Am J Pathol. 2008 Nov;173(5):1243-52. Epub 2008 Oct 2.

Physiological, pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation: living life on the edge of the wall.

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The anatomical and functional arrangement of the gastrointestinal tract suggests that this organ, beside its digestive and absorptive functions, regulates the trafficking of macromolecules between the environment and the host through a barrier mechanism. Under physiological circumstances, this trafficking is safeguarded by the competency of intercellular tight junctions, structures whose physiological modulation is mediated by, among others, the recently described protein zonulin. To prevent harm and minimize inflammation, the same paracellular pathway, in concert with the gut-associated lymphoid tissue and the neuroendocrine network, controls the equilibrium between tolerance and immunity to nonself antigens. The zonulin pathway has been exploited to deliver drugs, macromolecules, or vaccines that normally would not be absorbed through the gastrointestinal mucosal barrier. However, if the tightly regulated trafficking of macromolecules is jeopardized secondary to prolonged zonulin up-regulation, the excessive flow of nonself antigens in the intestinal submucosa can cause both intestinal and extraintestinal autoimmune disorders in genetically susceptible individuals. This new paradigm subverts traditional theories underlying the development of autoimmunity, which are based on molecular mimicry and/or the bystander effect, and suggests that the autoimmune process can be arrested if the interplay between genes and environmental triggers is prevented by re-establishing intestinal barrier competency. Understanding the role of zonulin-dependent intestinal barrier dysfunction in the pathogenesis of autoimmune diseases is an area of translational research that encompasses many fields.

PMID: 18832585 [PubMed - indexed for MEDLINE]

PMCID: PMC2570116

Supplemental Content

PLoS Pathog. 2008 Feb 8;4(2):e35.

Mucosal damage and neutropenia are required for Candida albicans dissemination.

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Comment in:

- Expert Rev Anti Infect Ther. 2008 Aug;6(4):441-5.

Candida albicans fungemia in cancer patients is thought to develop from initial gastrointestinal (GI) colonization with subsequent translocation into the bloodstream after administration of chemotherapy. It is unclear what components of the innate immune system are necessary for preventing C. albicans dissemination from the GI tract, but we have hypothesized that both neutropenia and GI mucosal damage are critical for allowing widespread invasive C. albicans disease. We investigated these parameters in a mouse model of C. albicans GI colonization that led to systemic spread after administration of immunosuppression and mucosal damage. After depleting resident GI intestinal flora with antibiotic treatment and achieving stable GI colonization levels of C. albicans, it was determined that systemic chemotherapy with cyclophosphamide led to 100% mortality, whereas selective neutrophil depletion, macrophage depletion, lymphopenia or GI mucosal disruption alone resulted in no mortality. Selective neutrophil depletion combined with GI mucosal disruption led to disseminated fungal infection and 100% mortality ensued. GI translocation and dissemination by C. albicans was also dependent on the organism's ability to transform from the yeast to the hyphal form. This mouse model of GI colonization and fungemia is useful for studying factors of innate host immunity needed to prevent invasive C. albicans disease as well as identifying virulence factors that are necessary for fungal GI colonization and dissemination. The model may also prove valuable for evaluating therapies to control C. albicans infections.

PMID: 18282097 [PubMed - indexed for MEDLINE]

PMCID: PMC2242836

- CYCLOPHOSPHAMIDE - HSDB

Supplemental Content

J Am Coll Nutr. 2007 Dec;26(6):684S-90S.

Probiotics in irritable bowel syndrome: an immunomodulatory strategy?

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The clear delineation of a post-infective variety of IBS, as well as the description, in a number of studies, of evidence of low-grade inflammation and immune activation in IBS, suggest a role for a dysfunctional relationship between the indigenous flora and the host in IBS and, accordingly, provide a clear rationale for the use of probiotics in this disorder. Other modes of action, including bacterial displacement and alterations in luminal contents, are also plausible. While clinical evidence of efficacy is now beginning to emerge, a review of available trials emphasizes the importance of a clear definition of strain selection, dose and viability. The possible roles of co-therapy or sequential therapy with antibiotics, probiotics, prokinetics, or other agents also deserves further study. The role of the enteric flora is evidently an area of great potential in IBS; we are on the threshold of a new era of research and therapy for this common disorder.

PMID: 18187434 [PubMed - indexed for MEDLINE]

Supplemental Content

Aliment Pharmacol Ther. 2002 Aug;16(8):1383-93.

The role of the gut flora in health and disease, and its modification as therapy.

Hart AL, Stagg AJ, Frame M, Graffner H, Glise H, Falk P, Kamm MA.

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The gut flora is a vast interior ecosystem whose nature is only beginning to be unravelled, due to the emergence of sophisticated molecular tools. Techniques such as 16S ribosomal RNA analysis, polymerase chain reaction amplification and the use of DNA microarrays now facilitate rapid identification and characterization of species resistant to conventional culture and possibly unknown species. Life-long cross-talk between the host and the gut flora determines whether health is maintained or disease intervenes. An understanding of these bacteria-bacteria and bacteria-host immune and epithelial cell interactions is likely to lead to a greater insight into disease pathogenesis. Studies of single organism-epithelial interactions have revealed the large range of metabolic processes that gut bacteria may influence. In inflammatory bowel diseases, bacteria drive the inflammatory process, and genetic predisposition to disease identified to date, such as the recently described NOD2/CARD15

gene variants, may relate to altered bacterial recognition. Extra-intestinal disorders, such as atopy and arthritis, may also have an altered gut milieu as their basis. Clinical evidence is emerging that the modification of this internal environment, using either antibiotics or probiotic bacteria, is beneficial in preventing and treating disease. This natural and apparently safe approach holds great appeal.

