E. COLI HEAT-STABLE ENTEROTOXIN AND GUANYLYL CYCLASE C: NEW FUNCTIONS AND UNSUSPECTED ACTIONS

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ABSTRACT

Some *E. coli* cause diarrhea by elaborating heat-labile and heat-stable (ST) enterotoxins which stimulate intestinal secretion. *E. coli* ST's are small peptides which bind to intestinal luminal epithelial cell receptors. The ST receptor, one of a family of receptor-cyclases called guanylyl cyclase C (GC-C), is a membrane spanning protein containing an extracellular binding domain and intracellular protein kinase and catalytic domains. The intestine synthesizes and secretes homologous peptides, guanylin and uroguanylin. The kidney also synthesizes uroguanylin. ST, guanylin or uroguanylin binding to GC-C results in increased cGMP, phosphorylation of the CFTR Cl^- channel and secretion. Proguanylin and prouroguanylin circulate in blood and bind to receptors in intestine, kidney, liver, brain etc. In the kidney, they stimulate the excretion of Na^+ and K^+. Study of GC-C "knock-out" mice reveal that GC-C is important to intestinal salt and water secretion, duodenal bicarbonate secretion, recovery from CCl_4-induced liver injury, and to intestinal polyp formation in Min mice lacking GC-C.

INTRODUCTION

E. coli cause diarrhea and man and animals by a variety of mechanisms (1). These include the elaboration of enterotoxins, invasion of the intestinal mucosa and causing an intense inflammatory reaction, alteration of the cytoskeleton of the epithelial cells, by the elaboration of cytotoxins which damage cells and interfere with protein synthesis, and by other poorly understood mechanisms. The enterotoxin-elaborating *E. coli*, called enterotoxigenic *E. coli* or ETEC are the common-

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est *E. coli* pathogenic for man. These strains may elaborate one or more enterotoxins which include a heat-labile enterotoxin (LT), a heat-stable enterotoxin (ST) and a Shiga-like toxin (STEC). These toxins have different mechanisms of action. LT's activate adenylyl cyclase to stimulate cAMP production which activates a cAMP-dependent kinase (PKA). PKA phosphorylates a Cl^- channel (CFTR) and results in stimulation of Cl^- secretion and the inhibition of Na^+ absorption. ST's stimulate guanylyl cyclase, specifically the guanylyl cyclase C isoform (GC-C) to increase cGMP which induces intestinal secretion (see below). The mechanisms whereby STEC stimulate secretion are poorly understood but these toxins have cytotoxic properties and damage epithelial cells and endothelial cells by virtue of inhibiting protein synthesis (1).

Of the various enterotoxin-elaborating *E. coli*, the most common are *E. coli* which elaborate a heat-stable enterotoxins generally referred to as ST. These are responsible for both sporadic diarrhea and are a common cause of traveler's diarrhea (1–2). *E. coli* heat-stable enterotoxins, their mechanism of action, their receptor, GC-C, and their effects on the gastrointestinal tract and other organs are reviewed in this chapter (1–5). While initially it was shown that guanylyl cyclase C was a major regulator of Cl^- secretion in the gastrointestinal tract (6,7), we have come to learn that the receptor-ligand system may have diverse, important biologic functions not only in the gastrointestinal tract but also in the kidney and in other organs. Investigation of this receptor-ligand system, has shown that this system may also be involved in duodenal bicarbonate secretion, cell proliferation and repair from cell injury. These hypotheses are also briefly discussed.

E. COLI HEAT-STABLE ENTEROTOXINS AND OTHER RELATED TOXINS

The *E. coli* heat-stable enterotoxins have been purified and their sequence determined (3). *E. coli* elaborate either an 18 or 19 amino acid peptide (Figure 1) which are identical in their carboxy-terminal 13 amino acids (3,4,8). These toxins are unusual in that 6 of the amino acids are cysteines and all are involved in forming 3 disulfide bridges. This results in a highly internally bonded molecule and this tertiary structure may account for its relative resistance to intestinal proteases. Intact disulfide bridges are required for biologic activity. The amino-terminal 4 or 5 amino acids, of the 18 and 19 amino acid species, respectively are not required for biologic activity (9). Another class of pathogenic *E. coli*, the enteroaggregative *E. coli* elaborate a related

E.coli STa	NSSNYC **CELC**CNP**AC**T**GC**Y
	NTFYC **CELC**CNP**AC**A**GC**Y
Guanylin	PGT**CEIC**AYA**AC**T**GC**
Uroguanylin	NDD**CELC**VNV**AC**T**GC**L

FIG. 1. Comparison of primary structures of *E. coli* heat-stable enterotoxins and their mammalian homologs, guanylin and uroguanylin. The darkened areas are locations of amino acid identity to all.

toxin referred to as EAST (5). This toxin retains a high degree of homology in the carboxy-terminal portion to the above described enterotoxigenic *E. coli*.

