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ORIGINAL ARTICLE

Commensal bacteria do translocate across the intestinal barrier in surgical patients ☆

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Summary
Background: The ''gut origin of sepsis'' hypothesis proposes that enteric bacteria may cause sepsis at distant extra-intestinal sites. Whilst there is much circumstantial evidence to support this hypothesis, there is no conclusive proof in humans. The nature of translocating bacteria remains unclear. The aim of this study was to establish the origin of *Escherichia coli* (*E. coli*) cultured from mesenteric lymph nodes (MLN) and determine if they belonged to any recognized pathotypes known to cause infections in humans.
Methods: MLN and faecal samples were obtained from 98 patients undergoing colonic resection. *E. coli* were isolated from 9/98 MLN samples. DNA fingerprints of MLN isolates were compared with faecal isolates from the same patient. MLN isolates were tested for adherence and invasion using HEp-2 epithelial cells, and screened for DNA markers indicative of different pathotypes of *E. coli*. MLN isolates were also tested for internalisation into Caco-2 cells.
Results: All the nine *E. coli* cultured from MLNs were found to have identical DNA fingerprints to at least one and often several *E. coli* isolates cultured from faecal samples of the same patient. 8/9 (89%) MLN isolates were weakly adherent and 2/9 (22.2%) were invasive. 8/9 (89%) tested negative for DNA markers. All the nine MLN strains were internalised by Caco-2 cells.
Conclusion: This study confirms the gut origin of translocating bacteria. Most translocating *E. coli* do not belong to any recognised pathotype and are therefore normal commensal

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microflora. Our results suggest that bacterial translocation is more dependent upon the gut epithelium rather than the virulence properties of resident enteric bacteria.

Introduction

Bacterial translocation is defined as the passage of viable bacteria or endotoxins across the intestinal epithelium to mesenteric lymph nodes (MLN) and beyond, possibly contributing to sepsis at distant extra-intestinal sites.[1] Most of the evidence supporting the gut as the origin of these bacteria is derived from animal and *in vitro* models.[2] In humans, the identification of enteric bacteria as a frequent cause of sepsis is regarded as indirect evidence to propose the gastrointestinal tract as an undrained abscess of multiorgan failure[3] and support of the gut origin of sepsis hypothesis.[4]

The nature of these translocating bacteria remains unclear. Some authors have suggested that these organisms must have specific properties that predispose to translocation or belong to an invasive pathotype.[5,6] Others however, have demonstrated that non-pathogenic commensal bacteria were able to translocate across an intact gut epithelium.[7,8]

The aims of this study therefore were twofold; firstly to confirm that the *Escherichia coli* (*E. coli*) cultured from MLN had originated from the lumen of the gastrointestinal tract, which by definition would confirm translocation across the gut barrier and secondly, to determine whether or not these bacteria have any specific attributes that predispose to translocation, such as adherence, invasion and DNA markers indicative of pathogenicity.

Patients and methods

This was a prospective study conducted with the approval of the Scarborough Hospital's Local Research Ethics Committee. A consecutive series of 98 elective surgical patients undergoing colectomy were recruited. Patients were excluded if they had evidence of intra-peritoneal sepsis or contamination and if sampling of lymph nodes was impractical or clinically inappropriate. All patients received intravenous cefuroxime and metronidazole as prophylactic antibiotics on induction of anaesthesia.

To confirm bacterial translocation, bacterial phenotypes obtained from MLN were compared to isolates obtained simultaneously from the lumen of the gastrointestinal tract. Virulence properties of bacteria were assessed by evaluating adherence properties, invasive abilities and by identifying DNA markers indicative of pathogenicity. In order to confirm that these bacteria could passively internalise into gastrointestinal epithelial cells, we used a 3 h co-incubation assay with a Caco-2 cell line.

Lymph node and stool sampling

Our objective was to identify bacterial phenotypes from MLN isolates and compare them with isolates obtained simultaneously from the lumen of the gastrointestinal tract. The methodology of MLN harvesting has been published previously.[4,6,9,10] In all patients, a sample of faeces was obtained from within the lumen of the resected colectomy specimen immediately after it was removed from the operative field. These faecal samples were homogenised in tryptone soya broth containing 15% glycerol (v/v) as cryoprotectant and stored at −70 °C until culture.

Isolation of bacteria from lymph node and stool samples

Lymph node samples were thoroughly rinsed in saline before analysis to remove any surface contamination, and then homogenized in 0.5–1 ml of peptone water. The homogenate was inoculated onto Cystine-Lactose-Electrolyte Deficient (CLED) agar and Columbia blood agar (Oxoid, Basingstoke UK) for incubation with and without air. All plates were incubated at 37 °C for 48 h. Faecal samples were serially diluted in phosphate buffered saline (PBS), and then plated onto MacConkey agar for incubation at 37 °C for 24 h. Four *E. coli* colonies were selected at random from amongst those cultured from each faecal sample. The identity of MLN and faecal isolates morphologically resembling *E. coli* was confirmed using API 20E identification strips (BioMerieux, Lyon, France), and checking for β-glucoronidase activity on TBX agar (Oxoid, Basingstoke, UK) at 44 °C.

DNA fingerprinting

To compare *E. coli* isolates, DNA fingerprints were generated by amplifying regions of DNA with consensus primers for Enterobacterial Repetitive Intergenic Consensus (ERIC) and Repetitive Extragenic Palindromic (REP) sequences as described by Versalovic et al.[11] Amplification was performed in 25 μl reaction volumes comprising 1.5u *Taq* polymerase, 1x Thermo Pol II buffer (New England Biolabs, Hertfordshire, UK), 1.5 mM Mg^{2+}, 1.25 mM dNTPs (Invitrogen, Paisley, UK), 10% dimethyl sulfoxide, and 50 pm of primers (REPIR-1, or ERICIR plus ERIC2).[11] Amplification reactions involved an initial denaturation (95 °C, 7 min) followed by 30 cycles of denaturation (90 °C, 30 s), annealing (42 °C for REP, 52 °C for ERIC, 1 min) and extension (65 °C, 8 min), then a single final extension of 65 °C for 16 min. Amplification products were resolved by running 10 μl aliquots on 1.5% agarose gels containing 1 × TAE buffer, and visualised after staining in ethidium bromide. When comparing patterns, strains were not considered to match each other if the profiles differed by more than two bands.