# The Effect of Glucocorticoid Administration on Bacterial Translocation

## *Evidence for an Acquired Mucosal Immunodeficient State*

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**Adherence of bacteria to intestinal epithelial cells may be the crucial initiating event for translocation and is normally prevented by both specific (secretory IgA) and nonspecific (mucus, bacterial antagonism, desquamation) mucosal defense mechanisms. The purpose of this study was to examine the effect of dexamethasone administration on mucosal immunity; specifically bacterial adherence and IgA. Twenty Fischer rats were randomly assigned to two groups of 10 animals each. Group I received 0.5 mL saline injection intraperitoneally (IP); and group II, 0.8 mg/150 g body weight dexamethasone IP per day for 2 consecutive days. The cecum, mesenteric lymph nodes, and bile were aseptically collected, and bacterial adherence, bacterial translocation, and IgA concentration were determined. Results indicate that, compared with saline-treated animals, dexamethasone-treated animals had a fall in IgA (54 ± 24 *versus* 232 ± 41 μg/mg protein), an increase in bacterial adherence (8.2 ± 0.5 *versus* 3.4 ± 0.6 cfu ($log^{10}$)/g cecum), and an increased incidence of bacterial translocation to the mesenteric lymph nodes (60% *versus* 0%). These data suggest that glucocorticoids may promote bacterial translocation by impairment of mucosal IgA synthesis.**

SECRETORY IMMUNOGLOBULIN A, THE MOST abundant immunoglobulin on mucosal surfaces, is the principle component of the mucosal immune system. Synthesis and expression of s-IgA into secretions prevents the adherence of bacteria and viruses to mucosal epithelial cells, thereby hindering their pathogenicity and shielding the systemic immune system from further activation.[1] Immunoglobulin A (IgA) is synthesized by plasma cells in the gut lamina propria and is expressed onto the mucosal epithelial surface and into the lumen in association with J chain and secretory component. Secretory component acts as a receptor on the basolateral surface of enterocytes, binding to dimeric IgA and facilitating its transport to the lumen.

We have previously reported that total parenteral nutrition promotes bacterial translocation to mesenteric lymph nodes in association with a marked diminution in both biliary secretory IgA as well as marked depletion of gut lamina propria plasma cells of the IgA isotype.[2,3] Bacterial translocation has been reported after several models of catabolic stress, including burns, shock, sepsis, and trauma.[4] Burn models result in a 90% reduction in biliary IgA concentration as early as 18 hours after the burn injury.[5] Glucocorticoids administered at physiologic doses result in significant decreases in IgA and IgG at the mucosal surface.[6] Because endogenous glucocorticoid release can be demonstrated in virtually all animal models involving a severe catabolic stress,[7] it was the purpose of this study to examine the effect of dexamethasone, a pure glucocorticoid, on mucosal IgA, bacterial adherence to the mucosa, and bacterial translocation.

### Materials and Methods

Twenty female Fischer rats weighing between 100 and 150 g were randomly assigned to two groups of 10 animals each. Group I (SALINE) received 0.5 mL 0.9% NaCl injection intraperitoneally (IP)/day in two divided doses for 2 consecutive days. Group II (DEX) received 0.8 mg/150 g body weight of dexamethasone IP/day in two divided doses for 2 consecutive days. Animals were allowed *ad libitum* access to water during the study period but were otherwise made *nulla per os* (NPO). Forty-eight hours after the initiation of the study, animals were anesthetized with pentobarbital (50 mg/kg IP) and ketamine (25 mg/kg IP), and sterilely prepared and shaved for abdominal exploration using 30% alcohol. The common bile duct was skeletonized just proximal to the mesoduodenum and cannulated using Silastic tubing as previously described.[8] Bile was collected into a sterile container for 15 minutes

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from five animals in each group. Next all animals underwent an *en bloc* excision of the mesenteric lymph nodes by aseptically dissecting the mesenteric chain of lymph nodes from the ileocecal valve to the root of the mesentery. The cecum was excised and its contents (stool) were removed for culture. The cecum was placed in a sterile petri dish and vigorously washed using a jet stream of 0.9% NaCl until the wash effluent was clear. The washed cecum was submitted for culture.

