

## **ENTERIC BACTERIAL TRANSLOCATION: CURRENT PERSPECTIVES FROM *IN VIVO* AND *IN VITRO* MODELS**

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### **SUMMARY**

Bacterial translocation was initially viewed with scepticism, but it is now commonly accepted that this process is associated with a wide variety of clinical conditions. There is evidence that bacterial movement across the intestinal epithelium has a role in the induction of normal immune response, as well as in the induction of inflammatory bowel disease, reactive arthritis, endotoxaemia, and sepsis. The term bacterial translocation has been generally applied to the process by which normal intestinal microbes migrate out of the intestinal tract and cause systemic infections in high-risk patients. Patients at greatest risk for these complicating infections include post-surgical patients, trauma patients, and immunosuppressed patients such as organ transplant recipients and cancer patients. In the past decade, studies involving humans and laboratory animals have helped to clarify some of the clinical conditions associated with bacterial translocation. Results from human studies have strongly implicated normal intestinal bacteria as aetiologic agents of systemic disease. Laboratory animal models have helped to clarify the factors that either increase or decrease the recovery of translocating bacteria from extra-intestinal tissues, and translocating microbes have been observed in the cytoplasm of intact intestinal epithelial cells. Thus, the absorptive enterocyte appears to be at least one portal of entry for translocating bacteria, and cultured enterocytes appear to be a relevant model to dissect the initial events involved in bacterial adherence and uptake by the intestinal epithelium. Future correlations of results from *in vivo* and *in vitro* models should provide information that will not only further clarify the physiological factors controlling bacterial translocation, but will suggest new treatment regimens to reduce the costly morbidity associated with complicating infections caused by translocating bacteria.

### **INTRODUCTION**

In its broadest terms, intestinal bacterial translocation can be defined as the passage of bacteria (both live and dead), and bacterial products (such as exotoxins, endotoxins, and cell wall fragments), from the intestinal lumen to extra-intestinal sites. The term bacterial translocation has been most often used

to describe the process by which normal flora microbes migrate out of the intestinal lumen and cause complicating systemic infections and/or endotoxaemia in hospitalised patients. High-risk patients include post-surgical patients, trauma patients, and immunosuppressed patients such as cancer patients and organ transplant recipients. To target new treatment regimens and reduce costly morbidity, investigators are attempting to clarify the clinical conditions and physiological mechanisms involved in bacterial translocation. Clarification of these factors may have broad implications, because the process of bacterial movement from the intestinal lumen is likely inherent in a number of physiological processes, as described below.

#### **Enteric bacteria and induction of intestinal immune responses**

The intestinal epithelium is subject to a constant barrage of antigenic stimuli, originating not only from food antigens, but from the many antigens in a complex microflora that includes over 400 different species of bacteria (Moore and Holdeman, 1975). These bacteria are relatively sparse ( $10^2$  to  $10^3$  per ml) in the proximal small bowel and become extremely dense (approximately  $10^{12}$  per gram) in the colon (Gebbers and Laissue, 1984). The obvious site of host interaction with this antigenic burden is the intestinal epithelium.

According to current concepts for induction of mucosal immunity, soluble and particulate antigens, including bacteria and their products, penetrate the intestinal epithelium to be processed by antigen presenting cells, such as tissue macrophages. These processed antigens are presented to uncommitted gut lymphoblasts which migrate through the thoracic duct and "home" to the intestinal mucosa (via selective endothelial recognition mechanisms) as mature lymphocytes (Bland and Kambarage,

1991). As a result, approximately 80% of all immunoglobulin-producing cells in the human body are found in the intestinal mucosa (Brandtzaeg et al., 1991). In addition, it may not be coincidental that animal studies of bacterial translocation repeatedly culture viable intestinal bacteria from the draining mesenteric lymph nodes of a small percentage of normal animals (Wells and Erlandsen, 1992b).

Although initial investigations implicated Peyer's patch M cells as the primary site of antigen uptake (Sneller and Strober, 1986), there is accumulating evidence that other cell types may not only participate in this process, but may be the primary site of antigen uptake. Peyer's patches have been reported to take up inert particles (carbon, India ink, latex beads) and viable pathogenic bacteria, such as *Salmonella* spp., *Listeria monocytogenes*, and *Vibrio cholerae* (Wells et al., 1988c). However, there is growing evidence that absorptive enterocytes can also function as fixed phagocytes for the uptake of particulate antigens (Falkow et al., 1992). Inert particles (ferritin and latex beads) and viable microbes (*Salmonella* spp., *L. monocytogenes*, *Escherichia coli*, *Proteus mirabilis*, *Enterococcus faecalis*, *Candida albicans*) have been observed within enterocytes (Wells and Erlandsen, 1992b). Interestingly, major histocompatibility complex class II glycoproteins have been localised in the apical cytoplasm of absorptive enterocytes, suggesting that enterocytes can function as antigen presenting cells (Mayer and Shlien, 1987; Bland et al., 1991). By sheer numbers, absorptive enterocytes may be the most efficient route available for the uptake and processing of luminal antigens. At the risk of further complicating this discussion, it should be mentioned that Paneth cells have been shown to ingest and degrade certain bacteria and protozoa, and may

also function as fixed phagocytes in the intestinal epithelium (Erlandsen and Chase, 1972a, 1972b).

Mayer and Shlien (1987) suggested that M cells are the site of antigen sampling only for those antigens with specific receptors on M cells. Curiously, the bacteria reported to be taken up by M cells are frankly pathogenic species, such as *Salmonella* spp. and *L. monocytogenes*. No species of normal microbial flora has been observed to penetrate into Peyer's patches, but definitive studies have not been done.

### **Enteric bacteria and inflammatory bowel disease**

Although the aetiology of inflammatory bowel disease remains elusive, there is evidence that an infectious agent is at least partially involved. To date, no animal model for ulcerative colitis or Crohn's disease perfectly reproduces the human disease, but several models have characteristics of one or both diseases and might provide insights into the disease aetiology.

