Leading article – Tropical infection of the gastrointestinal tract and liver series

The shigella paradigm and colitis due to enterohaemorrhagic *Escherichia coli*

Colitis may be broadly defined as any inflammatory process involving the colon. In Europe and the USA the aetiology of colitis is not usually considered to be infectious, unless associated with overseas travel. Rather, it falls into the category of 'inflammatory bowel disease', a group of conditions of unknown aetiology, with an immunological component in many. In the developing world, however, colitis is often due to infection, especially *Shigella* sp among the bacteria, or *Entamoeba histolytica* among the protozoa. The inability to find an aetiological agent in most cases in Western countries is intriguing and raises the interesting speculation whether colitis can be precipitated by infections that are either not searched for, are present only transiently, or cannot be identified by current laboratory methods.

The two most common bacterial causes of colitis are shigella and enterohaemorrhagic *Escherichia coli*. With the exception of enterohaemorrhagic *E coli*, colitis causing bacteria are invasive, including shigella, salmonella, campylobacter, and yersinia. Enterohaemorrhagic *E coli* is not invasive, but causes structural damage to enterocytes and resembles shigella in producing related cytotoxins. This article focuses on recent advances in understanding the molecular pathogenesis of shigella and enterohaemorrhagic *E coli* infections.

Shigella

The four species of *Shigella* (*dysenteriae*, *flexneri*, *boydii*, and *sonnei*) all cause colitis, albeit with diminishing severity from the first to the last. Invasion of the colonic mucosa, probably proceeding by a common mechanism among the four species, is a critical virulence determinant in the pathogenesis of shigellosis. *S dysenteriae* is the only shigella producing significant levels of the cytotoxin, shiga toxin, and its role in pathogenesis is controversial. Hypotoxigenic mutants of *S dysenteriae* produce a less severe and less prolonged illness in humans compared with toxin positive controls.¹ Infection of rhesus monkeys with a toxin gene deletion mutant of *S dysenteriae* type 1 resulted in dysentery although the parent toxin positive strain caused a more bloody diarrhoea, with more capillary damage seen histologically.²

Irrespective of the role of shiga toxin, shigella dysentery is characterised by an invasive bacterial colitis. The importance of mucosal invasion is underscored by the extremely low dose of organisms needed to cause disease in humans, with as few as 10 *S dysenteriae* type 1 able to cause dysentery in adults.³ The invasive process has been dissected using *S flexneri* almost exclusively, and extrapolated to the other species. Shigella pathogenesis is complex.⁴ The organisms presumably first adhere to and then invade the gut mucosa, although whether there is a separable adherence step, and whether they invade epithelial cells or M cells remains controversial. The intracellular bacteria multiply and subsequently invade the underlying lamina propria, however this invasion is comparatively limited in relation to the degree of the inflammatory response elicited. Are there causes of inflammation

other than invasion in itself? Inflammation results in focal abscess and ulcer formation, and when severe enough, ileus, toxic megacolon, gross haemorrhage, or perforation. Systemic complications such as leukaemoid reactions and haemolytic uraemic syndrome are common in *S dysenteriae* type 1 infection and thus may be related to shiga toxin.

The molecular mechanisms participating in the pathogenesis of shigella may be divided into four events: (a) cellular invasion; (b) intracellular multiplication; (c) intracellular and cell to cell spread of organisms; (d) and host cell killing.⁴ *S flexneri* is internalised by a bacterium directed endocytic process, requiring energy produced by both microbe and host cells, and is associated with a 220–240 Kb plasmid. Invasion is temperature regulated; organisms are non-invasive at 30°C but fully invasive at 37°C.⁵ Multiple genes control entry of shigella and they seem to lie in five independent contiguous loci within a 30 Kb segment of the invasion plasmid. A positive regulatory gene (*virB*), under the control of a second regulatory gene (*virF*), induces invasion genes contained in other loci. The most studied of these is locus 2, which encodes four invasion plasmid antigens (ipa A, B, C, and D). These antigens induce antibodies detectable in convalescent human serum samples, indicating they are made in vivo. Selective ipa deletion mutants show, however, that ipaA is not essential to pathogenesis in animal models. Certain chromosomal loci are also essential for shigella virulence, but we do not propose to discuss them here.

