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PROMOTION OR INHIBITION OF BACTERIAL TRANSLOCATION FROM THE GI TRACT BY BACTERIAL COMPONENTS

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SUMMARY

Bacterial translocation is defined as the passage of viable bacteria from the gastrointestinal (GI) tract across the mucosal epithelium to extra-intestinal sites. Bacterial translocation does not normally occur in the healthy adult animal due to the host immune defences, the physical barrier of an intact mucosa, and the indigenous microflora maintaining an ecological balance in the GI tract. Disruption of any of these defence mechanisms allows indigenous bacteria to translocate from the GI tract to the mesenteric lymph nodes (MLN) and other organs. The disruption of more than one mechanism allows the translocating bacteria to spread systemically to cause lethal sepsis. Various bacterial components can either promote or inhibit bacterial translocation. Bacterial endotoxin injected once intraperitoneally (i.p.) promotes the translocation of certain indigenous bacteria from the GI tract to the MLN. Histologic examination of the intestinal mucosa from these endotoxin-challenged mice reveals physical disruption of the mucosal barrier. Oral gavage prior to endotoxin injection with allopurinol, a competitive inhibitor of xanthine oxidase activity, decreases endotoxin-induced mucosal injury and subsequent bacterial translocation. Inactivation of xanthine oxidase activity due to placing mice on a molybdenum-free, tungstate diet for 14 days prior to endotoxin challenge also reduces mucosal injury and bacterial translocation. Thus, it appears that a primary mechanism promoting bacterial translocation from the GI tract after endotoxin challenge is mucosal injury caused by xanthine oxidase-generated oxidants.

Other bacterial components can inhibit bacterial translocation from the GI tract. One intraperitoneal injection of formalin-killed *Propionibacterium acnes*, a non-specific immunomodulator, inhibits the translocation of various Gram-negative, enteric bacilli, such as *E. coli*, from the GI tract to the MLN. Interestingly, *P. acnes* vaccination does not inhibit *E. coli* translocation in gnotobiotic mice monoassociated with *E. coli. P. acnes* vaccination inhibits *E. coli* translocation in 8-week-old gnotobiotic mice if they are colonised with the entire GI microflora within 1 week after birth, but not if they are colonised with the microflora beginning at 2 or 3 weeks after birth. Thus, it appears that antigenic stimulation by the indigenous GI microflora is required to prime the host immune response so that a subsequent *P. acnes* vaccination can activate macrophages to inhibit bacterial translocation. These experimental models utilising various bacterial components have proved useful in attempts to determine the pathophysiology of bacterial translocation and to delineate immune defence mechanisms.

Table 1: Mechanisms promoting bacterial translocation in various animal models

Animal model	Intestinal bacterial Overgrowth	Gut mucosal injury	Immunocompromised host defences
Oral antibiotics	+	-	-
Intestinal obstruction	+	+	?
Endotoxin	-	+	+
Thermal injury	-	±	+
Haemorrhagic shock	-		?
Diabetes	±	±	+
Leukaemia	-	-	+
Protein malnutrition	±	±	±

INTRODUCTION

Indigenous bacteria normally remain confined to the gastrointestinal (GI) tract in healthy, adult mice. However, under certain circumstances these indigenous bacteria cross the gut mucosal barrier to appear in extraintestinal sites, such as the mesenteric lymph node complex (MLN), spleen, liver, kidney, and blood. We have termed this passage of indigenous bacteria from the GI tract to other sites as bacterial translocation (*Berg* and *Garlington*, 1979). *Keller* and *Engley* (1958) appear to be the first to have used the term translocation when they described the passage of bacteriophage particles across the intestinal epithelium. Bacterial translocation was subsequently used to describe the passage of bacteria from the GI tract to the MLN in rats (*Wolochow* et al., 1966) and to the liver in chickens (*Fuller* and *Jayne-Williams*, 1970).

