MINIREVIEW

Escherichia coli STb toxin and colibacillosis: knowing is half the battle

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Abstract

Expression of both adherence and enterotoxin expression are required for enterotoxigenic *Escherichia coli* (ETEC) strains to cause colibacillosis. ETEC strains are responsible for diarrhea in humans and animals by production of various enterotoxins. For many years, the role of the heat-stable *E. coli* enterotoxin STb as a diarrhea-causing toxin in animals, and in particular in swine, has been controversial. In fact, although the presence of STb-positive *E. coli* strains and diarrhea in animals is frequently observed, the difficulty of reproducing the pathology in an animal model was interpreted as a lack of toxicity. Recently, new light was shed on the activity of STb in intestinal ligated loops and in pigs orally inoculated with STb-positive *E. coli* strains. This minireview revisits the effects of STb on the intestinal epithelium and enlightens the significance of STb in swine colibacillosis. The interaction of STb toxin with other *E. coli* enterotoxins and dual ETEC/enteropathogenic *E. coli* or ETEC/attaching effacing *E. coli* infections are also discussed.

Introduction

Enterotoxigenic *Escherichia coli* (ETEC) strains have important implications for the farming industry, as a single etiological agent, where it is a major pathogen of cattle and neonatal and postweaning piglets (Nagy & Fekete, 1999). ETEC provokes severe and sudden onset of symptoms, frequently followed by death. Piglets older than 1 week of age experience lower mortality rates but significant weight loss and their growth is retarded. Thus, the age of the animal in ETEC colibacillosis (a diarrheal disease caused by *E. coli*) influences the outcome of the disease.

ETEC causes diarrhea in animals and humans by first colonizing the small intestine and then producing toxins responsible for fluid secretion (Quadri *et al.*, 2005; Turner *et al.*, 2006b). Adherence is mediated by colonization factors. These can be filamentous surface appendages called fimbriae on which adhesins, specific proteins involved in binding to eukaryotic cells, are found. Afimbrial adhesins are also found on *E. coli* (Fekete & Nagy, 1999). Loss of colonization factors from the bacteria leaves them unable to colonize and cause disease (Gaastra & Svennerholm, 1996). Numerous types of fimbriae are expressed by ETEC strains causing diarrhea in pigs. These include F4 (K88), F5 (K99), F6 (987P), F7 (F41) and F18. In addition, fimbrial adhesins F4 and F18 occur in several antigenic forms. The F4 and F18 variants are, respectively, F4ab, F4ac and F4ad, and F18ab and F18ac (Francis, 2002). Colonization of the intestinal mucosa allows for the localized delivery of enterotoxins. The *E. coli* enterotoxins are classified based on their thermal stability. They are divided into heat-labile (LT-I and LT-II) and heat-stable (STa, STb and EAST1) toxins. Colibacillosis results from ingestion of ETEC strain(s) that possess these virulence factors (Fig. 1).

LT, an 88-kDa molecule, belongs to the AB_5 class of bacterial toxins. LT shows 80% identity with the cholera toxin (CT) in the primary (Dallas & Falkow, 1980; Spicer *et al.*, 1981) and superimposable tertiary structures (Sixma *et al.*, 1991). Two subtypes of LT, LT-I and LT-II, are known. Furthermore, these subtypes can be divided into, respectively, LT-Ih (human) and LT-Ip (pig), and LT-IIa and LT-IIb. Whereas LT-I is associated with human disease, LT-II is associated primarly with animal-specific ETEC. LT-I and LT-II have similar biological activities but are immunologically distinct.

EAST1, a 38-amino-acid toxin, is less well characterized structurally and functionally than LT. Although originally isolated from an enteroaggregative *E. coli* strain (EAEC strain 17–2), it has now been found in other pathotypes

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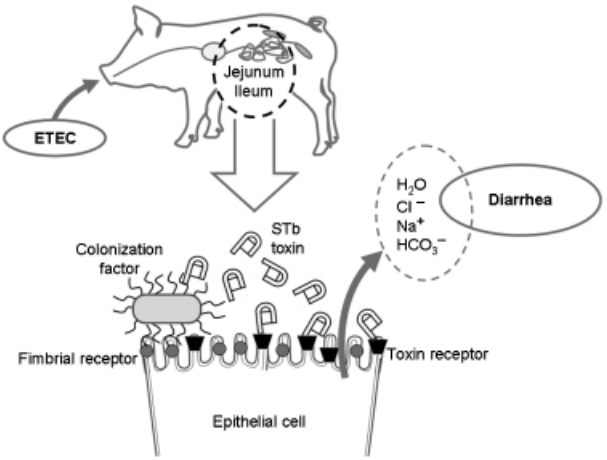

