

MECHANISMS PROMOTING BACTERIAL TRANSLOCATION FROM THE GASTROINTESTINAL TRACT

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SUMMARY

Bacterial translocation is defined as the passage of viable bacteria from the gastro-intestinal tract to extra-intestinal sites, such as the mesenteric lymph node complex (MLN), liver, spleen, kidney, and bloodstream. Three primary mechanisms promote bacterial translocation from the GI tract: (a) intestinal bacterial overgrowth, (b) immunodeficiencies, and (c) increased intestinal permeability. These mechanisms can act in concert to promote synergistically translocation and the systemic spread of the translocating bacteria to cause lethal sepsis. In animal models of translocation with a normal intestinal epithelium, indigenous bacteria translocate by an intracellular route through the epithelial cells lining the intestines and travel via the lymph to the MLN. In animal models exhibiting increased intestinal permeability or physical damage to the mucosal epithelium, indigenous bacteria translocate intercellularly between epithelial cells to directly access the lymph and blood. The indigenous bacteria found to translocate most readily in animal models also are the bacterial types most commonly causing septicaemia in hospitalised patients. Indigenous GI bacteria also have been cultured directly from the MLN of patients with haemorrhagic shock, bowel cancer, bowel obstruction, Crohn's disease, ulcerative colitis, inflammatory bowel disease, or trauma. Thus, evidence is accumulating that translocation from the GI tract is an important early step in the pathogenesis of opportunistic infections caused by the indigenous GI microflora. Furthermore, bacterial translocation is strongly suspected in the pathogenesis of human septicaemia, acute respiratory death syndrome (ARDS), and multiple organ failure syndrome (MODS). The study of translocation of indigenous bacteria from the GI tract is becoming even more relevant with the dramatic rise in numbers of hospitalised patients with compromised immune systems and/or increased intestinal permeability, such as the elderly, and those with cancer, diabetes, transplants, invasive devices, trauma, or AIDS.

INTRODUCTION

We define bacterial translocation as the passage of viable indigenous bacteria from the gastro-intestinal (GI) tract to extra-intestinal sites, such as the mes-

enteric lymph node complex (MLN), liver, spleen, kidney, and bloodstream (*Berg and Garlington, 1979*). Translocation is an appropriate term since it simply describes the relocation of bacteria from one site (intestinal) to another (extra-intestinal) without implying the mechanisms. In the healthy adult host, indigenous bacteria are "spontaneously" translocating across the intestinal epithelial barrier at a low rate (*Berg, 1980; 1981a*). The translocating bacteria are killed, however, by innate host immune defences during transit through the intestinal lamina propria, the draining lymph, and in reticulo-endothelial organs, such as the MLN. Thus, only rarely are viable indigenous GI bacteria cultured from the MLN or other extra-intestinal organs of normal adult rodents with an intact intestinal barrier and a competent immune system (*Berg 1980*).

Indigenous bacteria translocate primarily through the intestinal epithelial cells (intracellular route) rather than between the epithelial cells (intercellular route) in animals exhibiting a normal intestinal epithelium (*Berg, 1981a; 1985*). Thus, the epithelial cells lining the intestinal tract can be considered "non-professional phagocytes" readily taking in any bacterium that comes in

close contact. The translocating bacteria pass through the individual epithelial cells and are transported via the lymphatics to the MLN and then spread to other extra-intestinal sites, such as the liver, spleen, peritoneal cavity, and even bloodstream.

Indigenous bacteria translocate intercellularly between intestinal epithelial cells when there is increased intestinal permeability or physical damage to the intestinal mucosa. For example, indigenous bacteria translocate intercellularly or through ulcerations left by denuded epithelial cells in rodents subjected to haemorrhagic shock or endotoxic shock (*Deitch et al., 1988*). In this case, the translocating indigenous bacteria may travel directly to the blood bypassing the MLN.

In 1980, we identified three primary mechanisms promoting bacterial translocation from the GI tract: (a) intestinal bacterial overgrowth, (b) immunodeficiencies, and (c) increased intestinal permeability (*Berg, 1980*). Since that time, no other mechanisms promoting bacterial translocation have been added to this list. Thus, one or more of these three primary promotion mechanisms is operating in all the animal models of translocation examined to date.

