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BACTERIAL TRANSLOCATION AND IMMUNITY

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SUMMARY

Bacterial translocation is defined as the passage of viable bacteria from the GI tract to extra-intestinal sites, such as the MLN, spleen, liver, kidney and bloodstream. In healthy adult rodents, normal flora bacteria are not normally crossing the mucosal barrier or they are translocating at a rate low enough to be eliminated by the host defences. Translocation readily occurs, however, when the mucosal barrier is physically disrupted, bacterial overgrowth occurs in the ileum or caecum, or the host immune defences are compromised. Little is known about the exact anatomical route by which bacteria translocate from the GI tract to the MLN and other organs. Most likely, except in the case of disrupted mucosal epithelium, the bacteria translocate intracellularly by endocytosis through the epithelial cells lining the intestinal tract. Also, very little is known concerning the immune mechanisms inhibiting bacterial translocation. Preliminary experiments demonstrate that bacterial translocation readily occurs in athymic (nu/nu) mice and neonatally thymectomised mice, but is inhibited in heterozygous nu/+ mice and in thymus-grafted (nu/nu) mice. Bacterial translocation is also promoted by injection with immunosuppressive agents, such as cyclophosphamide or prednisone. On the other hand, bacterial translocation is inhibited by vaccination with formalin-killed *Propionibacterium acnes*, a non-specific immunomodulator of macrophages. It is important to determine the relative roles of mucosal immunity (especially secretory-IgA), systemic immunity (serum IgG and IgM), and cell-mediated immunity (macrophages and T-cells) in preventing bacterial translocation. By elucidating the various immune mechanisms inhibiting bacterial translocation, strategies can be devised to reduce life-threatening opportunistic infections originating from the GI tract.

INTRODUCTION

The term translocation has been used by *Keller* and *Engley* (1958) and by *Hildebrand* and *Wolochow* (1962) to describe the passage of virus particles across the GI mucosal barrier. The term translocation was used subsequently by *Wolochow* et al. (1966) to describe the passage of viable bacteria from the GI tract to the lymph and mesenteric lymph nodes (MLN) of rats, and by *Fuller* and

Jayne-Williams (1970) for the passage of bacteria from the GI tract to the liver of chickens. Consequently, we employed the term bacterial translocation to describe the phenomenon of the passage of viable normal flora bacteria from the GI tract to extra-intestinal sites, such as the MLN, spleen, liver, kidney and bloodstream (*Berg* and *Garlington*, 1979).

In the healthy adult mouse, normal flora bacteria are not usually cultured from extra-intestinal sites, such as the MLN (*Berg* and *Garlington*, 1979). The bacteria either do not translocate across the mucosal barrier or they cross the mucosa but are killed in route or *in situ* in reticulo-endothelial organs. Most likely, normal flora bacteria are continuously translocating from the GI tract at very low numbers and are killed by the host's immune defences and, therefore, are not cultured from extra-intestinal sites, such as the MLN. However, very little information is available concerning spontaneous bacterial translocation in the healthy animal. For example, even the exact anatomical route is not known by which normal flora bacteria translocate across the intestinal mucosa. *Takeuchi* (1967) observed by electron microscopy *Salmonella typhimurium* translocating intra-cellularly through GI epithelial cells in pre-starved, opium-treated guinea pigs. *Staley* et al. (1968) and *Murata* et al. (1979) also found enteropathogenic *Escherichia coli* to translocate by endocytosis through the mucosal epithelial cells rather than translocating between the epithelial cells. Thus, it is likely that the relatively non-pathogenic normal flora bacteria also translocate through mucosal epithelial cells by endocytosis (an intracellular route) rather than by interrupting the tight junctions between epithelial cells (an inter-cellular route). However, if the mucosal barrier is disrupted by injury, then bacteria can easily pass through the denuded or ulcerated areas of the mucosa. It also is not known whether normal flora bacteria translocate primarily through the mucosa of the small intestine or caecum or whether they translocate with equal efficiency at all sites in the GI tract.