Since the identification of the E. coli ST's, various enteric bacteria have been shown to elaborate identical (to the 18 or 19 amino acid *E. coli* ST's described above) or highly homologous to *E. coli* ST's. Some of these bacteria include *Yersinia enterocolitica, Citrobacter freundii, Klebsiella pneumoniae,* and NAG-Vibrios. The ST's secreted by these bacteria vary in total length but retain a high degree of homology to the carboxy-terminal, biologically-active portion, of ST (3). All the ST's activate guanylyl cyclase C (GC-C), a membrane-bound form of guanylyl cyclase.

RECEPTOR FOR ST'S

While it has been appreciated for some time that ST stimulates guanylyl cyclase (GC) (6), how it does so has only recently been elucidated. Our laboratory demonstrated that receptors for ST exist on the luminal or brush border surface of small intestinal and colonic epithelial cells and that binding to these receptors activated guanylyl cyclase (10). The relationship between the binding to the receptor and activation of guanylate cyclase was not known until Garbers et al. (11) cloned a guanylyl cyclase and showed that it was a receptor-cyclase which spanned the cell membrane once, had an extracellular domain, a small transmembrane domain and cytoplasmic catalytic and kinase-like domains (Figure 2). The first such cloned guanylyl cyclase was shown to be the receptor of atrial natrurietc peptide (ANP) and was called GC-A. Subsequently, many members of this family have been cloned but only 3 members have known ligands, i.e., GC-A, GC-B, and GC-C (12,13). As mentioned, GC-A is the receptor for ANP, GC-B the receptor for CNP and BNP, while GC-C was shown to be the receptor for *E. coli* heat-stable enterotoxin (ST) (11). The intracellular domains of these 3 receptor cyclases are highly homologous while the extracellular binding domains are divergent (12). GC-C is a glycoprotein (11,15–17) but

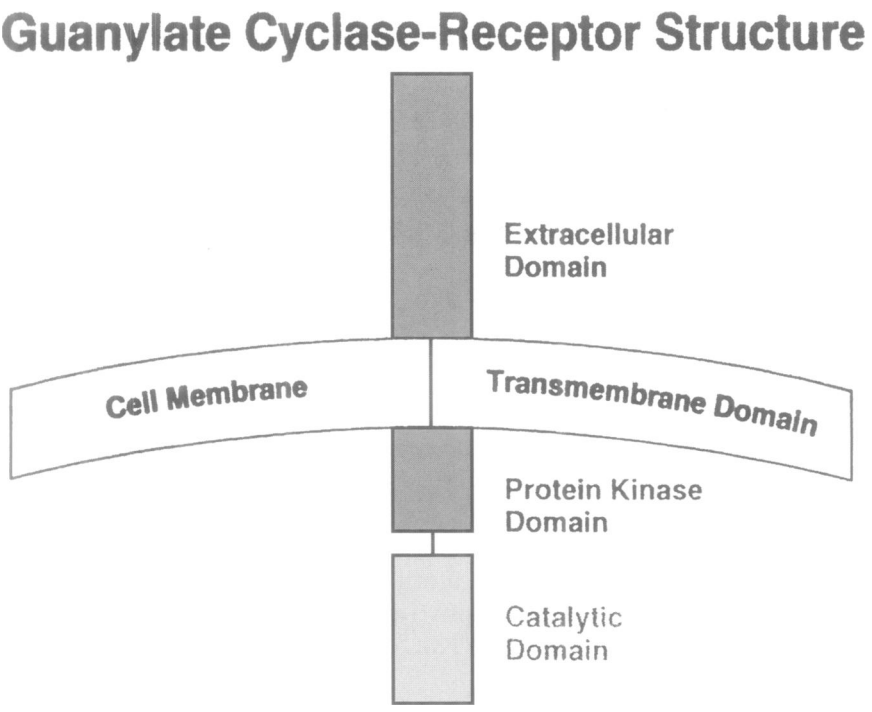

FIG. 2. Postulated structure of membrane-associated receptor-guanylyl cyclases. Protein spans the cell membrane once and has an extracellular domain, a short transmembrane domain, and an intracellular domain comprised of a catalytic and protein kinase-like domains.

