

Dissecting the Molecular Mechanisms of Intestinal Bacterial Translocation to Facilitate Definition of its Proposed Role in Systemic Sepsis

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Abstract

Intestinal translocation of bacteria is defined as the ingress of gastrointestinal microflora across the lamina propria to local mesenteric lymph nodes and thence to extranodal sites. Bacterial translocation has been long been considered as a possible direct cause of sepsis when under certain conditions bacteria cross the intestinal barrier, enter the systemic circulation and cause ageneralised inflammatory response syndrome. While this is an attractive hypothesis, which finds support from experimental models, evidence from clinical studies is equivocal in confirming that bacterial translocation is the primary cause of sepsis. Moreover, the underlying mechanisms by which gut bacteria gain entry to the systemic circulation are not well defined. This review provides a brief overview of bacterial translocation in the intestine, discusses our current understanding of the role it plays in the development of sepsis syndrome and suggests areas for future research to determine the molecular mechanism(s) involved in the aetiology of disease.

Keywords Bacterial translocation; mesenteric lymph node; gastrointestinal microflora; enteric bacteria; sepsis

List of abbreviation: MLN = mesenteric lymph nodes, E. coli = *Escherichia coli*, MODS = multiple organ dysfunction syndrome.

Introduction

An important function of the mammalian intestinal epithelial barrier is to prevent gut bacteria from invading systemic organs and tissues. However, in specific circumstances, gut bacteria may cross the epithelial barrier and appear in mesenteric lymph nodes (MLN) and possibly other organs. This movement is called bacterial translocation⁽¹⁾.

The anatomical site of bacterial translocation has been investigated in many studies with the consensus view that the rate of passage is greater in the small intestine (composed of the duodenum, jejunum and ileum) than it is in the

large intestine (caecum, colon, rectum, and anus)^(2,3). This is related to the former location's role in food digestion and absorption of nutrients.

Three Primary Mechanisms Promoting Bacterial Translocation

As determined by investigation in animal models, translocation of enteric bacteria is considered to depend primarily on three influences:

1. *Disturbance of the ecological equilibrium of the indigenous microflora.* This may be through factors that regulate bacterial population size, such as impaired coordination of gene expression via quorum sensing or exposure to ingested antibiotics and other chemotherapeutic agents, in each

case resulting in overgrowth of certain bacteria, including opportunistic pathogens⁽⁴⁾. In mice with normal intestinal barrier function, bacterial translocation may occur if certain enteric bacteria reach or exceed a local population density of 10^{9-10} bacteria/gram of caecal content or stool⁽⁵⁾.

2. *Physical disruption of the gut mucosal barrier.* Increased permeability of the epithelial barrier may be a consequence of a breakdown of tight junctions or loss of cell integrity, thus increasing bacterial passage to underlying tissue structures⁽⁶⁾.
3. *Impaired host immunity.* The large and diverse microbial communities that exist in the gastrointestinal tract repeatedly challenge the mucosal immune system. This is a complex, multi-factorial network that interacts to maintain organisms at a normal level and/or eradicate potential pathogens that may cross the protective barrier⁽⁷⁾. Thus, a breakdown in immune function can lead to survival and overgrowth of bacteria that usually would be maintained at a healthily balanced population density⁽⁸⁾.

When all three factors occur simultaneously, bacterial translocation occurs at a higher rate than when only one or two components are present⁽⁹⁾. In addition to these host-related conditions, bacteria may be implicated directly in this process. Species that are able to control the expression of virulence genes, along with amassing an overwhelming cell population density, both have a significant survival advantage and possess the capability to overrun an immune response before it is fully initiated^(10,11).

Additionally, there are a number of physical events, which promote translocation. Accumulating evidence from human and animal studies indicates that events such as haemorrhagic stress, burn injury, trauma, endotoxaemia, malnutrition, fasting and intestinal obstruction promote bacterial translocation⁽¹²⁻¹⁶⁾. It is presumed that the stress response of the host leads to an increase in the three conditions discussed above, thereby

causing an enhanced rate and/or absolute level of bacterial translocation.

