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BACTERIAL TOXINS: NEW ASPECTS ON THEIR ROLE IN GASTROINTESTINAL INFECTIONS

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INTRODUCTION

Already Theodor Escherich at the end of the century suspected that certain strains of the indigenous intestinal microflora of young children produced soluble toxins causing diarrhoea and other gastrointestinal diseases. Only about a decade later the major symptoms of both cholera and shigellosis were proposed by other investigators to be caused by extracellular protein toxins (*Guerrant*, 1985; *Wadström* et al., 1986; *Wadström*, 1988).

Despite these early observations it was not until the early seventies, after the characterisation of cholera toxin and cholera-like *Escherichia coli* heat-labile (LT) enterotoxins, that it was demonstrated that many gastrointestinal pathogens produce enterotoxins.

The toxins produced by gastrointestinal pathogens can be divided into three major classes (Table 1):

1: Cytotonic enterotoxins not inducing cell damage of target enterocytes but stimulating cell secretory mechanisms.
2: Cytotoxic enterotoxins taken up into cells and causing cell damage.
3: Cytolytic toxins / membrane damaging toxins.

Toxins of class 1 activate the adenylate and guanylate cyclase systems of enterocytes. *E. coli* STa, belongs to this class while the mode of action of the STb toxin is still not known. However STb producing strains are rarely isolated from human infections but are common in enterovirulent strains of young pigs and calves (*Flock*, personal communication). These cytotonic enterotoxins induce cholera-like watery diarrhoea commonly defined as enterotoxic enteropaties to discriminate these infections from infections with class 2 and 3 pathogens causing epithelial cell damage and inflammation (*Guerrant*, 1985; *Cane* and *Guerrant*, 1989). Interestingly *C. difficile* and *C. jejuni* infections as well as salmonellosis and *Aeromonas* induced diarrhoea are also characterised by an initial phase with watery cholera-like stools probably via yet undefined class 1 toxins.

However *Keusch* and colleagues (1991) described that also the cytotoxic Shiga toxin may act on small bowel epithelium in an early stage of intestinal colonisation to induce watery stools typical of class 1 toxins. Later stages are characterised by epithelial cell invasion and cell death, after intracellular multiplication and synthesis of cytotoxic Shiga toxin of class 2.

We now want to compare and discuss the possible roles of toxins produced by various enteric microbes as additional virulence factors to induce mucosal barrier damage in various stages of acute and chronic intestinal infections and inflammatory bowel diseases such as ulcerative colitis. However, first some aspects on toxigenic *Clostridium botulinum* and other toxin producing microbes in the gastrointestinal tract of young infants will be discussed.

Certain strains of *Clostridium botulinum* in infant food, such as bee honey, can colonise the intestinal tract of young infants with a poorly developed indigenous microflora (*Arnon*, 1990; *Popoff*, 1990) and cause flaccid paralysis of certain muscles and sudden infant death syndrome (SIDS) (*Akotories* and *Just*, 1990).

More recent studies in Japan also suggest that haemagglutinating strains of *C. botulinum* are able to colonise the human intestinal tract while non-haemagglutinating strains seem to lack adhesins to colonise the human gut and induce SIDS (*Tabita* et al., 1991).
Bettelheim and colleagues (1990) have recently reported that Shiga-like toxin (Syn. Verotoxin) producing strains of *E. coli* are commonly isolated from children with SIDS in Australia but not from age-matched control children. Studies in England have shown that strains of coagulase-negative staphylococci (CNS) producing a delta-haemolysin-like cytolytic toxin (class 3 toxin) are commonly isolated from children with SIDS and also from children with certain forms of nicrotising colitis (*Scheifele* and *Bjornson*, 1988). These findings are interesting in the perspective that it has not been ruled out whether potent staphylococcal immunomodulating toxins such as toxic shock toxin 1 (TSS 1) (*Newbould* et al., 1989) and the related enterotoxins within the same toxin superfamily may under certain conditions be associated with SIDS and similar fulminating toxicoses later in life. These toxins are classified today into a new class of toxins often called immunomodulating toxins or superantigens (*Alouf*, 1986; 1991).