PMID: 12182739 [PubMed - indexed for MEDLINE]

Supplemental Content

Am J Pathol. 2008 Dec;173(6):1714-23. Epub 2008 Oct 30.

NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora.

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Improved hygiene has been suggested to influence certain autoimmune disorders, such as multiple sclerosis. In this study, we addressed whether altering the composition of gut flora may affect susceptibility to experimental autoimmune encephalomyelitis (EAE), an animal model of MS. We administered a mixture of non-absorbing antibiotics, kanamycin, colistin, and vancomycin (KCV), orally to mice induced to develop EAE. The antibiotic treatment, beginning 1 week prior to sensitization, altered the composition of gut flora and, intriguingly, also ameliorated the development of EAE. While this result was associated with a reduced production of pro-inflammatory cytokines from the draining lymph node cells, a reduction of mesenteric Th17 cells was found to correlate with disease suppression. In addition, we found that Valpha14 invariant NKT (iNKT) cells were necessary for maintaining the mesenteric Th17 cells. The homologous effects of KCV treatment and iNKT cell depletion led us to speculate that KCV treatment may suppress EAE by altering the function of iNKT cells. Consistent with this hypothesis, KCV treatment did not suppress EAE that was induced in iNKT cell-deficient mice, although it was efficacious in mice that lacked Valpha19 mucosal-associated invariant T cells. Thus, gut flora may influence the development of EAE in a way that is dependent on iNKT cells, which has significant implications for the prevention and treatment of autoimmune diseases.

PMID: 18974295 [PubMed - indexed for MEDLINE]

PMCID: PMC2626383

Supplemental Content

Clin Microbiol Infect. 2008 Jan;14(1):41-8. Epub 2007 Nov 13.

Changes in Escherichia coli resistance patterns during and after antibiotic therapy: a longitudinal study among outpatients in Germany.

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There is worldwide concern about the appearance and rise of bacterial resistance to commonly used antibiotics. Although the gut is an important reservoir for resistant Escherichia coli, data from large-scale epidemiological studies concerning the colonisation dynamics of the normal gut flora with resistant E. coli during and after antibiotic therapy are sparse. Accordingly, a large community-based study was conducted to ascertain changes in the prevalence of resistant E. coli during and after antibiotic treatment. Stool samples before, during and after antibiotic therapy were obtained from 541 patients (aged >/=40 years) with a febrile infection who attended a general practitioner in southern Germany. The MICs of commonly prescribed antibiotics for E. coli isolates from the stools were determined. The prevalence of resistance to the corresponding antibiotics rose from 18% to 38%, from 29% to 58% and from 33% to 67% during treatment with beta-lactam antibiotics, doxycycline and co-trimoxazole, respectively. Prevalences of resistance in the E. coli isolates also rose for other antibiotic classes. With the exception of co-trimoxazole resistance, prevalences of resistance returned to baseline levels in <2 weeks after the cessation of antibiotic therapy. Thus, there was a substantial, but rapidly reversible, increase in the prevalence of resistant E. coli isolates during antibiotic treatment.

PMID: 18005177 [PubMed - indexed for MEDLINE]

Supplemental Content

Surgery. 2007 Apr;141(4):470-80. Epub 2007 Jan 25.

Modification of intestinal flora with multispecies probiotics reduces bacterial

translocation and improves clinical course in a rat model of acute pancreatitis.

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BACKGROUND: Infection of pancreatic necrosis by gut bacteria is a major cause of morbidity and mortality in patients with severe acute pancreatitis. Use of prophylactic antibiotics remains controversial. The aim of this experiment was assess if modification of intestinal flora with specifically designed multispecies probiotics reduces bacterial translocation or improves outcome in a rat model of acute pancreatitis. METHODS: Male Sprague-Dawley rats were allocated into 3 groups: (1) controls (sham-operated, no treatment), (2) pancreatitis and placebo, and (3) pancreatitis and probiotics. Acute pancreatitis was induced by intraductal glycodeoxycholate and intravenous cerulein infusion. Daily probiotics or placebo was administered intragastrically from 5 days prior until 7 days after induction of pancreatitis. Tissue and fluid samples were collected for microbiologic and quantitative real-time PCR analysis of bacterial translocation. RESULTS: Probiotics reduced duodenal bacterial overgrowth of potential pathogens (Log(10) colony-forming units [CFU]/g 5.0 +/- 0.7 [placebo] vs 3.5 +/- 0.3 CFU/g [probiotics], $P < .05$), resulting in reduced bacterial translocation to extraintestinal sites, including the pancreas (5.38 +/- 1.0 CFU/g [placebo] vs 3.1 +/- 0.5 CFU/g [probiotics], $P < .05$). Accordingly, health scores were better and late phase mortality was reduced: 27% (4/15, placebo) versus 0% (0/13, probiotics), respectively, $P < .05$. CONCLUSIONS: This experiment supports the hypothesis that modification of intestinal flora with multispecies probiotics results in reduced bacterial translocation, morbidity, and mortality in the course of experimental acute pancreatitis.