Bacterial translocation from the GI tract readily occurs when: (a) the mucosal barrier is physically disrupted, (b) the host immune defences are compromised, or (c) there is bacterial overgrowth in the GI tract (reviewed in *Berg*, 1980; 1981; 1983; 1985; *Berg* and *Itoh*, 1986). Deficiencies in host defence mechanisms can act synergistically to promote bacterial translocation from the GI tract, as demonstrated by animal models with multiple alterations in host defences. For example, bacterial translocation occurs to a greater degree in mice receiving the combination of an immunosuppressive agent (cyclophosphamide or prednisone) plus an oral antibiotic (penicillin or clindamycin) to cause bacterial overgrowth, than in mice receiving only the immunosuppressive agent or only the oral antibiotic (*Berg* et al., 1988). Thus, an immunosuppressive agent plus an oral antibiotic acts synergistically to promote translocation and subsequent lethal sepsis by the normal flora bacteria. Other animal models exhibiting multiple alterations in the defence against bacterial translocation from the GI tract include streptozotocin-induced diabetes (*Berg* 1985), endotoxaemia (*Deitch* and *Berg* 1987), thermal injury (*Maejima* et al., 1984), and haemorrhagic shock (*Baker* et al., 1988).

The pathogenesis of bacterial translocation from the GI tract appears to occur in several discrete stages (Figure 1). In the healthy animal, spontaneous bacterial translocation is likely occurring continuously at a very low rate but these low numbers of translocating bacteria are killed by the host immune defences.

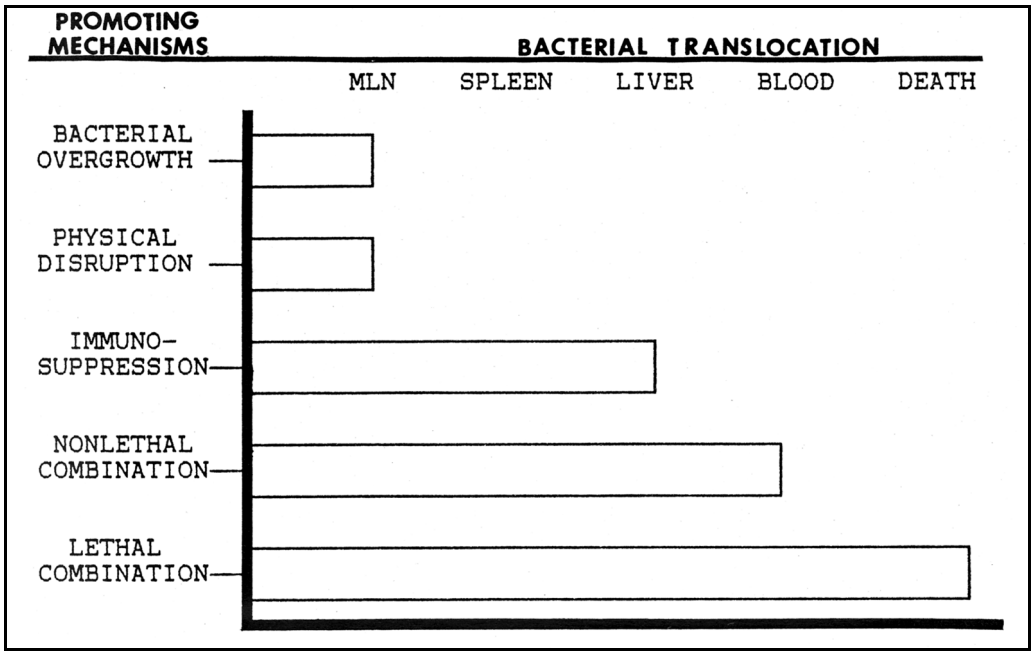

Figure 1: Mechanisms promoting bacterial translocation to extra-intestinal sites.

The administration of an oral antibiotic, however, disrupts the ecologic equilibrium in the GI tract to allow intestinal overgrowth by certain bacteria and the concomitant translocation of these bacteria from the GI tract (*Berg*, 1981). Although these bacteria readily translocate from the GI tract to the MLN in this model, they rarely spread from the MLN to other organs and sites because of the intact host immune defences. Within one day after the oral antibiotic is discontinued, translocating bacteria are eliminated from the MLN (*Berg* and *Owens*, 1979). Thus, the translocating bacteria do not multiply in the MLN but a persistent infection of the MLN is maintained by continuously translocating bacteria.

Immunosuppression promotes the next stage of bacterial translocation. For example, after the injection of an immunosuppressive agent, the host can no longer confine translocating bacteria only to the MLN. The translocating bacteria spread systemically from the MLN to the spleen, liver, kidney and even the bloodstream (*Berg*, 1983). Depending on the degree of immunosuppression and the pathogenic properties of the translocating bacteria, the host may control the infection or the infection may proceed to lethal sepsis. Thus, multiple alterations in host defences can lead to bacterial translocation and lethal sepsis by opportunistic normal flora bacteria residing in the GI tract or by recently acquired exogenous pathogenic bacteria. The host immune defences appear critical in determining whether the translocating bacteria will establish a temporary local infection of the MLN or spread systemically to cause lethal sepsis.