It is yet too early to speculate on the possible role of toxins produced by other enteric organisms than haemagglutinating *C. botulinum* to induce SIDS and maybe also similar fulminant toxicoses in older children and adults. However it is most likely that certain strains of potent toxigenic staphylococci

Table 1: Classification of toxins produced by gastrointestinal pathogens

Class 1	Cytotonic enterotoxin	Prototype toxins Cholera toxin *Escherichia coli* LT and ST[1]
Class 2	Cytotoxic toxins	Shiga toxin Shiga-like toxins (Syn. Vero-like toxins) *H. pylori* vacuolising toxin *Clostridium difficile* toxin A[2]
Class 3	Cytolytic toxins (Cell membrane damaging toxins, haemolysins)	Aeromonas alpha and beta toxins *E. coli a*nd Shigella haemolysin *H. pylori* haemolysin[3] *C. difficile* toxin B

[1]Other members of this class are *Salmonella* LT like toxins (*Prasad* et al., 1990; *Stephen*, 1991), and *Campylobacter jejuni* enterotoxin(s). See also: *Cane* and Guerrant, 1989.
[2]Shiga-like toxins or Vero-like cytotoxins is a family of toxins produced by 0157:H 7 and certain other specific serotypes of *E. coli* (*Karmali*, 1989; *Wadström* and *Ljungh*, 1990; *Keusch* et al., 1991)
[3]*Helicobacter pylori* may produce a number of toxins not yet defined, as well as cytotoxic phospholipase A2 and C (*Raedsh* et al., 1989; *Slomiany* et al., 1987; *Slomiany* et al., 1989).

and other "abnormal" organisms in the gastrointestinal tract may be able to colonise when the gut flora has been disturbed by e.g. antibiotic therapy. This field is certainly now open for more investigations.

CLOSTRIDIUM DIFFICILE INFECTIONS

Dubos-Ramare and *Corthier* (1990) reported on the influence of a "low protein protective diet" on toxin production by *Clostridium difficile* in gnotobiotic mice. These studies confirm previous observations that the diet can influence the intestinal microflora and that proteolytic digestion by certain microflora members may be necessary for toxigenic *C. difficile* strains to colonise the gut and produce toxins.

Studies by *Borriello* (1989) indicate that specific surface fimbriae may be the intestinal colonisation factors and necessary for a successful colonisation of the colon to allow toxin delivery at the epithelium level similar to how fimbrial colonisation factors (CFAI, CFAII etc.) allow enterotoxigenic *E. coli* (ETEC) to colonise the small bowel.

It has been speculated that the low sensitivity of children under 2 to 4 years of age to *C. difficile* toxin induced diarrhoea may be due to lack of toxin receptors of immature enterocytes while intestinal colonisation is possible due to specific receptors in the colonised mucosa for *C. difficile* surface adhesins (*Borriello*, 1989). Further research in this area may reveal a new strategy to prevent *C. difficile* infections by oral feeding with nontoxigenic strains with good ability to colonise the colon mucosa.

STAPHYLOCOCCAL ENTEROCOLITIS

Before the discovery of *C. difficile* as a common cause of antibiotic associated enterocolitis, certain strains of *S. aureus* were suggested as the major cause of nosocomial enterocolitis (*Kapral*, 1986). However, studies in recent years have not confirmed the role of enterotoxigenic *S. aureus* strains as a common cause of enterocolitis but recent observations that certain strains of coagulase negative staphylococci producing not yet defined cytolytic toxins (*Scheifele* and *Bjornson*, 1988) suggest that further research in this area is needed.