Bacterial translocation occurs when: (a) the host immune defences are compromised (*Berg*, 1983a; *Owens* and *Berg*, 1980; 1983), (b) the mucosal barrier is physically disrupted (*Morehouse* et al., 1986; *Deitch* et al., 1989a), or (c) there is intestinal bacterial overgrowth due to alterations in the ecologic equilibrium of the indigenous microflora (*Berg* and *Owens*, 1979; *Berg*, 1980a,b,c; *Steffen* and *Berg*, 1983). We have demonstrated bacterial translocation in a variety of animal models including rodents subjected to streptozotocin-induced diabetes (*Berg*, 1985), thermal injury (*Maejima* et al., 1984a,b), leukaemia (*Penn* et al., 1986), endotoxaemia (*Deitch* and *Berg*, 1987; *Deitch* et al., 1987a), haemorrhagic shock (*Baker* et al., 1987), malnutrition (*Deitch* et al., 1987b), intestinal obstruction (*Deitch* et al., 1990a) or bile ligation (*Deitch*, et al., 1990b). The mechanisms responsible for promoting bacterial translocation in these animal models are presented in Table 1 (*Berg*, 1980b, 1981b, 1983b, 1985). In some models, the host exhibits multiple deficiencies in defence mechanisms resulting in bacteraemia and lethal sepsis by translocating indigenous bacteria. Thus, the translocation of indigenous bacteria is a clinically important event even though the indigenous bacteria are relatively nonpathogenic, i.e. opportunistic pathogens.

Not all species of indigenous bacteria translocate from the GI tract with equal efficiency. The bacterial species found to translocate most readily from the GI tract in rodent models, namely the Gram-negative, facultatively anaerobic, enteric bacilli, such as *Escherichia coli, Proteus mirabilis,*

Klebsiella pneumoniae, Enterobacter cloacae, and aerobic *Pseudomonas aeruginosa* (*Steffen* et al., 1988), also are the bacterial species recognised as causing a large proportion of septicaemia in hospitalised patients. Interestingly, the strictly anaerobic bacteria, such as *Bacteroides fragilis*, *Clostridium*, and *Eubacterium*, translocate at the lowest rate of any of the bacteria tested to date even though they colonise the GI tract at extremely high levels (10^{10-11}/g caecum).

PROMOTION OF BACTERIAL TRANSLOCATION BY ENDOTOXIN

Large amounts of endotoxin, the lipopolysaccharide component of Gram-negative bacteria, are normally present in the GI tract since the GI tract harbours tremendous populations of Gram-negative, indigenous bacteria. Endotoxin is released during bacterial cell growth and particularly upon bacterial cell death and lysis. The lethal dose of endotoxin varies from species to species, ranging from 10 μg/kg body weight in the goat to 100 μg/kg in the rat. The lethal dose for humans is not known. However, the human is much more sensitive to the effects of endotoxin than are most other animals; 10^{-12} g/kg endotoxin produces symptoms in humans.

Only small amounts of endotoxin are absorbed from the healthy GI tract. Two mechanisms have been proposed to account for this poor endotoxin absorption: (a) the intestinal mucosa is relatively impermeable to endotoxin absorption and (b) bile salts bind directly to endotoxin in the GI lumen to form detergent-like complexes that are poorly absorbed (*Cahill* et al., 1987). The very small amounts of endotoxin that are absorbed from the healthy GI tract are detoxified by Kuppfer cells in the liver (*Nolan*, 1981). However, in severely ill or injured patients, bacteria and endotoxin can cross the gut mucosal barrier and gain access to tissues and the systemic circulation. Thus, endotoxin absorption from the GI tract is promoted by conditions that increase mucosal permeability or decrease bile output.