DISRUPTION OF PHYSICAL AND MECHANICAL BARRIER

One of the most important defences against bacterial translocation from the GI tract is the physical barrier of the GI mucosa. Increased intestinal permeability promotes the pathogenesis of various inflammatory as well as infectious diseases. *Schweinburg* et al. (1950) reported that *E. coli* can translocate from the GI tract in dogs injected intra-peritoneally with 5% mono-ethanolamine oleate to induce peritonitis and increased intestinal permeability. Irradiation also promotes bacterial translocation by damaging the architecture of the GI mucosa (*Gordon* et al., 1955). Bacterial translocation also appears to occur to a greater degree in neonates prior to intestinal closure than in mature animals (*Fuller* and *Jayne-Williams*, 1970).

We have found that ricinoleic acid, (12-hydroxy-9-octadecenoic acid) the pharmacologically active constituent of castor oil, given orally to mice severely damages the intestinal mucosa to allow bacterial translocation from the GI tract (*Morehouse* et al., 1986). A single dose of ricinoleic acid administered intragastrically to mice produces significant alterations in the mucosa of the proximal small intestine. Two hours after administration, the duodenal villi are shortened with massive exfoliation of columnar and goblet cells resulting in continuity between the intestinal lumen and the lamina propria. Because of this loss of the mucosal barrier, both strictly anaerobic and facultatively anaerobic bacteria of the normal flora translocate from the GI tract to the MLN, spleen, and liver. The peak incidence of bacterial translocation occurs 4 days after the ricinoleic acid treatment. The mucosa begins to regenerate by 4 hours after a single dose of ricinoleic acid and bacterial translocation ceases by 7 days following treatment.

We have examined other animal models in which mucosal injury appears to be particularly important in promoting bacterial translocation. For example, bacterial translocation readily occurs after the intra-peritoneal injection of endotoxin (*Deitch* et al., 1987). The ileal and caecal mucosa appear relatively intact, although there are sporadic areas where the lymphatic lacteals of the lamina propria are congested and the mucosa is exfoliated. It has been demonstrated previously that endotoxin treatment disrupts the intercellular tight junctions between intestinal epithelial cells (*Walker* and *Porvaznik*, 1978). Consequently, endotoxin may increase GI mucosal permeability due to the local action of humoral mediators acting within the gut wall.

Protein malnutrition also produces histologic atrophy of the small bowel and caecal mucosa, but the epithelial barrier remains intact and bacterial translocation does not occur (*Deitch* et al., 1987). However, the combination of protein malnutrition plus endotoxin injection produces a spectrum of histologic changes ranging from areas of moderate villus oedema to areas of ulceration and increased bacterial translocation. Similarly, physical damage to the mucosal barrier is important in the synergistic promotion of bacterial translocation after a 30% total body surface area burn and endotoxin injection (*Deitch* and *Berg*, 1987).

Haemorrhagic shock of rats for 90 minutes also produces necrosis of the ileal mucosa and subsequent bacterial translocation from the GI tract (*Baker* et al., 1988). Allopurinol, a competitive inhibitor of xanthine oxidase, administered orally prior to haemorrhagic shock reduces the mucosal damage and bacterial translocation (*Deitch* et al., 1988). Rats fed a tungsten-supplemented molybdenum-free diet to inactivate xanthine

oxidase prior to haemorrhagic shock exhibit reduced mucosal damage and bacterial translocation compared to controls fed a regular diet. Thus, bacterial translocation occurring after haemorrhagic shock appears to be due to mucosal damage mediated by oxidants generated by activation of the xanthine oxidase system. Oxygen-free radicals generated during the period of intestinal reperfusion are particularly important in the mucosal damage and associated increase in bacterial translocation. However, other factors are probably also involved, such as ischaemia-induced tissue hypoxia, when gut hypofusion persists for long periods.

These experimental animal models demonstrate conclusively that the intestinal mucosa provides a very important physical and mechanical defence against bacterial translocation, especially in conditions where there are multiple alterations in host defences. Thus, patients suffering from severe trauma with associated haemorrhagic shock or thermally injured patients may be particularly susceptible to infections originating from the GI tract due to a breakdown of the physical mucosal barrier.