OTHER AETIOLOGIES FOR ACUTE AND CHRONIC ENTEROCOLITIS

C. difficile as well as other enteric pathogens such as *C. jejuni* and *Aeromonas hydrophila* have been reported to cause both acute and chronic infections of the small and large bowel of children and adults. Both *C. jejuni* and *A. hydrophila* infections are commonly associated with consumption of certain foods. Acute *Aeromonas* cholera-like infantile diarrhoea is common in many developing countries (*Ljungh*, 1987; *Wadström* and *Ljungh*, 1990) associated with high bacterial counts in certain waters, especially during the warm seasons. We know very little about the environmental reservoirs of enterotoxin and cytotoxin producing *Aeromonas* (*A. hydrophila, A. sobria, A. caviae* and a few more species) in water and food products in countries

with temperate climates and low hygienic standards (*Wadström* and *Ljungh*, 1991). Moreover, certain cytotoxin producing as well as non-cytotoxin producing *Aeromonas* belonging to the indigenous gut microflora of pigs may also colonise humans. However few gut microflora studies of humans involving selective search for these oxidase positive "haemolytic *E. coli*-like organisms" have yet been carried out. Interestingly, a recent report from England suggest that certain strains of *Aeromonas hydrophila* are associated with chronic colitis (*Grimminger*, 1990; *Willoughby* et al. 1989). Recent observations that certain *Bacteroides* strains in both animal and man can produce enterotoxins indicate that more studies have to be performed to diagnose possible new pathogens in the aetiology of acute, sub-acute as well as relapsing forms of enterocolitis, especially after travelling to countries with warm climates. We have initiated a study in Lund to define the possible role of toxigenic aerobes in patients with intestinal symptoms that remain for more than a week after returning home from travels in southern Europe or other continents.

TOXIGENIC ENTERIC BACTERIA AND ULCERATIVE COLITIS

Studies in the sixties showed that haemolytic *E. coli* were commonly isolated from young pigs with diarrhoea (*Thayer*, 1987). Such strains were later shown to produce also heat-labile and heat-stable (LT and ST) enterotoxins (*Järnerot*, 1986; *Wadström* and *Ljungh*, 1990).

Early studies on the human faecal flora showed that haemolytic *E. coli* strains were more commonly isolated from patients with chronic colitis and patients with subacute phase of ulcerative colitis (*Fiocchi*, 1986). More recently, tests for cytotoxins in stools of such patients and isolated *E. coli* strains revealed that strains produced also the non-haemolytic Shiga-like toxin (*Ljungh* and *Wadström*, 1988; *Ljungh* et al., 1991). Apart from direct toxic effects on enteric cells, this group of toxins may induce e.g. platelet aggregation which can be important in the pathogenesis of ulcerative colitis (*Rose* et al., 1985). Interestingly, further studies on surface properties of these toxigenic strains revealed specific heat and protease sensitive structures binding to various subepithelial extracellular matrix (ECM) components such as fibronectin, vitronectin and various collagens (*Ljungh* and *Wadström*, 1988; *Ljungh* et al., l991). Certain strains of *E. coli* and maybe also other enteric micro-organisms may thus be able to colonise in mucosal lesions of ulcerative colitis. However, despite the fact that antibiotics seem not to have effects on relaps-

Table 2: Putative effects of *Helicobacter pylori* on the human gastric epithelium

Phospholipases:	- Destruction of the hydrophobic lining on the mucus layer (*Raedsch* et al., 1989; *Slomiany* et al., 1987, 1989)
	- cytotoxic effects on the epithelium
Ammonia produced close to the cell surface	
Cytotoxins	
Vacuolising toxin(s)	
Cytolytic toxin(s)[1]	

[1] No isolation procedure for cell associated cytolytic toxin(s) has yet been published; neither it has been published whether certain strains produce extracellular as well as cell associated toxins.

ing ulcerative colitis (*McLaren* and *Gutnick*, 1982) it seems tempting to speculate that certain toxigenic *E. coli* and other organisms may precipitate relapses by causing damage of the mucosal barrier.

