Bugs and guts: it's good to talk?

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The association between man and microbes in the alimentary tract has been present from the earliest stages of human evolution. About $10^{14}$ bacteria inhabit the human alimentary tract, which is 10 times more than the total of host eukaryote cells in the human organism. This association did not occur by chance, and from an evolutionary standpoint is likely to be mutually advantageous.

For the bacterial prokaryote, the human alimentary tract offers a relatively non-hostile environment with a regular and usually plentiful supply of nutrients. The physicochemical milieu along the tract varies, providing environmental niches for aerobic and for both facultative and obligatory anaerobic organisms. The human host benefits from products of bacterial metabolism such as short chain fatty acids (SCFAs), particularly butyrate which acts as an energy fuel for colonic epithelial cells, and from the important contributions made by the bacterial flora to immune and non-immune host defence.

However, not all bacteria in the alimentary tract are friendly. Closely related organisms can be both commensal and pathogen, the switch being initiated by genetic material commonly localised in a region of the bacterial chromosome or plasmid, a so-called pathogenicity island or locus, which encodes for one or more virulence factors necessary for the expression of human disease. Although virulent, disease-producing organisms are greatly outnumbered by their commensal cousins, and pathogenicity is a rare event, enteric infection nevertheless constitutes a major infectious disease burden for humans worldwide, following closely on malaria and tuberculosis. The emergence of virulent, disease-producing strains of enteric bacteria favours survival of the organism by ensuring continued transmission by diarrhoeic stools not only of the virulent organism but also of the many other components of the commensal enteric flora to other humans and possibly animals.

The human organism is thus a eukaryote-prokaryote consortium with a high degree of interdependency. There is abundant evidence of sophisticated 'cross-talk' between enteropathogens and the many cell systems that make up the gastrointestinal (GI) tract; enteropathogens have 'learnt' to subvert the host's cytoskeletal machinery and signal transduction systems to colonise and create a favourable environment in the gut. The lines of communication between commensal organisms and host cells are less well established, although there is good evidence that commensal bacteria influence epithelial cell turnover in the gut, drive the normal gut mucosal immune response, and contribute a variety of essential nutrients to host epithelial cell metabolism. Thus, 'bugs and guts: it's good to talk' describes a dilemma for the human host, its commensals and its commensals turned pathogens. It is good to talk, but words are not always sweet, and the messages received are sometimes uninvited and not desired.

Symbiosis and evolution

Symbiosis is the peaceful and profitable coexistence between two organisms. A symbiotic state exists between humans and the commensal microflora of the GI tract. Humans appeared on the earth about 400 million years ago, but the association between a prokaryotic intestinal microflora and the eukaryotic alimentary tract of animals began 1,000–2,000 million years ago (Fig 1). Symbiosis in the intestine is thus an ancient phenomenon. Eukaryotic cells evolved as multiples of prokaryotes by a process of serial endosymbiosis. The typical bacterial prokaryote has a nucleoid (or genophore) and thus lacks a nucleus with nuclear membranes; it also lacks mitochondria, large ribosomes and endoplasmic reticulum, and has a simple flagellum. The basic eukaryotic cell was formed from a series of prokaryotic 'add-ons' (Fig 2):

- the nucleus of the eukaryote evolved from endosymbiotic fermenting thermophilic eubacteria
- mitochondria originated from aerobic eubacteria
- internalised spirochaetes provided the motile cytoskeletal proteins that are vital for mitosis and meiosis
- other external spirochaetes formed the basis of the eukaryotic undulipodium

More recently, an alternative biochemically based hypothesis, the 'hydrogen hypothesis', has been put forward to explain the transition from pro- to eukaryotic state. This still depends on the principle of symbiosis, and suggests that the host (an anaerobic, strictly hydrogen-dependent, autotrophic archaeabacterium) became associated with a eubacterium symbiont that was able to respire, but which generated hydrogen as a waste product of anaerobic heterotrophic metabolism. It is proposed that the host's dependence on molecular hydrogen acted as a selective principle for the emerging eukaryotic state.

Whatever theory is eventually shown to be the most
likely explanation for this aspect of cellular evolution, human evolution has, from the earliest times, been totally dependent on prokaryotic endosymbiosis, and even today continues to rely on a symbiotic relationship with its enteric microflora.