the sugar residues are not required for ST binding (16). The binding site has been localized to the extracellular domain and the specific amino acids required for binding have been defined (18,19). In order to be active, the ST/guanylin/uroguanylin receptor must exist as a homodimer (20). Two or more wild type monomers are required for guanylyl cyclase activity and signal transduction (21). Activation of GC-C is regulated by a novel protein called IKEPP, intestinal and kidney-enriched PDZ protein. This protein inhibits ST activation of GC-C (22). We have cloned the cDNA for the human ST receptor from CaCo2 cells and shown that the encoded protein is identical to that encoded by the cDNA cloned from human intestine save for a single amino acid (14,15).

Initially, it was that that GC-C was only expressed in the small and large intestine (10,11,23). Subsequently, by RT-PCR and other techniques we now know that GC-C is expressed in many organs including the stomach, kidney, pancreas, salivary glands, liver, pituitary, adrenal gland, lung, etc. (12,13,24–30). With the exception of the kidney,

which is further discussed below, the function of GC-C in these organs is unknown.

In the intestine GC-C is found only in the villous and crypt epithelial cells and not in other layers of the small or large intestine (31–33). The subcellular location of GC-C in the small intestine is the brush border, or luminal membrane. GC-C was not detected on basolateral membranes of small intestinal villous cells. Subsequently, we have shown that in the rat, an ST binding species resides on the basolateral membrane of the colonocyte but that binding to this membrane does not result in the production of cGMP (34). Thus, it is likely that this "receptor" is not GC-C.

We have demonstrated that GC-C expression in both rat and human intestine is developmentally regulated. GC-C receptor number is very high at birth and in the neonatal period but then falls to a lower number which remains stable through life (35,36). In the human, this transition occurs at approximately 1–2 months of age. Laney et al also showed that GC-C was highly expressed, by northern analysis, in newborn liver but was undetectable in adult liver (24). With damage to the liver, however, either by partial hepatic resection or with CCl_4-induced injury, GC-C was upregulated and easily detected (37). This has subsequently been confirmed by Scheving et al. (38).

STIMULUS-SECRETION COUPLING

The mechanisms by which cGMP synthesized in response to ST/guanylin/uroguanylin induces secretion has been clearly defined. Studies in intact intestine, by deJonge et al. (7,39,40) demonstrate that cGMP activation of a specific isoform of cGMP-dependent protein kinase II (cGK II or PKG) is sufficient and necessary to activate Cl^- secretion. Pfeifer et al have demonstrated that the intestine of the cGK II knock out mouse is not responsive to ST, guanylin, or cGMP (41). These studies clearly demonstrate that in vivo the primary activation of cGK II mediates ST/guanylin/uroguanylin-induced secretion. However, in the intestinal cell line, T_{84}, very high levels of intracellular cGMP cross activate protein kinase A (PKA) which alters Cl^- channels to induce Cl^- secretion (42,43). The pathway through which ST/guanylin evoke Cl^- secretion is also clear. Chao et al. (42) demonstrated that ST_a, guanylin, or cGMP could induce Cl^- secretion only in cells expressing CFTR and Cuthbert et al. (44) reported that while guanylin induces Cl^- secretion in normal mouse colon, it is inactive in the colon of transgenic cystic fibrosis mice which lack a functional CFTR. Further, humans with cystic fibrosis do not respond to ST (45). Thus, these

findings strongly support the view that cGMP activates the CFTR Cl channel (46). These pathways are shown schematically in Figure 3.

It is also likely that ST/guanylin/uroguanylin receptors may be coupled to signal transduction systems other than guanylyl cyclase. This hypothesis is supported by the observations that ST stimulates PIP_2 hydrolysis and stimulates phosphorylation of several BBM proteins that can be inhibited by protein kinase C (PKC) inhibitors (47,48), that ST stimulates duodenal mucosal HCO_3^- secretion via a Ca^{++}-activated mechanism (49), and that uroguanylin stimulates a cGMP-independent, G protein pathway in kidney (30). Thus, it is likely that the cellular actions of the various GC-C ligands might involve an interrelationship among Ca^{++}, PI cycling, PKC, cGMP, and G proteins (30,50,51). This is an area that requires further investigation.