It has been proposed that Crohn's disease might be caused by an increase in mucosal permeability, causing luminal antigens to enter the lamina propria. Consequently, Morris et al. (1989) instilled a hapten (to elicit an inflammatory response) suspended in 50% ethanol (to increase mucosal permeability) into the rat colon; the resulting chronic inflammatory response had a thickened bowel wall, granulomas, and inflammatory infiltrate. Mee et al. (1979) irritated the rabbit colon with formalin, and parenterally injected the common enterobacterial antigen, followed by soluble immune complexes; chronic inflammation occurred only in the colonic regions treated with formalin. Sartor et al. (1985) produced chronic, granulomatous inflammation after surgical injection of *Streptococcus* (*Enterococcus*) *faecium* cell wall frag-

ments into the rat bowel wall. Sartor et al. (1988) subsequently noted that peptidoglycan-polysaccharide complexes (the primary structural component of the cell walls of nearly all bacterial species) could initiate or enhance intestinal inflammation. Local infusion of N-formyl-methionyl-leucyl-phenylalanine (an inflammatory peptide produced *in vitro* by all species of intestinal bacteria investigated to date) resulted in experimental colitis (Chester et al., 1985).

Highlighting the importance of the intestinal flora in colitis, oral carageenan caused caecal ulcerations in conventionally reared, but not germfree guinea pigs (Onderdonk et al., 1977). Parenteral immunisation and oral feeding of *Bacteroides vulgatus* augmented this carageenan-induced colitis, and the outer membrane antigens of *B. vulgatus* appeared responsible for the effect (Breeling et al., 1988). Mycobacteria may also be involved in Crohn's disease. *Mycobacterium paratuberculosis* has been isolated from several patients with Crohn's disease and is the causative agent of John's disease, a chronic enteritis of ruminants with clinical and histopathological features in common with Crohn's disease. Hamilton et al. (1989) inoculated *M. paratuberculosis* into germfree athymic mice and functionally normal controls; a progressive infection, characterised by the presence of acid-fast bacilli and granulomas in the intestinal mucosa and the liver, was noted only in the athymic mice.

It may be unrealistic to propose a single aetiology for Crohn's disease or ulcerative colitis. Both diseases are heterogeneous and vary greatly in their clinical presentation. Evidence is accumulating that both diseases may result from a complex interplay of mechanical damage to the bowel wall, the presence of bacterial antigens within the intestinal mucosa (possibly causing an overstimulation of T helper cells in the lam-

ina propria), and an inherent immune defect in the host. Thus, inflammatory bowel disease may be associated with increased intestinal permeability, coupled with mucosal penetration of whole bacteria, bacterial fragments, or bacterial products.

### **Enteric bacteria and reactive arthritis**

The microbes involved in septic arthritis are many and diverse (Calin, 1987), e.g., *Mycoplasma* spp., *Chlamydia trachomatis*, fungal agents, and viral agents. Taxonomically diverse bacterial agents have been implicated in septic arthritis, and include *Hemophilus influenza*, *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *N. meningitidis*, *Streptococcus pneumoniae*, *Salmonella* spp., *Moraxella osloensis*, *Kingella kingae*, *Streptobacillus moniliformis*, *Pseudomonas* spp., *Borrelia burgdorferi* (Lyme disease), *Peptostreptococcus* spp., *Bacteroides* spp., *Clostridium* spp., *Yersinia enterocolitica*, and *Mycobacterium* spp.

Reactive arthritis can be defined as arthritis triggered by prior infection. There is evidence that the synovitis associated with both Reiter's syndrome and ankylosing spondylitis is related to the presence of tissue damaging immune complexes within the synovium, possibly associated with microbial antigens (Keat, 1983). Reiter's syndrome is often preceded by enteric infection with *Shigella* spp., *Salmonella* spp., *Y. enterocolitica*, or *Campylobacter jejuni*, although viable bacteria are not cultured from arthritic joints. Ankylosing spondylitis is associated with *Klebsiella pneumoniae* in the bowel flora (Ogasawara, 1986). Approximately 70-90% of ankylosing spondylitis and Reiter's syndrome patients is HLA-B27<sup>+</sup>, while only 10% of normal Caucasians have this marker. Antibody to HLA-B27 cross-reacts with certain *K. pneumoniae*

strains, and there is evidence that *K. pneumoniae* can modify the lymphocytes of asymptomatic HLA-B27<sup>+</sup> persons resulting in active ankylosing spondylitis. There is also an association between HLA-B27, *Y. enterocolitica* enterocolitis, and reactive arthritis (Toivanan et al., 1985).

Similar to inflammatory bowel disease, there is evidence that arthritis can be induced or augmented by cell wall fragments of normal intestinal bacteria. Stimpson et al. (1988) induced arthritis in rats by simple intraperitoneal injection of cell wall fragments of *S. faecium* and *Peptostreptococcus productus*, two prominent members of the normal intestinal flora; in further studies, it was shown that parenteral lipopolysaccharide could reactivate the arthritis produced by these cell wall fragments. Another common bacterial antigen, termed the 65K protein, has also been linked to arthritis (Kaufman, 1988). This bacterial cell wall protein is found on a variety of *Mycobacterium* spp. and many Gram-positive and Gram-negative, pathogenic and non-pathogenic species, including *E. coli*. The wide distribution of the 65K protein suggests that a variety of bacteria, rather than a single species, may be involved in the induction of arthritis.

Thus, in a scenario similar to that described above for inflammatory bowel disease, there is evidence that certain arthritic syndromes may involve a leakage of bacterial cell wall components across the intestinal mucosa, coupled with gradual accumulation of bacterial cell wall fragments or immune complexes in the tissues of a genetically predisposed host.