**The gastrointestinal microflora**

More than 500 species of bacteria colonise the alimentary tract, with marked regional differences both in the type of bacteria and the total number of organisms. All the bacteria in the GI tract enter through the mouth, either with food and drink or by direct person-to-person contact. The mouth has a unique flora containing both aerobes and anaerobes, some of which survive in special locations such as dental plaque. Although many bacteria are swallowed every day, the low intragastric pH restricts their number in gastric fluid (10³/ml), most of them being lactobacilli. *Helicobacter pylori* has resided in the human stomach for about 300 million years, and for longer in some small mammals and other animals. Peptic ulcer disease began to appear only during the last century; it has therefore been suggested that *H. pylori* has been a commensal, and possibly even a symbiont, for the vast majority of the time that humans have existed on the planet. The initial increase in gastric acid production associated with *H. pylori* infection and the inflammatory response in the gastric antrum may both be factors which improve host defence against other enteropathogens. In addition, there is some evidence that *H. pylori* infection protects against the development of gastro-oesophageal reflux disease. Thus, at the same time – and possibly even in the same patient – *H. pylori* can be both pathogen and commensal.

Bacterial counts rise to 10⁴/ml in the duodenum and, as in the stomach, are predominantly lactobacilli. In the distal ileum, there is a sharp rise to 10⁶ organisms/ml, with streptococci appearing in addition to the lactobacilli. In the caecum, bacterial counts rise steeply to 10¹²/g faeces, with the appearance of large numbers of anaerobic organisms such as bacteroides, clostridia and methanogenic bacteria.

The GI tract of the neonate is sterile but, within hours, becomes colonised by bacteria acquired from the mother and other environmental sources. The neonatal intestine contains predominantly lactobacilli and bifidobacteria. As the infant is weaned, the microflora begins to reflect more closely that of the adult.

**Influence of the intestinal microflora on gut structure and function**

The intestinal microflora plays a major role in the maintenance of mucosal structure. Studies in germ-free animals have shown that introduction of a normal microflora results in increase in mucosal mass, intestinal surface area and epithelial cell turnover. These structural alterations almost certainly have functional implications for nutrient, fluid and electrolyte absorption, and for epithelial regeneration and repair.
Microflora and host defence. The intestine of germ-free animals contains relatively few immunocytes and other inflammatory cells, a situation reversed by introducing a normal microflora. The population of immune competent cells within the epithelium and lamina propria is the front line of immune host defence against invading enteropathogens. It therefore seems likely that the resident microflora is important for maintaining a background, non-specific immune host defence. There is increasing evidence that the commensal microflora plays a direct role in suppressing gut enteropathogens.

Louis Pasteur noted the antagonism between certain bacterial strains, while his colleague and professional sparring partner, Metchnikoff, proposed the possible therapeutic use of lactic acid-producing bacteria. Increasing evidence indicates that the commensal microflora plays a direct role in suppressing the colonisation of the gut by enteropathogens. Lactobacilli produce lactic acid and SCFAs which reduce intraluminal pH and inhibit the growth of acid intolerant organisms. These effects can be demonstrated in vitro, and there is some emerging clinical evidence to suggest that certain strains of lactobacilli can reduce the risk of acquiring intestinal infection. Bifidobacteria, a major commensal of the neonate, have been shown dramatically to reduce the risk of rotavirus infection in this age group.

The concept of administering a harmless organism to prevent infection has become known as probiotics. Attempts have also been made to use prebiotics, dietary substrates such as fructose-containing oligosaccharides (found in large quantities in onions, carrots and garlic), to promote the growth of resident probiotics such as bifidobacteria.

Nutrition. Commensal enteric bacteria make many nutritional contributions to their animal hosts. The rumen of some herbivores contains a highly specialised microflora able to digest cellulose and other plant structural molecules, making them available to the host as energy substrates. In the human colon, many bacterial species metabolise fibre and other unabsorbed carbohydrates to produce SCFAs. This liberates, and allows retrieval of, energy substrates which would otherwise be lost in the faeces. Butyrate, in particular, is an important fuel for the colonocyte: in addition to its direct effects on cell metabolism, it may have preventive effects against neoplasia. Bacteria may also contribute directly to host nutritional status by synthesising vitamins such as vitamins K and B12.