PMID: 17383524 [PubMed - indexed for MEDLINE]

Supplemental Content

Clin Microbiol Infect. 2006 Oct;12(10):974-9.

Risk-factors for gastrointestinal colonisation with resistant Enterobacteriaceae among hospitalised patients: a prospective study.

Lepelletier D, Caroff N, Riochet D, Bizouarn P, Bourdeau A, Le Gallou F, Espaze E, Reynaud A, Richet H.

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This study assessed the incidence of gastrointestinal colonisation by resistant Enterobacteriaceae among hospitalised patients, and identified risk-factors for ceftazidime and ofloxacin resistance. A prospective cohort study was performed in five wards in a French teaching hospital during a 2-year period. Patients hospitalised for > 48 h were enrolled between 17 April 2000 and 30 April 2002. A rectal swab was taken at admission, then once-weekly and/or on the day of discharge. In total, 933 patients were investigated and 585 amoxycillin-resistant isolates were obtained. Resistance rates for ceftazidime and ofloxacin were 9.4% and 4.8%, respectively. Multivariate analysis indicated that previous hospitalisation ($p < 0.004$) and exposure to amoxycillin-clavulanate ($p < 0.003$) and ceftriaxone ($p < 0.002$) were associated significantly with ceftazidime resistance. Hospitalisation in the urology ward ($p < 0.02$) and previous exposure to fluoroquinolones ($p < 0.01$) were the two independent risk-factors associated with ofloxacin resistance. The results of the study confirmed that antibiotic use selected resistant Enterobacteriaceae from the gut flora. Resistance was observed mostly in patients with previous antibiotic exposure and previous hospitalisation in wards with a high antibiotic selection pressure.

PMID: 16961633 [PubMed - indexed for MEDLINE]

Supplemental Content

Z Gastroenterol. 2006 Feb;44(2):193-204.

[Antibiotic-associated diarrhea]

[Article in German]

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The incidence of antibiotic-associated diarrhea (AAD) differs with the antibiotic and varies from 15 - 25 %. Most cases of AAD are directly or indirectly caused by alterations of gut microflora by the antibiotics resulting in clinically mild AAD cases due to functional disturbances of intestinal carbohydrate or bile acid metabolism. Alternatively, changes in the gut flora allow pathogens to proliferate. Clostridium difficile is responsible for 10 - 15 % of all cases of AAD and almost of all cases of antibiotic-associated pseudomembraneous colitis. There is also a growing body of evidence which supports the responsibility of Klebsiella oxytoca for the development of antibiotic-associated hemorrhagic colitis. Diagnosing Clostridium difficile-associated diarrhea should be based both on fecal-cytotoxin detection and culture. With respect to specific therapy, metronidazol has become the first choice

whereas treatment with oral vancomycin should be reserved for patients who have contraindications or intolerance to or who have failed to respond to metronidazole. Probiotics such as Sacharomyces boulardii can reduce the risk of development. Restrictive antibiotic policies (e. g. restricting clindamycin and cephalosporins) and the implementation of a comprehensive hospital infection control have also been shown to be effective in reducing the incidence of AAD.

PMID: 16456762 [PubMed - indexed for MEDLINE]

Supplemental Content

Infect Immun. 2006 Jan;74(1):192-201.

Enterocyte cytoskeleton changes are crucial for enhanced translocation of nonpathogenic Escherichia coli across metabolically stressed gut epithelia.

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Substantial data implicate the commensal flora as triggers for the initiation of enteric inflammation or inflammatory disease relapse. We have shown that enteric epithelia under metabolic stress respond to nonpathogenic bacteria by increases in epithelial paracellular permeability and bacterial translocation. Here we assessed the structural basis of these findings. Confluent filter-grown monolayers of the human colonic T84 epithelial cell line were treated with 0.1 mM dinitrophenol (which uncouples oxidative phosphorylation) and noninvasive, nonpathogenic Escherichia coli (strain HB101, 10(6) CFU) with or without pretreatment with various pharmacological agents. At 24 h later, apoptosis, tight-junction protein expression, transepithelial resistance (TER; a marker of paracellular permeability), and bacterial internalization and translocation were assessed. Treatment with stabilizers of microtubules (i.e., colchicine), microfilaments (i.e., jasplakinolide) and clathrin-coated pit endocytosis (i.e., phenylarsine oxide) all failed to block DNP+E. coli HB101-induced reductions in TER but effectively prevented bacterial internalization and translocation. Neither the TER defect nor the enhanced bacterial translocations were a consequence of increased apoptosis. These data show that epithelial paracellular and transcellular (i.e., bacterial internalization) permeation pathways are controlled by different mechanisms. Thus, epithelia under metabolic stress increase their endocytotic activity that can result in a microtubule-, microfilament-dependent internalization and transcytosis of bacteria. We speculate that similar events in vivo would allow excess unprocessed antigen and bacteria into

the mucosa and could evoke an inflammatory response by, for example, the activation of resident or recruited immune cells.