INTESTINAL BACTERIAL OVERGROWTH

Intestinal bacterial overgrowth is the most effective of the three primary translocation promoting mechanisms in initiating bacterial translocation from the GI tract to the MLN (*Berg, 1998*). That is, a greater percentage of MLN cultures are positive in animal models exhibiting only intestinal bacterial overgrowth than in animal models exhibiting only immunodeficiency or only increased intestinal permeability. Intestinal bacterial overgrowth promotes bacterial translocation in the animal models listed in Table 1.

Bacterial translocation is directly promoted by an increase in GI population levels. That is, there is a "cause and effect" relationship between the GI population level of a particular bacterial species and its translocation from the GI tract. For example, *E. coli* attains caecal populations of $10^{9-10}/g$ in *E. coli*-mono-associated gnotobiotic mice (ex-germ-free), and 100% of the MLN are positive for translocating *E. coli* (*Berg and Owens, 1979*). If these *E. coli*-mono-associated mice are then inoculated with

Table 1: Promotion of bacterial translocation by intestinal bacterial overgrowth.

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- Oral antibiotics
 - Associated gnotobiotic (ex-germfree) mice
 - Endotoxic shock
 - Zymosan shock
 - Starvation
 - Protein malnutrition
 - Parenteral (i.v.) alimentation
 - Enteral (oral) alimentation
 - Bowel obstruction
 - Bile duct ligation
 - Streptozotocin-induced diabetes
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an indigenous microflora (i.e. the whole caecal contents from conventional mice), the *E. coli* caecal population decreases 1000-fold and *E. coli* no longer translocates to the MLN. If the obligate anaerobes of the indigenous microflora antagonistic to *E. coli* are removed by antibiotic treatment, *E. coli* again increases in intestinal population levels and readily translocates from the GI tract to the MLN.

This relationship is even more dramatically demonstrated by comparing caecal populations of three different species of *Enterobacteriaceae* in tri-associated gnotobiotic mice with their translocation rates to the MLN. Germ-free mice were tri-associated with indigenous *E. coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* and the proportion of each of the three bacterial species in the caecum compared to the proportion of each of the three bacterial species translocating to the MLN (Steffen and Berg, 1983). Thus, *P. mirabilis* comprised 10% of the caecal bacteria and 14% of the bacteria translocating to the MLN, *K. pneumoniae* comprised 66% of the caecal bacteria and 54% of the MLN bacteria, and *E. coli* comprised 25% of the caecal bacteria and 32% of the MLN bacteria. Thus, the proportion of each of the three bacterial species in the caecum is statistically

similar to the proportion of each of the three bacterial species in the MLN, demonstrating again a direct relationship between intestinal bacterial overgrowth and bacterial translocation.

In the intestinal bacterial overgrowth model, the translocating indigenous bacteria do not establish an "infection" in the MLN. That is, the indigenous bacteria are continuously translocating from the GI tract to the MLN rather than multiplying within the MLN. For example, the numbers of indigenous *E. coli* translocating to the MLN of *E. coli*-mono-associated gnotobiotic mice is similar after either 2 days of mono-association or 100 days of mono-association (Berg and Owens, 1979). *E. coli* maintains constant level of 10^{9-10} /g caecum throughout the entire 100-day test period. If the *E. coli*-mono-associated gnotobiotics are treated orally with a non-absorbable antibiotic to remove *E. coli* from the GI tract, MLN cultures become negative within 24 hours for translocating *E. coli*. These results suggest translocating *E. coli* do not establish in the MLN but rather continuously seed the MLN from the GI tract.

The intestinal bacterial overgrowth model has several advantages over other translocation models. As already mentioned, intestinal bacterial overgrowth is more efficient in promoting initial bacte-

rial translocation from the GI tract than either immunodeficiency or increased intestinal permeability. Most importantly, promotion of bacterial translocation in the intestinal overgrowth model is due directly to bacterial overgrowth and not confounded by other variables, such as increased intestinal permeability. Another advantage of the intestinal overgrowth model is that bacterial challenge is by the "natural" oral route rather than by "artificial" intravenous or intraperitoneal injection as is often the case in bacterial pathogenesis studies. Bacterial translocation to the MLN and other organs also is extremely sensitive. Theoretically, a single translocating bacterium will produce a positive MLN culture. In fact, culture of the MLN for viable translocating bacteria has proved more sensitive in detecting bacterial translocation than tests employing fluorescein-labelled or even radiolabelled bacteria (Alexander et al., 1990).