Factors which modify the gastrointestinal microflora

Gastric acid. Many factors influence the gut microflora both qualitatively and quantitatively. Gastric acid presents a formidable barrier to organisms entering the alimentary tract, but bacteria still exist in reasonable numbers in the upper GI tract. Reduction in gastric acid secretion, either as a result of gastric atrophy or by pharmacological inhibition with acid inhibitory drugs, increases bacterial counts within the stomach and upper small intestine and also the risk of acquiring an intestinal infection.

Motility disorders. In the proximal small intestine, high bacterial counts increase intestinal motility disorders, particularly those associated with diabetes mellitus, systemic sclerosis, and chronic idiopathic intestinal pseudo-obstruction due either to a visceral myopathy or a neuropathy. In these conditions, normal peristalsis is impaired, enabling higher numbers of bacteria to proliferate and colonise the proximal small intestine.

Diet. The intestinal microflora is modified by dietary influences. Poorly absorbed carbohydrates, such as fibre and resistant starches, increase the numbers of certain species of colonic bacteria (bacteroides and bifidobacteria), and it has been proposed that certain dietary manipulations might be important in reducing the risk of colorectal cancer.

Antimicrobial agents. Administration of broad-spectrum antimicrobial agents is perhaps the most obvious way to disturb the normal human intestinal microflora. Diarrhoea associated with antibiotic therapy is common, at least 70% of which can be attributed to emergence of Clostridium difficile. It is presumed that this organism is present in many healthy adults, but is kept in check by other components of the resident microflora. When this ‘host defence’ is disturbed, C. difficile proliferates, producing diarrhoea and pseudomembranous colitis. Despite the acute, clinically dramatic effects of short-term broad spectrum antibiotic
therapy, it is virtually impossible to make any long-term impact since the microflora rapidly returns to baseline once antibiotic therapy is discontinued.

The microflora turns ‘pathogen’

Some intestinal bacteria are able to deconjugate bile salts. When this occurs in the small intestine in conditions that promote bacterial overgrowth, malabsorption of fat can result due both to reduced concentrations of conjugated bile salts in the small intestine and to the membranotoxic effects of unconjugated bile salts on the small intestinal epithelium. The normal intestinal flora actively participates in the metabolic pathways of a variety of endogenous steroid hormones and in the excretion profiles of many drugs. Colonic bacteria with azoreductase activity are thought to be involved in the conversion of dietary procarcinogens to carcinogens in the intestine, thereby having a role in the pathogenesis of colorectal cancer.

The presence of commensal microorganisms in the intestinal tract is thought to be important in promoting the background inflammatory response that forms part of normal host immune defence, but which may get out of control in certain circumstances such as inflammatory bowel disease (IBD). One hypothesis for the aetiology of IBD centres around the concept that immune tolerance to the host’s normal microflora is lost in a susceptible individual, resulting in an excessive and damaging inflammatory response. A similar situation may exist in the tropics where some healthy individuals have mild or moderate partial villous atrophy (so-called tropical enteropathy). This may be a result of mucosal T cell activation driven by either the normal microflora (total bacterial counts are often increased compared to Western controls) or repeated intestinal infection. In immunocompromised situations, harmless species like Candida albicans can become invasive enteropathogens and produce conditions such as candida oesophagitis associated with HIV/AIDS.

Commensal turns pathogen: the molecular genetic determinants

Escherichia coli

One of the most common bacterial species in the human alimentary tract is Escherichia coli. These organisms are almost inevitably harmless commensals in healthy humans, and have a symbiotic relationship with the host. During the past 50 years, however, a variety of subtypes has been characterised that are pathogenic to humans and animals, producing an extensive spectrum of clinical disease, including acute watery diarrhoea, dysentery and persistent diarrhoea, sometimes with nutrient malabsorption (Table 1). Although the E. coli organism remains fundamentally the same, the pathogenic subtypes possess additional genetic material that encodes for specific virulence factors which directly determine the nature of the intestinal disease.