PMID: 16368973 [PubMed - indexed for MEDLINE]

PMCID: PMC1346602

Supplemental Content

Gastroenterology. 2004 Nov;127(5):1474-87.

Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition.

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BACKGROUND AND AIMS: The extent and severity of mucosal injury in inflammatory bowel diseases are determined by the disequilibrium between 2 opposing processes: reparative and cytoprotective mechanisms vs. inflammation-induced injury. Probiotics may provide clinical benefit by ameliorating colitis; however, their mechanisms of action remain largely unknown. Our objective was to investigate microbial-epithelial interactions that could explain the beneficial therapeutic effects of probiotics. METHODS: The effect of VSL#3-conditioned media on the nuclear factor-kappaB pathway in young adult mouse colonic epithelial cells was assessed by using monocyte chemoattractant protein-1 enzyme-linked immunosorbent assays; IkappaBalpha, IkappaBbeta, and p105 immunoblot analysis; and nuclear factor-kappaB luciferase reporter gene and proteasome assays. Effects on heat shock proteins were determined by electrophoretic mobility shift assay and immunoblot for heat shock proteins 25 and 72 in young adult mouse colonic cells. Cytoprotection against oxidant injury was determined by chromium 51 release and filamentous and globular actin assays. RESULTS: VSL#3 produces soluble factors that inhibit the chymotrypsin-like activity of the proteasome in gut epithelial cells. Proteasome inhibition is an early event that begins almost immediately after exposure of the epithelial cells to the probiotic-conditioned media. In addition, these bacteria inhibit the proinflammatory nuclear factor-kappaB pathway through a mechanism different from the type III secretory mechanisms described for other nonpathogenic enteric flora. They also induce the expression of cytoprotective heat shock proteins in intestinal epithelial cells. CONCLUSIONS: The resulting inhibition of nuclear factor-kappaB and increased expression of heat shock proteins may account for the anti-inflammatory and cytoprotective effects reported for probiotics and may be a novel

mechanism of microbial-epithelial interaction. These effects seem to be mediated through the common unifying mechanism of proteasome inhibition.

PMID: 15521016 [PubMed - indexed for MEDLINE]

Supplemental Content

Blood. 2004 Jan 1;103(1):340-6. Epub 2003 Sep 11.

Reduced stem cell mobilization in mice receiving antibiotic modulation of the intestinal flora: involvement of endotoxins as cofactors in mobilization.

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Since endotoxins are potent inducers of stem cell mobilization, we hypothesized that their presence in the gut may play a role in cytokine-induced mobilization. To address this possibility we added ciprofloxacin and polymyxin B to the drinking water of Balb/c mice mobilized with either interleukin-8 (IL-8), granulocyte colony-stimulating factor (G-CSF), or flt3 ligand (FL). The yield of colony-forming units (CFUs) was significantly reduced in all mice treated with these antibiotics when compared with controls (IL-8: 192 +/- 61 vs 290 +/- 64, P <.05; G-CSF: 1925 +/- 1216 vs 3371 +/- 1214, P <.05; FL: 562 +/- 213 vs 1068 +/- 528, P <.05). Treatment with ciprofloxacin eliminated only aerobic Gram-negative bacteria from the feces without effect on mobilization. Polymyxin B treatment did not result in decontamination but significantly reduced the number of mobilized hematopoietic progenitor cells (HPCs) most likely due to the endotoxin binding capacity of polymyxin B. More than 90% of the gastrointestinal flora consists of anaerobic bacteria. Elimination of the anaerobic flora by metronidazol led to a significantly reduced number of mobilized HPCs when compared with controls (IL-8: 55 +/- 66 vs 538 +/- 216, P <.05). Germ-free OF1 mice showed a significantly reduced mobilization compared with their wild-type controls (IL-8 controls: 378 +/- 182, IL-8 germ free: 157 +/- 53, P <.05). Finally, we performed reconstitution experiments adding Escherichia coli-derived endotoxins to the drinking water of decontaminated mice. This resulted in partial restoration of the IL-8-induced mobilization (67 +/- 28 vs 190 +/- 98.1, P <.01). Our results indicate that endotoxins serve as cofactors in cytokine-induced mobilization. Modification of the endotoxin content by antibiotic treatment may affect the yield of cytokine-induced mobilization.

PMID: 12969972 [PubMed - indexed for MEDLINE]

Supplemental Content

Inflamm Bowel Dis. 2008 Nov;14(11):1585-96.

Mechanisms of probiotic action: Implications for therapeutic applications in inflammatory bowel diseases.

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Probiotics are defined as nonpathogenic living microorganisms, including some commensal bacterial flora, which have beneficial effects on host health and disease prevention and/or treatment. Clinical trials have shown beneficial effects of probiotics on several human diseases, such as inflammatory bowel diseases (IBDs), which are among the most-studied diseases testing probiotics as a potential therapy. However, a significant question regarding clinical use of probiotics is the mechanism underlying the wide range of actions. Studies discussed in this review suggest 3 distinct cellular and molecular mechanisms for probiotic regulation in IBD therapy: 1) Probiotics block pathogenic bacterial effects by producing bactericidal substances and competing with pathogens and toxins for adherence to the intestinal epithelium; 2) Probiotics regulate immune responses by enhancing the innate immunity and modulating pathogen-induced inflammation via toll-like receptor-regulated signaling pathways; and 3) Probiotics regulate intestinal epithelial homeostasis by promoting intestinal epithelial cell survival, enhancing barrier function, and stimulating protective responses. Probiotics modulate host cell signaling pathways, including Akt, mitogen-activated protein kinases, and nuclear factor-kappaB to mediate these intestinal epithelial functions. It is hoped that developing a mechanistic understanding of probiotic action will provide the rationale to support the development of new hypothesis-driven studies to define the clinical efficacy in preventive, adjunctive, or alternative treatments for IBD.

