SESSION II: COLONIC MICROBIOTA: LUMINAL AND SYSTEMIC INFLUENCE ON DISEASE

COLONIC MICROBIOTA: LUMINAL AND SYSTEMIC INFLUENCE ON DISEASE

PROBIOTIC POTENTIAL OF A COMMENSAL BACTERIUM IN AN ANIMAL MODEL OF COLITIS

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The Western world is experiencing a growing medical crisis. Epidemiologic and clinical reports reveal a dramatic increase in immune disorders: inflammatory bowel disease, asthma, type 1 diabetes, and multiple sclerosis. Emboldened by the 'hygiene hypothesis' proposed two decades ago, scientists have speculated that lifestyle changes (vaccination, sanitation, antibiotics) have predisposed developed societies to these disorders by reducing bacterial infections. However, the hypothesis remains without explanation as our exposure to most bacteria does not result in disease. Mammals are colonized for life with 100 trillion indigenous bacteria, creating a diverse ecosystem whose contributions to human health remain poorly understood. In recent years, there has been a revolution in biology toward understanding how (and more importantly, why) mammals harbor multitudes of symbiotic bacteria. We have recently demonstrated for the first time that intestinal bacteria direct universal development of the immune system; thus fundamental aspects of mammalian health are inextricably dependent on microbial symbiosis. Furthermore, it is now clear that all of the diseases in question astonishingly involve a common immunologic defect found in the absence of symbiotic bacteria. As we have co-evolved with our microbial partners for eons, have strategies used against infectious agents reduced our exposure to health-promoting bacteria, ultimately leading to increased disease? We propose that the human genome does not encode all functions required for health, and we depend on crucial interactions with products of our microbiome (collective genomes of our gut bacterial species). Ultimately, understanding the immune mechanisms of these symbiosis factors may lead to natural therapeutics for human diseases based on entirely novel biological principles.

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MICROBIAL TRANSLOCATION AND DISEASE PROGRESSION IN HIV AND OTHER PRIMATE LENTIVIRAL INFECTIONS

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The chronic phase of HIV infection is marked by pathological activation of the immune system, the extent of which better predicts disease progression than either plasma viral load or CD4+ T cell count. Recently, translocation of microbial products from the gastrointestinal tract has been proposed as one underlying cause of this immune activation, based on indirect evidence including the detection of microbial products and specific immune responses in the plasma of chronically HIV-infected humans or SIV-infected Asian macaques. We analyzed tissues from SIV-infected rhesus macaques (RM) to provide direct in situ evidence for translocation of microbial constituents from the lumen of the intestine into the lamina propria and to draining and peripheral lymph nodes and liver, accompanied by local tissue responses. In chronically SIV-infected RM this translocation is associated with breakdown of the tight epithelial barrier of the gastrointestinal (GI) tract and apparent inability of lamina propria macrophages to effectively phagocytose all translocated microbial constituents. Because immune activation is characteristic of the chronic phase of progressive HIV/SIV infections, these findings suggest that increased microbial translocation from the GI tract, in excess of capacity to clear the translocated microbial constituents, helps drive pathological immune activation. Moreover, the degree to which microbial products, as compared to viral replication, underlie immune activation, and disease progression is underscored by studies of pigtailed macaques who have more microbial translocation prior to infection and who rapidly progress to AIDS after infection. Novel therapeutic approaches to inhibit microbial translocation or attenuate chronic immune activation in HIV-infected individuals may complement treatments aimed at direct suppression of viral replication.