Mesenteric lymph nodes (MLN) were weighed, transferred to a sterile 2.0 Ten Broek tissue grinder (Corning Glass Works, Corning, NY), and diluted 1:10 (wt/vol) with sterile 0.85 NaCl. After manual grinding, each sample was spread plated on five TSA II blood Agar plates (BBL, Becton Dickson, Co., Cockeyesville, MD). Representative colonies were expressed as colony-forming units per gram of organ tissue (cfu/g) and converted to $\log_{10}$. Tissues were considered to be "positive" for bacteria if greater than 100 cfu/g of tissue was found and at least three of the five plates were growing bacteria.

The cecum and stool of each rat was also weighed, transferred to sterile tissue grinders, and diluted as above. Homogenized samples then were serially diluted 10-fold in sterile 0.85 NaCl. Nine spread plates of each dilution were made on EMB Agar (Difco Labs, Detroit, MI). Representative colonies from the EMB plates were identified by the hospital clinical laboratory using standard techniques. Results were expressed in cfu/g of cecum($\log_{10}$). Colony counts from EMB plates were used to distinguish two groups of gram-negative bacteria, non–lactose- and lactose-fermenting bacteria.

Bile was assayed for IgA by enzyme-linked immunosorbent assay (ELISA) as previously described.[9]

Protein was assayed using the Lowry method (Sigma Chemical Co., St. Louis, MO). Total IgA in bile was expressed as the concentration of IgA per milligram bile protein ($\mu$g/mg protein) to normalize for variability in bile flow or liver blood flow between animals.

Cecal stool was diluted and microscopically examined for IgA coating of bacteria using fluorescein isothiocyanate (FITC)-labeled specific antisera using a modification of the method described by Vidhichamnong et al.[10] Briefly, stool was diluted 1:10 (wt/vol) in phosphate-buffered saline (PBS) and the heavy debris was allowed to settle at 30 C. One hundred microliters of the supernatant was removed and placed on a glass slide, and the bacteria was fixed to the slide by heating. The slide then was incubated with several dilutions of FITC-labeled anti-IgA antisera, washed several times with PBS, and then allowed to dry in the refrigerator. Using both phase contrast and fluorescent microscopy, rods were identified and evaluated as to their fluorescence on a scale of 1 to 5. Fluorescence was expressed as intensity units (IU) per high-power field (hpf).

## Statistical Analysis

Translocation rates were tested for level of significance using a chi-square analysis with a Yates correction for continuity. Culture results were compared between groups using a Mann-Whitney U test because of the nonparametric nature of these data. Data for IgA were tested for significance using a the Mann-Whitney sum rank analysis.

## Results

All animals survived the 2-day study period. Animals that received dexamethasone developed a mild septic appearance manifested by piloerection, conjunctivitis, and a slight degree of hypoactivity.

Animals receiving saline for the 2-day study period appeared healthy without any signs of illness. Dexamethasone-treated animals (Group II) had a statistically significant decrease ($p < 0.01$) in biliary IgA compared with the saline-treated animals (DEX-54 ± 24 *versus* SALINE-232 ± 41 $\mu$g/mg protein ± standard error of the mean [SEM]). In addition a statistically significant decrease ($p < 0.01$) in IgA coating of bacteria was observed in the dexamethasone-treated animals compared with the saline group (DEX, 1.4 ± 0.5 *versus* SALINE, 3.8 ± 1.1 IU/hpf ± SD) (Fig. 1). Dexamethasone-treated animals developed a statistically significant increase ($p < 0.05$) in the incidence of bacterial translocation to the mesenteric lymph nodes compared with saline-treated animals (DEX, 60% *versus* SALINE, 0%) (Fig. 2). The most common bacteria cultured in the MLNs was *Escherichia coli*, followed by *Proteus vulgaris*. This increased translocation rate was accompanied by a statistically significant increase in both the gram-negative bacterial concentration in stool (STOOL) and the gram-negative bacterial concentration adherent to the cecal wall (CECUM); (STOOL: DEX, 9.2 ± 0.4 *versus* SALINE, 7.7 ± 0.9 cfu ($\log_{10}$)/g stool ± stan-