The various species of indigenous bacteria do not all translocate at the same rate. The Gram-negative, facultatively anaerobic *Enterobacteriaceae*, such as *E. coli*, *K. pneumoniae*, and *P. mirabilis*, translocate most readily from the GI tract (Steffen et al., 1988). The Gram-positive, oxygen-tolerant bacteria, such as *Staphylococcus epidermidis*, translocate at an intermediate rate. Surprisingly, the strict anaerobes, such

as *Bacteroides*, *Clostridium*, and *Fusobacterium*, translocate at the lowest rate even though they normally maintain very high GI population levels (10^{10-11} /g caecum). There is some evidence that oxygen sensitivity may be a limiting factor in the translocation of strictly anaerobic bacteria (Berg and Itoh, 1986).

Intestinal bacterial overgrowth is not just a laboratory phenomenon, but occurs readily in a variety of patients including those receiving antibiotic therapy. Patients treated with antibiotics often exhibit intestinal overgrowth by antibiotic-resistant indigenous bacteria or are colonised by more pathogenic, antibiotic-resistant, exogenous bacteria. This clinical situation is readily produced in rodent models given oral antibiotics (Berg, 1981b). Parenteral alimentation, enteral alimentation, protein malnutrition, starvation, bowel obstruction, diabetes, and endotoxic shock also induce intestinal bacterial overgrowth and subsequently promote translocation (Berg, 1983b; 1985; 1992a; 1992b). Interestingly, the bacterial types that translocate the most readily from the GI tract following intestinal bacterial overgrowth, namely the *Enterobacteriaceae*, also are the bacterial types that most commonly cause septicaemia in hospitalised patients, with *E. coli* being the most prominent (Donnenberg et al., 1994).

IMMUNODEFICIENCY

Translocating bacteria are cultured from the MLN and other extra-intestinal sites only if they survive transit through the intestinal lamina propria and are not killed by immune mechanisms in the lymph or other extra-intestinal sites. If the translocating bacteria are killed in route, cultures of the MLN and other organs will be negative and bacterial translocation will not have taken place

even though the bacteria crossed the intestinal epithelial barrier. Host immune defences are therefore integral components in the dynamics of bacterial translocation.

Table 2 lists animal models in which deficiencies in host immune defences allow bacterial translocation. The injection of mice with immunosuppressive agents, such as cyclophosphamide,

Table 2: Promotion of bacterial translocation by immunodeficiency

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- Injection of immunosuppressive agents
 - Genetically athymic (*nu/nu*) mice
 - Beige/nude (*bg/bg;nu/nu*) mice
 - Thymectomised (*nu/+*) mice
 - T-cell-depleted (anti-CD4/anti-CD8) mice
 - Lymphoma
 - Leukaemia
 - Streptozotocin-induced diabetes
 - Thermal injury
 - Endotoxic shock
 - Haemorrhagic shock
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prednisolone, methotrexate, 5-fluorouracil, or cytosine arabinoside, readily promotes translocation of indigenous bacteria from the GI tract to the MLN, spleen, liver, and kidneys (Berg, 1983a). Bacterial translocation due to immunodeficiency also occurs in animal models of diabetes (Berg, 1985), leukaemia (Penn et al., 1986), endotoxaemia (Deitch and Berg, 1987), thermal injury (Maejima et al., 1984), and haemorrhagic shock (Baker et al., 1987). Likely all components of the host immune system, such as systemic immunity (serum immunoglobulins), mucosal immunity (secretory IgA), and cell-mediated immunity (T-cells and macrophages) are involved in immune defence against bacterial translocation. Very little research has been conducted to date, however, to delineate the relative roles of these immune compartments or to identify the immuno-effector mechanisms responsible for killing the translocating bacteria.