Fig. 1. Colibacillosis resulting from an enterotoxigenic *Escherichia coli* infection in piglets. The animal is contaminated by ingestion of ETEC. These bacteria colonize the small intestine (jejunum and ileum) through specific interactions between colonization factors and receptors on the epithelial intestinal cells. Enterotoxins are elaborated by ETEC (STb toxin is shown) and released in the intestinal lumen. These molecules bind to receptors and activate pathways specific to each toxin. The end result is the secretion of water and electrolytes in the feces and the condition known as secretory diarrhea.

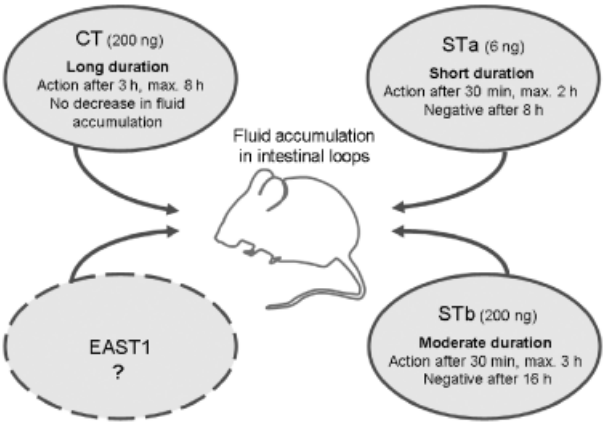

Fig. 2. Comparison of the potency of toxins produced by ETEC as evaluated using mice intestinal loops (Hitotsubashi *et al.*, 1992a). This study indicated that in ligated intestinal loops, the action of each toxin was observed after varying time periods and lasted from a short (STa) to longer period (STb and CT). For CT (a toxin with similar toxicity to LT), no decrease of fluid was observed after the maximal action was observed whereas re-absorption of fluid was seen for STa and STb after 8 and 16 h, respectively. The amount of toxin added to observe comparable fluid secretion is indicated in the corresponding ovals. For EAST1, no test was done as the toxin was only recently purified (Ménard *et al.*, 2004).

including ETEC strains of human and animal origin (Veilleux & Dubreuil, 2006).

STa and STb can be categorized on the basis of their solubility in methanol and protease resistance. STa is methanol soluble, protease resistant whereas STb is methanol insoluble and protease sensitive (Burgess *et al.*, 1978). On the other hand, STb shares no homology with STa either at the structural or the functional level. STa has been characterized according to the host from which the ETEC strain was isolated. STh (19 amino acids) is produced by ETEC strains infecting solely humans whereas STp (18 amino acids), initially from a strain infecting pigs, has also been observed in strains of bovine and human origin (Nair & Takeda, 1998).

STb, comprising 48 amino acids, is a toxin primarily associated with porcine ETEC strains (Handl & Flock, 1992). STb is rapidly acting, inducing a diarrhea of moderate duration. In mouse intestinal loops, STb elicits a fluid response within 30 min and accumulation is maximal in *c.* 3 h (Hitotsubashi *et al.*, 1992b). STb was considered to be inactive in the intestine of animals other than pigs. This conclusion was due to inactivation of the toxin by trysin (and trypsin-like) proteases found in the gut of animals (Whipp, 1987). Addition of a protease inhibitor, as soybean trypsin inhibitor, protected STb from proteolysis and so the toxin activity could be observed in rats and mice (Whipp, 1987; Dubreuil *et al.*, 1991; Fujii *et al.*, 1991; Hitotsubashi *et al.*, 1992a). In piglets less than one week old, the number of strains producing STb only is lower (Moon *et al.*, 1986). In general, the number of strains in which STb is present increases with the age of the animal (Dubreuil, 1997). Hitotsubashi *et al.* (1992a) showed that to induce a comparable fluid accumulation in the mouse intestinal loop assay, the quantity of STb toxin needed was *c.* 35 times more than STa. For LT (CT was used as it shows a similar action and it was available as pure toxin) and STb the same quantity of toxin induced the same fluid accumulation (Fig. 2).

