Annotations

Human *Escherichia coli* diarrhoea

Striking advances have been made in the last decade in elucidating the mechanisms of diarrhoea induced by *Escherichia coli*. This has resulted in a reclassification of these organisms that is based on their pathogenic properties.

Enterotoxigenic *E coli*

Enterotoxins. Enterotoxigenic *E coli* cause cholera like diarrhoea by releasing polypeptides (enterotoxins) that induce intestinal secretion of fluid and electrolytes. Two enterotoxins have been identified, designated LT (heat labile) and ST (heat stable).¹

LT (molecular weight 73 000) shows striking homology with the enterotoxin of *Vibrio cholerae* in protein structure, immunological cross reaction, brush border receptor, and mode of action. LT attaches to the brush border of the small intestine by the binding of five identical (B) subunits of the toxin to galactose residues of the brush border glycolipid GM₁ ganglioside, with enzyme like specificity. The toxic (A) subunit of the LT molecule then enters the enterocyte via 'pores' created in the brush border membrane by a ring of five B subunits. The A subunit, itself an enzyme, interacts with the enzyme adenylate cyclase (AC) located in the basal and lateral membranes of the enterocyte in such a manner that AC is permanently 'switched on' and intracellular concentrations of cyclic AMP (cAMP) rise. The mechanisms by which raised cAMP concentrations cause massive losses of electrolytes (especially Na⁺, Cl⁻, HCO₃⁻) and water across the brush border into the intestinal lumen are still controversial. The net result, however, is that the capacity of the colon to reabsorb electrolytes and water is overwhelmed by small bowel secretion and cholera-like diarrhoea occurs.²

ST is an 18 amino acid peptide that owes its remarkable structural stability to 6 cystine molecules which hold the peptide chain together by disulphide bonds.³ Its mode of action is to activate the enzyme guanylate cyclase in the enterocyte brush border and this blocks neutral NaCl absorption.^{1 2}

Colonisation factors. Enterotoxigenic *E coli* require virulence factors additional to enterotoxins in order to cause diarrhoea. To colonise the small bowel these organisms adhere to the microvilli of the brush

border and resist expulsion by peristalsis.⁴ Filamentous protein structures on the bacterial surface, termed fimbriae or pili, have been identified which allow specific adherence of enterotoxigenic *E coli* to human small intestinal (but not colonic) epithelial cells.⁵ Two separate fimbrial 'colonisation factors' have been described so far, designated CFA/I and CFA/II. CFA/II has been shown recently to consist of three immunologically and morphologically distinct proteins (CS1 and CS2, filamentous; CS3, fibrillar) that may be produced in varying combinations by enterotoxigenic *E coli*.⁶

Genetic control of virulence factors. The genes coding for LT, ST, CFA/I, and CFA/II are all found on species of extrachromosomal DNA called plasmids.¹ Plasmids can be transferred from one strain of *E coli* to another so that an individual strain can gain (or lose) the properties that define enterotoxigenic *E coli*.

The recent construction of radiolabelled 'probes' for these genes makes possible the accurate identification of enterotoxigenic *E coli* in stools without the need for culture or bioassay.⁷ This will greatly increase the potential scale and accuracy of epidemiological surveys.

Enteroinvasive *E coli*

There is a group of *E coli* that is closely related to shigellae, both biochemically and in the diarrhoeal disease that it induces. Enteroinvasive *E coli* cause dysenteric disease by invading and destroying the mucosal cells of the distal intestine resulting in ulceration and the passage of bloody stools with pus and mucus.

Less is known about the pathogenic mechanisms of enteroinvasive *E coli*, but specific mucosal attachment mechanisms⁸ and toxin production have been described⁹ and it is becoming clear that both plasmid and chromosomal genes may be involved in the expression of virulence determinants.¹⁰

Enteropathogenic *E coli*

Serotypes. These organisms, identified by somatic antigen (0) serotyping, are familiar to senior paediatricians as the organisms that were linked in

epidemiological studies to serious outbreaks of 'summer diarrhoea' in the 1950s.¹¹ These organisms do not produce LT, ST, CFA/I or CFA/II and they do not invade the mucosa.^{11 12} The absence of demonstrable virulence properties led some workers, until recently, to reject enteropathogenic *E coli* as true pathogens, in spite of the fact that epidemiological evidence continued to associate various serogroups with outbreaks of diarrhoea. Furthermore the cholera-like diarrhoea which could still be produced by strains of enteropathogenic *E coli* remained convincing evidence of pathogenicity to adult volunteers and their medical attendants.¹²

Mucosal attachment. The breakthrough came with the description of a specific ultrastructural appearance of the brush border at sites of mucosal attachment of enteropathogenic *E coli*. These organisms seem to be capable of destroying brush border microvilli and fashioning for themselves in the process a cup-like pedestal of bare plasma membrane in which individual bacteria sit like eggs in egg cups.¹³ All enteropathogenic *E coli* serogroups that have been tested so far, in vivo in animals¹⁴ and in vitro using human intestinal mucosal organ culture,¹¹ induce an identical lesion.

Adhesion of an enteropathogenic *E coli* strain to human mucosa was shown some years ago to be mediated by a 60 Md plasmid.¹⁵ This plasmid has recently been shown to be ubiquitous in enteropathogenic *E coli* and, using cultured human epithelial cells as a model for gastrointestinal adhesion, the genes coding for adhesion have been identified.¹⁶

Enterotoxins. When cultured in iron depleted media certain strains of enteropathogenic *E coli* synthesise an enterotoxin identical to that produced by *Shigella dysenteriae* I.¹¹ This protein, molecular weight 70 000, is capable of producing gut damage and fluid secretion, death of human epithelial cells in culture, and paralysis and death in mice. Its role in diarrhoeal disease is still not proved.

Summary

Using the microbiological techniques that are currently available, it has been shown that enterotoxigenic *E coli* are the most common cause of travellers' diarrhoea, and that enterotoxigenic *E coli*, enteropathogenic *E coli*, and to a lesser extent enteroinvasive *E coli* are important causes of diarrhoeal disease in infants in developing countries. Further elucidation of pathogenic mechanisms at the

molecular level will lead to the production of gene probes and the possibility of accurate epidemiological studies. It will also bring the possibility of effective oral vaccines one step nearer.

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