Serum immunoglobulins act as opsonins to increase the effectiveness of phagocytosis and killing of bacteria by macrophages and polymorphonuclear leukocytes. Surprisingly, i.v. or i.p. injection with anti-*E. coli* antibodies do not decrease the numbers of *E. coli* translocating from the GI tract to the MLN (Gautreaux et al., 1990). Anti-*E. coli* antibodies, however, reduce the

spread of the translocating *E. coli* from the MLN to the spleen, liver, or kidney. Thus, serum antibodies appear to be more effective in reducing the spread of translocating bacteria that have already penetrated the intestinal barrier than inhibiting the initial translocation of bacteria across the intestinal mucosa to the MLN. Nonetheless, systemic immunity has not been studied sufficiently to draw any firm conclusions concerning its relative role in translocation defence.

It seems likely that specific anti-bacterial secretory IgA on intestinal mucosal surfaces would be a major factor in the defence against translocation. Indigenous bacteria must come in close contact with the intestinal epithelial cells prior to their translocation across the intestinal mucosa and secretory IgA is known to inhibit bacterial adherence to mucosal surfaces. For example, specific anti-bacterial secretory IgA inhibits association with the intestinal epithelium of certain pathogenic bacteria, such as *Vibrio cholerae* and *Salmonella typhimurium* (Winner et al., 1991; Michetti et al., 1992). However, secretory IgA has not been demonstrated to decrease association of indigenous bacteria with the intestinal epithelium nor is there any information concerning the importance of secretory IgA in defence against indigenous bacterial translocation.

Thymectomy of neonatal mice pro-

motes the translocation of indigenous bacteria from the GI tract to 46% of MLN, spleen, liver, and kidneys compared with only 5% positive organs in control sham-thymectomised mice (*Owens and Berg, 1982*). Athymic (nu/nu) mice exhibit "spontaneous" translocation of aerobic, facultative, and obligately anaerobic bacteria to 50% of the MLN, spleen, liver, and kidneys compared with 5% positive organs in control euthymic (nu/+) mice (*Owens and Berg, 1980*). Since IgA development is T-cell-dependent, athymic mice lack intestinal secretory IgA. Neonatal thymuses grafted from heterozygous donor (nu/+) mice to homozygous (nu/nu) recipients decrease translocation in the recipients from 58% to 8% of these organs. These results suggest T-cell-mediated immunity contributes to host defence against translocation and is especially effective in preventing the spread of translocating bacteria from the MLN to other extra-intestinal sites.

The importance of T-cells in host defence against translocation is confirmed in mice depleted of T-cells to promote bacterial translocation and then adoptively transferred with T-cells to inhibit translocation. Thymectomised mice depleted of CD4+ and/or CD8+ T-cells via injections of specific anti-T-cell monoclonal antibodies exhibit increased bacterial translocation (*Gautreaux et al., 1993*). As demonstrated by flow cytometry, the T-cell depletion regimen depletes 100% of the CD4+ and CD8+ T-cells in the spleen, MLN, intestinal lamina propria, and intestinal epithelium. CD4+ and/or CD8+ T-cells harvested from donor mice and adoptively transferred to the T-cell-depleted mice inhibit bacterial translocation (*Gautreaux et al., 1995*). The adoptive T-cells (Thy-1.1+) migrated to the sites of interest, namely the MLN and intestinal lamina propria, in the Thy-1.2+ recipients.

The fact that either CD4+ or CD8+ adoptively-transferred T-cells reduces bacterial translocation suggests an effector function common to both subsets of T-cells. Direct cytotoxicity by T-cells as a defence against bacterial translocation is unlikely because both CD4+ and CD8+ T-cells kill only MHC-restricted targets. Phagocytic cells are likely the ultimate immune effector cells in the defence against bacterial translocation. Consequently, it is possible that the translocating bacteria are engulfed by MHC I and II-expressing macrophages. In this case, both CD4+ and CD8+ T-cell subsets would provide protective function. CD4+ and CD8+ T-cells also secrete gamma interferon and granulocyte/macrophage colony-stimulating factor, both of which activate phagocytic cells.