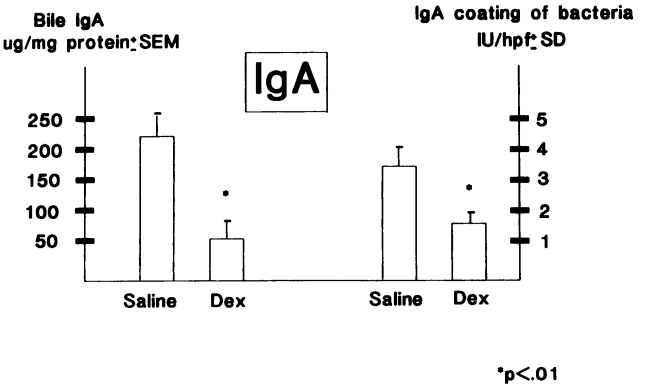


FIG. 1. IgA concentration in bile and IgA coating of bacteria. A statistically significant decrease ($p < 0.01$, Mann-Whitney) was achieved between biliary concentration of IgA and IgA coating of bacteria in the dexamethasone-treated animals.

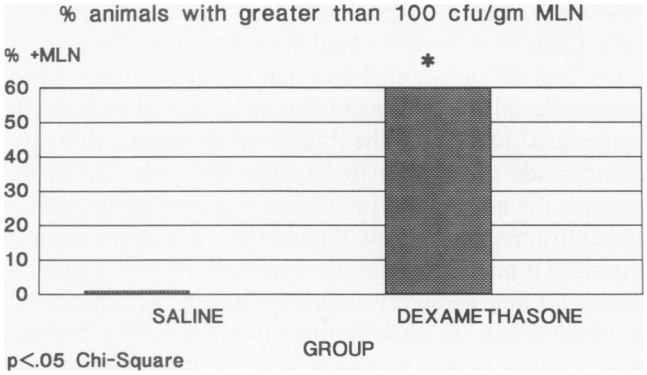


FIG. 2. Incidence of bacterial translocation to mesenteric lymph nodes (MLN) between groups. A statistically significant difference ($p < 0.05$ chi square) between translocation rates can be seen.

dard deviation [SD]; CECUM: DEX, 8.2 ± 0.5 *versus* SALINE, 3.4 ± 0.6 cfu ($log_{10}$)/g cecum ± SD) (Fig. 3).

## Discussion

Bacterial translocation from the gut, the extraintestinal relocation of the indigenous intestinal microflora, can occur to a significant degree after a variety of stresses, which include burns, trauma, shock, anaerobic decontamination, parenteral nutrition, radiation, and chemotherapy.[11] Two basic findings common among these animal models appear to be alteration of the normal intestinal microbial balance and physical or immunologic disruption of the normal epithelial barrier to bacteria.[12]

Normal enteric bacteria are confined to the intestinal lumen by a complex interplay of luminal, mucosal, cellular, and immunologic/inflammatory mechanisms. By this reasoning interference of bacterial migration can occur intraluminally, at the mucosal surface, or in passage by a complex repertoire of bactericidal activity present in all layers of the intestines. Intestinal immune function

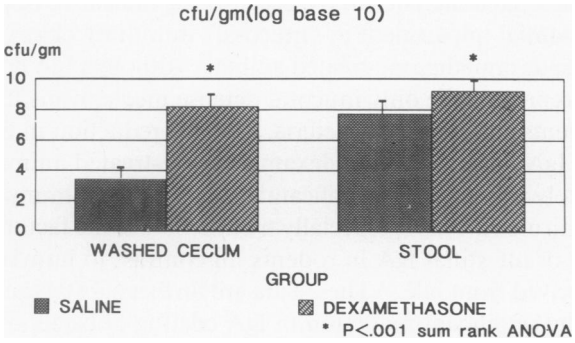


FIG. 3. Cecal and stool culture results. Stool and ceca were homogenized and plated on EMB agar, which is selective for gram-negative bacteria. A statistically significant increase ($p < 0.001$, Mann-Whitney) was observed in both cecal and stool bacterial concentrations in the dexamethasone-treated animals. This increase appeared particularly striking in the washed cecum.