A relationship among shock, intestinal ischaemia, and endotoxaemia was first proposed by *Ravin* and *Fine* (1962). More recently, it is hypothesised that gut barrier failure in conjunction with heptatic dysfunction promote or potentiate the newly recognised multiple organ failure syndrome (MOF) (*Carrico* et al., 1986). MOF is a common final pathway leading to death in a variety of patients. Conditions such as shock, infection or immunosuppression increase gut mucosal permeability resulting in increased translocation of bacteria and bacterial products (e.g. endotoxin) from the GI tract to the portal and systemic circulations. Translocated endotoxin then activates various plasma protein cascades, resident macrophages, and circulating neutrophils releasing monokines and proteins that in turn further increase gut mucosal permeability.

Endotoxin is known to increase gut mucosal permeability and to decrease host immune defences. Since endotoxaemia is common in a variety of patients, we performed studies in rodent models to determine whether endotoxin also could promote the translocation of indigenous bacteria from the GI tract. Specific pathogen-free (SPF) mice were injected once intraperitoneally (i.p.), with *E. coli* O26:B6 endotoxin and the peritoneum, MLN, spleen, liver, and blood tested 24 hr later for

translocating indigenous bacteria (*Deitch* et al., 1987a). Endotoxin promoted bacterial translocation to the MLN in a dose-dependent fashion; 88% of the MLN were positive after i.p. injection with 2 mg endotoxin. The spleen and liver cultures were negative at all endotoxin doses. Thus, one i.p. injection of endotoxin promotes bacterial translocation from the GI tract to the MLN. However, the translocating bacteria remain confined to the MLN and do not spread to other organs, such as the spleen or liver.

Polymyxin B interferes with the biologic action of endotoxin by stoichiometrically binding to the endotoxin molecule (*Jacobs* and *Morrison*, 1977). Consequently, to demonstrate the specificity of endotoxin-induced bacterial translocation, mice were injected with *E. coli* O26:B6 or *E. coli* O111:B4 endotoxin that had been incubated with polymyxin B for 3 hr at 20°C and compared to other mice injected with endotoxin not reacted with polymyxin B. Mice injected with *E. coli* O26:B6 endotoxin exhibited 71% incidence of bacterial translocation to the MLN whereas mice injected with the polymyxin B-*E. coli* O26:B6 endotoxin mixture exhibited only a 29% translocation incidence (*Deitch* et al., 1989a). Mice injected with *E. coli* O111:B4 had 75% positive MLN cultures compared to 13% positive cultures in mice injected with the polymyxin B-*E. coli* O111:B4 endotoxin mixture. Gentamicin exhibits a similar bactericidal spectrum as polymyxin B but does not bind to endotoxin. Incubation of *E. coli* O111:B4 endotoxin with gentamicin rather than polymyxin B prior to injection did not decrease endotoxin-induced translocation. These results further confirm the specificity of endotoxin-induced bacterial translocation.

Interestingly, the caecal populations of Gram-negative, enteric bacilli, such as *E. coli*, increased 100-fold 24 hr following one i.p. endotoxin injection (*Deitch* et al., 1987a). By 48 hr following endotoxin injection, the caecal population levels of these bacteria returned to normal and concomitantly both the incidence and levels of bacterial translocation to the MLN also decreased. The mechanisms whereby endotoxin injection influences caecal population levels of indigenous bacteria are unknown, although endotoxin is known to reduce GI motility and to cause transient intestinal ischaemia. Zymosan, a yeast cell wall product unrelated to endotoxin that is inflammatory due to its activation of complement, also increased caecal population levels of indigenous enteric bacilli and promoted bacterial translocation to the MLN when injected i.p. (*Deitch* et al., 1992).