Translocating bacteria are always cultured from the MLN prior to their appearance in organs, such as the liver, spleen, kidney, or bloodstream (*Berg, 1992c; 1995*). Thus, resident macrophages in the MLN are strategically located along the translocation route from the GI tract. It is not surprising, therefore, that non-specific immunostimulation of macrophages by vaccination with formalin-killed *Propionibacterium acnes* (formerly classified as *Corynebacterium parvum*) inhibits translocation of indigenous bacteria to the MLN (*Fuller and Berg, 1985*).

P. acnes vaccination induces splenomegaly, a lymphoreticular response commonly reported to indicate macrophage activation. Furthermore, plastic-adherent spleen or MLN cells (predominantly macrophages) from *P. acnes*-vaccinated mice adoptively transferred to non-vaccinated recipients inhibit bacterial translocation whereas non-adherent control cells (predominantly lymphocytes) do not. (*Gautreaux et al., 1990*). These results suggest macrophages are important effector cells

Table 3: Promotion of bacterial translocation by increased intestinal permeability

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- Ricinoleic acid (castor oil)
 - Endotoxic shock
 - Zymosan (yeast polysaccharide) shock
 - Thermal injury
 - Haemorrhagic shock
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in the host defence against bacterial translocation. Non-specific activation of macrophages and polymorphonuclear leukocytes to more efficiently engulf and kill a variety of bacterial types would be a particularly effective defensive measure since it cannot be predicted with certainty which of the 400-500 indigenous bacterial species in the GI tract will translocate under particular clinical conditions.

Unexpectedly, *P. acnes* vaccination does not reduce *E. coli* translocation in gnotobiotic (ex-germfree) mice mono-associated with *E. coli* (Fuller and Berg, 1985). This is despite the fact that the gnotobiotic mice exhibit marked splenomegaly indicating immunologic stimulation. The only difference between the germfree and conventional mice is the presence of an indigenous microflora in conventional mice. Consequently, the indigenous microflora appears to "prime" the immune system of conventional mice so that subsequent *P. acnes* vaccination is effective in inhibiting *E. coli* translocation.

To test this hypothesis, adult germ-free mice were colonised for 8 weeks with the whole caecal microflora from conventional mice prior to *P. acnes* vaccination (Berg and Itoh, 1986). The mice then were decontaminated with oral antibiotics to remove the indigenous microflora, mono-associated with *E. coli*, vaccinated with killed *P. acnes*, and tested for inhibition of *E. coli* translocation. Surprisingly, *P. acnes* vaccination did not reduce *E. coli* translocation to the MLN in these adult gnotobiotics exposed to the entire in-

digenous GI microflora for 8 weeks. Further studies then revealed that the germfree mice must be colonised with the indigenous microflora within 1 week after birth in order for *P. acnes* vaccination at 8 weeks of age to inhibit *E. coli* translocation (Berg and Itoh, 1986). These results are another dramatic demonstration of the profound influence of the indigenous GI microflora on the immunologic development of the host (Berg, 1983b).

Activated macrophages and/or polymorphonuclear leukocytes appear to promote rather than inhibit bacterial translocation in mice with inflamed abdominal abscesses (Wells et al., 1987). It is suspected that polymorphonuclear leukocytes engulf the indigenous GI bacteria and carry them to the abdominal abscess. In this case, macrophages and polymorphonuclear leukocytes promote rather than defend against bacterial translocation.

Bacterial translocation from the GI tract to the MLN and liver is neither decreased nor increased in op/op mice genetically deficient in CSF-1-dependent macrophage populations (Feltis et al., 1994). Only a few op/op mice were tested, however, so it is difficult to draw firm conclusions from these experiments. Nonetheless, it seems likely that macrophages are important effector cells in the defence against translocation. More study is required to determine the exact role of macrophages in translocation defence and to determine if macrophages promote translocation under certain circumstances.

INCREASED INTESTINAL PERMEABILITY.