Bacterial Survival Strategies

After passing through the mucosal barrier, translocating microorganisms can either enter the portal circulation or be carried to MLN by macrophages⁽¹⁷⁾. The MLN-thoracic duct-circulation course was first demonstrated to be a major route of bacterial dissemination in a model of experimental acute pancreatitis⁽¹⁸⁾, a finding, which has found subsequent support in humans⁽¹⁹⁾. Since MLN are rich in lymphocytes and macrophages, they should be able to eliminate invading bacteria through phagocytosis. However, *Escherichia coli* (*E. coli*) strains can survive in MLN for several days⁽²⁰⁾. Factors that enhance such survival could include a reduced host immune function but other possible influences have yet to be elucidated. It is postulated that bacteria which display phase-variable surface proteins may have a better ability to circumvent the immune response. For example, Ag43 of *E. coli* is a cell surface protein that enhances immune avoidance, thus promoting *E. coli* survival and colonisation of immunocompetent cells⁽²¹⁾. Once growth is established, bacteria may then move from MLN via the blood to organs such as the spleen and liver⁽¹⁹⁾. The net outcome is a systemic inflammatory response, induced sepsis and multiple organ dysfunction syndrome.

Experimental colonisation of gnotobiotic mice⁽²²⁾ with single strains of bacteria has demonstrated clearly that not all bacteria are able to translocate at the same rate and that Gram-negative enteric bacilli translocate more efficiently to MLN than do Gram-positive cocci and obligate anaerobes. For example, in a mouse model 89% of Gram-negative isolates translocated to MLN between 1-3 weeks⁽²³⁾. These bacteria were also found in the spleen, liver, kidney and peritoneal cavity. In contrast, Gram-positive bacteria translocated to MLN in only 43% and 50% of mice after weeks 1 and 3, respectively, with translocation to the abdominal visceral organs and peritoneal cavity similar to

that of Gram-negative organisms ⁽²³⁾. Among Gram-negative enteric bacteria, *E. coli* strains translocate at a higher rate than do other gut organisms due to their ability to produce continuously new phenotypes that facilitate adaptation to a multiplicity of environments ⁽²⁴⁾. Pathogenic *E. coli* may be divided into groups that cause intestinal diseases and those that produce disease elsewhere in the body ^(6,25). Pathogenic *E. coli* are not only the major cause of gastroenteritis globally, but are responsible for almost 85% of community-acquired urinary tract infections, of which 50% are transmitted nosocomially. These bacteria are also one of the five leading causes of bloodstream infections and are the principal source of Gram-negative meningitis in neonates ⁽²⁴⁾. Disease is produced by strains that possess specific somatic (O-antigen) determinants and virulence-associated characteristics ⁽²⁴⁾. Virulence determinants of *E. coli* strains may be patho type-specific, such as toxin production and adhesive properties in the case of diarrhoeagenic strains, or include a variety of properties necessary for invasion and survival inside the human body ^(6,25).

Non-pathogenic *E. coli* form part of the normal flora of the gastrointestinal tract. When the gut is in homeostasis, these bacteria engage with other microorganisms to metabolise ingested food ⁽²⁶⁾. Although regarded as non-pathogenic, under conditions of host stress and/or bacterial overgrowth, they have the ability to adhere to the gut epithelium and translocate from the gastrointestinal tract to extra-intestinal sites ^(6,7). The trigger for, and mechanism by which, this apparent change in behaviour occurs are not understood. Neither is it clear whether strains of *E. coli* found in MLN of animal models or in the blood of septic patients with gut-associated bacteraemia are better able to cross the mucosal barrier or have an increased ability to survive in the hostile environment of lymphoid tissues.

Determining Mechanisms of Bacterial Translocation

In order to gain a better understanding of this process, several mechanisms require

investigation ⁽²⁷⁾. The route of translocation should be assessed to determine if it is a transcellular, paracellular or phagocytic cell-mediated occurrence. Mechanisms that promote translocation need to be elucidated. This includes utilising already known virulence characteristics of *E. coli* to ascertain if translocating strains possess any of these determinants. The survival and colonisation of an organism within MLN may be addressed to ascertain the mechanisms that enhance bacterial survival in this phagocyte-rich environment. Light may be shed on the mechanisms of bacterial translocation of *E. coli* and its survival in MLN by:

- a. identification of genes involved in adherence of these bacteria to intestinal epithelial cells and by elucidation of the process of translocation;
- b. identification of genes involved and mechanisms by which the translocating strains of *E. coli* survive the hostile environment of MLN;
- c. verification of the role of genes involved in adherence, translocation and survival of both wild-type and mutant strains using gnotobiotic and conventional mice;
- d. investigation of the presence of translocating genes among epidemiologically unrelated *E. coli* strains isolated from septic patients.

