V. cholerae. A mannose-sensitive lectin-like adhesin produced by motile *Aeromonas* strains was shown to coagglutinate with some *Salmonellae*[3]. Whether this can be of importance in the course of *Aeromonas*- or *Salmonella*-associated intestinal infections remains to be investigated.

Strains of *Aeromonas* were found to adhere to rabbit brush border cells and rabbit intestines in low numbers[14], whereas human *Aeromonas* isolates adhered in high numbers to isolated human intestinal cells (M. Lindahl, Å. Ljungh, unpublished). Furthermore, human and animal *Aeromonas* isolates commonly express pronounced surface hydrophobicity in contrast to strains isolated from water and shellfish which are often surrounded by a slimy, hydrophilic material.

In summary, *Aeromonas* strains produce surface proteins of fimbrial and non-fimbrial nature which can represent surface adhesins with different host specificity in analogy to the plethora of *E. coli* adhesins. They can also be involved in determining the level of adhesion in the gut.

Conclusions

The role of β-hemolysin in soft tissue infection is well established. In diarrheal disease, however, correlation to biotype is circumstantial and no correlation between presumptive virulence factor and infection has been shown. In animal feeding experiments as well as in human volunteer studies *Aeromonas* strains with defined characteristics failed to induce diarrhea[16, 19, 20].

Aeromonas intestinal infection may present as 1) toxigenic, rice-water, small intestinal diarrhea, 2) classical dysentery involving the large intestine, or as 3) combinations of the two extremes. It is likely that surface characteristics determine the level of adhesion and that the production of toxins and enzymes determines the severity of disease. Certain combinations of toxins, enzymes and surface factors are probably crucial for establishing infection. To study this, wild type strains and a panel of mutants devoid of selected virulence factors must be characterized and tested in appropriate models.

1 Adams, D., Atkinson, M.A., and Woods, W.H., J. clin. Microbiol. *17* (1983) 422.
2 Asao, T., Kinoshita, Y., Kozaki, S., Uemura, T., and Sakaguchi, G., Infect. Immun. *46* (1984) 122.
3 Atkinson, H.M., Adams, D., and Woods, W.H., FEMS Microbiol. Lett. *20* (1983) 311.
4 Atkinson, M.A., and Trust, T.J., Infect. Immun. *27* (1980) 938.
5 Bernheimer, A.W., and Avigad, L.S., Infect. Immun. *9* (1974) 1016.
6 Buckley, J.T., Halasa, L.N., Lund, K.D., and MacIntyre, S., Can. J. Biochem. *59* (1981) 430.
7 Burke, V., Cooper, M., Robinson, J., Gracey, M., Lesmana, M., Echeverria, P., and Janda, J.M., J. clin. Microbiol. *19* (1984) 39.
8 Campbell, J.D., and Houston, C.W., Curr. Microbiol. *12* (1985) 101.
9 Chakraborty, T., Montenegro, M.A., Sanyal, S.C., Helmuth, R., Bulling, E., and Timmis, K.N., Infect. Immun. *46* (1984) 435.
10 Howard, S.P., and Buckley, J.T., Biochemistry *21* (1982) 1662.
11 Hsu, T.C., Waltman, W.D., and Shotts, E.B., Dev. Biol. Stand. *49* (1981) 101.
12 James, C., Dibley, M., Burke, V., and Gracey, M., Clin. exp. Immun. *47* (1982) 34.
13 Lawson, M.A., Burke, V., and Chang, B.J., Infect. Immun. *47* (1985) 680.
14 Levett, P.N., and Daniel, R.R., J. gen. Microbiol. (1981) 167.
15 Ljungh, Å., Eneroth, P., and Wadström, T., Toxicon *20* (1982) 787.
16 Ljungh, Å., and Kronevi, T., Toxicon *20* (1981) 397.
17 Ljungh, Å., and Wadström, T., Toxic Rev. *1* (1982) 257.
18 Ljungh, Å., Wretlind, B., and Möllby, R., Acta path. microbiol. scand. *B89* (1981) 387.
19 Morgan, D.R., Johnson, P.C., DuPont, H.L., Satterwhite, T.K., and Wood, L.V., Infect. Immun. *50* (1985) 62.
20 Pitarangsi, C., Echeverria, P., Ehitmire, R., Tirapat, C., Formal, S., Dammin, A.J., and Tingtalapong, M., Infect. Immun. *35* (1982) 666.
21 Rigney, M.M., Zilinsky, J.W., and Rouf, M.A., Curr. Microbiol. *1* (1978) 175.
22 Sanyal, S.C., Agarwal, R.K., and Annapurna, E., Indian J. med. Res. *78* (1983) 324.
23 Shimada, T., Sakazaki, R., Horigome, K., and Uesaka, Y., Jap. J. med. Sci. Biol. *37* (1984) 141.
24 Stewart, G.A., Bundell, C.S., and Burke, V., J. Med. Microbiol. *21* (1986) 319.
25 Thelestam, M., and Ljungh, Å., Infect. Immun. *34* (1981) 949.