The intact intestinal mucosa provides a physical barrier preventing bacteria colonising the GI tract from translocating to extra-intestinal sites. Translocation of indigenous GI bacteria readily occurs, however, when the intestinal barrier is compromised as in the animal models presented in Table 3. Chemical agents can damage the mucosal barrier and promote bacterial translocation. For example, ricinoleic acid (12-hydroxy-9-octadecenoic acid), the pharmacologically active constituent of castor oil, when given to mice once intragastrically severely damages the intestinal mucosa and readily promotes the translocation of indigenous GI bacteria (*Morehouse et al.*, 1986). Massive exfoliation of columnar epithelial cells in the proximal small intestine occurs within 2 hrs after the ricinoleic acid administration. Both facultatively anaerobic and strictly anaerobic bacteria translocate to the MLN, spleen, and liver in the ricinoleic acid-treated mice. The incidence of bacterial translocation is greatest at 4 days following the ricinoleic acid administration and ceases completely by 7 days when the damaged mucosal epithelium has regenerated.

Shock with the accompanying ischaemia/reperfusion injury to the intestinal mucosa also readily promotes bacterial translocation from the GI tract. For example, mice injected once i.p. with *E. coli* O26:B6 endotoxin exhibit ischaemia/reperfusion injury and concomitant translocation of indigenous bacteria (*Deitch and Berg*, 1987). To determine which components of the endotoxin structure are involved in promoting bacterial translocation, mice were injected with endotoxin from six R-mutant strains of *Salmonella* all differing in their endotoxin composition (i.e. Ra, Rb, Rc, Rd, Re, or lipid A) (*Deitch et al.*, 1989). Injection of intact

Salmonella endotoxin (wild type), the Ra endotoxin fragment, or the Rb endotoxin fragment increased bacterial translocation to the MLN. Injection of the smaller endotoxin fragments, Rc, Rd, Re, or lipid A alone, did not promote bacterial translocation. Apparently, endotoxin must contain the terminal three sugars of the core polysaccharide in order to induce sufficient mucosal damage to promote translocation.

Both endotoxin and lipid A produce their toxic manifestations by stimulating host cells, especially macrophages, to release mediator substances that then act as second messengers to disrupt various homeostatic systems. For example, oxygen-free radicals generated by xanthine oxidase are released during ischaemia/reperfusion (*Parks et al.*, 1982). Endotoxin-challenged mice exhibit intestinal oedema and separation of the epithelium from the lamina propria. Injection of the translocation-promoting Ra endotoxin fragment increases ileal xanthine oxidase and xanthine dehydrogenase activities, whereas injection of the non-promoting Rc or Re endotoxin fragments does not (*Deitch et al.*, 1989b). Consequently, intestinal damage and subsequent bacterial translocation are reduced when animals are pretreated with allopurinol (xanthine oxidase inhibitor), dimethylsulphoxide (hydroxyl scavenger), or deferoxamine (iron chelator) (*Deitch et al.*, 1988; 1989). Intestinal mucosal damage and bacterial translocation also are inhibited in animals fed for 2 weeks prior to endotoxin challenge a tungsten-supplemented molybdenum-free diet to deplete intestinal xanthine oxidase (*Deitch et al.*, 1989). Thus, xanthine-generated hydroxyl radicals are responsible, in part at least, for the intestinal mucosal damage and increased bacterial translocation following endotoxic shock.

Haemorrhagic shock and the shock of thermal injury also induce intestinal ischaemia/reperfusion injury and promote bacterial translocation (Baker et al., 1987; 1988; Maejima et al., 1984). For example, rats submitted to haemorrhagic shock for 90 minutes exhibit ileal mucosa necrosis and increased bacterial translocation. The mucosal damage occurring in haemorrhagic shock and thermal injury also is due to oxidants generated by xanthine oxidase (Deitch et al., 1988; 1989). As with endotoxic shock, pre-treatment with allopurinol or maintenance on a tungsten-supplemented molybdenum-free diet ameliorates the damage due to xanthine oxidase and lessens bacterial translocation (Deitch et al., 1988; 1989).