PMID: 18623173 [PubMed - indexed for MEDLINE]

Supplemental Content

J Occup Health. 2009;51(1):64-73. Epub 2008 Dec 19.

Oral lead exposure induces dysbacteriosis in rats.

Sadykov R, Digel I, Artmann AT, Porst D, Linder P, Kayser P, Artmann G, Savitskaya I, Zhubanova A.

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OBJECTIVES: Lead's (Pb(II)) possible role in intestinal pathologies of microbial etiology remains mostly unknown. The aim of this study was to examine the effects of lead on the gut microbial community and its interactions with rat intestinal epithelium. METHODS: The lead-induced changes in different intestinal microbial groups (lactose-positive lac(+) and -negative lac(-) E.coli strains, lactobacilli and yeasts) were followed separately by the colony-forming unit (CFU) method. Samples were taken from outbred white rats subjected to different exposure schedules. Additionally, the impact of different lead doses on microbial adhesion to cultured intestinal cells (IEC-6) was investigated. Finally, the lead accumulation and distribution were measured by means of atomic absorption spectrometry. RESULTS: For the first time it was shown that oral lead exposure causes drastic changes in the gut microbial community. Proportional to the lead dose received, the relative number of lactose-negative E.coli cells increased dramatically (up to 1,000-fold) in comparison to the other microbial groups during 2 wk of exposure. Considering the number of microbes in the intestine, such a shift in intestinal microflora (dysbacteriosis) is very significant. Adhesion studies showed certain stimulating effects of lead on E. coli attachment to rat intestinal epithelium as compared to Lactobacillus attachment. CONCLUSIONS: The mechanisms providing the apparent competitive success of the lac(-) group are unclear but could be related to changes in surface interactions between microbial and host cells. This study may provide important clues for understanding the pathological effects of metal dietary toxins in human beings.

PMID: 19096199 [PubMed - indexed for MEDLINE]

Supplemental Content

PLoS Pathog. 2008 Aug 1;4(8):e1000112.

Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kappaB activation.

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Host defence against infection requires a range of innate and adaptive immune responses that may lead to tissue damage. Such immune-mediated pathologies can be controlled with appropriate T regulatory (Treg) activity. The aim of the present study was to determine the influence of gut microbiota composition on Treg cellular activity and NF-kappaB activation associated with infection. Mice consumed the commensal microbe Bifidobacterium infantis 35624 followed by infection with Salmonella typhimurium or injection with LPS. In vivo NF-kappaB activation was quantified using biophotonic imaging. CD4+CD25+Foxp3+ T cell phenotypes and cytokine levels were assessed using flow cytometry while CD4+ T cells were isolated using magnetic beads for adoptive transfer to naïve animals. In vivo imaging revealed profound inhibition of infection and LPS induced NF-kappaB activity that preceded a reduction in S. typhimurium numbers and murine sickness behaviour scores in B. infantis-fed mice. In addition, pro-inflammatory cytokine secretion, T cell proliferation, and dendritic cell co-stimulatory molecule expression were significantly reduced. In contrast, CD4+CD25+Foxp3+ T cell numbers were significantly increased in the mucosa and spleen of mice fed B. infantis. Adoptive transfer of CD4+CD25+ T cells transferred the NF-kappaB inhibitory activity. Consumption of a single commensal micro-organism drives the generation and function of Treg cells which control excessive NF-kappaB activation in vivo. These cellular interactions provide the basis for a more complete understanding of the commensal-host-pathogen trilogue that contribute to host homeostatic mechanisms underpinning protection against aberrant activation of the innate immune system in response to a translocating pathogen or systemic LPS.

PMID: 18670628 [PubMed - indexed for MEDLINE]

PMCID: PMC2474968

Supplemental Content

Alcohol. 2008 Aug;42(5):349-61. Epub 2008 May 27.

Alcohol, intestinal bacterial growth, intestinal permeability to endotoxin, and medical consequences: summary of a symposium.