This minireview revisits the action of STb on the intestinal epithelium and discusses recent knowledge regarding the importance of STb toxin in pig colibacillosis. It also considers the interactions of STb with other *E. coli* toxins *in vivo*.

Mechanism of action of ETEC toxins

LT toxins

Enterotoxins disrupt intestinal fluid homeostasis and cause hypersecretion of fluid and electrolytes through activation of specific pathways. The cell surface receptor to which LT toxin binds depends on the B subunit. The B subunit for LT-II is different to the B subunit of LT-I (Sprangler, 1992). The difference translates into the ability for LT-I to bind preferentially to GM1 (but also binds weakly to GM2 and asialo-GM1) whereas LT-IIa binds most efficiently to ganglioside GD1b and LT-IIb binds to GD1a (Fukuta *et al.*, 1988). The B subunit binds to the membrane receptor to mediate entry of the enzymatically active A subunit into the

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target cell. After the B subunit contacts the cell surface, the disulfide bond connecting the A to the B subunit is reduced, allowing its entry into the host cell. The A subunit is cleaved proteotically into A_1 and A_2 domains (Lencer *et al.*, 1997). Inside the cell the A_1 domain ADP-ribosylates a stimulatory G protein (Gsα), which is part of the adenylate cyclase complex (Spangler, 1992). LT thus activates adenylate cyclase in enterocytes of the small intestine causing an abnormal increase in intracellular concentrations of cyclic AMP (cAMP) (Nataro & Kaper, 1998). Then, activation of cAMP-dependent protein kinase A and phosphorylation of Cl^- channels, such as the cystic fibrosis transmembrane conductance regulatory chloride channel (CFTR), results in stimulation of Cl^- secretion from crypt epithelial cells and inhibition of Na^+ absorption by villous enterocytes, with resultant secretory diarrhea (Sprangler, 1992; Rappuoli *et al.*, 1999). Arachidonic acid metabolism with production of prostaglandin E_2 (PGE_2) and 5-hydroxytryptamine (5-HT, or serotonin) with stimulation of intestinal secretion was also reported (Nataro & Kaper, 1998).

STa toxin

STa binds to a trans-membrane guanylate cyclase type C receptor (GC-C), and activates the guanylate cyclase domain in enterocytes, resulting in increased intracellular concentrations of cyclic GMP (cGMP) (Vaandrager, 2002). Increased cGMP levels activate the CFTR through cGMP-dependent protein kinase II phosphorylation. This activation results in enhanced Cl^- and H_2O secretion and inhibition of Na^+ absorption in the small intestine (Goldstein *et al.*, 1994; Tien *et al.*, 1994; Sears & Kaper, 1996; Vaandrager, 2002).

EAST1 toxin

The role of EAST1 in mediating diarrhea remains controversial (Ngeleka *et al.*, 2003). *In vivo* assays have shown that it can induce fluid secretion in suckling mice and in rabbit ileal loops. The toxin also demonstrated biological activity in Ussing chambers (Savarino *et al.*, 1991, 1993). EAST1 was shown to increase cGMP concentrations within enterocytes (Savarino *et al.*, 1993). It is believed that this toxin, based on structural similarities with STa (the functional regions of STa and EAST1 share 50% identity) and the elevation of cGMP levels, might act in a similar way to STa toxin. The effects of EAST1 on induction of electrolyte loss from the intestine have not yet been determined (Berberov *et al.*, 2004).