Large populations of Gram-negative indigenous bacteria are normally present in the GI tract and endotoxin is continuously released during their cell growth and, particularly, during cell death and lysis. Fortunately, endotoxin is not readily absorbed from the GI tract. In certain clinical situations where intestinal permeability is increased, however,

there is increased bacterial translocation and endotoxin can be detected in the lymph and portal and systemic circulations. In these severely ill patients, bacteria and endotoxin readily cross the mucosal barrier to gain access to extra-intestinal tissues and the bloodstream. The translocated endotoxin activates plasma protein cascades, resident macrophages, and circulating neutrophils to release monokines and proteins that, in turn, further increase gut mucosal permeability. Thus, a cycle is initiated with increased translocation of both bacteria and endotoxin and increased damage to the intestinal barrier. In fact, it is hypothesised that failure of the intestinal barrier in conjunction with hepatic dysfunction promotes or potentiates multiple organ failure syndrome, a newly recognised syndrome leading to death in a variety of patients (Carrico et al., 1986). Thus, bacterial translocation from the GI tract may be an early step in the pathogenesis of acute respiratory death syndrome (ARDS) and multiple organ failure syndrome.

CONCLUSIONS

In complex animal models of translocation, such as endotoxic shock, haemorrhagic shock, thermal injury, and streptozotocin-induced diabetes, multiple mechanisms operate to promote bacterial translocation (Berg, 1996; 1998). In fact, in some models these promotion mechanisms act synergistically. This is easily demonstrated in mice given the combination of an oral antibiotic (e.g. penicillin) plus an immunosuppressive agent (e.g. prednisolone) (Berg et al., 1988). Oral penicillin disrupts the GI ecology allowing intestinal bacterial overgrowth by indigenous *Enterobacteriaceae* resistant to penicillin and thereby promotes *Enterobacteriaceae* translocation. The translocating

Enterobacteriaceae usually do not spread beyond the MLN to other extra-intestinal sites in these antibiotic-treated mice. Prednisolone given alone also promotes *Enterobacteriaceae* translocation to the MLN and, furthermore, allows spread of the translocating bacteria from the MLN to other organs, such as the spleen, liver, and kidney. The combination of the antibiotic plus the immunosuppressive agent is even more effective and synergistically promotes *Enterobacteriaceae* translocation. Thus, mice given the combination of penicillin plus prednisolone die within 2 weeks of lethal sepsis caused by the translocating indigenous bacteria.

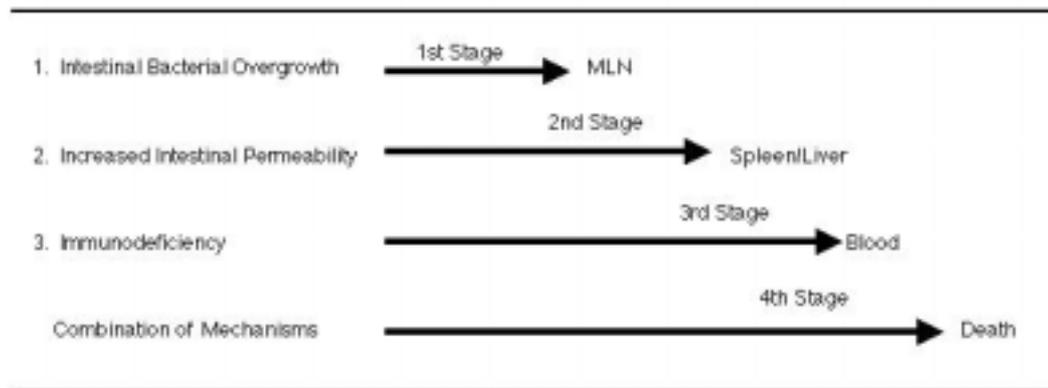


Figure 1: Stages in pathogenesis of bacterial translocation.

Other translocation promotion mechanisms also can act synergistically. For example, protein malnutrition alone produces histologic atrophy of the mucosa of the small bowel and caecum but the mucosal barrier remains intact and bacterial translocation does not occur (Deitch et al., 1987). The combination of protein malnutrition plus one endotoxin injection, however, produces intestinal ulcerations with concomitant bacterial translocation (Ma et al., 1989). Similarly, a 30% total body surface area burn plus one IP injection with endotoxin causes intestinal mucosal damage and synergistically promotes bacterial translocation (Deitch and Berg, 1987).