0014-4754/87/040367-02$1.50 + 0.20/0

Enterotoxins of *Aeromonas* species

by V. Burke, J. Robinson and M. Gracey

Gastroenterology and Nutrition Research Unit, Princess Margaret Children's Medical Research Foundation, and Department of Child Health, University of Western Australia, Perth, W.A. (Australia)

Key words. Aeromonas; enterotoxin; cytotoxin; haemolysin; cholera toxin.

Case reports and epidemiological studies[6] suggest that *Aeromonas* spp. may cause diarrhoea. Identification of virulence factors has been complicated by the multiplicity of *Aeromonas* exoproteins and lack of any experimental model for *Aeromonas*-associated diarrhoea.

Enterotoxin production of *Aeromonas* spp. was suggested by Sanyal et al.[25] and confirmed, using cell free preparations, by Wadström et al.[29]. Enterotoxins of *Aeromonas* spp. may by cytotoxic[23] or cytotonic[24] (table).

Cytotonic enterotoxin

Ljungh et al.[19] reported that *Aeromonas* spp. produce a heat-labile cytotonic enterotoxin (MW about 15000) which produces fluid accumulation without mucosal damage in ileal loops of rabbits, rats and mice but not in suckling mice. Like cholera toxin (CT), this enterotoxin is cytotonic in Y1 cells and increases intracellular cAMP but it does not cross-react immunologically with CT[19]; it is not cytotoxic or haemolytic.

Chakraborty et al.[8] have cloned an *Aeromonas* cytotonic enterotoxin without haemolytic or cytotoxic activity which, unlike the enterotoxin described by Ljungh et al.[19], is positive in suckling mice. There was no DNA homology between the *Aeromonas* cytotonic enterotoxin and *E. coli* LT or ST.

While some laboratories have found no cross-reactivity between *Aeromonas* exotoxins and CT[2, 23] others have reported neutralisation of *Aeromonas* enterotoxin by antiserum to CT or LT[12, 16, 17]. More recently cross-reactivity between CT and *Aeromonas* exotoxin, in systems such as ELISA, has been reported[7, 14, 27].

We have now purified this CT cross-reactive material using affinity chromatography[24]. This protein produces fluid accumulation in rat ileal loops and infant mice and is cytotonic in Y1

Properties of *Aeromonas* enterotoxins

	MW	Suckling mice	Ileal loops	Cytotonic	Cytotoxic	Haemolytic	CT-cross reaction
Cytotonic	> 27000	+	+	+	−	−	+
Cytotoxic	63000	+	+	−	+	+	−

cells. Like CT, it inhibits platelet aggregation, an effect dependent on elevation of intracellular cAMP[13]. Using SDS-PAGE, molecular weight was estimated to be greater than 27,000 Da, possibly with separation into subunits as reported for LT in a similar system[10,11].