ENDOGENOUS ST-LIKE PEPTIDES

The role of ST receptors or GC-C in the intestine was not known until Currie et al identified a peptide synthesized in the rat small intestine that bound to GC-C and stimulated guanylyl cyclase to produce cGMP. When purified and sequenced, this small peptide proved to be highly homologous to *E. coli* ST. Because of its ability to activate guanylyl cyclase activity, it was named guanylin (52–54). Subse-

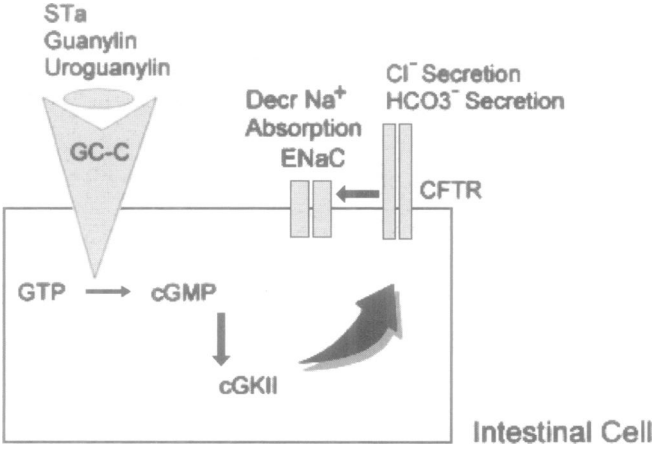

FIG. 3. Proposed mechanism of GC-C action. ST, guanylin, or uroguanylin bind to GC-C in the plasma membrane. Binding activates the catalytic domain of GC-C to effect the conversion of GTP to cGMP. cGMP then activates a specific cGMP-dependent kinase isoform (cKGII) which phosphorylates the chloride channel, CFTR. Activation of CFTR channels results in Cl- and HCO_3-secretion and also causes the decreased absorption of Na+ by unclear mechanisms.

quently, a second homologous peptide was found in urine and was called uroguanylin (55–57). The relative potencies of these three peptides with regard to activating guanylyl cyclase or stimulating secretion are ST>uroguanylin>guanylin. The comparative structures of *E. coli* ST, guanylin, and uroguanylin are shown in Figure 1. Subsequently, it was also shown that uroguanylin was also synthesized in the intestine but with a different proximal-distal gradient than guanylin. Uroguanylin is synthesized primarily in the duodenum and proximal small intestine while guanylin is synthesized in the distal ileum and proximal colon (58). Uroguanylin is also synthesized in the kidney (59,60). Both guanylin and uroguanylin are excreted in urine (59,60). When synthesized in the intestine, both guanylin and uroguanylin are secreted into both the lumen and blood stream. Secretion by the intestinal epithelium is regulated and the majority secreted luminally with a minority being secreted into blood (61). Both peptides are synthesized as pro-compounds, i.e. proguanylin and prouroguanylin and both circulate in blood. Increased circulating levels and urinary levels of these "hormones" are found in renal failure, congestive heart failure, and with carcinoid tumors (67,68). Guanylin is also expressed pancreas, salivary glands, trachea, and adrenal gland (25–28,54,62) while uroguanylin is also expressed in kidney, pancreas, and salivary glands (25–28,58).

The role of these peptides in intestinal function has not been directly demonstrated but it is highly likely that the GC-C/uroguanylin/guanylin system is a major regulator of electrolyte secretion and, as discussed below, of duodenal HCO_3^- secretion. The role of these peptides in renal function has received more study. When guanylin, ST, or uroguanylin are infused intravenously or into the isolated perfused kidney a diuresis, natriuresis, and kaliuresis are induced (63–65). Interestingly, in mice lacking GC-C, intravenously administered uroguanylin continues to induce a diuresis and natriuresis suggesting the existence of an additional, non-GC-C receptor in the kidney (64). Direct evidence for a second, non-GC-C, non-cGMP-dependent receptor in human renal proximal tubular cells has recently been reported by Sindice et al. (30). Furthermore, mRNA for uroguanylin and guanylin are responsive to Na^+ in the diet of the rat and mouse (66,68). A low salt diet caused both the upregulation of guanylin and uroguanylin in the intestine while a high Na^+ diet had the opposite effect. Similarly, a high salt diet causes the upregulation of uroguanylin mRNA in mouse kidney (66).