Enterotoxigenic E. coli (ETEC), for example, possess two major virulence factors, both plasmid-encoded. First, they have highly specialised attachment organelles called pili (or fimbriae) which mediate adherence to host epithelium. These pili demonstrate selectivity in their binding characteristics, such that human, porcine and bovine E. coli adhere most efficiently to their natural host, causing little or no disease in other mammalian hosts. Plus gene deletant mutants are unable to colonise and produce clinical disease, which emphasises the importance of this virulence factor in colonisation. ETEC also possess genes that encode for the secretory enterotoxins, heat-labile toxins I and II (LT-I, LT-II) and heat-stable toxin (Sta). Once ETEC have adhered to and colonised the small intestine, intestinal secretion is induced by promoting chloride ion (Cl) secretion from the small intestinal epithelial cells.

Enteropathogenic E. coli (EPEC) and enterohaemorrhagic E. coli (EHEC) have a pathogenicity locus that encodes for several genes which enable these organisms first to attach to the intestinal epithelium, and then to disrupt the brush border membrane to produce the so-called attaching-effacing lesion characteristic of these infections. EHEC, in addition, have a chromosomal gene that encodes for Shiga-like toxins I and II, also known as verocytotoxins, which have close sequence homology to Shiga toxin and similar inhibitory effects on protein synthesis. These cytoxins contribute to the inflammatory colitis that accompanies this infection.

Enteroinvasive E. coli (EIEC) differ from the other types in possessing several surface proteins that permit direct invasion of the bacterium into the host epithelial cell; these invasion plasmid antigens (Ipa A-D) are identical with those

| Type                  | Major clinical syndrome | Virulence factors                  |
|-----------------------|-------------------------|------------------------------------|
| Enteropathogenic      | Persistent diarrhoea    | Bundle forming pili Esp A,B,D Intimin Tir |
| (EPEC)                |                         |                                    |
| Enterotoxigenic       | Acute watery diarrhoea  | Pili LT-I, LT-II Sta                |
| (ETEC)                |                         |                                    |
| Enterohaemorrhagic    | Dyentery                | As for EPEC plus SLT-I, SLT-II      |
| (EHEC)                |                         |                                    |
| Enteroinvasive        | Dyentery                | Ipa ShET II                        |
| (EIEC)                |                         |                                    |
| Enteroaggregative     | Persistent diarrhoea    | Bundle forming pili EAST I          |
| (EspEC)               |                         |                                    |

EAST: enteroaggregative E. coli heat-stable toxin; Esp: EPEC secreted protein; Ipa: invasion plasmid antigen; LT: heat-labile toxin; ShET: Shigella enterotoxin; SLT: Shiga-like toxin; Sta: heat-stable toxin; Tir: translocated intimin receptor.
found in *Shigella* sp, making EIEC and *Shigella* close relatives both genetically and in the human disease they produce.

Thus, the difference between *E. coli* as commensals and as pathogens depends entirely on the presence of additional genetic material encoding for one or more of a series of virulence factors. These virulence factors are often located together on the bacterial chromosome or plasmid, suggesting that over time they have moved together to endow previous commensals with a complete 'pathogenicity package' or virulence cassette.

**It's good to talk: establishing the lines of communication**

Identification of the many interactions that occur between a pathogen and its host is clearly central to our understanding of the pathogenesis of disease, of immune and non-immune host defence mechanisms, and ultimately to the establishment of new therapeutic and prevention strategies. Enteric pathogens use a variety of lines of communication to secure a colonisation niche within the intestine, techniques which often involve subversion of host intracellular signalling pathways and utilisation of certain structural components of the host epithelial cell, particularly the cytoskeleton. Bacterial communication strategies can be classified into four major types depending on the final location of the bacterium:

1. The intestinal lumen.
2. Intimate adherence to, and disruption of, the apical membrane of the enterocyte.
3. Invasion into the epithelium and subepithelial structures.
4. Systemic effects, either by direct penetration into the circulation or through the action of distant signalling molecules such as cytokines.