STb toxin

STb is mostly associated with porcine ETEC isolates but it has also been reported in human isolates (Lortie *et al.*, 1991; Okamoto *et al.*, 1993). STb binds to sulfatide, an acidic glycoshingolipid, widely distributed on epithelial cells (Rousset *et al.*, 1998). The toxin has to be internalized to stimulate fluid secretion (Labrie *et al.*, 2002). Once inside the cell, a pertussis toxin-sensitive GTP-binding regulatory protein is stimulated (Dreyfus *et al.*, 1993), resulting in an influx of calcium through a receptor-dependent ligand-gated calcium channel activating calmodulin-dependent protein kinase II. This activates the opening of an intestinal ion channel and may also activate protein kinase C and consequently CFTR (Dreyfus *et al.*, 1993; Fujii *et al.*, 1997). The increased calcium levels could regulate the activities of phospholipases A_2 and C and release arachidonic acid from membrane phospholipids leading to the formation of PGE_2 and 5-HT, which mediate H_2O and electrolytes transport out of the intestinal cells (Hitotsubashi *et al.*, 1992b; Harville & Dreyfus, 1995; Peterson & Whipp, 1995). The quantity of PGE_2 is proportional to the volume of fluid released into the intestinal lumen. The level of PGE_2 in the intestinal intraluminal fluid increases as a result of the action of STb and prostaglandin synthesis inhibitors significantly reduce the response to STb (Fujii *et al.*, 1995; Dubreuil, 1999). STb stimulates secretion of HCO_3^- from enterocytes, which causes its accumulation, along with increased concentrations of Na^+ and Cl^-, in the intestinal lumen (Dubreuil, 1997). Another hypothesis concerns the activation of the enteric nervous system by PGE_2 and 5-HT (Sears & Kaper, 1996; Dubreuil, 1997). Thus, STb toxin induces diarrhea in animals without activating adenylate or guanylate cyclases (Hitotsubashi *et al.*, 1992b). For STb, the mechanism of action differs from that for LT and STa. This conclusion was made as a combination of maximal doses of STa and STb yielded additive effects on fluid accumulation (Peterson & Whipp, 1995).

ETEC are implicated in diarrheal disease in the young of a variety of animal species. Those implicated in diarrhea in pigs produce various combinations of LT, STa, STb and EAST1. STb-induced colibacillosis seems to depend on the age of the animal as before one week of age the number of strains producing only STb is lower than in older animals whereas the prevalence of STa is increased (Moon *et al.*, 1986; Handl & Flock, 1992). The incidence of STb is highest in pigs older than 1 week of age (Moon *et al.*, 1986; Söderlind *et al.*, 1988; Handl & Flock, 1992). The small intestine apparently becomes more susceptible to enterotoxins immediately after weaning (Stevens *et al.*, 1972).

Some aspects of the action of STb have been proposed and include mainly secretion of electrolytes due to activation of pathways without structurally affecting the epithelial cells of the intestine. This mode of action is debatable when we consider studies that have been conducted by various research teams with cell-free culture supernatants of STb-positive strains (Whipp *et al.*, 1985, 1987; Rose *et al.*, 1987). These experiments, conducted before purified STb toxin was

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available, showed histological alterations of the epithelium that could relate to the secretion observed as well as the decreased absorption. As these modifications can account, at least in part, for the diarrhea associated with STb, they are discussed in the next section and put into perspective given recent results obtained by our group.

STb and cellular alterations

STb is classified as a cytotonic toxin and by definition such toxin does not have to affect cellular structures to produce its action. Complying with such a definition, early studies by Kashiwazaki *et al.* (1981) indicated that STb-positive strains (T2 and UK/A) when tested in porcine ileal loops induced diarrhea. The toxin-treated tissue stained with hematoxylin and eosin, observed by light microscopy, revealed that villous and crypt epithelial cells remained apparently intact. Under the scanning electron microscope, bacteria were scattered over the villous epithelium in intestinal segments exposed to strain T2. In addition, Kennedy *et al.* (1984), using crude culture filtrates of STb-positive strains, noted no obvious differences in brush border, crypt cell or villous cell morphology. No evidence of cellular damage or inflammation was recognized.

Nevertheless, in 1985, the first report of induction of cellular alterations by STb was published (Whipp *et al.*, 1985). STb was reported to affect the villous length in pig jejunal loops but crypt depth was not affected by the toxin (Fig. 3). STb-positive cell-free filtrate markedly reduced the rate of net fluid absorption. The loss of villi, provoked experimentally by a virus (transmissible gastroenteritis virus) significantly decreased the response to STb-positive cell-free filtrate, indicating that differentiated villous epithelium is required to observe a maximal response to STb as this response may be mediated by the villous epithelium.