While the functions of guanylin and uroguanylin are not fully understood, since both peptides are synthesized by the intestine and are

released into the blood, as well as into the intestinal lumen, it is likely that the intestine can directly regulate the Na^+ and Cl^- absorptive behavior of the kidney (59). It seems likely that uroguanylin may serve as an intestinal natriuretic hormone linking the intestine and kidney in an endocrine axis.

GUANYLYL CYCLASE KNOCK-OUT MOUSE

Two laboratories have produced a mouse with inactivation of the GC-C gene by homologous recombination (70,71). These animals are referred to as GC-C knockout mice (KO). These animals do not express GC-C mRNA or protein and ST does not alter intestinal transport of Na^+ or Cl^- (70–72). These animals seem normal, live a normal life span, and are fertile. They have neither constipation nor diarrhea and have a normal appearing gastrointestinal tract and other organs. While, as discussed above, GC-C does modulate Na^+ and Cl^- transport in the intestine, it is obvious that this system is not essential and can be compensated for by other regulatory systems. KO mice are normally responsive to other intestinal secretory stimuli.

Additional characterization of the GC-C KO animals by Northern blot analysis of intestine revealed no up-regulation of guanylin or uroguanylin mRNA and Western blot analysis revealed no diminution of cGK II levels (70). Since cGMP regulates CFTR both via protein kinases (7,39,42,43,46,73) and by direct binding of cGMP to CFTR (74), it was possible that ablation of GC-C would alter the levels of and regulation of CFTR. Northern analysis with cDNA probes, and immunohistochemical studies with anti-CFTR antisera (kindly provided by Dr. Jon Cohen, Duke University) revealed markedly increased levels of CFTR mRNA and protein in the small intestine but not the colon (unpublished data). Interestingly, increased levels of Na^+ channel mRNA were seen in the colon.

Utilizing the GC-C KO mouse, we have demonstrated that there is greatly diminished, but not absent, ^{125}I-ST intestinal binding activity (70). There is statistically significant residual binding activity in both small intestine and colon. The residual binding activity has different properties than ST binding in wild type mice, i.e., a lower K_a of binding and a different pH profile of binding (75).

In collaboration with Charney et al. (72), we have characterized the transport behavior of the colon of KO mice mounted in Ussing chambers. We observed that net Cl^- absorption was lower in the colon of KO mice that controls and also the possibility that basal colonic HCO_3^- secretion was reduced in KO mice. This is an interesting possibility in

view of the abnormalities of duodenal HCO_3^- transport discussed below (49,76,77). As expected, the colon from KO animals were totally unresponsive to ST applied to either the mucosal or serosal surfaces but showed normal responsiveness to 8-Br-cGMP (72).

Continued study of the GC-C knockout mouse should help elucidate the significance of GC-C expression in extraintestinal sites, the existence of and role of additional receptors.

OTHER POSSIBLE RECEPTORS

A great deal of evidence suggests the existence of an additional ST/guanylin/uroguanylin receptor(s). Kinetic analysis of ST binding to intestinal membranes indicate both high and low affinity receptors (78). We reported that the IEC-6 rat crypt cell line possesses ^{125}I-ST binding activity but no ST-stimulated guanylyl cyclase activity, and no ST receptor (GC-C) mRNA was detected either by Northern or PCR analysis (14). We have demonstrated that highly purified basolateral membranes prepared from rat colonocytes bind ST but do not generate cGMP (34). Further, when ST was added to the mucosal surface of normal mouse colon mounted in Ussing chambers, there was a stimulation of short circuit current and a stimulation of Cl^- secretion. When added to the serosal surface, it was electrically silent and no transport effects were observed (72). These observations suggest that binding to the colonocyte basolateral "receptor" may subserve functions other than regulation of Na^+, Cl^-, or HCO_3^- transport. As mentioned above, we have shown that in the GC-C KO mouse lacking GC-C that ST binding activity persists in the intestine (70). With Isenberg et al. (unpublished observations), we have demonstrated that ST continues to stimulate a short circuit current response (although less than in wild type animals) in duodenum from GC-C KO animals mounted in Ussing Chambers. In collaboration with Greenberg et al. (64), we have demonstrated that intravenous infusion of ST to normal mice induces a diuresis and natriuresis and that this response persists in GC-C KO mice. Most recently, Sindice et al. (30) have reported that uroguanylin stimulates two distinct signaling pathways in human kidney (proximal tubules) and a human proximal tubule cell line. One involves GC-C and cGMP while the second is a non-GC-C, cGMP-independent pathway connected to a pertussis toxin-sensitive G protein (30). Taken together, these observations strongly support the existence of an alternative ST/guanylin/uroguanylin receptor. The function(s) of the alternative receptor is unknown but does not likely subserve Cl^- secretion, at least in the colon. In the duodenum, the

alternative receptor may be involved in the regulation of bicarbonate transport (79) although it may have other functions as well.