### **Enteric bacteria, endotoxaemia, and sepsis**

There is substantial evidence that translocating intestinal bacteria cause a significant proportion of complicating

systemic infections in hospitalised patients. Typical aetiologic agents include *E. coli*, *Proteus* spp., *Klebsiella* spp., other members of the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, enterococci, streptococci, and the yeast *Candida albicans*. Gram-negative bacteraemia is especially problematic because, despite advances in antimicrobial therapy, the mortality remains between 20% and 45% (*de la Torre* et al., 1985; *Gransden* et al., 1990; *Uzun* et al., 1992; *Geerdes* et al., 1992). Also, antibiotic-resistant *E. faecalis* is emerging as a major aetiologic agent of complicating nosocomial infections (*Moelling*, 1992), possibly due to widespread use of cephalosporins in hospitalised patients. Curiously, strictly anaerobic bacteria, such as *Bacteroides* spp. and *Clostridium* spp., outnumber other aerobic and facultative species by 100:1 or 1000:1 in the intestinal tract, yet anaerobic bacteria rarely cause complicating infections in high risk patients, and rarely translocate out of the intestinal tract in animal models of bacterial translocation.

Bacterial endotoxin appears to play a role in the pathogenesis of "sepsis syndrome", a clinical condition with the manifestations of systemic infection, but without successful isolation of a microbe from blood culture. Sepsis or sepsis syndrome is accompanied by hypertension and hypermetabolism that has been speculated to predispose to multiple organ failure, a poorly defined syndrome with a mortality of 50% to

75% (*Carrico* et al., 1986). According to one theory, the gut is a reservoir of potential pathogens; these potential pathogens, or their toxins, enter the circulation as a result of changes in the composition of the intestinal flora and/or altered barrier function.

Interest in the association between bacterial endotoxin and sepsis was renewed following a report by *Deitch* et al. (1987), who noted that parenteral endotoxin stimulated the translocation of normal flora bacteria from the intestinal lumen to the draining mesenteric lymph nodes of laboratory rodents. This finding had broad implications because alterations in gut barrier function, permitting leakage of endotoxin, could theoretically be initiated by any event facilitating intestinal ischaemia, e.g., shock, trauma. Investigators began to speculate that endotoxin-induced bacterial translocation might be the initial event in a wide variety of clinical conditions that predispose to sepsis, multiple organ failure, and death. The exact mechanism of endotoxin-induced bacterial translocation remains unclear. Parenteral endotoxin has been associated with intestinal bacterial overgrowth as well as alterations in intestinal histology, primarily villous oedema (*Deitch* et al., 1987; *Wells* et al., 1992c). There is recent evidence that even lethal doses of endotoxin do not consistently cause increased bacterial translocation, and that translocation may be associated with certain threshold level of intestinal bacterial overgrowth (*Wells* et al., 1992c).

## CLINICAL EVIDENCE OF BACTERIAL TRANSLOCATION

Clinical evidence of bacterial translocation is largely circumstantial and can be divided into three categories: (a) the use of prophylactic antibiotics designed to eliminate selected populations of intestinal bacteria in high risk patients, coupled with a decrease in complicating

infections in these patient populations; (b) prospective studies documenting that the predominant bacterial strain carried in the faecal flora is the same strain subsequently isolated from systemic infections; and (c) the recovery of viable intestinal bacteria from draining mesen-

teric lymph nodes, implying that bacteria can migrate out of the intestinal lumen. As noted below, there is substantial evidence that normal intestinal bacteria are important aetiologic agents of complicating infections in immunosuppressed, post-surgical, and trauma patients.

### **Selective antibiotic decontamination of the digestive tract**

In the early 1970's, *van der Waaij* and colleagues (1971, 1972a, 1972b) introduced the concept of "colonisation resistance". According to this theory, strictly anaerobic bacteria do not normally translocate, and function to limit the intestinal colonisation and translocation of potentially pathogenic species such as *E. coli*. Physicians began to administer prophylactic antibiotics designed to decontaminate the intestinal tract of high-risk patients.

Over the ensuing 20 years, a substantial number of clinical studies have concluded that selective antimicrobial decontamination, typically targeted at elimination of *Enterobacteriaceae*, results in a significant decrease in the incidence of complicating infection. An excellent review of this literature has been written by *van Saene* et al. (1990). Typically, the alimentary tract is decontaminated using a mixture of non-absorbable antimicrobials (such as polymyxin E, tobramycin, amphotericin B), with an initial short-term course of a parenteral agent, such as cefotaxime. Using this approach, fourteen out of fifteen controlled studies reported a significant reduction in infection. Six of ten studies reported decreased mortality; however, *van Saene* et al. (1990) emphasised that it is imperative to distinguish between overall mortality and infection-specific mortality, and that the relationship between infection and mortality still remains unclear.

### **Association between intestinal colonisation and development of sepsis**

Prospective studies have reported that a predominant strain of faecal bacteria is often the aetiologic agent of subsequent systemic infection. *Tancrede* and *Andreumont* (1985) identified and quantified bacteria in 4,347 stool specimens from 688 cancer patients receiving no antimicrobial therapy; 60 patients developed 64 episodes of Gram-negative bacteraemia that appeared to be caused by a dominant faecal organism that translocated from the intestinal tract during a period of severe granulocytopenia. *Wells* et al. (1987a) also found evidence of faecal carriage in organ transplant recipients with gram-negative bacteraemia.

### **Recovery of viable bacteria from lymph nodes draining the intestinal, reproductive, and respiratory tracts**

Direct evidence of bacterial movement across the intestinal mucosa is difficult to obtain in humans. Several investigators have cultured mesenteric lymph nodes obtained by surgery, attempting to recover viable bacteria in patients expected to have altered intestinal barrier function (Table 1). For ethical reasons, peri-operative antibiotics are typically used in these patients, thus underestimating the presence of viable bacteria; nonetheless, these results document the presence of viable intestinal bacteria in draining mesenteric lymph nodes.