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This report is a summary of the symposium on Alcohol, Intestinal Bacterial Growth, Intestinal Permeability to Endotoxin, and Medical Consequences, organized by National Institute on Alcohol Abuse and Alcoholism, Office of Dietary Supplements, and National Institute of Diabetes and Digestive and Kidney Diseases of National Institutes of Health in Rockville, Maryland, October 11, 2006. Alcohol exposure can promote the growth of Gram-negative bacteria in the intestine, which may result in accumulation of endotoxin. In addition, alcohol metabolism by Gram-negative bacteria and intestinal epithelial cells can result in accumulation of acetaldehyde, which in turn can increase intestinal permeability to endotoxin by increasing tyrosine phosphorylation of tight junction and adherens junction proteins. Alcohol-induced generation of nitric oxide may also contribute to increased permeability to endotoxin by reacting with tubulin, which may cause damage to microtubule cytoskeleton and subsequent disruption of intestinal barrier function. Increased intestinal permeability can lead to increased transfer of endotoxin from the intestine to the liver and general circulation where endotoxin may trigger inflammatory changes in the liver and other organs. Alcohol may also increase intestinal permeability to peptidoglycan, which can initiate inflammatory response in liver and other organs. In addition, acute alcohol exposure may potentiate the effect of burn injury on intestinal bacterial growth and permeability. Decreasing the number of Gram-negative bacteria in the intestine can result in decreased production of endotoxin as well as acetaldehyde which is expected to decrease intestinal permeability to endotoxin. In addition, intestinal permeability may be preserved by administering epidermal growth factor, l-glutamine, oats supplementation, or zinc, thereby preventing the transfer of endotoxin to the general circulation. Thus reducing the number of intestinal Gram-negative bacteria and preserving intestinal permeability to endotoxin may attenuate alcoholic liver and other organ injuries.

PMID: 18504085 [PubMed - indexed for MEDLINE]

PMCID: PMC2614138

Supplemental Content

Neuro Endocrinol Lett. 2008 Feb;29(1):117-24.

The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression.

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There is now evidence that major depression (MDD) is accompanied by an activation of the inflammatory response system (IRS) and that pro-inflammatory cytokines and lipopolysacharide (LPS) may induce depressive symptoms. The aim of the present study was to examine whether an increased gastrointestinal permeability with an increased translocation of LPS from gram negative bacteria may play a role in the pathophysiology of MDD. Toward this end, the present study examines the serum concentrations of IgM and IgA against LPS of the gram-negative enterobacteria, Hafnia Alvei, Pseudomonas Aeruginosa, Morganella Morganii, Pseudomonas Putida, Citrobacter Koseri, and Klebsielle Pneumoniae in MDD patients and normal controls. We found that the prevalences and median values for serum IgM and IgA against LPS of enterobacteria are significantly greater in patients with MDD than in normal volunteers. These differences are significant to the extent that a significant diagnostic performance is obtained, i.e. the area under the ROC curve is 90.1%. The symptom profiles of increased IgM and IgA levels are fatigue, autonomic and gastro-intestinal symptoms and a subjective feeling of infection. The results show that intestinal mucosal dysfunction characterized by an increased translocation of gram-negative bacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. It is suggested that the increased LPS translocation may mount an immune response and thus IRS activation in some patients with MDD and may induce specific "sickness behaviour" symptoms. It is suggested that patients with MDD should be checked for leaky gut by means of the IgM and IgA panel used in the present study and accordingly should be treated for leaky gut.

Immunity. 2008 Oct 17;29(4):637-49. Epub 2008 Oct 2.

Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses.

Hall JA, Bouladoux N, Sun CM, Wohlfert EA, Blank RB, Zhu Q, Grigg ME, Berzofsky JA, Belkaid Y.

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Comment in:

- Immunity. 2008 Oct 17;29(4):518-20.

The intestinal tract is in intimate contact with the commensal microflora. Nevertheless, how commensals communicate with cells to ensure immune homeostasis is still unclear. In this study, we found that gut flora DNA (gfDNA) plays a major role in intestinal homeostasis through Toll-like receptor 9 (TLR9) engagement. Tlr9(-/-) mice displayed increased frequencies of CD4(+)Foxp3(+) regulatory T (Treg) cells within intestinal effector sites and reduced constitutive IL-17- and IFN-gamma-producing effector T (Teff) cells. Complementing this, gfDNA limited lamina propria dendritic cell-induced Treg cell conversion in vitro. Further, Treg/Teff cell disequilibrium in Tlr9(-/-) mice led to impaired immune responses to oral infection and to oral vaccination. Impaired intestinal immune

responses were recapitulated in mice treated with antibiotics and were reversible after reconstitution with gfDNA. Together, these data point to gfDNA as a natural adjuvant for priming intestinal responses via modulation of Treg/Teff cell equilibrium.

PMID: 18835196 [PubMed - indexed for MEDLINE]

PMCID: PMC2712925

Supplemental Content

Indian J Med Microbiol. 2003 Jan-Mar;21(1):6-11.

Antibiotic associated diarrhoea: Infectious causes.

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Nearly 25% of antibiotic associated diarrhoeas (AAD) is caused by Clostridium difficile, making it the commonest identified and treatable pathogen. Other pathogens implicated infrequently include Clostridium perfringens, Staphylococcus aureus, Klebsiella oxytoca, Candida spp. and Salmonella spp. Most mild cases of AAD are due to non-infectious causes which include reduced break down of primary bile acids and decrease metabolism of carbohydrates, allergic or toxic effects of antibiotic on intestinal mucosa and pharmacological effect on gut motility. The antibiotics most frequently associated with C. difficile associated diarrhoea are clindamycin, cephalosporin, ampicillin and amoxicillin. Clinical presentation may vary from mild diarrhoea to severe colitis and pseudomembranous colitis associated with high morbidity and mortality. The most sensitive and specific diagnostic test for C. difficile infection is tissue culture assay for cytotoxicity of toxin B. Commercial ELISA kits are available. Though less sensitive, they are easy to perform and are rapid. Withdrawal of precipitating antibiotic is all that is needed for control of mild to moderate cases. For severe cases of AAD, oral metronidazole is the first line of treatment, and oral vancomycin is the second choice. Probiotics have been used for recurrent cases.