SYSTEMIC IMMUNITY

Serum immunoglobulins

Serum immunoglobulins serve as opsonising antibodies to facilitate phagocytosis and clearing of bacteria from the serum and tissues. Consequently, it is likely that serum immunoglobulins also facilitate the clearing of translocating bacteria from reticulo-endothelial organs, such as the MLN.

There is abundant evidence from the literature that the passive transfer of serum antibodies protects against subcutaneous or intra-peritoneal challenge with bacteria or parasites. *Kierszenbaum* (1980) protected athymic mice from *Trypansoma crusi* infection by adoptively transferring immune sera from vaccinated mice. *Tsay* and *Collins* (1984) demonstrated passive protection with anti-*Pseudomonas aeruginosa* polysaccharide IgG in burned and normal mice challenged with *P. aeruginosa*. *Cryz* et al. (1983a), using a murine burn wound sepsis model, passively transferred protection against *P. aeruginosa* PA220 by intravenously injecting homologous anti-*Pseudomonas* lipopolysaccharide IgG. *Cryz* et al. (1983b) also protected mice rendered leukopenic by cyclosphosphamide from *P. aeruginosa* challenge by adoptively transferring serotype specific anti-LPS IgG isolated from rabbit hyper-immune sera.

Serum antibodies are also found to be effective opsonins on mucosal surfaces. *Cooper* and *Rowley* (1979) reported that pre-opsonisation of bacteria with serum antibodies enhances the clearance of these bacteria from the lungs and peritoneum by macrophages. Interestingly, they found that pre-opsonisation with secretory-IgA (s-IgA) may actually delay clearance from the lungs because the Fc portion of s-IgA is incapable of binding to Fc receptors on the macrophages.

Serum immunity has also been demonstrated to affect adversely a protective mucosal immune response. *Pierce* (1980) showed that specific serum antibody to cholera toxin can actually suppress the intestinal immune response to cholera toxin in rats. Suppression is due largely to a direct effect of hyper-immune serum antibody on the interaction of absorbed enteric antigen with lymphoid tissue in Peyer's patches and possibly in the MLN. This effect occurred after the passive transfer of hyperimmune serum from immunised do-

nor rats to normal recipients.

Mucosal immunity, by inhibiting the association or adherence of bacteria to the intestinal epithelium, may be important in the defence against bacterial translocation. However, the systemic immune system must also be important in clearing bacteria that have already translocated across the mucosa. To date, protection against bacterial translocation systemic immunity (by either serum IgM and IgG) or mucosal immunity (s-IgA or s-IgM) has not been tested. Thus, an important area of future research is the role of systemic immunity (serum IgM or IgG) in clearing translocating bacteria that have crossed the intestinal epithelial barrier and to what extent systemic immunity might interfere with the mucosal immune system.

Secretory immunoglobulins

The daily production of s-IgA exceeds that of all other immunoglobulins combined (*Mestecky* et al., 1986). Consequently, s-IgA is important in protecting the mucosal surfaces of the digestive, respiratory, and genito-urinary tracts against potential pathogens. Secretory IgA binds antigen but normally does not fix complement by the classical pathway, does not function as an opsonin, and does not directly kill microorganisms. However, s-IgA exhibits several other important direct and indirect effector functions. The direct functions are inhibition of microbial adherence and colonisation (*Abraham* and *Beachey*, 1985), toxin and enzyme neutralisation (*Brown*,1986; *Holmgren* et al., 1972; *Russell-Jones* et al., 1981; *Ogra* et al., 1984), virus neutralisation (*Spikes* et al., 1975), and inhibition of antigen absorption (*Cummingham-Rundles* et al., 1978). The indirect effector functions include interactions with innate humoral defence factors (*Kilian* et al., 1988) and the ability to potentiate the effect of certain non-specific antibacterial factors such as lactoferrin and lactoperoxidase (*Arnold* et al., 1984; *Funashok* et al., 1982; *Tenovuo* et al., 1982). It appears that s-IgA can activate the alternate pathway of complement activation; but in such a manner that C3b does not become covalently bound nor is C5a released (*Hiemstra* et al., 1987; *Cooper*, 1987).

Appreciation and understanding of the immunological importance of s-IgA has been hampered by the seemingly normal status of IgA-deficient individuals. However, complete absence of s-IgA is rare and s-IgM may compensate for the lack of s-IgA. Even with s-IgM compensating for a lack of s-IgA, increased incidences of a number of pathologic conditions, such as respiratory tract infections and atopic and autoimmune diseases, have been associated with selective IgA deficiency (*Arman* and *Hong*, 1980). Intestinal absorption of intact proteins also appears to occur more readily in patients lacking s-IgA since patients with selective IgA deficiencies have a much higher incidence than normal individuals of antibodies to certain food antigens, such as milk antigens (*Walker* et al., 1972).