Bacterial translocation may occur in other sites in addition to the intestinal tract. Normal vaginal flora has been recovered from the uterine lymph nodes of 34% (n=83) of patients undergoing surgery for gynaecological tumours (*Wells* et al., 1990c). There is also evidence that mononuclear phagocytes can

**Table 1:** Clinical studies documenting recovery of viable intestinal bacteria from draining mesenteric lymph nodes (MLN) obtained at surgery

Reason for surgery	No. patients with MLN bacteria		Reference
	Total no. patients	(%)	
Crohn's disease	15/46	(33%)	<i>Ambrose et al. (1984)</i>
Other elective abdominal surgery	2/43	(5%)	
Crohn's disease	7/28	(25%)	<i>Laffineur et al. (1992)</i>
Colorectal cancer	13/20	(65%)	<i>Vincent et al. (1988)</i>
Other digestive diseases	6/20	(30%)	
Intestinal obstruction, without necrosis	10/17	(59%)	<i>Deitch (1989)</i>
other abdominal surgery	1/25	(4%)	
Laparotomy for intestinal disease	3/4	(75%)	<i>Peitzman et al. (1991)</i>
laparotomy following trauma	0/25	(0%)	

transport particles from the terminal alveolus of the canine lung to the draining tracheo-bronchial lymph node (*Harmsen et al., 1985*). These latter two studies

provide evidence for bacterial translocation in anatomical sites outside of the intestinal tract.

### **IN VIVO LABORATORY MODELS OF BACTERIAL TRANSLOCATION**

Because direct evidence is difficult to obtain from humans, many investigators have used laboratory animals to clarify the clinical situations and pathogenic mechanism associated with bacterial translocation. Information in this area is emerging rapidly, with over one hundred articles published in the past five years.

#### **Clinical conditions or treatments facilitating translocation**

A representative (not exhaustive) listing of the diverse clinical conditions facilitating bacterial translocation in animal models is presented in Table 2. Several caveats should be mentioned concerning these experimental studies: (a) Data concerning immunosuppressive effects is conflicting (*Maddaus et al.,*

1989), and there is evidence that the intestinal concentration and invasive ability of the colonising microbe may be more important than the immune status of the host (*Wells et al., 1991c; Jackson et al., 1991*); (b) Although several studies specifically monitor dietary influences, some animal models (such as parenteral endotoxin, burn wounds, surgery) have a malnutrition component that is typically not addressed; (c) Some models involve a histologically normal intestinal mucosa (e.g., antibiotic therapy), but other models induce alterations in intestinal histology (e.g., parenteral alimentation, burn wounds, parenteral endotoxin, intestinal ischaemia), and translocation may occur by different mechanisms across a normal or abnormal mucosa; (d) Insulin treatment of

**Table 2:** Selected references describing clinical conditions and treatments facilitating translocation of normal flora from the intestinal lumen to the draining mesenteric lymph nodes of experimental animals

Clinical condition/treatment	Reference
Antibiotic-induced intestinal bacterial overgrowth	<i>Berg</i> , 1981; <i>van der Waaij</i> et al., 1971, 1972a, 1972b; <i>Wells</i> et al., 1987b, 1988b, 1988d
Germfree animals colonised with aerobic /facultative bacteria	<i>Berg</i> and <i>Garlington</i> , 1979; <i>Steffen</i> et al., 1988; <i>Wells</i> et al., 1988a, 1991a
Subtherapeutic polymyxin B	<i>Dijkstra</i> et al., 1992
Neonatal animals	<i>Glode</i> et al., 1977; <i>Pluschke</i> et al., 1983
Cytomegalovirus	<i>Erickson</i> et al., 1991
Glucocorticoids	<i>Jones</i> et al., 1990a; <i>Alverdy</i> and <i>Aoys</i> , 1991
Interleukin-2	<i>Penn</i> et al., 1991
Surgery	<i>Salman</i> et al., 1992
Parenteral endotoxin	<i>Deitch</i> et al., 1987; <i>Wells</i> et al., 1992c
Experimentally induced diabetes	<i>Imai</i> and <i>Kurihara</i> , 1984; <i>Ohsugi</i> et al., 1991
Oral ricinoleic acid	<i>Morehouse</i> et al., 1986
Burn wounds	<i>Maejima</i> et al., 1984; <i>Alexander</i> et al., 1990; <i>Jones</i> et al., 1990a, 1990b, 1991; <i>O'Brien</i> et al., 1992; <i>Huang</i> et al., 1993
Obstructive jaundice/bile depletion	<i>Deitch</i> et al., 1990c / <i>Slocum</i> et al., 1992
Intestinal obstruction	<i>Maddaus</i> et al., 1989; <i>Deitch</i> et al., 1990b
Splenectomy/Hepatectomy	<i>Spaeth</i> et al., 1990c / <i>Wang</i> et al., 1992
Small bowel transplant	<i>Browne</i> et al., 1991; <i>Grant</i> et al., 1991
Haemorrhagic shock	<i>Baker</i> et al., 1988; <i>Rush</i> , et al., 1988; <i>Deitch</i> et al., 1990a
Mesenteric ischaemia	<i>Papa</i> et al., 1983; <i>Bennion</i> , et al., 1984; <i>Sheng</i> et al., 1992
Irradiation	<i>Brook</i> et al., 1984; <i>Souba</i> et al., 1990; <i>Kobayashi</i> et al., 1991
Immunosuppressive defects/agents	<i>Owens</i> and <i>Berg</i> , 1980; <i>Berg</i> , 1983; <i>Berg</i> et al., 1988
Parenteral alimentation	<i>Alverdy</i> et al., 1988; <i>Spaeth</i> et al., 1990a; <i>Keuppers</i> et al., 1993; <i>Helton</i> and <i>Garcia</i> , 1993
Oral liquid diet	<i>Alverdy</i> et al., 1990; <i>Spaeth</i> et al., 1990a, 1990b; <i>Mainous</i> et al., 1991; <i>Wells</i> et al., 1991b
Other dietary manipulations	<i>Li</i> et al., 1989; <i>Deitch</i> et al., 1990d; <i>Souba</i> et al., 1990; <i>Barton</i> et al., 1992; <i>Wells</i> et al., 1990d, 1992a
Intra-abdominal abscesses	<i>Wells</i> et al., 1986
Foreign materials	<i>Mora</i> , et al., 1991
Solid tumours	<i>Penn</i> et al., 1985