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DOMINANT "INDIGENOUS" BIFIDOBACTERIA ISOLATED FROM INFANT FAECES

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Background: Although the health promoting role of bifidobacteria is widely accepted, the diversity and stability of bifidobacteria among the intestinal microbiota is still poorly understood. In this work, we performed a census of dominant bifidobacterial population in infant microbiota over a 34-day period, in order to investigate the succession of "indigenous" bifidobacteria strains.
Methods: *Bifidobacterium* isolates were obtained from the dominant fecal microbiota of 15 healthy children (7-19 months old). Successive samples stools were collected at day 0 (D0), D10, D20 and D34. Quantitative and qualitative analysis of the fecal bifidobacteria was performed using culture methods. Ten randomly selected colonies, suspected to be bifidobacteria, were picked and identified at the genus level (PCR) and species level (PCR and sequencing of the 16S rRNA) for each individual and sampling time. *Xba*I macro-restriction PFGE analysis was performed to compare clone identity of consecutive isolates.
Results: Quantitative bifidobacteria colonization levels among children ranged from 7.3 to 10.3 $\log_{10}$ CFU/g of faeces. Dominant bifidobacteria species isolated included: *breve* (n=26), *longum* (n=22), *pseudocatenulatum* (n=9), *bifidum* (n=4), *adolescentis* (n=2) or *dentium* (n=1). Out of the fifteen infants, three showed the same *Bifidobacterium* species consecutively isolated and identified over time. The other twelve infants were colonized with two (n=7) or three (n=5) different dominant bifidobaceria species during the study. Moreover, five infants harbored two different dominant species at the same sampling time. Strain PFGE typing revealed that four infants conserved the same strain throughout the study. For the other infants, PFGE analysis showed genomic heterogeneity among strains which reflect intestinal bifidobacteria biodiversity for one individual.
Conclusion: This study reported the molecular survey of the successive dominant *Bifidobacterium* population which colonizes infants. It suggests that the bifidobacteria distribution depends on the turn over of the dominant *Bifidobacterium* species during a short period of time for the same individual. The fluctuation of the intestinal bifidobacteria population may question the link between specific bifidobacteria and beneficial effects which can not be restricted to one species or only one strain.

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CHARACTERIZING GENOMIC DIVERSITY OF *FUSOBACTERIUM NUCLEATUM* FROM THE HUMAN GUT TO EXPLAIN DIFFERENCES IN VIRULENCE

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The genus *Fusobacteria* comprises several species that are, in general, anaerobic, Gram negative members of the human microflora, including the Gastrointestinal tract (GIT). Several of these species are known to be pathogenic, and in particular, *Fusobacterium nucleatum* (Fn) has a well-characterized role in plaque formation and periodontitis in the oral setting. Fn has only recently been found to be a frequent resident of the intestinal mucosa and thus, little is known about the role of this species in the gut. Given its involvement in inflammatory disorders in the mouth, we sought to investigate a potential role for Fn in inflammatory bowel disease (IBD).

From an initial pilot study we determined that there is a significant correlation between recovery of Fn from intestinal biopsies and IBD status of the host; Fn was isolated from 50% of patients with IBD, versus only 17.6% of healthy controls (p=0.02). Using immunofluorescence microscopy of cultured Caco-2 cells, we have found that Fn isolates from IBD patients are in general more invasive *in vitro* than isolates from healthy controls (p= 0.01). Additionally we have found that these isolates also vary in their abilities to induce MUC2 gene upregulation and mucin secretion as well as a pro-inflammatory response in host cells both *in vitro* and *in vivo*, suggesting that some isolates are more virulent than others. In the oral niche, a difference in virulence between *F. nucleatum spp*. has similarly been noted. As yet, the reason why some isolates behave pathogenically whilst others do not is unknown. We are hypothesizing that the differences in virulence of these Fn isolates lies in genetic differences between the strains.

In collaboration with the Broad institute and the Human Microbiome Project, the genomes of 13 of our studied Fn isolates have been sequenced. We have used bioinformatic tools to try to identify virulence-associated loci and we present our comparative data. A bioinformatic approach will help elucidate a potential role for Fn as a pathogenic factor in cases of IBD.

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MOLECULAR-CELLULAR MECHANISMS INVOLVED IN HOMEOSTATIC REGULATION OF MUCOSAL BARRIERS CAUSED BY COMMENSAL BACTERIA AND THEIR ANTIGENS

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The cross-talk of the mucosal microbiota with host cells involves antigen-presenting cells, such as monocytes, macrophages, and dendritic cells that reside in epithelial regions. Both direct and cytokine-mediated interactions of epithelial and dendritic cells have been proposed as physiologically-important mechanisms to maintain the homeostasis of mucosal barrier function and immunity.