Since the primary function of secretory immunoglobulins, especially s-IgA, appears to be the inhibition of the adherence and absorption of certain bacteria and particulate antigens, it seems likely that s-IgA also will reduce bacterial translocation from the GI tract. Secretory IgA inhibits the adherence of certain pathogenic bacteria, such as *Vibrio cholerae* to intestinal epithelium and *Streptococcus mutans* to tooth surfaces. Secretory IgA has not, however, been shown to prevent the close association of normal flora bacteria with the intestinal mucosa.

To date, there have not been any studies focusing on the role of s-IgA in

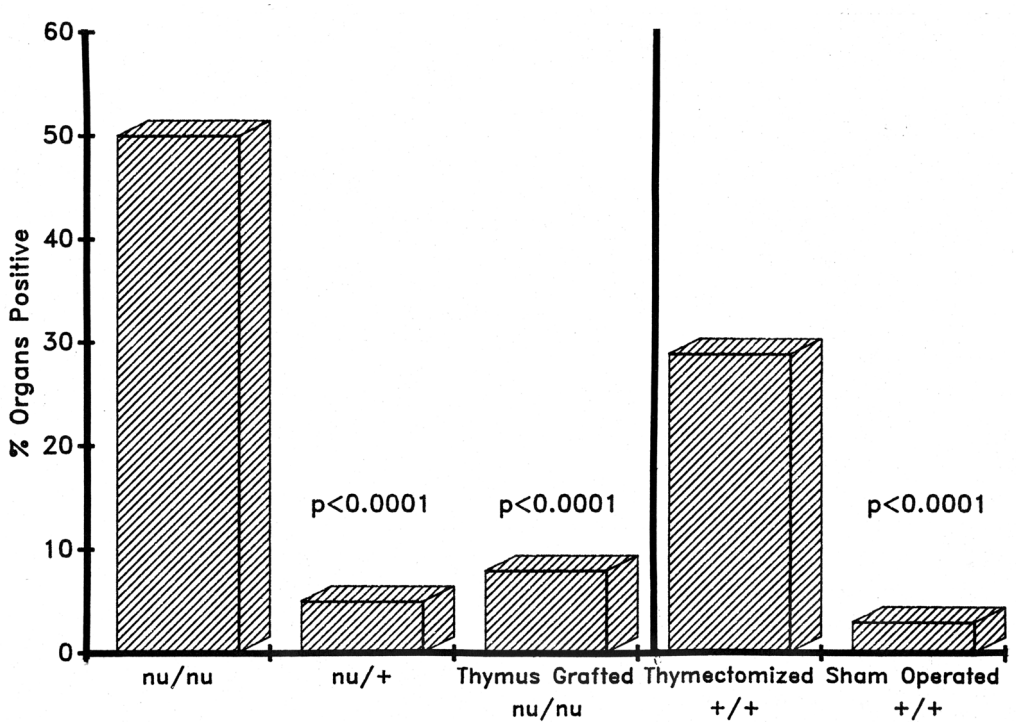

Figure 2: Inhibition of bacterial translocation by T-cell mediated immunity.
Percent positive organs represent percent of total MLNs, spleens, livers, and kidneys positive for translocating bacteria. Nu/nu percentages are compared to nu/+ ($p<.0001$) and thymus-grafted nu/nu percentages ($p<.0001$); thymectomised +/+ percentages compared to sham-operated +/+ percentages by Chi-square analysis.

the prevention of bacterial translocation from the GI tract. The bacterial translocation model seems particularly useful for studying the function of s-IgA, however, since bacteria certainly must associate closely with the intestinal epithelium prior to their translocation across the epithelial barrier.

T-CELL MEDIATED IMMUNITY

Congenitally athymic (nu/nu) mice provide a unique model for determining whether T-cell mediated immunity plays a role in preventing bacterial translocation from the GI tract. Nu/nu mice lack T-cell mediated immunity because their thymus fails to develop to produce functional T-lymphocytes (*Pantelouris*, 1971).

