blood. On the other hand, during an epidemic of gastroenteritis in Bangladesh, most patients displayed a dysentery-like disease distinguished by blood and fecal leukocytes (Huq et al. 1980). Unfortunately, the epidemic strains were not assayed for invasiveness. So, the possibility that an atypical clone of *V.fluvialis* disseminated in Bangladesh in that time is difficult to be ascertained. This supposition, however, would explain the differences in the clinical forms of *V.fluvialis*-linked enteritis found in Brazilian and Bangladesh patients. Brazilian strains are able to attach to, but lack the property of invasiveness.

TABLE

HeLa cell adherence and plasmids of environmental and clinical strains of *Vibrio fluvialis*

Strains[a]	Adherence[b]	Plasmids[c]
O-13	-	33 - 11
O-16	-	-
O-17	-	-
O-18	+	-
O-19	+	-
O-20	-	11
H-36	-	5 - 63 - 68
H-62	+	51
H-80	+	-
H-83	-	85
H-84	+	-
H-439	+	-

[a]: O = Oyster, H = Human; [b]: - = Absent, + = Present; [c]: = Kilobase.

Although most of our clinical isolates of *V.fluvialis* attached to HeLa cells (66.7%), several environmental strains (33.3%) were also able to do so; thus, adhesiveness could not distinguish the source of the strains, and its diarrheagenic potential was not clear. Rahman et al. (1992) also did not detect any distinction, concerning cell adherence, between one environmental and another clinical strain of *V.fluvialis*. Therefore, on this viewpoint, *V.fluvialis* behaves differently from *V.parahaemolyticus*, whose clinical isolates adhered faster and more efficiently to epithelial cell monolayers than did the environmental ones (Carruthers 1977, Hackney et al. 1980).

REFERENCES

Baumann P, Furniss AL, Lee JV 1984. Genus I. *Vibrio* Pacine 1854, p. 518-538. In NR Krieg, JG Holt (eds) *Bergey's Manual of Systematic Bacteriology*. The Williams and Wilkins, Baltimore.

Birnboim HC, Doly J 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res 7:* 1513-1523.

Brenner DJ, Hickman-Brenner FW, Lee JV, Steigerwalt AG, Fanning GR, Hollis DG, Farmer III JJ, Weaver RE, Joseph SW, Seidler RJ 1983. *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and the environment. *J Clin Microbiol 18:* 816-824.

Carruthers MM 1977. *In vitro* adherence of Kanagawa-positive *Vibrio parahaemolyticus* to epithelial cells. *J Infect Dis 136*: 588-592.

Chikahira M, Hamada K 1988. Enterotoxigenic substance and other toxins produced by *V.fluvialis* and *Vibrio furnissii*. *Jpn J Vet Sci 50*: 865-873.

Cravioto A, Gross RJ, Scotland SM, Rowe B 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. *Curr Microbiol 3*: 95-99.

Dupont HL, Pickering LK, 1980. *Infections of the gastroin-testinal tract*. Plenum Medical Book Company, New York, iv + 273 pp.

Hackney CR, Kleeman EG, Ray B, Speck ML 1980. Adherence as a method for differentiating virulent and avirulent strains of *Vibrio parahaemolyticus*. *Appl Environ Microbiol 40*: 652-658.

Huq MI, Alam AKMJ, Brenner DJ, Morris GK 1980. Isolation of *Vibrio*-like group, EF-6, from patients with diarrhea. *J Clin Microbiol 11*: 621-624.

Isberg RR 1991. Discrimination between intracellular uptake and surface adhesion of bacterial pathogens. *Science 252*: 934-938.

Jones GW, Rabert DK, Svinarich DM, Whitfield HJ 1982. Association of adhesive, invasive, and virulent phenotypes of *Salmonella typhimurium* with autonomous 60-megadalton plasmids. *Infect Immun 38*: 476-486.

Lee JV, Shread P, Furniss AL, Bryant TN 1981. Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). *J Appl Bacteriol 50*: 73-94.

Lockwood DE, Kreger AS, Richardson SH 1982. Detection of toxins produced by *Vibrio fluvialis*. *Infect Immun 35*: 702-708.

Rahman MM, Qadri F, Albert MJ, Hossain A, Mosihuzzaman M 1992. Lipopolysaccharide composition and virulence properties of clinical and environmental strains of *Vibrio fluvialis and Vibrio mimicus*. *Microbiol Immunol 36*: 327-338.

Sansonetti PJ, Kopecko DJ, Formal SB 1981. *Shigella sonnei* plasmids: evidence that a large plasmid is necessary for virulence. *Infect Immun 34*: 75-83.

Seidler RJ, Allen DA, Colwell RR, Joseph SW, Daily OP 1980. Biochemical characteristics and virulence of environmental group F bacteria isolated in the United States. *Appl environ Microbiol 40*: 715-720.

Small PLC, Isberg RR, Falkow S 1987. Comparison of the ability of enteroinvasive *Escherichia coli, Salmonella typhimurium, Yersinia pseudotuberculosis, and Yersinia enterocolitica* to enter and replicate within HEp-2 cells. *Infect Immun 55*: 1674-1679.

Vesikari T, Bromirska J, Maki M 1982. Enhancement of invasiveness of *Yersinia enterocolitica* and *Escherichia coli* in HEp-2 cells by centrifugation. *Infect Immun 36*: 834-836.

Wall VW, Kreger AS, Richardson SH 1984. Production and partial characterization of a *Vibrio fluvialis cytotoxin*. *Infect Immun 46*: 773-777.