While it is a logical first assumption that the apical surface of colonocytes is the primary route of entry for shigella, in certain tissue culture cell lines, such as Caco2 cells, invasion occurs at the basolateral surface of the cells in preference to the apical membrane.⁴ Indeed, in situ studies in human tissues suggest that colonocytes are not even the primary site of invasion, which may actually be M cells.⁴ This supposition is supported by data from experimental shigellosis in rabbits.⁶

Once organisms occupy an intracellular locus they multiply rapidly. In HeLa cells five organisms can multiply to 500 in four to five hours. Organisms enter cells within phagocytic vesicles, however vesicle lysis occurs shortly after phagocytosis and probably is a prerequisite for efficient intracellular multiplication. Shigella also require other genes for good intracellular growth; thus *aro* mutants grow significantly more slowly than wild type strains. This, and precedents in other organisms in which *aro* mutants are attenuated, has led to attempts to exploit such mutants as shigella vaccine strains. Deletions in *ompB*, an osmoregulatory locus, also limits intracellular growth and *ompB* mutants are less virulent in vivo.⁷

Movement of multiplying organisms within and between cells is also necessary for shigella pathogenesis. Within two hours of entry, intracellular shigella become coated with polymerised actin at one end of the bacterium. Continuous deposition of polymerised actin at one end of the rod shaped organism results in a propulsive forward movement. *TnPhoA* insertional mutations of the *icsA* gene, which encodes a 120 kDa outer membrane protein, are associated with loss of intracellular movement and

virulence.⁸ In vivo experiments in monkeys point to a crucial role for *icsA* in shigellosis.⁴ Additional mechanisms of movement also exist in shigella and seem to involve interactions with stress fibres within the host cell.⁹ Recent data using a *S flexneri* macrophage invasion model suggest that invading organisms induce apoptotic suicide in host cells,¹⁰ and implicate apoptosis as the underlying mechanism by which the organisms induce eukaryotic cell death.

While there is still much to be learned about the molecular pathogenesis of shigella infection these genetic studies have paved the way for creating new vaccine candidates based on the deletion of single or multiple virulence genes. To date, no successful candidate has emerged and further work is required.

Enterohaemorrhagic *Escherichia coli*

Enterohaemorrhagic *E coli* is non-invasive and resembles enteropathogenic *E coli* except for production of shiga like toxins. Population genetics strongly suggest that the most prominent enterohaemorrhagic *E coli*, serotype O157:H7, evolved from a classic enteropathogenic *E coli*, O55:H7, by the acquisition of toxin genes. The first well publicised outbreak with enterohaemorrhagic *E coli* was in 1982¹¹ in which a previously obscure *E coli* serotype, O157:H7, was isolated from haemorrhagic colitis patients in two American states. After the discovery, *E coli* O157:H7 was sought and found to be a common isolate and it is one of the most common causes of colitis in the USA and western Europe. A Canadian study in 1987 reported that enterohaemorrhagic *E coli* is the third most common bacterial pathogen found in routine stool samples from diarrhoea patients, trailing only salmonella and campylobacter.¹² Enterohaemorrhagic *E coli* causes a wide spectrum of clinical disease including bloody and non-bloody diarrhoea, and may be cultured from asymptomatic carriers as well.¹⁴ In contrast with shigellosis, fever may or may not be present in enterohaemorrhagic *E coli* infections, and faecal leucocytes are not usually detected. Low levels of suspicion among clinicians regarding this organism, coupled with the need for special microbiological studies to detect it, has resulted in problems other than underdiagnosis. For example, inappropriate treatment with sulphasalazine or methylprednisolone for presumed inflammatory bowel disease is reported, and barium enemas for presumed intussusception or even laparotomies for presumed ischaemic bowel¹⁴ have been done. Patients with presumptive classic ulcerative colitis have on occasion been found to be infected with shiga like toxin-producing *E coli*,¹⁵ 16 which raises the interesting question of its role: cause, provocateur, or unrelated?

Enterohaemorrhagic *E coli* is not only responsible for initiating colitis but often causes significant systemic complications as well, particularly haemolytic uraemic syndrome, now the commonest cause of acute renal failure in children in the USA. The fact that haemolytic uraemic syndrome is associated with two organisms that make shiga family toxins is the epidemiological basis for the hypothesis that toxin plays a part in pathogenesis. Two large outbreaks of *E coli*, O157:H7 infection occurred in the USA in early 1993, with multiple cases of haemolytic uraemic syndrome and several deaths, illustrating both the scope of the problem and the need to be aware of enterohaemorrhagic *E coli* as a rapidly emerging pathogen.

The precise mechanism by which enterohaemorrhagic *E coli* causes colitis and associated systemic complications are unknown. Enterohaemorrhagic *E coli* is not thought to be invasive,¹⁷ except perhaps for certain tissue culture cell lines.¹⁸ Enterohaemorrhagic *E coli* possesses other important virulence factors, however, including the ability

to attach to and induce effacing lesions on the apical membranes of mucosal cells, a change in the cell membrane with microvillus dissolution and formation of pedestal like regions underlying attached bacteria, and the ability to produce potent cytotoxins known as shiga like toxins in the USA because of their structural and functional resemblance to shiga toxin, or verotoxins in Europe because they are highly active in this cell line. Shiga like toxins encompass an ever increasing family of toxins, related both biochemically and biologically.¹⁹⁻²¹ The presence of shiga like toxins is one of the most consistent findings in both local and systemic enterohaemorrhagic *E coli* associated disease, as well as in the stools of patients colonised with enterohaemorrhagic *E coli*. Precisely how the toxins participate in the pathogenesis in vivo remains a mystery. Currently at least 50 different *E coli* serotypes, all of which make shiga like toxins, have been associated either with haemorrhagic colitis or haemolytic uraemic syndrome, or both.