Athymic (nu/nu) and heterozygous (nu/+) mice were tested for translocating bacteria cultured both aerobically and anaerobically (*Owens* and *Berg*, 1980). Fifty percent (50/100) of the MLN, spleen, liver, and kidney from athymic (nu/nu) mice contain viable bacteria compared to only 5% (5/96) of the same organs from nu/+ mice (Figure 2). While the incidences of translocating bacteria found in the MLN of nu/nu versus nu/+ mice are not statistically significant, significant differences in the

translocation incidences to the spleen, liver, and kidney are present between nu/nu and nu/+ mice.

Since congenitally athymic (nu/nu) mice might possess other unrecognised abnormalities in addition to the athymic condition that could influence bacterial translocation, thymectomised mice also were tested to add strength to the role of T-cell mediated immunity in preventing bacterial translocation (*Owens* and *Berg*, 1982). After tests demonstrated the depletion of T-cell immunologic functions in the thymectomised mice, it was determined that thymectomised mice exhibit higher incidences of bacterial translocation to the MLN, spleen, liver, and kidney than sham-thymectomised controls (29% vs. 3%) (Figure 2).

Once athymic (nu/nu) and thymectomised mice were found to exhibit greater incidences of bacterial translocation than euthymic mice (nu/+ or +/+), the definitive test for determining the role of T-cell dependent immunity was to graft thymuses from donor nu/+ mice to recipient nu/nu mice. Nu/nu mice, 21 days old, were grafted with thymuses from neonatal donor mice, 1-2 days old. Four weeks after receiving the thymic grafts, the nu/nu grafted mice were tested for T-cell dependent immune responsiveness against T-cell dependent sheep erythrocyte antigens. The grafted nu/nu mice responded similarly to heterozygous nu/+ mice with increases in specific serum haemagglutinins after the sheep erythrocyte vaccine. Histologic examination of recovered thymus grafts after sacrifice also revealed normal thymus architecture with well-defined cortex and medulla regions. At 8 weeks of age, the incidence of bacterial translocation to the MLN, spleen, liver, and kidney of thymus-grafted nu/nu mice was 8% (5/64), similar to the 5% incidence (5/96) exhibited by nu/+ mice (Figure 2).

Maddus et al. (1988) in a similar study did not detect increased bacterial translocation in athymic mice compared to heterozygotes. However, the translocation assay they employed required at least ten translocating bacteria to produce a positive culture result, whereas our translocation assay requires only one viable bacterium. Also, the strain of mice they used most likely harboured a different GI microflora than the mouse strain we employed. *Cantrell* and *Jutila* (1970) found bacteria, that presumably translocated from the GI tract, in the liver, spleen and blood of thymectomised BALB/c mice that also received injections of rabbit anti-mouse thymocyte sera. *Deitch* et al. (1986) also detected increased bacterial translocation in athymic (nu/nu) compared to euthymic (nu/+) mice. *Penn* et al. (unpublished observations) also found increased *E. coli* C25 translocation to the MLN, spleen and liver of BALB/c athymic mice mono-associated with *E. coli* C25 compared with *E. coli* C25 mono-associated heterozygotes. Thus, our results have been confirmed by others and suggest that T-cell mediated immunity contributes to the host defence against bacterial translocation and, particularly, against the spread of translocating bacteria from the MLN to other sites, such as the spleen and liver.

We also have tested whether T-cell mediated immunity plays a role in preventing the bacterial translocation that occurs after thermal injury (*Deitch* et al., 1986). Athymic (nu/nu), heterozygous (nu/+), and wild type (+/+) mice, with or without 30% total body surface area burns, were tested for the translocation of normal flora bacteria on post-burn days 1, 2 and 4. No translocating bacteria were cultured from the MLN, spleen, liver, peritoneal cavity, or blood of unburned or burned euthymic mice (+/+ or nu/+). In contrast, athymic mice (nu/nu) exhibited a low level of sponta-

neous bacterial translocation, even in the absence of thermal injury, similar to our previous results described above. By the 2nd post-burn day, the numbers of viable bacteria per gram tissue were tenfold higher in the spleens and almost 150-fold higher in the livers of burned athymic mice compared with the unburned controls. These translocating bacteria were identified as *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Staphylococcus epidermidis*, and *Streptococcus faecalis*. Bacterial overgrowth was not responsible for the increased translocation from the GI tract since the population levels of these bacteria in the ilea and caeca were not increased by thermal injury.

Thus, T-cell dependent immunity appear to be an important host defence mechanism inhibiting bacterial translocation from the GI tract, and may be even more important in preventing the systemic spread of translocating bacteria. Future research will focus on identifying the T-cell subpopulations and the specific immune mechanisms whereby these T-cells inhibit bacterial translocation.