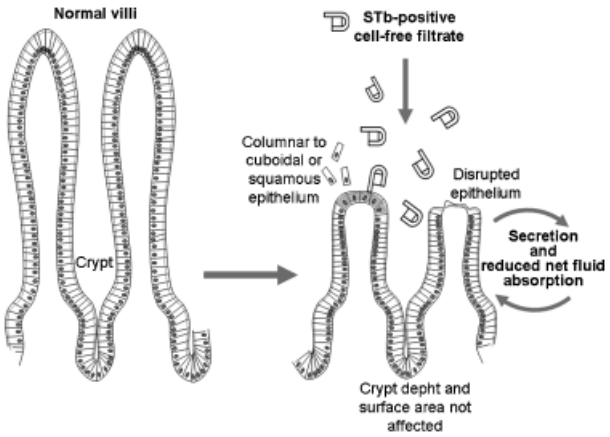

Fig. 3. Cellular structural alterations reported for STb-positive culture supernatant. In pig jejunal loops, addition of STb-positive cell-free culture filtrate affected the epithelial villi. Shortening of villi (between 11 and 19%) was observed with loss of absorptive cells. Normal columnar epithelial cells were replaced by cuboidal or squamous epithelial cells. The epithelium could also be disrupted at the villus tip. On the other hand, crypt depth and surface area were not affected by STb. These cellular microscopic alterations could account for the increased secretion and reduced net fluid absorption observed.

In another study, Whipp *et al.* (1986), using crude culture filtrates of STb-positive strains and jejunum intestinal loops, examined the effect of STb on the intestinal mucosa. Exposure of swine jejunum to STb induced microscopic structural alterations of the intestinal mucosa including shorter villi (11–19% decrease, i.e. loss of villous absorptive cells and partial atrophy), an increased number of sloughed epithelial cells in the lumen, a shift from columnar to cuboidal epithelium on the shorter villous tips and an increased occurrence of disrupted epithelium at the villous tips, consistent with a compromised absorptive capacity (Fig. 3). The STb preparation that caused the least microscopic alterations also induced the least change in net fluid movement.

A subsequent study confirmed the previous observations, i.e STb causes morphological lesions seen as a loss of villous epithelial cells and partial villous atrophy (Whipp *et al.*, 1987). Again, no detectable difference in crypt surface area was observed. The mucosal surface area in loops containing STb was about 20% less than in controls. Sucrase activity was also lower (*c.* 15%) and this observation was functionally correlated with villous atrophy. The histological alterations reported were subtle but highly reproducible. Taking the data from these studies together, we can conclude that the lesions observed could reflect a loss of absorptive cells, suggesting that STb could impair absorption as well as induce net secretion.

Using morphometric techniques, Rose *et al.* (1987) indicated that both villous epithelial surface area and mucosal volume were significantly smaller in STb-positive loops. Pig, rabbit and lamb ligated intestinal loops were tested for a response to STb. Positive responses were seen for pig and lamb but not for rabbit. Histological examination of the tissues from these three animal species revealed epithelial changes in porcine and ovine tissues only. In these two species, epithelium at the villous tips was cuboidal or squamous, or even absent. Some villi were partially denuded and gaps in the epithelium were also observed (Fig. 3). These changes were seen as early as after 30 min of incubation in pigs. Secretion and histological damage were both seen in pigs. The results support the idea that a species' ability to secrete in response to STb is a prerequisite for induction of damage by STb, corroborating the study of Whipp *et al.* (1986). No secretion was detected that was not accompanied by some level of villous atrophy. Although secretion did not occur in the absence of lesions, STb-induced morphological

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changes have been observed in pig loops in the absence of secretion (Whipp *et al.*, 1986).

Thus, despite the fact that histological damage to the small intestinal epithelium (mainly loss of villus epithelial cells and villus atrophy) has been associated with STb in the above studies, based on transmission electron microscopy observations, no mechanism to explain such histological damage was put forward. Nevertheless, it is clear that such damage could contribute to the host diarrheal response. No work has been conducted on this aspect since the toxin was purified and this situation suggests to us that the STb-containing culture filtrate used contained other molecules that may have been responsible for the lesions observed.

Microscopic histological damage to intestinal epithelium has been reported for STb. Using pure STb toxin, we recently determined that STb induces pore formation in brush border vesicles from pig jejunal intestinal tissue (Gonçalves *et al.*, 2007). These pores could account for the secretion of electrolytes observed in STb-provoked colibacillosis. The pores formed were nonspecific, allowing passage of anions and cations. As some pore-forming toxins can kill eukaryotic cells, STb could be responsible for cell death, resulting in observable alterations of the intestinal epithelium.

Relative importance of ETEC toxins in porcine colibacillosis

The contribution of STb to the pathogenesis of ETEC-induced diarrhea was of debate for many years as it is often found associated with other toxins (Moon *et al.*, 1986) and the diarrhea observed could be due to toxins other than STb.