Enteric pathogens have their primary interactions with the host epithelial cell or its specialised relative, the M cell covering the dome of Peyer's patches, but it is now increasingly clear that they also engage in cross-talk through other endogenous lines of communication, for example:

- the mucosal immune system
- inflammatory cells involved in host defence, such as polymorphonuclear neutrophils and mast cells
- the enteric nervous system.

*Communication' from the intestinal lumen*

Bacterial enteropathogens such as *Vibrio cholerae* and ETEC colonise the small intestine following pilus-mediated adherence to the enterocyte, and then cause acute watery diarrhoea by liberating enterotoxins (Fig 3).

**Cholera toxin and Escherichia coli heat-labile toxin.** These are closely related both structurally and immunogenically; they have about 85% sequence homology, the same A-B subunit substructure, one 'active' A subunit and five 'binding' B subunits (holotoxin, molecular weight ca 84,000 kDa) and the same GM_1 ganglioside receptor on the enterocyte apical membrane. Both activate adenylate cyclase located on the basolateral membrane of the enterocyte, which results in increased levels of intracellular cyclic AMP (cAMP), and ultimately in phosphorylation of the cystic fibrosis transmembrane regulator, the chloride channel, which leads to active Cl⁻ secretion and watery diarrhoea. *E. coli* STa is a much smaller molecule (ca 2,000 kDa) which binds to an apical membrane receptor linked to guanylate cyclase which, when activated, produces cyclic GMP. As yet, it has been impossible consistently to interfere with these pathways and inhibit secretion, although calmodulin antagonists such as zaldaride maleate can partially inhibit enterotoxin-mediated secretion by blocking the calcium-dependent component of the secretory process.

In recent years it has become evident that the classic adenylate cyclase-cAMP pathway of cholera toxin is not the only secretory mechanism, and that a neural reflex within the intestine may account for up to 50-60% of cholera toxin-induced fluid and electrolyte secretion. Cholera toxin is thought to release the potent secretagogue, 5-hydroxytryptamine (5-HT), from enterochromaffin cells of the intestinal epithelium. These cells are abundant in the intestinal tract and constitute the major reservoir of 5-HT in humans. The liberated 5-HT is thought to activate 5-HT_3 receptors located on a sensory afferent nerve which, through an interneuron in the myenteric plexus, then activates a vasoactive intestinal polypeptideergic (VIPergic) secretomotor afferent which releases the secretory neurotransmitter, VIP, at the basolateral membrane of the enterocyte to activate specific receptors and initiate intestinal secretion. 5-HT may also activate 5-HT_2 receptors in the lamina propria and on the enterocyte to promote synthesis and release of prostaglandins (PGs), particularly PGE_2, which also is a potent secretagogue.

The evidence for the presence of this pathway comes largely from studies in animals which have demonstrated both intestinal release of 5-HT and a close correlation between intraluminal 5-HT concentrations and the magnitude of the secretory response. In addition, the use of nerve blocking agents such as tetrodotoxin and lignocaine has clearly shown involvement of enteric nerves. Finally, the use of specific 5-HT and VIP antagonists has supported a direct role for them in the secretory process. Recent work in a human model of cholera secretion has demonstrated that the 5-HT_3 receptor antagonist, granisetron, can inhibit cholera secretion in humans. These antagonists may act not only by blocking neuronal 5-HT receptors but also by blocking autoreceptors on the enterochromaffin cell, thereby preventing further 5-HT release. *E. coli* LT and *E. coli* STa do not release 5-HT, and their related secretory states cannot be inhibited by 5-HT antagonists. The reason is not clear, but LT and ST secretion is inhibited by the local anaesthetic lignocaine and the sigma...
agonist, igmesine (thought to act via receptors on enteric nerves)\textsuperscript{19}, supporting the view that a neural reflex is also involved in the action of these toxins in the gut.

\textit{Vibrio cholerae}. It has recently been shown that \textit{V. cholerae} releases two other toxins:

- \textit{accessory cholera enterotoxin}, a secretory enterotoxin which augments short-circuit current in Ussing chambers, but the intracellular mechanisms of signal transduction have not yet been defined
- \textit{zonula occludens toxin} (ZOT), which activates an intracellular cascade involving activation of protein kinase C, actin polymerisation, and disruption of the protein complex at the ZO, including ZO1\textsuperscript{20}.