Endotoxin is composed of a core polysaccharide, a lipid-A component containing long-chain fatty acids linked to a glucosamine backbone, and polysaccharide side chains (the O-antigens). To determine which components of the endotoxin structure are important in promoting bacterial translocation, mice were injected with endotoxin from six R-mutant strains of *Salmonella* (Ra, Rb, Rc, Rd, Re, or lipid A). These R-mutants differ in their endotoxin compositions. Intact *Salmonella* endotoxin (wild type) and the Ra and Rb endotoxin fragments promoted bacterial translocation to the MLN whereas the *Salmonella* endotoxin fragments lacking the terminal-3 sugars of the core polysaccharide (Rc, Rd, Re, or lipid A) did not promote bacterial translocation (*Deitch* et al., 1989b). Thus, the terminal portion of the core polysaccharide of *Salmonella* endotoxin appears to be required to promote bacterial translocation. Only the endotoxin fragments that promoted bacterial translocation also

were associated with increased caecal population levels of Gram-negative, facultatively anaerobic, enteric bacilli. Also, injection of the translocation-promoting Ra fragment also increased ileal xanthine oxidase and xanthine dehydrogenase activities indicating mucosal injury, whereas the non-promoting Rc and Re fragments did not increase these enzymatic activities. Both lipid A and endotoxin appear to produce toxic manifestations by stimulating host cells, especially macrophages, to release mediator substances that then act as second messengers to disrupt various homeostatic systems. The finding that *Salmonella* lipid A and the Rc, Rd, or Re endotoxin fragments do not promote bacterial translocation suggests that endotoxin does not induce bacterial translocation primarily by inducing the liberation of macrophage products. This conclusion also is supported by our previous studies demonstrating that endotoxin injected i.p. also promotes bacterial translocation in C3H/HeJ mice that are genetically resistant to macrophage activation by endotoxin (*Deitch* et al., 1987a).

Endotoxin-sensitive C3HeB/FeJ mice and endotoxin-resistant C3H/HeJ mice were tested for bacterial translocation after endotoxin challenge to determine whether genetic sensitivity or resistance to endotoxin would be a factor in endotoxin-induced translocation (*Deitch* et al., 1987a). C3H/HeJ mice are known to exhibit a reduced response to endotoxin and, therefore, have been labelled endotoxin-resistant or hyporesponsive whereas C3HeB/FeJ mice exhibit an exaggerated physiologic or hyper-responsiveness to endotoxin. Endotoxin-sensitive C3HeB/FeJ mice did not exhibit spontaneous bacterial translocation nor did bacterial translocation occur after they received a low dose challenge with endotoxin (0.1 mg/kg i.p.) suggesting that this genetic sensitivity to endotoxin is not normally associated with spontaneous bacterial translocation. Also, translocation occurred at the same rate to the MLN in both endotoxin-resistant C3H/HeJ mice and outbred Ha/ICr CD-1 mice injected i.p. with 2 mg of endotoxin. Consequently, neither genetic sensitivity nor genetic resistance to endotoxin affected endotoxin-induced bacterial translocation suggesting that endotoxin does not promote translocation primarily by depressing or activating the immune system or by triggering increased activity of the lymphoproliferative system.

Histological examination of the GI tissue from endotoxin challenged mice revealed physical disruption of the mucosal barrier. The ileal and caecal lamina propria were oedematous and there was separation of the epithelium from the lamina propria in certain areas, such as at the tips of villi. The duodenal, jejunal, and colonic mucosa appeared normal. Thus, endotoxin-induced bacterial translocation appears to be due primarily to damage to the gut mucosal barrier.

Studies have implicated xanthine oxidase-generated, oxygen-free radicals as mediators of intestinal injury (*Parks* et al., 1982a). Consequently, we determined whether inhibition of xanthine oxidase activities by allopurinol or inactivation of xanthine oxidase by a sodium tungstate diet would prevent the mucosal damage and subsequent bacterial translocation occurring after endotoxin challenge. SPF CD-1 mice were antibiotic decontaminated for 4 days, monoassociated with streptomycin-resistant *E. coli* C25 and challenged once i.p. with *E. coli* O26:B6 endotoxin. The MLN were tested for translocating *E. coli* C25 24 hr later. One group of mice was given allopurinol by gastric lavage (50 mg/kg) 48 and 24 hr prior to i.p. endotoxin