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Supplemental Content

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Probiotic Escherichia coli Nissle 1917 inhibits leaky gut by enhancing mucosal integrity.

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BACKGROUND: Probiotics are proposed to positively modulate the intestinal epithelial barrier formed by intestinal epithelial cells (IECs) and intercellular junctions. Disruption of this border alters paracellular permeability and is a key mechanism for the development of enteric infections and inflammatory bowel diseases (IBDs). METHODOLOGY AND PRINCIPAL FINDINGS: To study the in vivo effect of probiotic Escherichia coli Nissle 1917 (EcN) on the stabilization of the intestinal barrier under healthy conditions, germfree mice were colonized with EcN or K12 E. coli strain MG1655. IECs were isolated and analyzed for gene and protein expression of the tight junction molecules ZO-1 and ZO-2. Then, in order to analyze beneficial effects of EcN under inflammatory conditions, the probiotic was orally administered to BALB/c mice with acute dextran sodium sulfate (DSS) induced colitis. Colonization of gnotobiotic mice with EcN resulted in an up-regulation of ZO-1 in IECs at both mRNA and protein levels. EcN administration to DSS-treated mice reduced the loss of body weight and colon shortening. In addition, infiltration of the colon with leukocytes was ameliorated in EcN inoculated mice. Acute DSS colitis did not result in an anion secretory defect, but abrogated the sodium absorptive function of the mucosa. Additionally, intestinal barrier function was severely affected as evidenced by a strong increase in the mucosal uptake of Evans blue in vivo. Concomitant administration of EcN to DSS treated animals resulted in a significant protection against intestinal barrier dysfunction and IECs isolated from these mice exhibited a more pronounced expression of ZO-1. CONCLUSION AND SIGNIFICANCE: This study convincingly demonstrates that probiotic EcN is able to mediate up-regulation of ZO-1 expression in murine IECs and confer protection from the DSS colitis-associated increase in mucosal permeability to luminal substances.

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Supplemental Content

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The "perfect storm" for type 1 diabetes: the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity.

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It is often stated that type 1 diabetes results from a complex interplay between varying degrees of genetic susceptibility and environmental factors. While agreeing with this principal, our desire is that this Perspectives article will highlight another complex interplay potentially associated with this disease involving facets related to the gut, one where individual factors that, upon their interaction with each another, form a "perfect storm" critical to the development of type 1 diabetes. This trio of factors includes an aberrant intestinal microbiota, a "leaky" intestinal mucosal barrier, and altered intestinal immune responsiveness. Studies examining the microecology of the gastrointestinal tract have identified specific microorganisms whose presence appears related (either quantitatively or qualitatively) to disease; in type 1 diabetes, a role for microflora in the pathogenesis of disease has recently been suggested. Increased intestinal permeability has also been observed in animal models of type 1 diabetes as well as in humans with or at increased-risk for the disease. Finally, an altered mucosal immune system has been associated with the disease and is likely a major contributor to the failure to form tolerance, resulting in the autoimmunity that underlies type 1 diabetes. Herein, we discuss the complex interplay between these factors and raise testable hypotheses that form a fertile area for future investigations as to the role of the gut in the pathogenesis and prevention of type 1 diabetes.

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Supplemental Content

Vet Res. 2009 Jul-Aug;40(4):40. Epub 2009 Apr 24.

The food contaminant fumonisin B(1) reduces the maturation of porcine CD11R1(+) intestinal antigen presenting

cells and antigen-specific immune responses, leading to a prolonged intestinal ETEC infection.

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Consumption of food or feed contaminated with fumonisin B(1) (FB(1)), a mycotoxin produced by Fusarium verticillioides, can lead to disease in humans and animals. The present study was conducted to examine the effect of FB1 intake on the intestinal immune system. Piglets were used as a target and as a model species for humans since their gastro-intestinal tract is very similar. The animals were orally exposed to a low dose of FB(1) (1 mg/kg body weight FB(1)) for 10 days which did not result in clinical signs. However, when compared to non-exposed animals, FB(1)-exposed animals showed a longer shedding of F4(+) enterotoxigenic Escherichia coli (ETEC) following infection and a lower induction of the antigen-specific immune response following oral immunization. Further analyses to elucidate the mechanisms behind these observations revealed a reduced intestinal expression of IL-12p40, an impaired function of intestinal antigen presenting cells (APC), with decreased upregulation of Major Histocompatibility Complex Class II molecule (MHC-II) and reduced T cell stimulatory capacity upon stimulation. Taken together, these results indicate an FB(1)-mediated reduction of in vivo APC maturation.

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Supplemental Content

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Both direct and indirect effects account for the pro-inflammatory activity of enteropathogenic mycotoxins on the human intestinal epithelium: stimulation of interleukin-8 secretion, potentiation of

interleukin-1beta effect and increase in the transepithelial passage of commensal bacteria.