A study on the role of STb was conducted by Casey *et al.* (1998) using F41-positive isogenic strains containing or lacking the STb gene. Strain 226M-pRas1 adhered and expressed STb *in vivo*, causing fluid secretion in ligated ileal loop in neonatal pigs. However, the same strain orally inoculated in neonatal pigs (less than 8 h old) caused very mild diarrhea with no weight loss compared with controls, 18 h following inoculation. One explanation for these results could be that STb plays a role in diarrhea in older and weaned pigs but is not as responsible for colibacillosis in newborn pigs (Vu-Khac *et al.*, 2006a,b). This fact could not be tested with the $F41^+$ ETEC strain used in the study as the F41 receptor is not present in older pigs. All strains (STb-positive and -negative) adhered similarly in ligated loops in neonatal pigs, suggesting that there could be differences in the bacterial growth rates in pigs orally inoculated compared with the parent strain containing different cloned enterotoxin genes. Another issue was the stability of the plasmids introduced into the parent strain. This effect was ruled out as 90% of the colonies retained the STb gene. Several hypotheses were proposed to account for the paradox of positive fluid accumulation in intestinal loops and the mild diarrhea observed in orally inoculated animals. First, the secretion caused by STb could be of shorter duration than that due to STa. It is also possible that STb toxin acts more slowly than STa and so the experiment in orally inoculated animals should be pursued for a longer period of time. At least in mice intestinal loops, Hitotsubashi *et al.* (1992a) indicated that STb was a toxin of short duration that acted more slowly than STa. Perhaps more importantly, the secretion caused by STb is reabsorbed in either the small intestine or the large intestine. This phenomenon, if occurring, could probably not be observed in confined intestinal loops.

More recently, Berberov *et al.* (2004) evaluated the relative importance of EAST1, LT and STb toxins in gnotobiotic piglets. The parent strain expressing these toxins was F4-positive and the piglets were expressing receptors for the F4 fimbriae. Sixty per cent of the piglets inoculated with an LT-negative mutant developed severe dehydrating diarrhea and septicemia compared with 100% of animals inoculated with the parent LT-positive strain. The mean rate of weight loss of animals inoculated with the LT-negative mutant was 67% lower. Nevertheless, inactivation of LT did not change the outcome of serum chemistry results, suggesting that the other toxins expressed by the ETEC (STb and EAST1) strain had considerable effects on water and electrolyte losses similar to those caused by LT. Moreover, this study demonstrated, via immunoblot or enzyme-linked immunosorbent assays, that EAST1, LT and STb could be concurrently expressed by porcine ETEC strains. Thus, EAST1 and STb contributed to the pathology observed, given that 60% of piglets receiving the LT-negative strain developed severe dehydrating diarrhea. The effect of LT was additive with those of STb and EAST1 in strains producing all three toxins. In humans, a similar conclusion was reached as Sjoling *et al.* (2006), who found that the genes for STa (*eastA*) and the B subunit of LT (*eltB*) were both expressed *in vivo*, as revealed by mRNA expression in bacteria directly isolated from patient stools, without subculturing. Compared with *in vitro* grown bacteria, there was no significant up- or down-regulation of the expression levels of the genes studied.

Zhang *et al.* (2006) examined the significance of LT and STb in porcine colibacillosis in an additive model of pathogenicity. Using an F4-positive ETEC strain, enterotoxin-positive and enterotoxin-negative isogenic strains were constructed. The parental strain used was *astA*-positive. All F4-positive strains constructed colonized the small intestine of piglets exhibiting the F4 receptor. Only LT-and STb-positive strains caused appreciable diarrhea. Piglets inoculated with the F4-positive *astA* LT-positive strain became dehydrated within 18 h, whereas those inoculated with the F4-positive *astA* STb-positive strain did not, although diarrhea developed in several piglets. Fifty gnotobiotic piglets were orally inoculated at 5 days of age and none of the piglets that