Table 2: Effect of inhibition of inactivation of xanthine oxidase activity on *E. coli* O26:B8 endotoxin-induced bacterial translocation

Group	Translocation Incidence to MLN	CFU/MLN
Control	0%	0
Allopurinol	0%	0
Tungsten diet	0%	0
Endotoxin	80%	1,607
Allopurinol plus endotoxin	31%*	220**
Tungsten diet plus endotoxin	17%*	144**

* $p<0.01$
** $p<0.05$

challenge. Another group of mice was placed on a diet supplemented with sodium tungstate (0.7 g/kg diet), but restricted in molybdenum (ICN Bichemical, Cleveland, OH). This diet contains normal levels of protein, vitamins, minerals, and other trace elements. To deplete intestinal levels of xanthine oxidase, the mice were main-tained on this diet a minimum of 14 days prior to endotoxin challenge. Intestinal xanthine oxidase and xanthine dehydrogenase activities of the control, endotoxin-challenged, and tungstate diet-treated mice were determined by the method of *Ward* and *Rajagopolan* (1976) measuring xanthine oxidase-dependent uric acid formation. Both the incidence and level of bacterial translocation to the MLN after endotoxin challenge were significantly reduced by pre-treatment with allopurinol or the tungstate diet (Table 2). Furthermore, there was no significant intestinal mucosal damage in endotoxin-challenged mice pre-treated with allopurinol or the tungstate diet. Ileal xanthine oxidase activity also was reduced in the mice fed the tungstate diet.

To further strengthen these findings, the effect of allopurinol pre-treatment on bacterial translocation was tested in a bacterial overgrowth model in which translocation is promoted by an increase in caecal population levels rather than by damage to the gut mucosal barrier as in endotoxin-induced translocation. Allopurinol pre-treatment should not decrease bacterial translocation in this overgrowth model. SPF mice were antibiotic decontaminated, monoassociated with *E. coli* C25, and tested 4 days later for *E. coli* C25 translocation to the MLN. There was no difference in the rate of *E. coli* C25 translocation between the group of mice pre-treated with allopurinol and the non-treated control group suggesting that the reduction of endotoxin-induced bacterial translocation by allopurinol in the earlier experiments described above is a specific response related to the ability of allopurinol to inhibit xanthine oxidase activity and thereby reduce mucosal damage (*Deitch* et al., 1989b).

It was also determined whether selective inhibitors of platelet-activating factor (PAF) could reduce endotoxin-induced bacterial translocation (*Deitch* et al., 1989). PAF is produced by many cells, such as macrophages, neutrophils, platelets, and endothelial cells. The biologic effects of PAF include increased vascular permeability, hypotension, and death (*Braquet* et al., 1987). PAF as been implicated as the mediator of bowel necrosis induced by endotoxin or tumour necrosis factor (TNF) (*Sun* et al., 1988). PAF antago-

nist, SRI 63-441 or BN 52021 (20 mg/kg), was administered i.p. 30 min prior to challenge with either *E. coli* O111:B4 or *E. coli* O26:B6 endotoxin. Neither endotoxin-induced mucosal damage nor endotoxin-induced bacterial translocation were blocked by the PAF antagonists (*Deitch* et al., 1989b). Thus, endotoxin challenge does not appear to promote bacterial translocation from the GI tract by inducing the synthesis and/or release of various pro-inflammatory mediators.

One i.p. injection of endotoxin promotes bacterial translocation to the MLN but the translocating bacteria remain confined to the MLN and do not spread systemically to other organs. This same dose of endotoxin given i.p. to thermally-injured mice (25% total body surface area burn) promotes bacterial translocation to the MLN, spleen, liver and blood, and the mice die of sepsis caused by their indigenous GI bacteria (*Deitch* and *Berg*, 1987). Furthermore, the mortality rate of mice receiving only endotoxin or only thermal injury is less than 10% whereas the mortality rate is 100% in mice receiving the combination of endotoxin plus thermal injury. Protein malnourished mice (21 days on a low-protein whey diet; Tekland Test Diets, Madison, WI) also are more susceptible to endotoxin-induced bacterial translocation than are normally nourished mice (*Li* et al., 1989).

