IV. Virulence factors

Aeromonas – Toxins and other virulence factors

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Aeromonas spp. produce a range of extracellular enzymes and toxins, some of which are potential virulence factors. The production of these factors varies with geographic location as well as source of isolation. So far, no single virulence factor has been implicated in the pathogenesis of gastrointestinal infection.

Cytotoxins

The majority of human isolates of *Aeromonas* are hemolytic, and many of the extraintestinal manifestations of *Aeromonas* infections are accompanied by soft tissue necrosis, indicating that hemolysin is a virulence factor. The most potent of the cytolytic toxins, aerolysin or β-hemolysin, has been purified and characterized[5,6,18]. It is a heat-labile protein with MW 50–51 kDa. It is cytotoxic with a broad cell specificity and binds rapidly to cell membranes also at 0 °C. In human embryonic lung fibroblasts labeled with markers of different MW, β-hemolysin causes release only of low MW markers (< 1000 Da)[25]. Murine erythrocytes which are among the most sensitive erythrocyte species have been shown to contain a glycophorin which was identified as the membrane receptor[10]. β-hemolysin is dermo-necrotic in rabbit skin and is lethal to rabbits and mice[18]. In the rabbit intestinal loop test (RIL) it causes accumulation of small amounts of sanguinolent fluid with high albumin and calcium content, indicating leakage of intracellular substances due to membrane damage, in contrast to the cytotonic enterotoxin (vide infra)[6].

Another hemolysin, alpha-hemolysin, is a weak hemolytic agent which causes incomplete hemolysis of ox erythrocytes with an enzymatic kinetics[25]. It is probably of minor importance in the pathogenesis of infections. Recently a heat-labile cytolytic protein called 'Asao'-toxin was purified and characterized[2]. The MW was 48–50 kDa. It is cytotoxic to Vero cells, hemolytic, lethal to mice and, in contrast to β-hemolysin causes fluid accumulation in RIL. It is antigenically not distinct from β-hemolysin.

Enterotoxins

The presence of an extracellular heat-labile enterotoxin in *Aeromonas* strains has been described by several groups[15]. Separated from hemolytic and enzymatic activities, purified enterotoxin induced intestinal fluid secretion without mucosal damage in rabbits and rats[16,18]. The intestinal fluid had a similar electrolyte composition to fluid secretion induced by cholera toxin, CT, and *E. coli* LT[16]. The onset of secretion was rapid, as for *E. coli* ST, and of shorter duration than with CT. The sensitivity of adrenal Y1 cells to *Aeromonas* enterotoxin was lower than for *E. coli* LT but was increased after treatment of the cells with β-galactosidase or β-glucosidase[17]. In adrenal Y1 cells *Aeromonas* enterotoxin induced cytotonic alterations accompanied by increased cAMP content of the cells and induction of steroidogenesis[17]. Neutralization tests in different assay systems as well as coagglutination tests with anti-LT failed to reveal any immunological relationship between *Aeromonas* enterotoxin and CT or *E. coli* LT. Furthermore, the enterotoxic activity was not inhibited by prior incubation with crude gangliosides, nor did *Aeromonas* bind in G_{M1}-ELISA[17]. It was hence concluded that strains of *Aeromonas* can produce a cytotonic enterotoxin which is immunologically unrelated to CT and does not share the same cell membrane receptor. These findings were confirmed by Chakraborty et al. who cloned a gene from *Aeromonas* coding for a cytotonic enterotoxin which is not neutralized by antiserum to CT[9].

However, recently Shimada et al. reported on the presence of an enterotoxin cross-reacting with CT in 4.5% of *A. hydrophila* (179 strains) but in none of 70 *A. caviae* strains[23]. A CT-cross-reacting factor was further demonstrated in a strain from Texas[8].

Whether or not *Aeromonas* produces a heat-stable enterotoxin has not been clarified. James et al. reported that the fluid secretion induced by heated (100 °C) culture filtrates of *Aeromonas* was inhibited in rats immunized with CT[12] but this secretagogue has not been further characterized.

Proteases

In *Aeromonas* strains at least one heat-labile and one heat-stable protease have been characterized[15]. The possible role of these in the pathogenesis of intestinal disease has not been elucidated, e.g. whether they are mucus-degrading or not.

Elastase, a well-established virulence factor in *Pseudomonas* infections, and staphylolytic enzyme were correlated with virulence in fish infections[11]. The role of these enzymes in human infections has not been studied. However, strains of *Aeromonas* as well as *Plesiomonas shigelloides* commonly produce elastase to varying extent (Å. Ljungh, unpublished).

The incidence of production of these extracellular proteins varies among the three motile *Aeromonas* sp. Generally, *A. caviae* is rarely hemolytic and the incidence of enterotoxin production is lower than for *A. hydrophila* and *A. sobria,* whereas no significant difference has been shown between the latter two species.

Endotoxin

Aeromonas produce an endotoxin, LPS, which has been suggested to enhance infections in frogs, red leg-disease[21]. Whether LPS enhances human infections has not been studied.

Enteroinvasivity

Though keratoconjunctivitis tests with *Aeromonas* strains are negative enteroinvasivity of *Aeromonas* has been shown by histological examination[20] and by invasivity in HEp-2 cells[13]. No common plasmid was detected.

Surface adhesins

Strains of *Aeromonas* isolated from human infections are commonly fimbriated and the number of fimbriae/cell is greater than in strains isolated from environmental samples (Å. Ljung, unpublished). Atkinson and Trust described five different patterns of hemagglutination in *Aeromonas*[4], which were correlated to outer membrane proteins and the presence of fimbriae. These typing schemes were later extended[1,7]. Good correlation was found between hemagglutination of human O erythrocytes in the presence of fucose, mannose and galactose, and biotype and source of isolation[7]. A different pattern of hemagglutination was reported in Indian strains[22]. A soluble hemagglutinin was characterized in strains of all three species[24]. It was inhibited by fetuin but not by simple sugars, and shared several properties in common with soluble hemagglutinin produced by strains of

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.

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Enterotoxins of *Aeromonas* species

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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