OTHER POSSIBLE FUNCTIONS OF THE GC-C/GUANYLIN/ UROGUANYLIN SYSTEM

As described above, the phenotype of the GC-C KO mouse was seemingly normal except for the lack of secretory response to ST. To further explore the possible role of GC-C, we decided to stress these animals to determine whether GC-C might be involved in the general processes of cell proliferation, cell injury, and cell repair. The hypothesis that GC-C might be involved in these cell proliferation or modulation of cell injury was suggested by several observations: a) GC-C expression is markedly higher in the new born and neonatal intestine and neonatal liver (both rapidly proliferating organs) than in adult life (35,36) b) GC-C is upregulated in the liver of animals subjected to partial hepatectomy, CCl_4-induced liver injury, or as an acute phase response (37,38) c) GC-C continues to be expressed in human adenocarcinoma of the colon (80,81) while guanylin is poorly or not expressed in these tumors (82) and that d) feeding of uroguanylin to Min mice (the mouse equivalent to adenomatous polyposis coli) reduces the number of intestinal polyps (90).

Effect of GC-C deficiency on CCL_4-induced liver injury and repair

We had previously shown that GC-C was not detectable in the adult liver by Northern analysis but became markedly upregulated in response to partial hepatic resection or to CCl_4-induced liver injury (37). Upregulation of GC-C in response to hepatic resection has been confirmed by Scheving and Russell (38). These data suggested the hypothesis that GC-C is involved in recovery from hepatic injury and/or in hepatocellular proliferation. To test this hypothesis, we compared the course, severity and duration of CCl_4-included liver injury in wild type and GC-C deficient mice (83). Our studies demonstrated that mortality to CCl_4 was significantly increased in GC-C deficient mice compared to controls (Figure 4). Further, although the degree of cell necrosis was the same in day one in both wild type and KO animals, the magnitude of hepatic injury was more severe and more long lasting and slower to recover in GC-C deficient mice compared to controls (Figure 5). These data suggest an important role for GC-C in modulating the response to hepatic injury and perhaps in hepatocyte proliferation, i.e., either initial cytotoxicity vs. recovery from injury (hepatocyte proliferation).

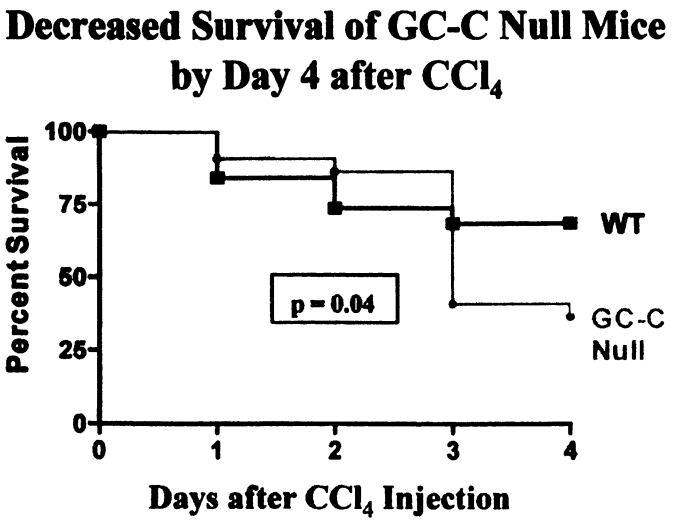

FIG. 4. Comparison of survival in CCl4-treated GC-C deficient (GC-C null) and normal (wt) mice. Statistically significant decreased survival is seen on day 4 in GC-C null animals.