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lacked F4 receptors developed any clinical signs of diarrhea. This study provides direct evidence that both LT and STb contribute to diarrhea resulting from infection with an ETEC strain. However, the effect of LT on the host was substantially greater than that of STb. Individual piglets showed variation in illness when orally inoculated with an STb-positive strain, ranging from no to moderate clinical signs. Piglets in the Berberov *et al.* (2004) study were 9 days old at challenge and developed severe dehydrating diarrhea following inoculation with an LT-negative mutant that expressed STb and EAST1. Whether EAST1 was expressed from the *astA* gene in the parent strain was not verified by Zhang *et al.* (2006). LT and STb expressed by F4-positive *E. coli* contribute significantly to dehydrating diarrhea in piglets. The clinical responses of 11 piglets to STb were variable, ranging from normal to watery diarrhea and moderate dehydration. Six of the 11 piglets developed diarrhea while five did not. The 11 animals challenged with LT-and STb-negative constructs remained clinically normal, showing neither diarrhea nor dehydration. Overall, the findings indicated that both LT and STb caused dehydration in challenged piglets, but the level of dehydration caused by the LT construct was greater than that caused by the STb construct.

These authoritative studies using isogenic strains and differential expression of ETEC toxins clearly demonstrated, for the first time, that STb was able to provoke diarrhea in piglets. Compared with LT, the magnitude of the disease caused by STb was less. On the other hand, given that the expression of EAST1 toxin was not tested and no diarrhea was associated with the parent strain containing the *astA* gene (Zhang *et al.*, 2006), we can conclude that the observed diarrhea is solely due to STb unless there is a synergistic effect for EAST1 and STb.

To support the importance of STb toxin, Chapman *et al.* (2006) compared the virulence gene profile of 75 *E. coli* strains isolated from healthy and diarrheic swine. They use a combination of uni-and multiplex PCR assays targeting virulence genes associated with *E. coli* strains causing intestinal and extraintestinal diseases in humans and other mammals. Commensal *E. coli* strains from healthy pigs and clinical isolates associated with neonatal and postweaning diarrhea were also used. The study indicated that EAST1, not previously identified as an important virulence gene, in clinical porcine isolates in Australia was associated with clinical isolates from diarrheic swine and not with commensal strains. Clearly, STb toxin originally associated with diarrhea was confirmed as an important virulence gene. The STb gene was found to be significant in distinguishing between commensal isolates and isolates from the diarrhea of newborn and postweaning pigs. The STb gene was associated with 25% of cases of neonatal diarrhea and 81.3% of postweaning diarrhea. No commensal strain tested was associated with STb.

Impact of dual ETEC/EPEC or ETEC/AEEC infections

The importance of microbial interactions in disease in humans and animals has been a subject of increasing interest recently. For example, dual infections involving ETEC/enteropathogenic *E. coli* (EPEC) are frequently noted in diarrhea in children (Adhikari *et al.*, 1985). A study by Crane *et al.* (2006) indicated a mutual enhancement of virulence by ETEC/EPEC infection tested *in vitro* on cell lines and in Ussing chambers. ETEC toxins could enhance, for example, one type of cellular damage caused by EPEC, namely ATP release. Possible ETEC/EPEC interactions are also observed in animals. Wada *et al.* (1996) reported a naturally occurring outbreak of unusually severe diarrhea among piglets which was due to a dual ETEC/attaching effacing *E. coli* (AEEC) infection. Thus, it is tempting to presume that STb toxin action could be potentiated by a dual infection with more than one *E. coli* pathotype. This could possibly explain why in the field the presence of STb is commonly associated with colibacillosis whereas reproducing the disease experimentally is difficult to achieve.

Concluding remarks

From the results discussed herein it is clear that new routes for STb toxin need to be explored to explain its mode of action. Alterations at the microscopic level of the intestinal epithelial cells could now be investigated using pure toxin either to confirm or to dispute the previously reported results using STb-positive cell-free culture supernatant. As some studies have shown that ETEC toxins are expressed concomitantly, the toxins expressed could synergistically augment the effect of STb toxin. The role and significance of STb toxin have been defined *in vivo* recently (Berberov *et al.*, 2004: Zhang *et al.*, 2006). It is now clear that STb is responsible for colibacillosis, although its has a lesser effect compared with LT. Recently, a polyphyletic origin was demonstrated for ETEC strains, implying that the genetic background is not conserved (Turner *et al.*, 2006a). This suggested that, for ETEC, the ability to cause disease has a genetic basis, like toxin genes including STb, which are encoded on plasmids. As immunization of swine with vaccine containing LT, STa and colonization factors (e.g. F4, F5 or F41) is achieved, the importance of these toxins will become negligible for pig producers. This situation could result in a blooming of STb-dependent colibacillosis. This status would probably remain until a vaccine for STb is designed and is used in the field.

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