The pathogenesis of indigenous bacterial translocation from the GI tract occurs in several stages (Figure 1). Indigenous bacteria are translocating continuously from the GI tract in low numbers in the healthy adult animal. However, the translocating bacteria are killed in route or in situ in the MLN by host immune defences and indigenous bacteria are not usually cultured from the MLN or other extra-intestinal organs. This low level of "spontaneous" translocation by indigenous bacteria to the MLN may actually be beneficial to the host by stimulating the host immune system to respond more rapidly and

more effectively to other more pathogenic exogenous micro-organisms.

In the first stage of translocation pathogenesis, bacteria translocate to the MLN but the host immune defences are able to confine the bacteria to the MLN and they do not spread to other sites. The second stage of translocation occurs when the host immune system is compromised and the translocating bacteria are allowed to spread from the MLN to organs, such as the liver, spleen, and kidney. Depending upon the degree of immunodeficiency and the virulence characteristics of the translocating bacteria, the host may still be able to confine the translocating bacteria to these organs. If host defences cannot control the translocating bacteria, they will spread systemically to the peritoneal cavity and bloodstream producing the third stage of translocation pathogenesis. Again, the host may recover depending on the degree of immunosuppression, the extent of intestinal mucosal damage, and the virulence properties of the translocating bacteria. The fourth and final stage occurs when the host succumbs to septic shock or multiple organ failure caused by the translocating bacteria. The fourth stage usually only occurs with a combination of promotion mechanisms, such as intestinal

bacterial overgrowth plus immunosuppression or intestinal bacterial overgrowth plus shock.

Indigenous bacteria also translocate from the GI tract in humans. Surveillance cultures of faecal samples from patients with leukaemia or other immunosuppressive disorders demonstrate an association between the bacterial biotype/serotype present at the highest population level in a patient's faeces and the bacterial biotype/serotype most likely to cause septicaemia in that patient (Trancrede and Andremont, 1985). Indigenous GI bacteria are detected in the blood or MLN of patients exhibiting haemorrhagic shock (Rush et al., 1988; Moore et al., 1991; 1992). Indigenous GI bacteria also have been cultured directly from the MLN of patients with bowel cancer (Vincent et al., 1988), bowel obstruction (Sedman et al., 1994;), trauma (Brathwaite et al., 1993; Reed et al., 1994), and from patients exhibiting increased intestinal permeability, such as those with Crohn's disease, ulcerative colitis, or inflammatory bowel disease (Ambrose et al., 1984; Peitzman et al., 1991; Sedman et al., 1994). A volunteer who ingested large quantities of *Candida albicans* exhibited *C. albicans* in large numbers in his urine and blood (Krause et al., 1969).

Thus, evidence is accumulating that translocation from the GI tract is likely an important early step in the pathogenesis of opportunistic infections caused by the indigenous GI microflora. Bacterial translocation is strongly suspected in the pathogenesis of human septicaemia, acute respiratory death syndrome (ARDS), and multiple organ failure syndrome. The translocation of indigenous bacteria from the GI tract is becoming even more relevant with the

dramatic rise in numbers of hospitalised patients with compromised immune systems and increased intestinal permeability, such as the elderly, and those with cancer, diabetes, transplants, invasive devices, trauma, or AIDS.

In animal models of bacterial translocation, the bacterial species most likely to translocate from the GI tract, namely *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and especially the Gram-negative enteric bacilli (i.e. *Enterobacteriaceae*) are also the most common causes of septicaemia in hospitalised patients. Selective antibiotic decontamination may eventually prove beneficial in certain types of patients to remove the indigenous *Enterobacteriaceae* but leave intact the indigenous obligate anaerobes to exert colonisation resistance against the indigenous *Enterobacteriaceae* and even more pathogenic exogenous bacteria. Oral and even systemic antibiotics, however, must be employed with caution, since intestinal bacterial overgrowth is an efficient mechanism promoting bacterial translocation. Maintaining a stable ecological balance in the GI tract therefore is a major defence mechanism preventing intestinal bacterial overgrowth and subsequent translocation. In this regard, the use of probiotics, such as *Lactobacillus acidophilus* or *Saccharomyces boulardii*, may have merit as an alternative to antibiotic therapy in maintaining an ecological balance. However, more information is required concerning the complex ecological interrelationships between the host and its indigenous GI microflora in order to design therapeutic measures effective against bacterial translocation but disruptive to the ecological GI balance.

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