therefore is a general term describing the prevention of passage of viable bacteria, viruses, or toxins from the gut lumen to any extraintestinal site and may involve immunoglobulins, oxygen radicals, macrophages, etc. Mucosal immune function, however, describes an earlier step in the translocation process and is defined as prevention of invasion of the surface epithelial cell by inhibition of adherence of luminal pathogens to the cell surface. Adherence of bacteria to the intestinal epithelial cell is thought to be the crucial initiating event for the establishment of mucosal invasion.[13] A vast array of well-described receptors for bacterial adhesins exists on mammalian cells, and interference with the adherence factors on pathogenic bacteria prevents their ability to cause mucosal disease (Table 1).[14] For example when adherence of diarrheagenic enteroinvasive bacteria has been prevented, they can no longer cause diarrhea even when introduced into the gastrointestinal tract in high concentration.[15] Bacteria have specialized pili or fimbriae that serve as the adhesin or virulence factor, permitting attachment to epithelial cell receptors. Bacterial adherence is normally prevented by both specific (secretory IgA) and nonspecific (mucus, bacterial antagonism, desquamation) mucosal defense mechanism.[16] Normally the anaerobic bacteria form a paste on the mucosal surface. Their presence has been shown to sterically inhibit the epithelial cell receptors for adhesins on gram-negative bacteria.[17] Anaerobic bacteria as a rule do not translocate across histologically normal intestine; however decontamination of the anaerobic flora with antibiotics results in translocation of gram-negative organisms.[18] Mucus is also an important physical barrier for bacteria, and its absence results in significant bacterial adherence.[19] If bacteria are able to breach these defense barriers, cell desquamation can help debride the bacteria. This apparently occurs only when the cell perceives a critical number of surface-colonizing bacteria.[20] Although these nonimmune mechanisms are important for mucosal defense, the only immune-specific mecha-

TABLE 1. *Examples of Adhesin/Receptor Interaction**

| Microorganism | Adhesin | Receptor |
|---|---|---|
| *Escherichia coli* | Type 1 fimbriae | D-mannose |
| | p-fimbriae | |
| | K88 fimbriae | alpha-D-Galp(1-4)-B-Galp |
| | K99 fimbriae | B-D-Gal(GM ganglioside) |
| | CFA 1 fimbriae | GM-2 ganglioside |
| | | GM-2 ganglioside |
| *Pseudomonas aeruginosa* | Fimbriae | Sialic acid residue |
| *Staphylococcus aureus* | Lipoteichoic acid | Fibronectin |

* To establish invasion, bacterial adhesions attach to epithelial cell receptors in lock-and-key fashion. These adhesion/receptor interactions account for the preponderance of potentially pathogenic resident flora to invade at specific sites.

nism for the prevention of bacterial adherence is the coating of bacteria by secretory IgA.

Secretory IgA (s-IgA) is a 360,000 MW immunoglobulin synthesized by gut lamina propria plasma cell originating as Peyer's patch B-cells.[1] Antigen-specific s-IgA is released into secretions after antigenic challenge in Peyer's patches and homing of B-cells to remote sites such as breast, salivary gland, intestine, and liver.