experimentally induced diabetes did not decrease the incidence of translocation, indicating that translocation may not result from diabetes (*Ohsugi* et al., 1991); (e) The effect of dietary manipulation is extremely variable, and while the studies listed in Table 2 reported a facilitating effect on bacterial translocation, other studies report no

effect using similar dietary additives/alterations (e.g., *Barber* et al., 1990; *Wells* et al., 1990b) and (f) When monitored, intestinal bacterial overgrowth (similar to that reported to induce translocation on its own) is a component of many animal models including some dietary manipulations, parenteral alimentation, parenteral endo-



toxin, intestinal obstruction, liver resection. (Curiously, intestinal overgrowth has recently been reported to facilitate the absorption of bacterial cell wall polymers, namely the peptidoglycan associated with reactive arthritis, providing further evidence for related aetiologies in reactive arthritis, inflammatory bowel disease, and bacterial translocation [Lichtman et al., 1991]).

### **Factors influencing translocation in burn wounds**

The burn wound model has received much attention in recent years, and this literature is too extensive to review here. Studies often attempt to address the mechanisms involved in bacterial translocation, and several findings deserve mentioning. Following burn wounds, increased bacterial translocation to the mesenteric lymph nodes is typically transient and diminishes after several days. There is evidence that the mechanism involved in bacterial translocation following burns is related to gut atrophy (Jones et al., 1990b; Huang et al., 1993) and mesenteric vasoconstriction (Herndon and Zeigler, 1993; Jones et al., 1991). The extent and duration of translocation can be increased with glucocorticoids (Alverdy and Aoy, 1991; Jones et al., 1990a), *P. aeruginosa* wound colonisation (Manson et al., 1992), cytomegalovirus (Erickson et al., 1991), and decreased with insulin-like growth factor 1 (Huang et al., 1993), a combination of fibroblast growth factor and sucralfate (Gianotti et al., 1993), prostaglandin E analogues (Fukushima et al., 1992), and inhibition of thromboxane synthetase (Tokuy et al., 1992). It is possible (even likely) that the physiological mechanisms involved in translocation associated with burn wounds, e.g., transient mesenteric ischaemia, may apply to other animal models listed in Table 2; if so, treatments modulating

translocation in the burned animal may apply to other clinical conditions as well.

### **Role of the mononuclear phagocyte**

Earlier studies from our laboratory demonstrated that mononuclear phagocytes could transport intestinal particles (1 $\mu$  latex beads and *E. coli*) from the small intestine to the draining mesenteric lymph nodes of experimental animals (Wells et al., 1988d). We then proposed a direct relationship between the ability of an enteric organism to translocate and its ability to interact with mononuclear phagocytes. This postulate seemed reasonable because pathogenic enteric bacteria that readily translocate, such as *Salmonella* spp. and *L. monocytogenes*, are reported to survive and replicate within mononuclear phagocytes (Moulder, 1985). Unlike normal flora, these enteric pathogens translocate after simple oral inoculation into normal animals.

We studied ten strains of enteric bacteria with a documented spectrum of ability to translocate in mice, including *S. typhimurium*, *L. monocytogenes*, *E. coli*, *P. mirabilis*, *E. faecalis*, *B. fragilis*, and *Bacteroides* species. Peritoneal exudate cells from the same mouse strain were then used to study bacterial interactions with mononuclear phagocytes. Differences in oral infectivity (translocating ability) did not consistently correlate with the ability of these strains to be ingested by, or to survive within, mononuclear phagocytes (Wells et al., 1993a).

The above results do not imply that intestinal bacteria can not translocate within mononuclear phagocytes, only that this method of bacterial transport may not be a rate-limiting factor modulating the incidence of translocation. Assuming some bacteria do translocate within phagocytes, translocation may be

augmented by conditions that interfere with phagocyte function. There are several reports of immunosuppression facilitating translocation (Berg, 1983; Berg et al., 1988; Gianotti et al, 1992). Factors facilitating bacterial translocation in laboratory animals often have concurrent immunosuppressive effects, e.g., burns, glucocorticoids, antibiotics. Thus, although transport by tissue phagocytes may not be a primary mechanism of bacterial translocation, bacterial interactions with phagocytes

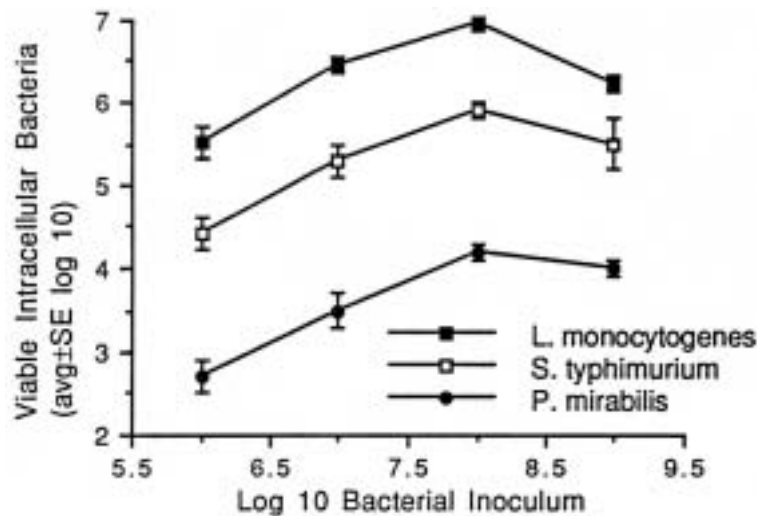
may play a significant role in some clinical conditions. This role may include limiting bacterial transport from the lumen to the draining lymph node, limiting systemic spread of bacteria that have translocated to the draining lymph node (lymphatic route) with its population of resident macrophages, or limiting the systemic spread of bacteria that have translocated to the liver (via the portal vein) with its population of Kupffer cells.