Still little is known of the interplay of these cellular and molecular interactions. Investigations conducted in this study have resulted in description of some humoral and cellular molecular mechanisms involved in host/bacterial interaction by using relevant animal models. In particular, it has been shown that: (i) the selected commensal bacteria exhibit a specific effect on opportunistic pathogens isolated in the clinical units, (ii) the two lactobacilli strains, but not Schaedler's *E. coli* and *M. morganii*, were able to translocate into the internal organs of IgA-deficient mice as compared to their wild type counterparts, (iii) colonisation of mice with Schaedler's *E. coli* and *M. morganii* prior to *L. salivarius* resulted in diminished translocation of lactobacilli, (iv) mono-association of germ-free severe combined-immunodeficient mice with the above-mentioned Gram-negative bacteria resulted in an increased level of NK cells amongst the intraepithelial lymphocytes that produced IFN-g locally, (v) increase of autocrine growth factor Reg III b/g expression was observed primarily in the caecum of colonized IgA deficient mice as compared to GF or *E. coli* monoassociated mice, (vi) gene expression of surfactant protein D is induced in the lung of IgA deficient mice, inoculated by both bacteria or Shaedler's *E. coli* alone, (vii) the number of macrophages and neutrophils were greatly increased in the bronchoalveolar lavage of IgA-deficient mice colonized with *L. salivarius* on the third day after inoculation, as compared to germ-free mice or those mono-associated with Schaedler's *E. coli*, (viii) B1 cells could be selectively stimulated *via* their BCR receptor locally in the nasal tissues of mice by TI-1 antigen bacterial phosphorylcholine of *H. influensae*, and T-cells are required for "bystander" effect in such specific immune response, (ix) *B. subtilis* induced secretion of IL-10 whereas IL-6, although in different levels, was stimulated by all the bacteria studied, (x) a human strain of *E. coli* 058 provoked synthesis of IL-23 as compared to the murine strains Schaedler's *E. coli* and *M. morganii* that stimulate secretion of IL-12, IL-8, IL-1b and activates NOD-2, Nalp3 and caspase-1 expression.

The crucial role of the selected commensal and normal microflora microorganisms, their derivates in the regulation of homeostasis of host mucosal surfaces is, therefore, clearly defined and confirmed.

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DO METHANOGENIC ARCHAEA PLAY A ROLE IN MIXED ANAEROBIC INFECTIONS?

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Increasing evidence suggests a role of methanogenic archaea in human health and disease. While *Methanobrevibacter smithii* seems to have an impact on the overall energy balance in the intestinal tract, possibly related to obesity, *Methanobrevibacter oralis* may promote the activity of pathogenic bacteria in oral diseases, such as periodontitis and endodontic infections. Our previous molecular-based studies have indicated the occurrence of an additional methanogenic phylotype at oral infected sites ("Phylotype 3"), however with less prevalence than *M. oralis*. Since the co-occurrence of methanogens with periodontal pathogens has not been investigated so far, the aim of this study was to identify possible syntrophic niche partners out of the so-called "red" and "orange" bacterial complexes associated with periodontal disease.
Methods: Subgingival plaque samples from 129 periodontitis patients were selected for this study. Periodontal pathogens were identified (and quantified) by 16S rRNA-based microarray analysis and included the species *Aggregatibacter actinomycetemcomitans*, *Actinomyces viscosus*, *Tannerella forsythia*, *Campylobacter rectus*, *Treponema denticola*, *Eikenella corrodens*, *Prevotella intermedia*, *Parvimonas micra*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*. Methanogenic archaea were detected by real-time quantitative PCR and sequencing analysis.
Results: In total 58 out of 129 samples were tested positive for methanogens with an average population size of 3.9×10^5 gene target molecule numbers per µl. Except for one case, 25 control samples from healthy individuals were tested negative for methanogens. *M. oralis* was more prevalent then Phylotype 3. Relative frequencies of the bacterial niche partners ranged from 26% (*A. actinomycetemcomitans*) to 100% (*F. nucleatum*). Positive associations and correlations could be ascertained which confirmed bacterial interactions within the so-called "red" and "orange complex" in periodontal disease. Methanogens were positively correlated with *T. forsythia,* one major pathogen of the "red complex". The population size of methanogens was elevated with increasing numbers of *T. forsythia* and also with the severity of periodontal disease
Conclusions: While the general importance of methanogens for mixed anaerobic infections remains unclear, the data of this study indicate a possible support of a periodontitis-associated consortium by methanogens driven through interactions with the major pathogen *T. forsythia*.