More study is required to define the relationships among endotoxaemia, xanthine oxidase activities, gut mucosal injury, bacterial translocation. and sepsis. Since endotoxin challenge also can reduce intestinal blood flow (*Morrison* and *Ryan*, 1987), it is possible that intestinal ischaemia induced by endotoxin could initiate the activation of xanthine oxidase (*Smith* et al., 1987). It also has been postulated that endotoxin increases intestinal permeability by the local action of vasoactive mediators acting within the gut wall (*Cuevas* and *Fine*, 1973). Another possibility is that endotoxin indirectly induces activation of xanthine oxidase through the liberation of secondary mediators, such as interferon.

INHIBITION OF BACTERIAL TRANSLOCATION BY NON-SPECIFIC MACROPHAGE STIMULATION

The host immune system is important in preventing bacterial translocation from the GI tract. It seems probable that the various compartments of the host immune system, such as secretory immunity, cell-mediated immunity, and serum immunity, all may be important to various degrees in protecting the host against bacterial translocation. Secretory IgA possibly could inhibit the close association or adherence of bacteria with the gut mucosa that must occur prior to their translocation across the mucosal barrier. However, the role of secretory IgA in preventing bacterial translocation has not been tested to date.

There is some evidence that T-cell mediated immunity is important in the immune defence against bacterial translocation. Spontaneous bacterial translocation from the GI tract to the MLN, spleen, liver and kidney readily occurs in genetically athymic (nu/nu) mice (*Owens* and *Berg*, 1980). Spontaneous bacterial translocation, however, is inhibited in adult nu/nu mice that have been grafted with thymuses when neonates (*Owens* and *Berg*, 1980). Bacterial translocation from the

Table 3: Inhibition of *E. coli* C25 translocation by *P. acnes* vaccination in antibiotic decontaminated mice monoassociated with *E. coli* C25

Mice	Spleen weight	CFU/g caecum	Translocation incidence	CFU/g MLN
Control	0.10	1.7×10^9	75% (23/29)	1,860
P. acnes	0.57	2.2×10^9	41%	304*

*$p<0.01$

GI tract also occurs in neonatally thymectomised mice (*Owens* and *Berg*, 1982). However, even though T-cell mediated immunity has been demonstrated to inhibit bacterial translocation, the mechanisms whereby this occurs have not been elucidated.

Serum immunoglobulins also are likely important in clearing translocating bacteria once they have entered the lamina propria, lymph, blood or reticulo-endothelial organs, such as the MLN. Certain indigenous bacteria readily translocate from the GI tract to the MLN, spleen, liver, and kidney. Mice injected intraperitoneally with immunosuppressive agents, such as prednisolone or cyclophosphamide, exhibited increased bacterial translocation to the MLN, spleen, liver, and kidney (*Berg*, 1983a). These mice also exhibited reduced spleen plaque-forming (PFC) responses to antigens of *E. coli.*

Resident macrophages in the MLN are ideally situated to clear translocating bacteria, since in most animal models demonstrating bacterial translocation, the translocating bacteria first appear in the MLN prior to their appearance in other organs, such as the liver or spleen (*Berg*, 1983b). Non-specific immunomodulators are available that non-specifically enhance the abilities of macrophages to engulf and kill bacteria. Since it cannot be predicted which of several species of bacteria will translocate in any particular clinical condition, it would be useful to develop an immunologic regimen that would inhibit the translocation of a broad range of bacterial species.