## IN VITRO MODELS OF BACTERIAL TRANSLOCATION

### Types of models

Mechanisms of pathogenesis of enteric bacteria are difficult to study *in vivo* because many factors in the intestinal environment confound interpretation of experimental results, such as peristalsis, epithelial sloughing, the presence of hundreds of different microbial species, an extensive 200 to 300 square meter surface area, etc. Using rodent models of microbial translocation, *C. albicans*, *E. coli*, *P. mirabilis*, and *E. faecalis* have been visualised within intact intestinal epithelial cells (Cole et al., 1988; Alexander et al., 1990; Wells et al., 1990a; Wells and Erlandsen, 1991a). Thus, the absorptive enterocyte may be at least one portal of entry for translocating bacteria, and bacterial uptake by "non-professional phagocytes" is becoming increasingly recognised as a mechanism of invasion for a wide variety of microbes (Falkow et al., 1992). However, direct observation of bacterial entry into the intestinal epithelium *in vivo* is difficult due to the typically low numbers of translocating microbes, and to the sampling problem inherent to the large surface area of the intestinal tract.

There are several alternatives to *in vivo* testing (Neutra and Louvard, 1989). Dispersed epithelial cells are readily obtained from the intestinal mucosa of experimental animals, but remain viable for an only few hours and are useful only for short-term physiologic experiments that do not require maintenance of polarity. Intact sheets of epithelium can be obtained by EDTA perfusion: Protein and glycoprotein synthesis continues, apical-basal polarity can be maintained, but basolateral surfaces lose their organisation within hours. Intestinal cell culture is an attractive alternative, and in recent years, numerous reports have described its use to study the interactions of enteric bacteria with intestinal epithelial cells. These reports have generally focused on frankly pathogenic bacteria such as *Salmonella* spp. and *L. monocytogenes*. Consequently, our understanding of the mechanisms involved in the epithelial adherence and uptake of these enteric pathogens has significantly expanded (Falkow et al., 1992). However, it is likely that this model can also be used to clarify the initial events involved in the translocation of normal enteric bacteria across the intestinal epithelium.



**Figure 1:** Internalisation of varying concentrations of *L. monocytogenes* ATCC 43249, *S. typhimurium* ATCC 10428, and *P. mirabilis* M13 incubated for one hour with Caco-2 cells, with intracellular bacteria quantified as described (Wells et al., 1993b). Each data point represent the results of three separate assays, each assay representing the average of triplicate determinations with a variability <20%; any two data points differing by  $\geq 1 \log_{10}$  are significantly different by  $p < 0.01$ , using a one-way analysis of variance followed by Scheffe's test for significant differences.

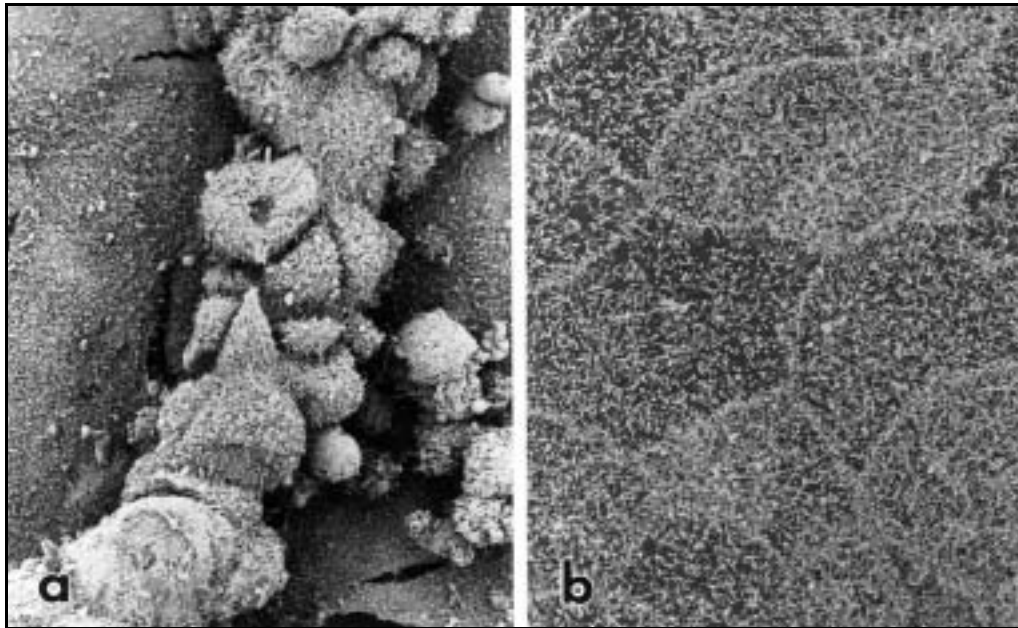
### Cultured enterocytes as a relevant model

Of the dozens of intestinal adenocarcinoma cell lines available, most do not differentiate under standard culture conditions. Important exceptions are Caco-2 and HT-29 cells, the two cell lines most widely used to study the interactions of enteric bacteria with enterocytes (Neutra and Louvard, 1989; Rousset, 1986). Caco-2 cells are well polarised, are joined by tight junctions, and have well-developed microvilli. Although derived from human colonic epithelium, Caco-2 cells express some of the disaccharidases and peptidases typical of villous cells from the small intestine. Caco-2 cells also transport water and ions toward the basolateral surface and form domes on impermeable substrates. When grown in medium containing glucose and normal serum, HT-29 cells do not have polarity or other characteristics of differentiated cells. However, when grown without glucose, HT-29 cells are

highly polarised, have several typical brush border enzymes, secrete an immunoreactive laminin, have transferrin receptors, and have histocompatibility antigen on the basolateral surface. Interestingly, fully differentiated HT-29 cultures contain two cell types resembling terminally differentiated absorptive cells and mucous-secreting goblet cells. Both Caco-2 cells and HT-29 cells are considered relevant models to study bacterial adherence and uptake by the intestinal epithelium.