MACROPHAGES

We have examined three non-specific immunomodulators, all activators of macrophages, for their abilities to inhibit bacterial translocation from the GI tract to the MLN in specific-pathogen free (SPF) mice antibiotic-decontaminated and subsequently mono-associated with *E. coli* C25. The three immunomodulators are muramyl dipeptide (MDP), glucan, and formalin-killed *Proprionibacterium acnes*.

MDP, a small molecular weight glycopeptide, is the minimal structure responsible for the adjuvant action of *Mycobacterium*. MDP is reported to activate macrophages directly (*Nagad* et al., 1979; *Takeda* et al., 1979; *Pabst* and *Johnson*, 1980). However, we found that MDP injected intra-peritoneally does not inhibit *E. coli* C25 translocation to the MLN in the mono-associated SPF mouse model. Since MDP did not inhibit translocation in our initial experiments, and because MDP is reported to be of limited usefulness in humans due to a short half-life in the bloodstream, we have not continued these studies with MDP.

Glucan, a polyglycan isolated from the inner cell wall of the yeast *Saccharomyces cerevisae*, is associated with enhancement of both humoral and cell-mediated immunity (*Wooles* and *DiLuzio*, 1962; 1963). In addition, glucan increases the activation and proliferation of macrophages. *Kimura* et al. (1983) reported that the antibacterial activity of glucan is primarily due to enhancement of bacterial digestion by macrophages.

We found that particulate glucan injected intra-peritoneally is ineffective in reducing the translocation of *E. coli* C25 from the GI tract to the MLN of mono-associated SPF mice. The glucan vaccine induces an immune response since splenomegaly occurs in the glucan-treated mice, demonstrating a lympho-reticular response, in agreement with other reports (*Burgaleta* and *Golde*, 1977; *Patchen* and *McVittie*, 1983). *Joyce* et al. (1978) found that the major effect of intra-peritoneally injected glucan is exerted in the peritoneal cavity. This conclusion is also supported by our results in which glucan vaccine injected intra-peritoneally reduces the mortality following intra-peritoneal challenge with 10^{10} viable *E. coli* C25. Interestingly, the particulate

glucan vaccine reduces mortality due to bacterial peritonitis induced by intraperitoneal challenge with 10^{10} *E. coli* but does not inhibit the low numbers of *E. coli* translocating from the GI tract to the MLN.

The third immunomodulator tested was formalin-killed *P. acnes* (formerly called *Corynebacterium parvum*, but reclassified). Vaccination with killed *P. acnes* exerts many effects on the immune system, the most important being the non-specific activation of macrophages (*Herbert* et al., 1983). *P. acnes* vaccination increases host resistance to a variety of pathogenic bacteria, including *Salmonella enteriditis* (*Collins* and *Scott*, 1974), *Salmonella typhimurium* (*Briles* et al., 1981), *Listeria monocytogenes* (*Miyata* et al., 1980) and *Staphylococcus aureus* (*Stinnett* et al., 1979).

P. acnes vaccination reduces significantly *E. coli* C25 translocation to the MLN in antibiotic-decontaminated SPF mice mono-associated with *E. coli* C25 (*Fuller* and *Berg*, 1985). The incidence of *E. coli* C25 translocation to the MLN decreased from 75% (50/67) in control non-vaccinated mice to 41% (28/68) in *P. acnes* vaccinated mice ($p = .002$). The mean numbers of translocating *E. coli* C25 per gram MLN also decreased from 1900 to 300 ($p = .009$). The caecal population levels of *E. coli* C25 were not altered by the *P. acnes* vaccine.

These experiments were repeated with indigenous *E. coli*, *Proteus mirabilis*, and *Enterobacter cloacae* with similar results, i.e. *P. acnes* vaccination decreased translocation of these bacteria to the MLN. Furthermore, the translocation of *E. coli* or *E. cloacae* was decreased even when the *P. acnes* vaccine was given to mice in whom these bacteria were already in the process of translocating to the MLN.