Future Research Directions

Both translocating and non-translocating strains of *E. coli* isolated from animal models for their adherence and translocation characteristics may be studied using conditioned monolayers of the polarised gut epithelial cell line Caco-2 ^(28,29). Originating from a colorectal adenocarcinoma, Caco-2, when grown to confluence, expresses properties similar to those expressed in the human gut, i.e. relevant membrane potential, ion conductance and permeability ⁽³⁰⁾. Cells polarise significantly, are joined by tight junctions, form domes on impermeable substrates (including apical to basal ion transport), have well developed apical microvilli

and express several disaccharides and peptidases typical of normal small intestinal villous cells. These properties make Caco-2 suitable for exploring intestinal functions and bacterial translocation^(31,32). The K12 strain of *E. coli*, known to be non-translocating, may serve as a negative control.

The adhesion of *E. coli* isolates to Caco-2 cells and the route and process of translocation may be observed by electron microscopy. Transmission electron microscopy is used to ascertain if translocation is an intracellular or extracellular phenomenon using confluent Caco-2 cells lines inoculated with bacteria⁽³³⁾. Adhesion and host cell structure may be observed by scanning electron microscopy⁽³⁴⁾.

Translocating strains may be subjected to a phagocytosis assay to identify their ability to survive in MLN^(9,35). Bacteria and phagocytic cells are grown in serum, after which extracellular bacteria and macrophages are separated by centrifugation. Macrophages are lysed and seeded onto plates to permit growth of internalised bacteria. The number of colony forming units is a representation of the surviving bacteria. This method also permits quantification of bacterial phagocytosis over time⁽³⁵⁾.

Comparative genome analysis, using the technique of next generation sequencing, may be utilised to investigate genetic differences between translocating and non-translocating *E. coli* strains. Whole genomic DNA is extracted (including both chromosomal and plasmid DNA), sequenced and assembled using publicly available assembly data⁽³⁶⁾. Publicly available *E. coli* genomic data (and also plasmid-specific databases) are used to analyse the sequence data of translocating strains and to compare them to non-translocating strains (*E. coli* JM109). In addition to identifying genes unique to translocating *E. coli*, such as strains HMLN-1 in humans, it is also possible to examine the sequences of genes involved in the translocation process⁽³⁷⁾. Once target genes have been identified, primers can be developed to isolate these and validate further their involvement in

translocation via PCR across many strains of translocating bacteria.

Finally, the presence of genes involved in translocation and/or survival of *E. coli* may be investigated among epidemiologically unrelated *E. coli* strains isolated from patients with septicaemia. Control groups comprise *E. coli* strains isolated from healthy human faeces and urinary tract infections. Hybridisation probes are designed for genes involved in translocating and/or survival and their presence in bacteria is determined by probing dot blots of translocating genes after digesting whole chromosomal DNA with specific endonuclease⁽³⁸⁾.

Clinical Significance

Mortality and morbidity that are due to systemic bacterial infections, especially among critically ill patients, represent a very significant public health problem. Neonatal sepsis caused by *E. coli* and other Gram-negative bacteria is a noted concern in Iraq and various Middle East countries⁽³⁹⁾. Hospitalised patients at highest risk for Gram-negative septicaemia include immunocompromised patients (e.g. cancer patients and organ transplant recipients), patients in post-surgical recovery and those suffering trauma. The mortality associated with systemic *E. coli* infection is consistently higher than that caused by other Gram-negative bacilli, ranging from 18-20% in cases of community-acquired infection to 23-40% in cases of hospital-acquired bacteraemia⁽⁴⁰⁾. In hospitals, patients in intensive therapy units, where there is heavy dependence on antibiotics, are at greater risk. These individuals can be colonised by naturally resistant microorganisms and develop a potentially life-threatening bacteraemia. In addition, immunocompromised patients readily develop infection with bacteria of low virulence, which may invade, often causing bacteraemia. For instance, around 30,000 blood isolates are reported from laboratories in the UK each year⁽⁴¹⁾. The source of most systemic infectious complications and the multiple organ dysfunction syndrome (MODS) in surgical and intensive therapy unit patients is now known to

be indigenous gut bacteria translocating to extra-intestinal sites by passing through the intestinal epithelial barrier⁽⁴²⁾.

Conclusions

While the role of bacterial translocation in pathogenesis of sepsis has received a great deal of attention, most translocation studies have focused on the function of the intestinal barrier and less research has been performed to elucidate the role and/or properties of microorganisms in this process⁽⁴³⁾. Future studies should aim to investigate bacterial properties that are directly involved in overcoming the function of the intestinal epithelium and immune defence mechanisms. Research to date suggests strongly that translocation, at least in *E. coli* strains, is principally a bacteria-related phenomenon rather than due to a host-associated mechanism. Identification of genes involved in this process should establish a platform for investigating further the relative importance of bacteria and of host defence to the translocation process. Furthermore, such insights may facilitate the adoption of an improved strategy for the management of bacterial sepsis.

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