### Interactions of normal enteric bacteria with cultured enterocytes

There is a rapidly expanding literature describing interactions of enteric pathogens with cultured enterocytes, but only few studies involve normal enteric flora. Most studies involving cultured epithelial cells and normal enteric bacteria utilise cells of non-intestinal origin, such as HEp-2 (human la-



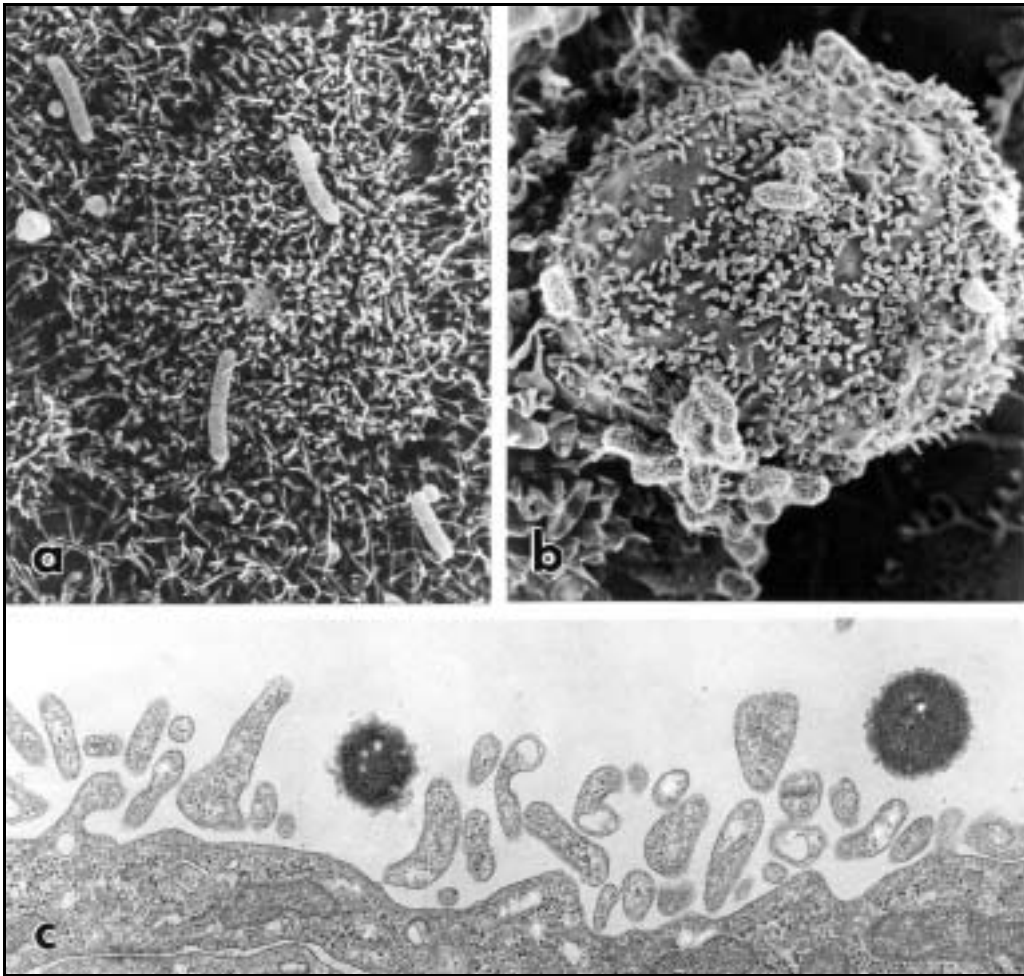
**Figure 2:** Low voltage scanning electron micrographs showing the variable surface topography of the intestinal epithelial cell line, Caco-2. a: Low magnification of enterocytes forming a three dimensional tunnel-like structure; b: Higher magnification of a monolayer area showing cellular outlines and relatively dense apical microvilli.

ryngeal epithelium), HeLa (human cervical epithelium), CHO (Chinese hamster ovary), and MDCK (Madin-Darby canine kidney) cells. The few studies involving normal enteric flora and cultured enterocytes, typically analyse only bacterial adherence and the process of bacterial internalisation is not studied.

Our laboratory is beginning to study the interactions of enteric bacteria with Caco-2 cells and HT-29 cells. Initial studies compared the Caco-2 internalisation of *L. monocytogenes*, *S. typhimurium*, *P. mirabilis*, *E. coli* (two strains), and *E. faecalis* (Wells et al., 1993b). As expected, *Salmonella* and *Listeria* were invasive in Caco-2 cells, but *P. mirabilis* and one of the two *E. coli* strains were also clearly internalised, albeit at significantly fewer numbers. Figure 1 presents the uptake of varying concentrations of *L. monocytogenes*, *S. typhimurium*, and *P. mirabilis*

following incubation with Caco-2 cells. Additional electron microscopic studies demonstrated that surface fimbriae and flagella mediated bacterial attachment to Caco-2 microvilli, although bacteria without surface appendages were also adherent to the enterocyte surface (Figures 2-4). Compared to *Salmonella* and *Listeria*, internalised *P. mirabilis* and *E. coli* were relatively difficult to locate, but were consistently within membrane-bound vacuoles in the apical cytoplasm of Caco-2 cells (Wells et al., 1993b).

Thus, cultured enterocytes can be used to study the interactions of normal enteric flora with intestinal epithelial cells. However, progress in this area will likely be more difficult than studies involving frank pathogens (e.g., *Salmonella* and *Listeria*) due to the comparative rarity of the interactions of normal flora with the intestinal epithe-



**Figure 3:** Surface interactions of enterobacteria with cultured Caco-2 cells. a and b: Low voltage scanning electron micrographs showing different patterns of bacterial adherence on the enterocyte surface: Diffuse adherence of *E. coli* C25 (a), and fimbriated and flagellated *P. mirabilis* M13 preferentially adherent on a single enterocyte (b); c: Transmission electron micrograph showing interactions of *E. coli* M21 with apical microvilli.