The studies above demonstrate that non-specifically stimulated macrophages can inhibit bacterial translocation. In contrast, it has been suggested that macrophages actually may be helpful in the translocation of bacteria and particles from the GI tract. *Joel* et al. (1978) found that macrophages containing carbon particles after long term oral exposure (2 months) were frequently seen in the sub-epithelial region of the Peyer's patch, in the intestinal lymphatics, and the sub-capsular sinus of the lymph node, suggesting that macrophages can carry the particles from the GI tract to the lymphatics. *Harmsen* et al. (1985) using red or green fluorescent microspheres also demonstrated that particles can be carried to the tracheo-bronchial lymph nodes by lung macrophages. Since the lung macrophages contained either all red or all green microspheres, the microspheres did not travel to the lymph nodes to be phagocytised but instead were engulfed by lung macrophages and transported to the nodes. *Wells* et al. (1987) also presented preliminary evidence that macrophages may play a role in the translocation of bacteria and particles from the GI tract. They noted lower translocation rates in macrophage defective, endotoxin-resistant C3H/HeJ mice than in endotoxin-sensitive C3H/HeN mice. They also attempted experiments similar to that of *Harmsen* et al. (1985) described above except that red or green fluorescent microspheres were injected into ligated intestinal loops rather than inoculated in individual lobes of the lungs. However, since the ligated intestinal loop is subjected to internal pressure, it is not known if the transport of engulfed particles by macrophages in this model represents the normal situation in the GI tract. Furthermore, unlike the lung, the GI tract is designed for absorption and, therefore, transport of bacteria by macrophages may not play a significant role in bacterial translocation from the GI tract.

Our results suggest that the macrophage may be an important effector cell for the immunologic prevention of bacterial translocation from the GI tract since translocation is inhibited by vaccination with killed *P. acnes*, a non-specific stimulator of macrophage function. However, adoptive transfer of *P. acnes* stimulated macrophages to non-vaccinated recipient mice to prevent bacterial translocation would considerably strengthen this hypothesis.

CONCLUSION

In healthy adult rodents, normal flora bacteria are not usually cultured from extra-intestinal sites, such as the MLN, spleen, liver, or bloodstream. Either the bacteria are not crossing the mucosal barrier or they are translocating at such low numbers that they are eliminated by the host immune defences. Certain normal flora bacteria readily translocate, however, when the mucosal barrier is physically disrupted, when bacterial overgrowth occurs in the ileum and caecum, or when the host immune defences are compromised. The host might not suffer any ill effects from the translocating bacteria depending on the extent of mucosal injury, the extent of immunosuppression and on the pathogenic properties of the translocating bacteria. However, under certain circumstances the translocating bacteria spread rapidly from the MLN to infect other sites to cause fatal sepsis.

Little is known concerning the exact anatomical route by which normal flora bacteria translocate from the GI tract to the MLN and other organs. Most likely these bacteria translocate intracellularly through the epithelial cells lining the intestinal mucosa by a process of endocytosis and then exocytosis into the lamina propria. The fate of the translocating bacteria in the lamina propria is not known. For example, it is not known whether the translocating bacteria are carried free in the lymph to the MLN or whether macrophages engulf and transport the translocating bacteria to the MLN. Neither is it known if translocating bacteria are killed by the host immune defences in route through the mucosa or are cleared primarily in the MLN and other reticulo-endothelial organs. More information is required concerning the translocation route if we are to understand the initial events in the pathogenesis of bacterial translocation from the GI tract.

This paper describes experimental animal studies suggesting that T-cell mediated immunity and macrophages are important in the host defence against bacterial translocation. However, it has not yet been demonstrated that certain populations of T-cells can inhibit bacterial translocation nor have the specific inhibitory mechanism of T-cells been identified. Vaccination with the non-specific immunomodulator, killed *P. acnes*, inhibits bacterial translocation from the GI tract to the MLN presumably by activating fixed macrophages. However, the macrophage has not, as yet, been conclusively demonstrated to be the effector cell responsible for reducing translocation in this model.

Secretory immunoglobulins have not been tested as to their supposed abilities of reducing bacterial translocation by inhibiting the adherence or close association of normal flora bacteria with the intestinal mucosa. Neither have serum immunoglobulins been tested as to their effectiveness in clearing translocating bacteria from the lamina propria, MLN, spleen, liver, or even bloodstream. Consequently, it is of interest to determine the relative roles of mucosal im-

munity (especially s-IgA), systemic immunity (serum IgG and IgM), in preventing bacterial translocation and cell-mediated immunity (macrophages and/or T-cells).

The GI tract is undoubtedly a reservoir for opportunistic bacterial infections in compromised patients, such as those with AIDS, leukaemia, and haemorrhagic shock, and those suffering from severe trauma. Elucidation of the immune mechanisms inhibiting bacterial translocation would provide opportunities for devising strategies to reduce these life-threatening opportunistic GI infections.

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