POSSIBLE ROLE OF THE *LACTOBACILLI* AND OTHER MEMBERS OF THE GUT FLORA TO COMBAT INTESTINAL DAMAGE BY TOXIGENIC MICROBES

Studies in recent years in Uppsala and Copenhagen (*Tvede* and *Rask-Madsen*, 1990) showed that faecal enemas have a dramatic effect on intestinal symptoms in patients with acute and relapsing *C. difficile* enterocolitis. *Streptomyces boulardii* may also combat toxigenic *C difficile* and aid in restoring a normal colonic microflora. It is also likely that more recent research on antibiotic-like bacteriocins of various lactobacilli will make it possible to design one or two strains for successful therapy of *C. difficile* infections.

It is yet too early to speculate about the possibility to use such organisms in the treatment of ulcerative colitis.

HELICOBACTER PYLORI - A TOXIGENIC GASTRIC PATHOGEN

Marshall and *Warren* rediscovered spiral gastric pathogens in 1983 (*Rathbone* and *Hartley*, 1989; *Peterson*, 1991) with the first successful culture of a micro-aerophilic pathogen initially called *Campylobacter pylori*, now known as *Helicobacter pylori*. Organisms closely related to *H. pylori* have been isolated more recently also from primates, ferrets *(H. mustelae)* and cats (*H. felis)*. High motility and high urease production are necessary for the microbe to colonise the gastric mucosa and to induce so called "type B gastritis". The formation of ammonia from urea at the gastric epithelium (*Smoot* et al., 1990; *Turbett* et al., 1991), which the organism colonises by specific surface adhesins, is very toxic for the cells. Close cell adherent *H. pylori* can probably deliver ammonia directly on the epithelial cells but may also damage the intercellular tight junction during invasion down to subepithelial tissues and survive as coccoidal forms (*Jones* and *Curry*, 1990).

Several investigators have described the following toxins produced by strains of *H. pylori* isolated from gastric and duodenal ulcer lesions (Table 2):

1. Haemolysins also cytolytic for tissue culture cells (*Gregor* et al., 1990, *Wadström* and *Ljungh*, unpublished observations)
2. Vacuolising toxins (*Blaser*, 1990; *Cover* et al., 1990; *Leunk* et al., 1990).
   It is not clear whether the toxins described by these investigators are identical.
3. Phospholipase A2 and C: The possible cytolytic effects of these cell membrane active cell toxic enzymes (*Möllby*, 1978; *Raedsch* et al., 1989) have not yet been studied while they have been proposed to destroy the normal hydrophobic mucosal cell lining of the gastric epithelium (*Wadström* and *Aleljung*, 1990).
   H. pylori toxins have also been proposed as important virulence factors in the development of stomach and duodenal ulcer diseases (*Rathbone*, 1989) and for development of the