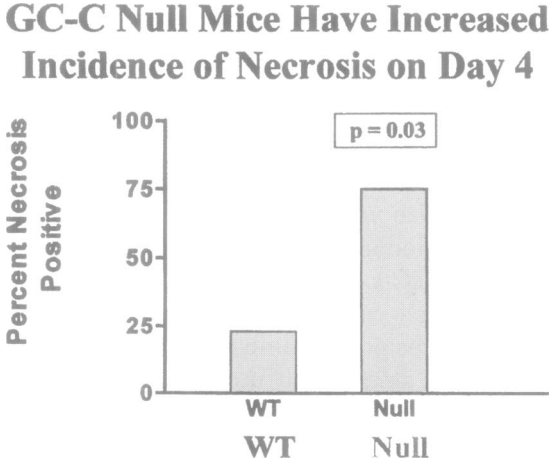

FIG. 5. Comparison of degree of liver cell necrosis in CCl4-treated mice. Wt = normal mice. Null = GC-C deficient mice.

Effect of GC-C deficiency on expression of intestinal tumors in Min/+ mice

The biologic significance of the GC-C/guanylin/uroguanylin system assumed added importance when it was shown by Carrithers et al. (80,81) that GC-C was expressed in primary colonic adenocarcinomas

and in their metastases. Interestingly, subsequently, Cohen et al. demonstrated that guanylin was down regulated in colon polyps and adenocarcinomas (82,85). Park et al. have recently demonstrated that some esophageal and gastric adenocarcinomas also may express GC-C (86). The expression of GC-C in colonic adenocarcinomas has led to attempts to target and visualize these tumors utilizing ST analogues coupled to ^{111}In by scanning (87,88).

Two studies have demonstrated that GC-C agonists inhibit proliferation in colon cancer cell lines (89,90). The exact mechanism remains in question as Shailubhai et al. (90) saw induction of apoptosis in the T84 cell line upon uroguanylin treatment while, in contrast, Pitari et al. (89) showed that ST addition delayed DNA synthesis and prolonged the cell cycle in the absence of cell death. Thus, the mechanism(s) by which ST or uroguanylin may alter cell proliferation is uncertain and needs to be further examined.

In order to help understand the possible role of GC-C in intestinal polyp and cancer formation/progression the min/+ mouse model has been used. This model is the equivalent of human adenomatous polyposis coli (APC). Min mice have the same gene defect as do patients with APC. Long term oral administration of uroguanylin led to a significant decrease in the number of polyps seen in Min/+ mice (90). These authors suggested that uroguanylin reduced the number of polyps via GC-C.

We reasoned that if GC-C was indeed integral to tumor development/progression processes, mating min/+ mice with the GC-C deficient mouse should cause an alteration in intestinal polyps. Paradoxically, mating of GC-C KO mice with *min*/+ mice significantly reduces the number of intestinal adenomas (84) in the resultant GC-C−/−/min+ mice (Figure 6). Thus, GC-C does not seem to be essential in reducing polyp number and clearly can occur via a non-GC-C mechanism. Perhaps uroguanylin reduces polyp number via a non-GC-C or alternative receptor. In an initial attempt to understand how GC-C might be interacting with polyp formation, we examined the role of B-catenin in the transcriptional regulation of the human GC-C gene. We have shown that the GC-C gene is transcriptionally regulated by B-catenin/TCF (91).

Duodenal bicarbonate secretion

In collaboration with Isenberg et al. (49,76,77,92), we have been studying duodenal HCO_3^- transport in KO and normal mice. Duodenal mucosa is mounted in Ussing chambers and studied under short circuit conditions. Guanylin has been shown to be a potent stimulant of

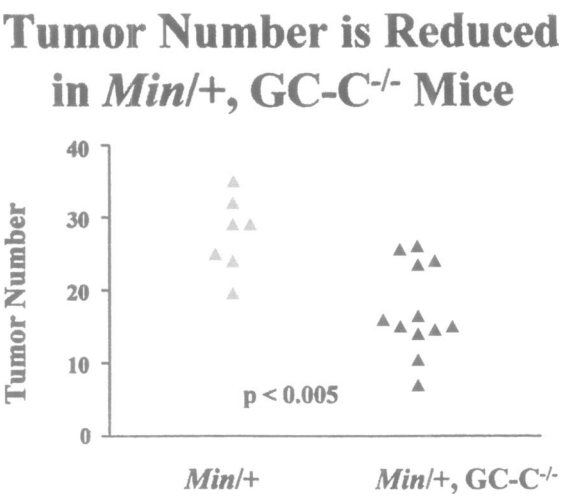

FIG. 6. Comparison in intestinal polyp number in min/+ mice compared to min/+ mice lacking GC-C (min/+, GC-C−/−). There is a statistically significant reduction in number of adenomatous polyps in mice lacking GC-C.