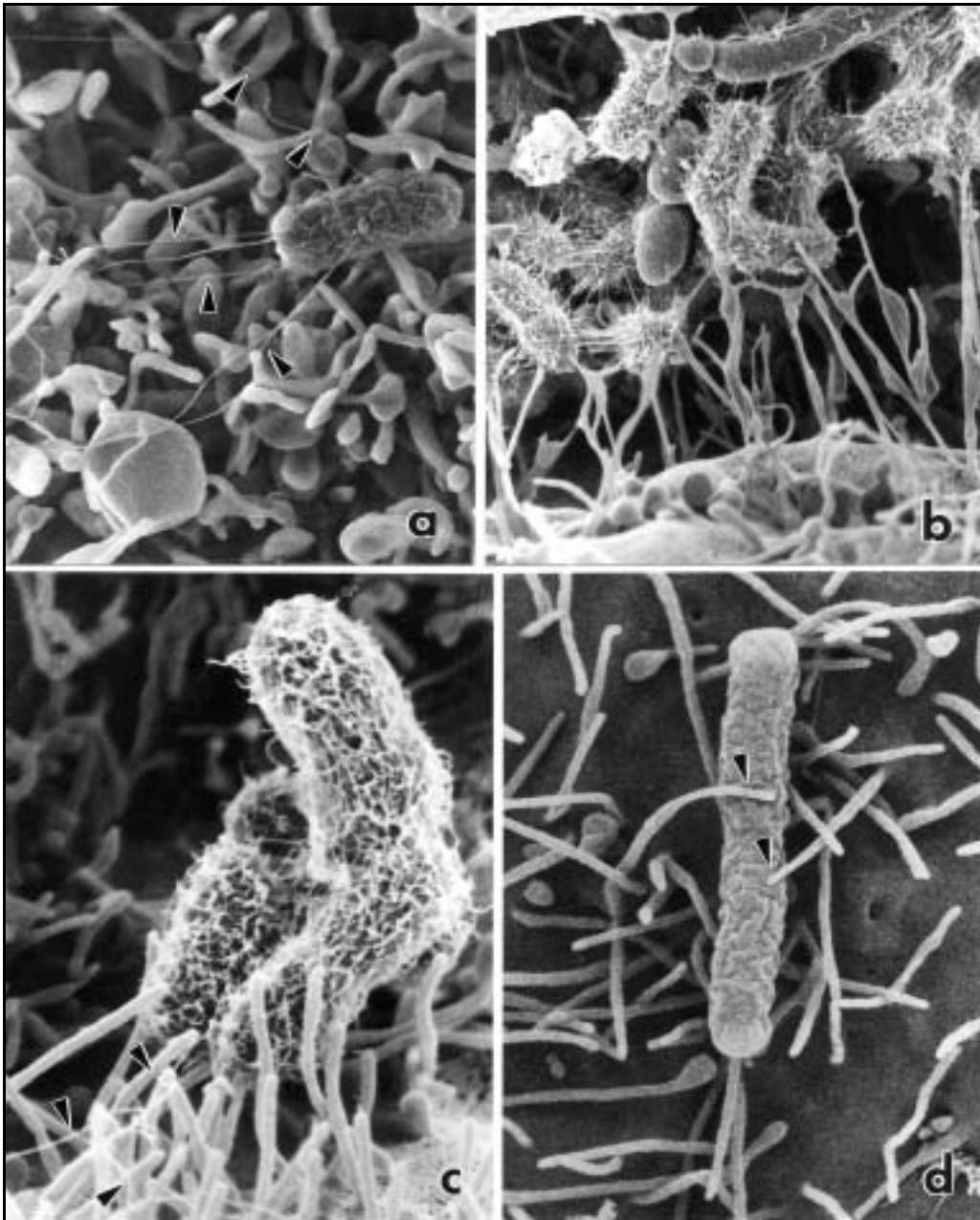
lium. Nonetheless, it is anticipated that this *in vitro* model will be useful in clarifying the initial events involved in

epithelial adherence and internalisation of translocating bacteria.

### CONCLUSION

Bacterial translocation has evolved from a process whose existence was viewed with scepticism, to a process that is clearly associated with a variety

of clinical conditions. Bacterial movement across the intestinal epithelium is inherent in induction of normal immune response, as well as in induction of in-



**Figure 4:** Low voltage scanning electron micrographs showing the interactions of enteric bacterial cell walls and surface appendages with apical microvilli of Caco-2 cells. a: Flagella (arrowheads) of *E. coli* M21 appear to aid in anchoring the bacterium to the enterocyte surface; b: Fimbriae and flagella of *P. mirabilis* M13 appear to mediate attachment of bacterial cells to one another, while microvilli appear to suspend bacteria above the enterocyte surface; note the variable distribution of surface appendages among individual bacteria in this pure culture of *P. mirabilis*; c: Higher magnification showing interactions of *P. mirabilis* flagella (arrowheads) and fimbriae with enterocyte microvilli; d: *E. coli* C25 adherent to the enterocyte microvillous surface, demonstrating bacterial adherence in the absence of surface appendages (note contact of individual microvilli [arrowheads] with bacterial surface).

inflammatory bowel disease, reactive arthritis, endotoxaemia, and sepsis. Experiments using humans and laboratory animals have provided much information concerning the clinical conditions associated with bacterial translocation, but mechanistic information is limited by the complex physiology of the intestinal tract. Cultured enterocytes appear to be a relevant model that may be used to apply the techniques of basic cell biology to the *in vivo* phenomenon of

bacterial translocation. Correlation of results from *in vivo* and *in vitro* models should aid in clarifying the mechanisms involved in bacterial translocation. These results may have broad implications because current evidence indicates that the process of bacterial translocation may not be limited to the intestinal tract, but may occur at other mucosal surfaces such as those of the reproductive and respiratory tracts.

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