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NOVEL LACHNOSPIRACEAE SPECIES ISOLATED FROM THE HUMAN GUT: PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

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The Lachnospiraceae represent a diverse family of Gram positive anaerobic bacteria within the Clostridiales order. Many species in this family are residents of the human GI tract, and recently the flagellins of unknown bacteria belonging to the Lachnospiraceae family have been implicated as biomarkers in Crohn's Disease (CD), an inflammatory bowel disease (IBD). In a recent microbiological survey of biopsy samples taken from a small cohort of IBD patients and healthy (colon cancer screen) controls, we recovered 25 Lachnospiraceae isolates and characterized these phenotypically and genotypically.

By 16S rRNA gene analysis, 8 strains represent novel genera and 4 strains novel species. Additionally, 13 examples of previously described species were recovered. All but one of our recovered Lachnospiraceae strains were isolated from IBD patients, and occasionally more than one isolate was recovered from the same individual, illustrating a potential positive correlation between the presence of these members of the Lachnospiraceae family and IBD.

Phenotypically, representative cells of each novel species vary from 500nm to 1μm in width, with length varying from 1μm to 3.5μm, and several species are flagellate. Transmission electron microscopy has revealed an interesting cellular morphology for most of these novel species resembling a lowercase theta (θ) shape, whereby the body of the cell bears a unique pseudoseptum; the function of this pseudoseptum is unknown. Biochemical and phenotypic tests are generally unhelpful in delineating species or aligning with close relatives. The strains have been submitted to the Broad Institute for whole genome sequencing as part of the Human Microbiome Project (HMP), in order to enable genome comparisons that may be helpful in predicting species-specific genetic traits within the Lachnospiraceae.

Since most of our isolates were obtained from IBD patients, and there is a previously noted association between *Lachnospiraceae flagella* and CD, we assayed the flagellins of several strains for the ability to elicit IL-8 release in a TLR5-dependent Caco-2 cell model. We found that, in comparison to *E. coli* flagellin, the flagellins from the Lachnospiraceae were 100- to 1000-fold less potent at eliciting IL-8 release, suggesting reduced agonist activity for TLR5.

Our novel isolates, along with other gut-associated Lachnospiraceae members, may be of hitherto unrecognized clinical importance, and we are initiating experiments to study this further.

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POLYSACCHARIDE BIOSYNTHESIS IN *BACTEROIDES FRAGILIS*

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Bacteroides fragilis is a Gram-negative, obligate anaerobe and a commensal of the human gastrointestinal (GI) tract. It performs a number of important functions such as the breakdown of complex polysaccharides, stimulation of anti-microbial peptides and competitive inhibition of pathogens. *B. fragilis* is also the most commonly isolated Gram-negative anaerobic bacterium from human infections such as peritonitis. Infection usually occurs in conjunction with trauma to the GI tract, e.g. following gastrointestinal surgery, and can lead to abscess formation, soft tissue infection and bacteraemia. *B. fragilis* produces a range of extracellular polysaccharides including large, small and micro-capsules which play a role in protecting the bacterial cell from environmental insults. The micro-capsule is antigenically variable and is composed of high molecular mass polysaccharides which we hypothesise form the outer domain of *B. fragilis* lipopolysaccharide (LPS). This is very different to the O-antigen found in the LPS of other Gram-negative bacteria such as *E.coli*, which are typically made of short chain polysaccharides. The inter-strain variation of the extracellular polysaccharides in *B. fragilis* is due to the presence of multiple loci implicated in their production. In contrast, there is generally only one polysaccharide biosynthesis locus present in *E.coli*. Multiple polysaccharide loci have been identified in *B. fragilis* NCTC9343, eight of which are associated with the micro-capsule. Of these eight loci, seven can be switched on and off by invertible DNA promoters. Although a single Polysaccharide Co-Polymerase has been found in *B. fragilis*, the gene encoding the protein necessary to ligate the polysaccharide to the lipid-A core has yet to be identified. Using gene deletion techniques, we have created mutants to test the hypothesis that the micro-capsule is anchored to lipid-A and to further analyse the genes involved in *B. fragilis* polysaccharide production.