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Mycotoxins are fungal secondary metabolites responsible of food-mediated intoxication in animals and humans. Deoxynivalenol, ochratoxin A and patulin are the best known enteropathogenic mycotoxins able to alter intestinal functions resulting in malnutrition, diarrhea, vomiting and intestinal inflammation in vivo. Although their effects on intestinal barrier and transport activities have been extensively characterized, the mechanisms responsible for their pro-inflammatory effect are still poorly understood. Here we investigated if mycotoxin-induced intestinal inflammation results from a direct and/or indirect pro-inflammatory activity of these mycotoxins on human intestinal epithelial cells, using differentiated Caco-2 cells as model and interleukin 8 (IL-8) as an indicator of intestinal inflammation. Deoxynivalenol was the only mycotoxin able to directly increase IL-8 secretion (10- to 15-fold increase). We also investigated if these mycotoxins could indirectly stimulate IL-8 secretion through: (i) a modulation of the action of pro-inflammatory molecules such as the interleukin-1beta (IL-1beta), and/or (ii) an increase in the transepithelial passage of non-invasive commensal Escherichia coli. We found that deoxynivalenol, ochratoxin A and patulin all potentiated the effect of IL-1beta on IL-8 secretion (ranging from 35% to 138% increase) and increased the transepithelial passage of commensal bacteria (ranging from 12- to 1544-fold increase). In addition to potentially exacerbate established intestinal inflammation, these mycotoxins may thus participate in the induction of sepsis and intestinal inflammation in vivo. Taken together, our results suggest that the pro-inflammatory activity of enteropathogenic mycotoxins is mediated by both direct and indirect effects.

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Supplemental Content

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Activated macrophages inhibit enterocyte gap junctions via the release of nitric oxide.

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Enterocytes exist in close association with tissue macrophages, whose activation during inflammatory processes leads to the release of nitric oxide (NO). Repair from mucosal injury requires the migration of enterocytes into the mucosal defect, a process that requires connexin43 (Cx43)-mediated gap junction communication between adjacent enterocytes. Enterocyte migration is inhibited during inflammatory conditions including necrotizing enterocolitis, in part, through impaired gap junction communication. We now hypothesize that activated macrophages inhibit gap junctions of adjacent enterocytes and seek to determine whether NO release from macrophages was involved. Using a coculture system of enterocytes and macrophages, we now demonstrate that "activation" of macrophages with lipopolysaccharide and interferon reduces the phosphorylation of Cx43 in adjacent enterocytes, an event known to inhibit gap junction communication. The effects of macrophages on enterocyte gap junctions could be reversed by treatment of macrophages with the inducible nitric oxide synthase (iNOS) inhibitor l-Lysine omega-acetamidine hydrochloride (l-NIL) and by incubation with macrophages from iNOS(-/-) mice, implicating NO in the process. Activated macrophages also caused a NO-dependent redistribution of connexin43 in adjacent enterocytes from the cell surface to an intracellular location, further suggesting NO release may inhibit gap junction function. Treatment of enterocytes with the NO donor S-nitroso-N-acetylpenicillamine (SNAP) markedly inhibited gap junction communication as determined using single cell microinjection of the gap junction tracer Lucifer yellow. Strikingly, activated macrophages inhibited enterocyte migration into a scraped wound, which was reversed by l-NIL pretreatment. These results implicate enterocyte gap junctions as a target of the NO-mediated effects of macrophages during intestinal inflammation, particularly where enterocyte migration is impaired.

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Supplemental Content

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Increased arginase activity and endothelial dysfunction in human inflammatory bowel disease.

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Nitric oxide (.NO) generation from conversion of l-arginine to citrulline by nitric oxide synthase isoforms plays a critical role in vascular homeostasis. Loss of .NO is linked to vascular pathophysiology and is decreased in chronically inflamed gut blood vessels in inflammatory bowel disease (IBD; Crohn's disease and ulcerative colitis). Mechanisms underlying decreased .NO production in IBD gut microvessels are not fully characterized. Loss of .NO generation may result from increased arginase (AR) activity, which enzymatically competes with nitric oxide synthase for the common substrate l-arginine. We characterized AR expression in IBD microvessels and endothelial cells and its contribution to decreased .NO production. AR expression was assessed in resected gut tissues and human intestinal microvascular endothelial cells (HIMEC). AR expression significantly increased in both ulcerative colitis and Crohn's disease microvessels and submucosal tissues compared with normal. TNF-alpha/lipopolysaccharide increased AR activity, mRNA and protein expression in HIMEC in a time-dependent fashion. RhoA/ROCK pathway, a negative regulator of .NO generation in endothelial cells, was examined. The RhoA inhibitor C3 exoenzyme and the ROCK inhibitor Y-27632 both attenuated TNF-alpha/lipopolysaccharide-induced MAPK activation and blocked AR expression in HIMEC. A significantly higher AR activity and increased RhoA activity were observed in IBD submucosal tissues surrounding microvessels compared with normal control gut tissue. Functionally, inhibition of AR activity decreased leukocyte binding to HIMEC in an adhesion assay. Loss of .NO production in IBD microvessels is linked to enhanced levels of AR in intestinal endothelial cells exposed to chronic inflammation in vivo.

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Supplemental Content