S-IgA coats bacteria and prevents bacterial adherence by an unknown mechanism. S-IgA is resistant to proteolysis, contains J-chain and secretory pieces, and is held together by several disulfide bridges, permitting its survival at varying temperatures and pHs, such as are encountered in the gastrointestinal lumen. This antigen-specific immunoglobulin appears to have a particular affinity for the gram-negative bacteria that posses the necessary adhesins for attachment to human and animal gut mucosa. IgA coating of bacteria has been demonstrated to be selective for the gram-negative enteric flora in humans and rodents.[21] A careful study by Van der Waaj and Berghuis[22] demonstrated that approximately 60% to 80% of the gram-negative enteric organisms in the human and rodent gastrointestinal tract are IgA coated, whereas only 10% to 20% of gram-positive organisms were coated.[22]

Data from the present study indicate that the most common translocating organism was *E. coli.* In addition cecal homogenates grown on media selective for gram-negative bacteria demonstrate that significant adherence of gram-negative bacteria occurred. It is interesting that in virtually all the human and animal reports where bacterial translocation has been demonstrated, the most common organisms isolated are the *enterobacteriaceae.*[23] Furthermore both IgA concentration in fluids and IgA coating of bacteria has been demonstrated to be decreased in many of these models, such as parenteral nutrition, radiation, and chemotherapy.[22] It is attractive to speculate that IgA deficiency may lead to adherence of gram-negative bacteria to the mucosal epithelial cell and that this is the crucial initiating event for bacterial translocation. The causal relationship between bacterial translocation and IgA, however, remains to be proven.

Data from the present study demonstrate that dexamethasone administration results in a significant decrease in biliary IgA and IgA coating of cecal bacteria associated with bacterial adherence to the cecum and bacterial translocation to mesenteric lymph nodes. Wira has recently reported that dexamethasone administration, in physiologic doses, results in a marked decrease in IgA in saliva and vaginal secretions of rats.[6] In addition antigen-specific IgA production after oral antigenic challenge was significantly attenuated in dexamethasone-treated animals.[6] These findings were associated with a significant increase in serum polymeric IgA. These investigators suggested that endogenous glucocorticoids might enlist antibodies at the mucosal surface to confer immune protection systemically. Data from the Wira study and data from the present study beg the question: Is the fall in mucosal IgA from stress-induced glucocorticoid release beneficial or harmful to the host? Data from the present study suggest that glucocorticoids may result in harm to the host, especially because the animals in the dexamethasone group developed chromodacryorrhea. Bacterial translocation to mesenteric lymph nodes may be a sample process whereby the host is able to survey enteric pathogens with potential to disseminate. In this manner induction of a backup serum immune response such as a rise in polymeric IgA, as Wira would suggest, occurs. In the process of bacterial translocation to the mesenteric lymph nodes, however, release of macrophage-derived cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) can be released and result in a severe systemic inflammatory response.[24] To what extent the translocation of bacteria is helpful or harmful to the host is therefore not yet clear. Just as low levels of TNF may be important for the induction of the inflammatory response to invading pathogens, low levels of bacteria in the mesenteric lymph nodes may be appropriate during the initial phase of the catabolic response to sepsis. Persistence or dissemination of bacteria within the mesenteric lymph nodes, however, may be harmful.

Because bacterial translocation can be demonstrated in such a wide variety of animal models of stress, data from the present study suggest that glucocorticoid release may be a common stimulus in these models. Furthermore because a significant diminution in IgA can be demonstrated in many models of stress, such as burn injury, the mechanism of glucocorticoid-induced bacterial translocation may be by impairment of IgA synthesis and function.[7] Data from Figure 3 demonstrate that differences in bacterial adherence between the groups was much more dramatic than the differences in stool bacterial counts. A near five order of magnitude difference in the $\log_{10}$ cfu/g of bacterial adherence to the cecal wall is indicative of the substantial impairment in "mucosal" immunity observed in the dexamethasone-treated animals. Although IgA does not represent the only mucosal defense mechanisms that prevents adherence of bacteria, the 76% reduction in biliary IgA observed in the dexamethasone-treated animals is likely to contribute significantly to the mucosal immune impairment. This is especially true in view of the fact that 90% of intestinal IgA in rodents, in contrast to humans, is derived from bile.[25] These data are further substantiated by the significant reduction in IgA coating of bacteria in the dexamethasone-treated animals.

In summary dexamethasone-treated animals develop bacterial translocation to the mesenteric lymph nodes in association with a marked decrease in IgA concentration in bile. Whether patients with multiple organ failure states

die *of* or *with* bacterial translocation remains an important focus of future studies in humans.

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