Again, disturbance of the host cytoskeleton by a bacterial product contributes to disease pathogenesis by increasing permeability of the tight junctions.

\textit{Communication} at the enterocyte apical membrane

\textit{Enteropathogenic Escherichia coli}. Some enteropathogens adhere intimately to the enterocyte microvillus membrane and activate host intracellular signalling pathways. The mechanism by which EPEC produces the typical ‘attaching and effacing’ lesion is now well understood\textsuperscript{21,22}. A three-stage model has been developed to describe the process:

1. \textit{Non-intimate attachment}, which was thought to be mediated by a bundle-forming pilus, but recent work suggests that the bundle-forming pilus functions only to attach EPEC to each other, and that adhesion to the enterocyte is mediated by another filamentous organelle which contains an EPEC-secreted protein, EspA, exported by a type III secretion system\textsuperscript{22}.

2. \textit{Signal transduction and cytoskeletal rearrangement in the host cell}, initiated by other secretory proteins, EspB and EspD, which are translocated into the infected host cell.

3. \textit{Intimate bacterial adhesion}, accompanied by actin accumulation in the host cell beneath the organism, followed by pedestal formation which requires an outer membrane protein adhesin, intimin, which binds to a protein receptor (formally HP90), originally thought to be of host origin but now known to be an EPEC secreted protein, the translocated intimin receptor (Tir)\textsuperscript{21}. The genes for EspA, EspB and EspD, and for intimin and Tir are contained in a chromosomal pathogenicity island, designated the locus for enterocyte effacement (LEE).

\textit{Enterohaemorrhagic Escherichia coli}. A similar ‘attaching and effacing’ lesion is produced by EHEC with the same pathogenicity locus. Thus, these organisms are able to rearrange the host cytoskeleton, presumably to create an effective environment for colonisation. It is extraordinary that these organisms synthesise an attachment ligand together with its specific receptor, which is then secreted and inserted into the host cell membrane.

\textit{Intestinal protozoa}. Intestinal protozoa can also signal to host cells, again presumably to create a more advantageous environment. \textit{Cryptosporidium parvum} secretes a phospholipase which is apparently essential in the attachment process, and may also be involved in initiating intracellular signals that lead to cytoskeletal rearrangements\textsuperscript{23}. \textit{Giardia intestinalis}, an exclusively extracellular protozoa, also appears to be able to signal to the host cell through cell-cell surface contact and, by an as yet unidentified pathway, to stimulate the enzyme ornithine decarboxylase, a marker of cell proliferation\textsuperscript{24}.

\begin{figure}[h]
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\caption{Classic mechanisms of action of cholera toxin and \textit{Escherichia coli} enterotoxin (AC: adenylate cyclase; ATP: adenosine triphosphate; CaM: calmodulin; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; CT: cholera toxin; GC: guanylate cyclase; GTP: guanosine triphosphate; LT: heat-labile toxin; PK: protein kinase; ST: heat-stable toxin).}
\end{figure}
Entamoeba histolytica. Ulceration and inflammation in the colon is produced by *E. histolytica*, but it is not primarily an invasive organism. It produces its cytolethal effects through cell-cell contact; initial engagement depends on a surface membrane-associated galactose-binding lectin which mediates adherence to the host epithelial cell, followed by the release of a variety of hydrolytic enzymes and pore-forming proteins. A protein of particular interest, amoebapore, is released and inserted into the host cell membrane, producing high conductance ion channels leading to a rapid increase in intracellular calcium and cell death. *E. histolytica* has also been shown to contain and release 5-HT, which could theoretically activate neural reflexes, as described above for cholera toxin. As yet, there is no evidence that this is an important mechanism of diarrhoea in human infection.

‘Communication’ by invasion

The mechanism by which invasive organisms such as *Salmonella, Shigella* and EIEC enter the host epithelial cell depends on their ability to trigger a signalling cascade which leads to cytoskeletal rearrangements that permit entry of the organism into the host epithelial cell in an endocytotic vesicle (Fig 4). Following contact of the organism with the host surface membrane, there is localised membrane ruffling, rearrangement of actin filaments and capping of host surface proteins.