Like the cytotonic enterotoxin cloned by Chakraborty et al.[8], the toxin we isolated caused fluid accumulation in suckling mice. However, those authors found no DNA homology with LT while we found cross-reactivity with CT which is well recognised to be similar to LT immunologically. The lower molecular weight cytotonic enterotoxin described by Ljungh and Kronevi[19] did not cross-react with CT and was negative in suckling mice. We isolated the CT cross-reactive toxin by affinity chromatography and it is possibile that there may be other cytotonic enterotoxins of *Aeromonas* spp. not cross-reactive with CT.

In a study of 201 *Aeromonas* spp. isolated from faeces of children with or without diarrhoea, CT cross-reactive material detected in ELISA was found in 25.8% of *A. sobria*, 20.0% of *A. hydrophila* and 24.3% of *A. caviae*[24]. This contrasts with the findings of Shimada et al.[27] who reported CT-like activity in *A. hydrophila*, a classification which would include *A. sobria* and *A. hydrophila*[22], but not in *A. caviae*. Most strains positive in ELISA also produced cytotoxin and, overall, diarrhoea correlated with production of cytotoxic, not cytotonic toxin[24]. Of 11 strains of non-cytotoxic *A. caviae* positive in ELISA, one was isolated from the same faecal specimen as *Salmonella* spp. in a child with diarrhoea but the other 10 children had no diarrhoea. These findings suggest that the CT cross-reactive toxin alone is not a virulence factor in *Aeromonas* spp.

Cytotoxic enterotoxin

Several reports have documented correlation between haemolysis, cytotoxicity and enterotoxicity of *Aeromonas* spp.[3,9,18]. However, haemolysins of *Aeromonas* spp. without enterotoxic activity have been described[21]. We used affinity chromatography with monoclonal antibodies to characterise the cytotoxin of *Aeromonas* spp.[23]. In SDS-PAGE the purified exotoxin gave a single protein band (MW 63000) which showed no immunological cross-reactivity with CT. This protein caused fluid accumulation in rat ileal loops and infant mice, was cytotoxic in cell culture and produced haemolysis of human erythrocytes. These activities were heat-labile.

These properties were similar to those described by Hostacka et al.[15] and Asao et al.[1] for *Aeromonas* cytotoxin purified using biochemical techniques and are consistent with haemolytic, cytotoxic and enterotoxic activity as properties of a single protein.

The failure of Ljungh et al.[20] to find enterotoxicity associated with the *Aeromonas* haemolysins they isolated is unexplained. One problem in interpreting their results is in their reports of negative suckling mouse tests. Other laboratories have found suckling mouse tests positive with *Aeromonas* cytotoxic[2,3,18,28] and cytotonic[8,14,24] enterotoxins and the reason for this discrepancy is not clear.

In our experience, cytotoxic enterotoxin is most commonly associated with *A. sobria*, less frequently with *A. hydrophila* and not with *A. caviae*[4]. Most children with *Aeromonas*-associated diarrhoea have *A. sobria* isolated; *A. hydrophila* is the predominant species isolated from water[5]. We find *A. caviae* with about equal frequency in children with and without diarrhoea and we would agree that *A. caviae* is of low virulence, as previously suggested[26]. Our data imply that cytotoxic enterotoxin is more likely than cytotonic enterotoxin to be a virulence factor in *Aeromonas*-associated diarrhoea. No doubt other potential virulence factors such as invasiveness and colonisation factors must also be considered.

Clarification of the relative importance of these cytotonic and cytotoxic enterotoxins needs the availability of a simple experimental model for *Aeromonas*-associated diarrhoea.