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THE ROLE OF AN EXTRACYTOPLASMIC FUNCTION SIGMA FACTOR IN THE OXIDATIVE STRESS RESPONSE OF *BACTEROIDES FRAGILIS*

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The anaerobe *Bacteroides fragilis* is a highly-aerotolerant, opportunistic pathogen that resides principally in the gastro-intestinal tract of humans and animals. Its ability to persist in infections and in intra-abdominal abscesses under oxidizing conditions is important for its pathogenicity. Previous studies revealed that a dynamic change in gene expression is responsible for its oxidative stress response, which is important for its infectivity. Among the genes induced by oxidative stress are a large number of transcriptional regulators including a set of extracytoplasmic function (ECF) sigma factors.

ECF sigma factors are known to transmit changes in environmental signals to specific responses in gene expression. Upon induction, they bind to core RNA polymerase and initiate the transcription of specific response genes from their respective promoters. There are 43 ECF Sigma factors coded by the *B. fragilis* genome but none have been characterized.

An ECF sigma factor SigOD, was used as a model to study the role of ECF sigma factors in the oxidative stress response of *B. fragilis*. We show that expression of *sigOD* is induced by oxidative stress, and that deletion of this gene significantly affects the sensitivity of *B. fragilis* to several oxidative stressors and the ability to survive aerobic exposure. We also show from genetic and biochemical experiments that the antisigma factor, AtsOD, found in the same operon as SigOD, interacts with and regulates the activity of the SigOD. An expression microarray experiment was carried out to explore the target genes regulated by SigOD. *B. fragilis* mutants lacking *sigOD* and *atsOD*, and strains in which *sigOD* was controlled by an inducible promoter were used to generate a data set in which we could define a set of genes under control of SigOD. Preliminary analyses suggest that SigOD and AtsOD appear to function in the same pathway that regulates a subset of oxidative stress response genes.

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ASSOCIATION OF *HELICOBACTER PYLORI* INFECTION WITH DIARRHEAL ILLNESS IN CHILDREN

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Helicobacter pylori is a Gram-negative microaerophilic bacterium adapted to survive in the stomach of humans where it can cause a variety of clinical conditions, among them peptic ulcer disease, gastric cancer, MALT lymphoma and other gastro-intestinal or extragastric manifestations. Since the infection is acquired mainly in early childhood, we examined the possible association of *H. pylori* with specific diarrheal illness in hospitalized children.

The study was conducted between July and August 2009 in two pediatric hospital departments in Athens. Fecal samples were obtained and examined from 85 subjects (44 boys and 41 girls) admitted to the hospitals, aged from 1 month to 12 years (median: 2 years). Among them 34 suffered from acute diarrheal illness and 51 from other non-diarrheal diseases. Detection of *H. pylori* antigen in stool was performed by the immunochromatograhic assay of Novamed Ltd (Jerusalem, Israel).

Of the 34 children suffered from acute diarrheal illness, 18 were found positive in *H. pylori* infection (52.9%). On the other hand, only 7 (13.7%) out of 51 children with other non-diarrheal diseases were *H. pylori* positive. The results showed a strong association between diarrheal diseases and *H. pylori* infection. ($p < 0.001$). No significant differences were found in *H. pylori* detection between patients suffered from various diarrheal syndromes (bacterial or viral gastroenteritis, parasitic diseases etc.). There was also no association of the *H. pylori* infection with any specific enteric pathogen.