Consequently, we examined the effectiveness in preventing bacterial translocation of three such non-specific immunomodulators, namely glucan, muramyl dipeptide and killed *Propionibacterium acnes* (formerly classified as *Corynebacterium parvum*). Glucan is a polyglycan derived from the cell wall of *Saccharomyces cerevisiae* (*Hassid* et al., 1941) and increases both cell-mediated and humoral immunity (*Wooles* and *DiLuzio*, 1962). Muramyl dipeptide is a small molecular weight glycopeptide responsible for the adjuvant action of *Mycobacterium*. *P. acnes* is known to exert a multitude of effects on the immune system, the most important being the activation of macrophages. Formalin-killed *P. acnes* when injected into mice increases their resistance to a variety of pathogenic bacteria, such as *Salmonella* (*Collins* and *Scott*, 1974), *Listeria* (*Miyata* et al., 1980) and *Staphylococcus* (*Stinnett* et al., 1979).

SPF mice were antibiotic decontaminated with oral streptomycin (1 mg/ml) and penicillin G (100 U/ml) in their drinking water for 7 days. One group of mice then was injected once i.p. with 0.2 ml (1.4 mg) of formalin-killed *P. acnes* (Burroughs-Wellcome, Research Triangle, NC). Another group of mice was injected i.p. with 0.2 ml (0.4 mg) of particulate glucan-P (Accurate Chemical and Scientific Corp., Westbury, NY) on days 10 and 12 following antibiotic decontamination. A

Table 4: Lack of inhibition of *P. acnes* vaccination on the translocation of *E. coli* C25 in gnotobiotic mice monoassociated with *E. coli* C25

Mice	Spleen weight	CFU/g caecum	Translocation incidence	CFU/g MLN
Controls	0.10	4.6×10^8	100% (20/20)	2,650
P. acnes	0.71	5.4×10^8	100% (20/20)	1,730

third group of mice was injected i.p. with muramyl dipeptide. On day 10, the mice were challenged by giving *E. coli* C25 (1×10^9/ml) in their drinking water. The mice were sacrificed on day 14 and the MLN cultured for translocating E. *coli* C25. The population levels of *E. coli* C25 in the caecum also were determined. Vaccination with killed *P. acnes* reduced both the incidence and numbers of *E. coli* C25 translocating to the MLN when compared with control mice injected with saline (Table 3). Neither glucan nor muramyl dipetide inhibited *E. coli* C25 translocation to the MLN. The caecal levels of *E. coli* C25 were similar in all groups of mice. Splenomegaly, as an indicator of the stimulatory effect of the immunomodulator, was more pronounced after injection with *P. acnes* than after injection with glucan. In other experiments, vaccination with *P. acnes* also decreased the translocation of *P. mirabilis* and *E. cloacae* to the MLN. Since killed *P. acnes* vaccination is reported to non-specifically activate macrophages (*Herbert* et al., 1983), it appears that macrophages are important effector cells in the host defence against translocation by Gram-negative, enteric bacilli.

The effectiveness of *P. acnes* vaccination in decreasing *E. coli* C25 translocation also was tested in monoassociated gnotobiotic mice. Germfree mice were injected intraperitoneally with 1.4 mg of formalin-killed *P. acnes* and monoassociated with *E. coli* C25 3 days later by placing an overnight culture in their drinking water (1×10^9/ml). On day 7, the mice were sacrificed and the MLN cultured for translocating *E. coli* C25 as described previously (*Berg* and *Garlington*, 1979). The splenic index of 7.1 (spleen weight of experimentals/spleen weight of controls) demonstrated that the monoassociated gnotobiotes were immunologically stimulated by the *P. acnes* vaccination (Table 4). However, there was not a decrease in the incidence or the numbers of *E. coli* C25 translocating to the MLN as compared with control, non-vaccinated gnotobiotes monoassociated with *E. coli* C25. Thus, it appears that the host immune system must first be "primed" by the indigenous GI microflora before a later second "stimulation" by *P. acnes* vaccine activates macrophages sufficient to inhibit bacterial translocation.