1 Asao, T., Kinoshita, Y., Kozaki, S., Uemura, T., and Sakaguchi, G., Infect. Immun. *46* (1984) 122.
2 Boulanger, Y., Lallier, R., and Cousineau, G., Can. J. Microbiol. *23* (1977) 1161.
3 Burke, V., Robinson, J., Atkinson, H. M., Dibley, M., Berry, R. J., and Gracey, M., Aust. J. exp. Biol. med. Sci. *59* (1981) 753.
4 Burke, V., Robinson, J., Cooper, M., Beaman, J., Partridge, K., Peterson, D., and Gracey, M., Appl. envir. Microbiol. *47* (1984) 1146.
5 Burke, V., Cooper, M., Robinson, J., Gracey, M., Lesmana, M., Echeverria, P., and Janda, J. M., J. clin. Microbiol. *19* (1984) 39.
6 Burke, V., and Gracey, M., J. Gastroent. Hepat. *1* (1986) 237.
7 Campbell, J. D., and Houston, C. W., Curr. Microbiol. *12* (1985) 101.
8 Chakraborty, T., Montenegro, M. A., Sanyal, S. C., Helmuth, R., Bulling, E., and Timmis, K. N., Infect. Immun. *46* (1984) 435.
9 Cumberbatch, N., Gurwith, M. C., Langston, C., Sack, R. B., and Brunton, J. L., Infect. Immun. *23* (1979) 829.
10 Dafni, Z., and Robbins, J. B., J. infect. Dis. *133* (1976) S138.
11 Dafni, Z., Sack, R. B., and Craig, J. P., Infect. Immun. *22* (1978) 852.
12 Dobrescu, L., Zentrbl. VetMed. (B) *25* (1978) 713.
13 Fumarola, D., and Miragliotta, G., ICRS med. Sci. *9* (1981) 1033.
14 Honda, T., Sato, M., Nishimura, T., Higashitsutsumi, M., Fukai, K., and Miwatani, T., Infect. Immun. *50* (1985) 322.
15 Hostacka, A., Cizenaar, I., Korych, B., and Karolcek, J., Zentbl. Bakt. ParasitKde, I Abt. Orig. A. *252* (1982) 525.
16 James, C., Dibley, M., Burke, V., Robinson, J., and Gracey, M., Clin. exp. Immun. *47* (1982) 34.
17 Jiwa, S. F. H., Vet. Microbiol. *8* (1983) 17.
18 Johnson, W. M., and Lior, H., Can. J. Microbiol. *27* (1981) 1019.
19 Ljungh, Å., and Kronevi, T., Toxicon *20* (1982) 397.
20 Ljungh, Å., Eneroth, P., and Wadström, T., Toxicon *20* (1982) 787.
21 Ljungh, Å., Wretlind, B., and Mollby, R., Acta path. microbiol. scand. Sect. B *89* (1981) 387.
22 Popoff, M., in: Bergey's Manual of Systematic Bacteriology, vol 1, 9th edn, pp. 545–548. Eds N. R. Krieg and J. G. Holt. Williams and Wilkins Co., Baltimore/London 1984.
23 Potomski, J., Burke, V., Watson, I., and Gracey, M., J. med. Microbiol. in press.
24 Potomski, J., Burke, V., Robinson, J., Fumarola, D., and Miragliotta, G., J. med. Microbiol., in press.
25 Sanyal, S. C., Singh, S. J., and Sen, P. C., J. med. Microbiol. *8* (1975) 95.
26 Schubert, R. H. W., Arch. Hyg. Bakt. *150* (1967) 1709.
27 Shimada, T., Sakazaki, R., Horigome, K., Uesaka, Y., and Niwano, K., Jap. J. Med. *37* (1984) 141.
28 Turnbull, P. C. B., Lee, J. V., Miliotis, M. D., van de Walle, S., Koornhof, J., Jeffrey, L., and Bryant, T. N., J. clin. Microbiol. *19* (1984) 175.
29 Wadström, T., Ljungh, Å., Wretlind, B., Acta path. microbiol. scand. Sect. B *84* (1976) 112.

0014-4754/87/040368-02$1.50 + 0.20/0