duodenal HCO_3^- secretion (93) and we wondered whether this might be altered in the GC-C KO mouse. Our findings demonstrate that duodenal HCO_3^- secretion is markedly reduced in KO mice (76,92) (Figure 7) and that HCO_3^- secretion in response to carbachol and

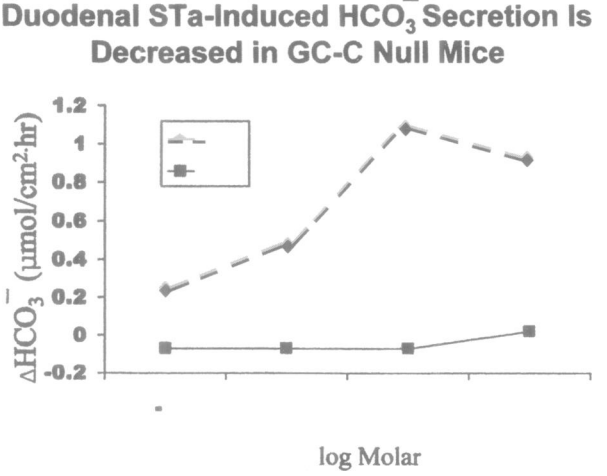

FIG. 7. Comparison of ST-stimulated HCO3- secretion in duodenal mucosa of normal and GC-C deficient mice. The upper curve represents normal mice while the lower curve represents GC-C deficient mice.

PGE2 are also diminished (49,76). Interestingly, ST administration to the duodenal mucosa of KO mice continues to stimulate, although less than in wild type mice, a short circuit current response (unpublished data). We have also shown that duodenal HCO_3^- secretion in response to duodenal acidification in vivo, the normal physiological stimulus to HCO_3^- secretion, is also blunted (77). The biochemical mechanisms by which lack of GC-C alters duodenal HCO_3 secretion are unknown. We have preliminary evidence that some of these effects may be a consequence of reduced MAPK activation (77).

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DISCUSSION

DuPont, Houston: Ralph, very nice, and thank you for that presentation. Enterotoxigenic *E. coli* is the principle cause of diarrhea in travelers and military populations, and prevention of this disease is really important. There are three virulence properties, the cholera-like heat labile toxin, the heat stable toxin and colonization fimbriae. Vaccines are being developed against the LT and against the fimbriae. But ST being non-antigenic we don't have a good handle on that. Can you think of novel ways to treat or prevent ST producing disease employing GCC? It appears that ST is the most important ETEC toxin clinically.

Giannella, Cincinnati: There are several possible approaches. While ST being a small molecule by itself is not particularly immunogenic, if you conjugate it to a larger carrier molecule you can make it immunogenic. And you can make antibodies. I'm not sure if that strategy is useful in creating human vaccine or not, but it certainly works in animals and you can protect pigs for example which we've done with Dr. Harley Moon by that particular approach. I think understanding the pathway as to how ST works and what it activates will expose various potential targets for therapeutic intervention. The problem is one of specificity. The system is not just in the gut, the system is not just activated in diarrheal disease, and as I have tried to show you it may be involved in quite

a number of important processes. So I am not so sure that the strategy of interrupting a protein kinase for example, a protein kinase G inhibitor, would be a particularly useful tool, unless you could target it very specifically to where you wanted it.

LaMont, Boston: Ralph, my question relates to those other important processes you just mentioned. Normally the gut doesn't secrete anything, it absorbs. Yet we have a system with a ligand and a receptor, guanylin and GCC, that seems to regulate secretion. What's the physiological role of guanylin? Are there any states or animal models where you can up regulate the concentration or the release of the ligand?

Giannella: That's a good question for which I don't have a good answer. The physiologic role of guanylin in the gut is not particularly clear. I mean it's loosely said that it's probably important to keep the contents liquid, and to facilitate digestion and like phenomena. I don't find that particularly satisfying answer, but I can't do any better than that at the moment.

Frohman, Chicago: Ralph, that was a lovely presentation. Along the lines of an earlier question, what about the possibility of developing an antagonist to GCC, such as a non-absorbable antagonist that might be used to block its effects? Granted it's not a prophylactic treatment, but one that might effectively inhibit this entire signal transduction mechanism.

Giannella: I think, Larry, that is a theoretical possibility particularly for a non-absorbable compound, would be confined to the gut and could be specific to GCC. I think that is quite a reasonable strategy to pursue. I am unaware of it being pursued, but it's a good idea.