A possible limitation of this study is that the socioeconomic status of the children had not been examined.

Our findings suggest that gastric infection with *Helicobacter pylori* might increase the risk of diarrheal disease in children and/or both conditions might have the same route of transmission.

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ANTI-INFLAMMATORY EFFECT OF VARIOUS *BIFIDOBACTERIUM* STRAINS ON COLON EPITHELIUM-LIKE HT-29 CELLS

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Aim of research: Several groups have reported recently that certain probiotic *Bifidobacterium* strains are capable of secreting low molecular weight metabolites with anti-inflammatory activity. However neither the chemical nature nor precise mechanism of their action has been determined. Here we report on a survey of similar anti-inflammatory properties in several well studied laboratory *Bifidobacterium* strains and several fresh clinical *Bifidobacterium* isolates from healthy infant faeces.
Materials and Methods: Conditioned media were obtained using several well studied laboratory *Bifidobacterium* strains and several fresh *Bifidobacterium* isolates from healthy infant faeces. Strain from other genera of gram-positive bacteria were used as controls. Spent supernatants from stationary cultures were applied onto HT-29 colon epithelium-like cell culture simultaneously with TNF-α and LPS stimulation. Production of cytokines was measured using ELISA. Messenger RNAs were quantitated using real-time RT-PCR.
Results: It was found that conditioned media of a majority of tested strains was capable of attenuating albeit to varying extent TNF-α- and LPS-induced production of IL-8 and LPS-induced production of TNF-α in cultured colon epithelium-like HT-29 cells. In contrast, neither of *E. coli* *Corynebacterium glutamicum*, *Lactococcus lactis* and *Lactobacillus casei* strains significantly decreased production of IL-8 and TNF-α. The anti-inflammatory effect of bifidobacterial culture supernatants was dose-dependent and was accompanied by down-regulation of IL-8 and IκB mRNA. Some of the strains also significantly up-regulated expression of $p21^{CIP}$ mRNA. No changes were observed in levels of IL-10 and TGF-β mRNAs. Interestingly three out of four *Bifidobacterium bifidum* strains tested showed specific inhibition of LPS-dependent but not TNF-dependent IL-8 response.
Conclusion: Our findings indicate that most *Bifidobacterium* strains are able to inhibit inflammatory response in HT-29 cells and that the putative anti-inflammatory metabolites from bifidobacteria act directly by inhibiting the NF-κB pathway rather than indirectly through induction of anti-inflammatory cytokines.

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BACTEROIDES FRAGILIS TOXIN (BFT) ISOTYPES SECRETED BY ENTEROTOXIGENIC *B. FRAGILIS* (ETBF) DIFFER IN TUMOR-INDUCING POTENTIAL

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The intestinal flora is hypothesized to contribute to Inflammatory Bowel Disease (IBD) and formation of colonic tumor in humans. We recently identified that enterotoxigenic *Bacteroides fragilis* (ETBF) colonizes and causes colitis in mice. Notably, we demonstrated that ETBF colonization induces colonic tumor formation in Multiple Intestinal Neoplasia (Min, *Apc*+/-) mice through a selective T helper type 17 (Th17)-dependent mechanism. ETBF strains secrete a 20-kDa heat-labile zinc-dependent metalloprotease toxin termed the *B. fragilis* toxin (BFT). ETBF secretes one of three highly related BFT isoforms, known as BFT-1, BFT-2, and BFT-3. The relationship between BFT isoforms and disease development is not clear. The aim of this study was to investigate this relationship using our ETBF-Min mouse model.