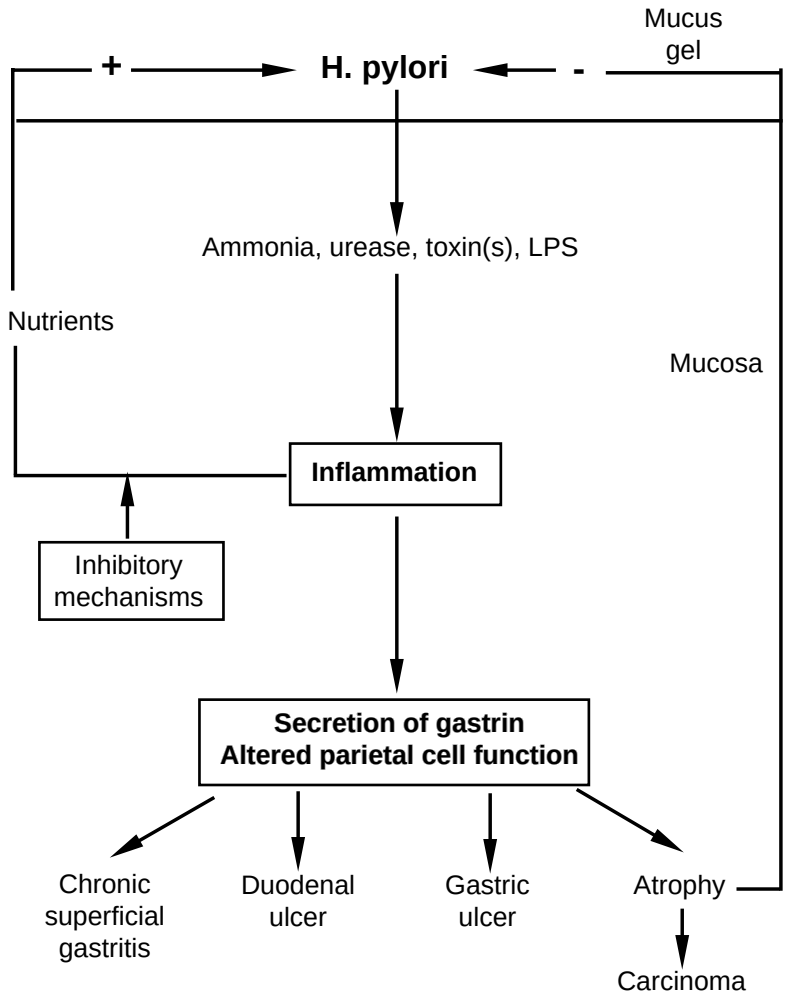

Figure 1: Model for the relationship between *Helicobacter pylori* products, inflammation and gastroduodenal pathology (modified after *Blaser*, 1990).

lesions in oesophagitis.

4. Very low levels of extracellular toxin production seems common among *H. pylori* strains as reported by several investigators (*Blaser*, 1990; *Leunk* et al., 1990). However, it is too early to speculate if *in vivo*-like growth conditions, such as adding gastric mucins in fractions to culture medium, may enhance toxin production. It is also possible that certain cells of the human gastric epithelium are more susceptible to the toxin(s) than HeLa cells or other *in vitro* tissue culture cells used for screening for *H. pylori* toxins. Our own studies confirm a recent report by *Smoot* and co-workers (1990) that the vacuolising cytotoxin of *H. pylori* does not produce drastic effects on the cell membrane and changes in membrane permeability. The possible role of this toxin and cytolytic (haemolytic) toxins in the pathogenesis of *H. pylori* infections is thus still quite obscure. However, strains which are

associated with stomach and duodenal ulcer disease may be more potent toxin producers than strains isolated from patients with mild and often symptom free acute or chronic gastritis. Interestingly, patients also produced antibodies to cytolytic toxins described by *Figura* and *Blaser*, but a high serum antibody titre does not seem to influence the cause of the disease (*Leunk* et al., 1990; *Cover* et al., 1990; *Wadström*, in preparation). It thus seems likely that toxin formation may be important just in the first initial stages of gastritis and development of acute duodenal and stomach ulcer disease.

Studies in Brussels indicate that more than half of the patients with *H. pylori* infections respond with an antibody titre to crude *H. pylori* toxin(s) as determined in toxin neutralisation tests (NT) in tissue culture assays (*Goosens*, personal communication). Moreover, biotyping of *H. pylori* strains from various geographic regions suggests that toxin(s) are commonly produced by different biotypes of strains. However, studies in Lund on strains isolated from patients with gastritis and ulcer disease show that toxin production occurs only at low levels in Swedish strains (titre <1/8-1/16). Work is now in progress in several laboratories to explore how to enhance toxin production in laboratory cultures in order to purify toxin(s) and phospholipase C which, like other bacterial phospholipases, is probably per se cytolytic.

A model for the relationship between *H. pylori* products, inflammation and gastroduodenal pathology is presented in Figure 1.

FUTURE PROSPECTIVE

The discoveries in the last two decades of a number of new toxins and produced by "old and new" enteropathogens or putative enteropathogens such as *Aeromonas hydrophila* will stimulate further studies on how to prevent gut colonisation and diseases induced by such microbes. The great complexity of toxins and toxin families (such as Shiga-like toxins) indicate that vaccines seem less likely as prophylaxis for these infections but that new strategies to develop the probiotic concept to prevent and combat these infections by various toxigenic enteric organisms is now a fruitful area to explore for both human and animal medicine.

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