Interestingly, *P. acnes* vaccination does not reduce *E. coli* translocation to the MLN even if adult germfree mice are monoassociated with *E. coli* C25 or colonised with the whole indigenous GI microflora for 8 weeks prior to *P. acnes* vaccination (*Berg* and *Itoh*, 1986). However, if the germfree mice are colonised with the whole indigenous GI microflora within 1 week after birth, then a subsequent *P. acnes* vaccination at 8 weeks of age inhibits *E. coli* C25 translocation in these mice. Thus, exposure to the indigenous microflora is not a prerequisite for splenomegaly induced by *P. acnes* vaccination but is required for inhibition of bacterial translocation.

Other investigators also have reported that the indigenous microflora is

required for the normal development of cell-mediated immunity. *Starling* and *Balish* (1981) found that germfree rats have fewer pulmonary macrophages than conventional rats and that exposure to the indigenous microflora increases alveolar macrophage proliferation and activity. There also are reports that colonisation of germfree animals with the indigenous microflora increases the activities of peritoneal macrophages (*Morland* and *Midtvedt*, 1984) and natural killer cells (*Bartizal* et al., 1983). *Pabst* et al. (1982) suggest that human monocytes do not function optimally unless they have been exposed to the indigenous microflora.

Scott (1972), *Bash* (1978), *Wells* and *Balish* (1979), and *Johnson* and *Balish* (1980) found that prior exposure to the indigenous microflora influences the effectiveness of *P. acnes* vaccination. *P. acnes* given i.p. to conventional rats stimulated the splenic PFC response to sheep erythrocytes whereas *P. acnes* vaccination inhibited this spleen PFC response in germfree rats or gnotobiotic rats monoassociated with *P. acnes*. It does not seem unusual that the indigenous microflora affects the development of the host immune response since the indigenous microflora also is known to profoundly influence the anatomic and physiologic development of the host.

CONCLUSION

Animal models have been developed whereby bacterial components can either promote or inhibit bacterial translocation from the GI tract. One i.p. injection of endotoxin causes histologic damage to the intestinal mucosa and promotes the translocation of indigenous bacteria from the GI tract to the MLN. Inhibition or inactivation of xanthine oxidase or dehydrogenase activities reduces the extent of endotoxin-induced mucosal damage and also decreases bacterial translocation. Thus, a primary mechanism of endotoxin-induced bacterial translocation appears to be the production of xanthine oxidase-generated oxidants which damage the gut mucosa. It also is possible that the translocating bacteria and their products (e.g. endotoxin) further exacerbate the initial mucosal injury caused by endotoxic shock. Studies are in progress to delineate the relationships among endotoxic shock, mucosal injury, mucosal permeability, the translocation of bacteria across the mucosal barrier, and lethal sepsis.

In order to design therapeutic regimens to prevent or decrease bacterial translocation, much more knowledge is required concerning the role of the host immune defence against bacterial translocation. The results described here demonstrating that *P. acnes* vaccination inhibits the translocation of *E. coli* from the GI tract to the MLN suggest that macrophages are important immune effector cells in the host defence. The fact that macrophages can be activated non-specifically to inhibit the translocation of a variety of bacteria is of practical significance, since it cannot be predicted with certainty which of the many indigenous bacterial species will translocate from the GI tract in any given clinical situation. The described studies utilising athymic mice suggest that T-cell mediated immunity also is important in the immune defence against translocation. Studies are in progress to determine the relative roles in preventing bacterial translocation of various compartments of the host immune system, such as secretory

immunity on mucosal surfaces, cell-mediated immunity (macrophages, neutrophils, and T-cell subpopulations), and systemic immunity (serum IgG and IgM).

Bacterial translocation involves complex interactions between the host defence mechanisms and the abilities of bacteria to translocate mucosal barriers and to survive in hostile environments. Delineation of the immune mechanisms important in inhibiting bacterial translocation will provide information for devising rational approaches for the control of opportunistic infections originating from the GI tract in debilitated patients, such as those with endotoxaemia, thermal injury, trauma, and immunosuppressive disorders such as AIDS.

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