*Salmonella*. Invasion with *Salmonella* is rapid, with internalisation of bacteria in vacuoles within minutes, and associated with calcium influx and activation of the inositol phosphate transduction pathways. Regulation of the cytoskeletal rearrangement appears to be under the control of CDC 42, one of the ras-related superfamilies of small GTPases. *Salmonella* has a chromosomal pathogenicity island, SPI-1, containing several invasion operons including inv/spa which are homologous with invasion genes of other invasive bacteria.

*Shigella*. Invasion mechanisms used by *Shigella* are similar to those used by *Salmonella*, again involving actin polymerisation, in this case dependent on the small GTPase Rho. Invasion is mediated by three surface effector proteins, IpaB, IpaC and IpaD, which trigger the host endocytotic process followed by release of *Shigella* from the vacuole mediated by IpaB. These invasion mechanisms again exemplify the stealth of enteropathogenic bacteria in using their own surface or secreted proteins to subvert host cell structures to advance the colonisation process.

Intimate attachment and invasion of enteropathogens into the host epithelial cell also stimulate the synthesis and release of pro-inflammatory cytokines. Many organisms such as EHEC, *Shigella, Salmonella* and *C. parvum* stimulate intestinal epithelial cells to produce the potent chemotactant interleukin (IL)-8 which promotes a rapid influx of neutrophils into the lamina propria of infected intestines. This is an appropriate host response to limit the progress of an infecting microorganism, but the presence of large numbers of neutrophils enhances the inflammatory cascade and ultimately increases tissue damage and the secretory response within the epithelium. Inhibition of neutrophil influx by administration of an antibody to the cell adhesion molecule CD18 not only reduces the mucosal inflammatory response and structural damage but also diminishes the epithelial secretion of fluid and electrolytes.

‘Communication’ by distant signalling molecules

Microorganisms that invade the epithelium, and in some cases penetrate the systemic circulation, can have far-reaching effects beyond the structural and functional
derangement that occurs in the intestine (Fig 5) – effects on host nutrition mediated through pathways in the central nervous system. Organisms that produce a chronic inflammatory process within the intestine produce systemic effects such as fever, anorexia and, if infection is prolonged, undernutrition. These infections result in the release of pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor-α, which mediate the effects of systemic immune activation and are probably the prime effectors of anorexia and undernutrition.36,37

Transgenic mice which overexpress IL-6 are 50–70% the size of non-transgenic litter mates and have reduced circulating concentrations of immunoglobulin IGF-1 but normal growth hormone levels. Administration of a monoclonal antibody directed towards the murine IL-6 receptor partially reverses the growth defect, indicating that growth failure and the decrease in IGF-1 are mediated by IL-6.38

In models of chronic intestinal inflammation associated with anorexia and weight loss, the pro-inflammatory cytokines produced in inflamed gut have a dual role, both reducing appetite and food intake and directly impairing growth and development by a mechanism independent of food intake. These cytokines, particularly IL-1 and IL-6, have central effects in hypothalamic nuclei which are responsible for control of feeding behaviour. In rat models of experimental colitis, IL-6 has been implicated as an aetiologic factor in the impaired bone growth, and the reduction in food intake and the associated impaired weight gain are thought to be related to inappropriate responses of hypothalamic neuropeptide Y and 5-HT, neurotransmitters with established central roles in the control of feeding behaviour.

Implications of pathogen-host cross-talk

The lines of communication are varied and complex between microorganisms within the GI tract and host epithelial cells, enteric nerves and a variety of inflammatory cells. Enteropathogens utilise host intracellular machinery to optimise the colonisation process, and probably to ensure wider dissemination of the species to secure survival. Identification of these pathways has already offered new possibilities for therapeutic interventions. These include the development of new anti-secretory drugs for the treatment of enterotoxin-mediated diarrhoea, and the potential importance of anticytokine antibodies in inhibiting inflammation, thereby decreasing the impact of infective intestinal inflammatory disease on appetite and nutritional status, particularly because of the secondary effects on growth and development in children.

Enhanced understanding of these processes is also likely to continue to provide new therapeutic targets for vaccine development in the future.

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