Colonic abnormality in patients infected with *E coli* O157:H7 shows oedema, fibrin deposition, and haemorrhage in the submucosa, along with mucosal ulceration and haemorrhage, neutrophil infiltration, and microvascular thrombi.²² How much of this damage is caused by shiga like toxins is unknown. How the shiga like toxins can get from the gut lumen, where produced, to the submucosal tissues is also unknown. Preliminary data from our laboratory (Acheson *et al.*, unpublished) suggest that shiga like toxins translocate across differentiated intestinal tissue culture cell lines such as CaCo2 or T84 cells, which have formed tight junctions as determined by measurement of mucosal resistance. The bacteria themselves, in the formation of attaching and effacing lesions, may also participate in toxin uptake. Attaching and effacing lesions are associated with polymerisation of intracellular actin, changes in calcium, phosphorylation of several intracellular proteins,²³ 24 and a change in transepithelial conductance.²⁵

Endothelial cells may be a major target in both local and systemic complications of enterohaemorrhagic *E coli* infection, associated with a thrombotic microangiopathy. Shiga like toxins act directly on endothelial cells, suspected to be a primary target for the toxin's action.²⁶ 27 Shiga like toxins are cytotoxic to human endothelial cells,²⁸ especially in the presence of tumour necrosis factor, and the cells reduce prostacyclin release.²⁹ The glycolipid receptor for shiga like toxins, globotriaosylceramide (Gb3) is present in kidney and cultured human endothelial cells. In the second, Gb3 is also induced by tumour necrosis factor α .³⁰ In view of the common association of endotoxaemia with systemic effects in shigellosis, and the extremely potent upregulation of tumour necrosis factor and other cytokines by bacterial endotoxin, it is possible that endotoxaemia sensitises endothelial cells to the effects of the shiga like toxins.³¹

It is now becoming clear that interactions of bacterial cells themselves with epithelial cells may initiate a cascade of events including the release of inflammatory cytokines.³² In humans, the development of colitis after infection with enterohaemorrhagic *E coli* can be explained by a combination of factors including attaching and effacing lesions, subsequent changes in epithelial cell metabolism, and the interaction of shiga like toxins with the capillary endothelial cells in the intestine. The last may be exaggerated by cytokines released either directly from intestinal epithelial cells or systemically secondary to endotoxaemia.

Enterohaemorrhagic *E coli* is now one of the most commonly diagnosed bacterial causes of colitis in the USA and Europe. O157:H7 is the serotype most frequently

detected because it is the only shiga like toxin-producing *E coli* that consistently fails to ferment sorbitol, a property that permits its identification in the laboratory. This will miss sorbitol positive shiga like toxin-producing *E coli*, however, and there is substantial evidence to support the notion that many other shiga like toxin-producing serotypes cause disease in humans.³³ It may be more productive to search for toxin production, and toxin based tests are being developed but currently are only available on a research basis.^{34 35} Widespread use of such tests may change our appreciation of the epidemiology of enterohaemorrhagic *E coli* infections.

An as yet unanswered speculation is that the invasive property of the shigella might provide it with enhanced capability to deliver shiga toxin systemically compared with the non-invasive enterohaemorrhagic *E coli*. The second is more often associated with grossly bloody diarrhoea, however, pointing to greater capillary destruction, which frequently leads to systemic complications such as thrombotic microangiopathy and haemolytic uraemic syndrome. These findings show that the molecular mechanisms involved in the pathogenesis of shigella and enterohaemorrhagic *E coli* are far more complex than our current understanding.

In conclusion, we believe that a search for shiga like toxin producing *E coli* in patients presenting with bloody diarrhoea should be done as a routine investigation, along with microbiological studies for shigella, salmonella, campylobacter, and perhaps yersinia. Critics of this approach question the value of knowing the causative organism in the case of enterohaemorrhagic *E coli* because there are no clear guidelines for specific treatment decisions. For example, there are no good data regarding even the clinical value of eradicating enterohaemorrhagic *E coli* with antibiotics, tempered by the speculation that antibiotics may provoke more severe illness. Knowing that a subject is infected with enterohaemorrhagic *E coli*, however, should alert the attending physician to the possibility of systemic complications such as haemolytic uraemic syndrome, and is critical in relation to epidemiological and containment considerations, given the potential of the organism to cause significant outbreaks.

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