In this study, we colonized Min mice with each of three wild-type ETBF strains: VPI 13784 (BFT-1-secreting; aka VPI), 86-5443-2-2 (BFT-2-secreting; aka 86), and 570 (BFT-3-secreting) for 2 weeks to assess colonic inflammation or for 2-3 months to examine colon tumor formation. Our results show that fecal ETBF colonization rapidly achieves a maximum in 3 days (~10^{8-9} CFU/gm feces) and persists up to 3 months. Mucosal ETBF adherence to colon mucosa was: $2.9 \times 10^5 \pm 6.0 \times 10^4$, $2.5 \times 10^5 \pm 5.4 \times 10^4$ and $1.7 \times 10^5 \pm 4.5 \times 10^4$ CFU/mg tissue (mean ± SEM) for strain VPI, 86 and 570, respectively, at 2 weeks. In vivo expression of the *bft* isoforms was similar as assessed by quantitative real-time PCR of fecal RNA. Colonic mucosal IL-17 expression stimulated by strain VPI, 86 or 570 increased 6.2 ± 3.6, 31.4 ± 33.7, or 17.2 ± 5.3 fold (median ± SEM), respectively, at 2 weeks, based on real-time PCR. Strain 86 induced significantly greater IL-17 expression when compared to strain VPI ($P<0.02$). Colon tumors were quantified in Min mice colonized with ETBF for 2-3 months using a dissecting microscope after the colons were stained with methylene blue. All three ETBF strains significantly promote colonic tumor formation in Min mice. The average number of colon tumors is: 7 ± 1, 18 ± 2 and 9 ± 2 (mean ± SEM) for strain VPI, 86, and 570, respectively. Among these ETBF strains, 86 is the most potent tumor-producer ($P<0.01$, 86 vs. VPI; $P<0.05$, 86 vs. 570). In summary, our data suggest that wild-type ETBF secreting discrete BFT isoforms vary in their ability to induce colon IL-17 and to promote colon tumor formation in Min mice. Our data do not suggest that differences in ETBF colon mucosal colonization and/or BFT expression in the mouse colon explain the tumor-inducing potential of these ETBF strains. It is possible that wild-type ETBF strains possess differing ancillary virulence genes that contribute to oncogenesis.

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DEVELOPMENT OF A CHEMOSTAT MODEL TO STUDY POPULATION SHIFTS IN THE HUMAN GUT MICROFLORA IN RESPONSE TO NOREPINEPHRINE

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Both experimental and clinical evidence propose the commensal microbial community (microbiota) of the gut as a factor in Inflammatory Bowel Disease (IBD). IBD is a chronic, relapsing and remitting inflammatory disease of the intestine, which includes ulcerative colitis (UC) and Crohn's disease. Patients with UC possess a less diverse microbiota which may be more prone to environmental effects, such as dysbiosis. Stress has been proposed to provoke or exacerbate IBD; however, much of the evidence is anecdotal or inferential. Interestingly, the stress hormone, norepinephrine (NE), has been shown to elicit wide ranging effects including growth stimulation and upregulation of gene expression patterns of many bacterial species in pure culture, including some key anaerobic pathogens. We hypothesize that NE has a direct effect on the balance of the largely anaerobic gut microbiota as a community, and that the microbiota from individuals with IBD are more susceptible to NE-induced dysbiosis than the microbiota from healthy people.

The bacterial population from the distal gut contains many bacterial species that are refractive to axenic culture, but as a group these can be modeled *in vitro* using continuous culture vessels called 'chemostats', in which communities seeded from fresh feces reach a steady-state that closely resembles the *in vivo* consortium. Being a host-free system, chemostats supporting the gut microbiota should make ideal vessels in which to study microbial perturbations that directly result from the addition of exogenous stimuli, such as NE. To validate this approach, we set up two identical chemostat vessels using conditions set to mimic the distal gut, and seeded with fresh fecal material from a healthy volunteer. The bacterial consortium within each chemostat was monitored by profiling the 16S rRNA gene content of the communities using PCR and Denaturing Gradient Gel Electrophoresis. Using this profiling method, we found that the chemostat communities within each vessel reached steady-state within 5 days of inoculation, and the profiles from each vessel closely matched each other as well as the fecal inoculum. Preliminary data from these continuing studies suggests the addition of NE to this system can directly affect the community profile by shifting the abundance of distinct sub-populations within the consortium.

The use of chemostat models represents a valid approach to study the response of the human gut microbiota to NE stimulation. The model lends itself well to the study of microbial population shifts within the gut microbiota as a response to many exogenous stimuli that may be important clinically.