

## **The role of *Fusobacterium nucleatum* in IBD**

Our studies this year have extended our initial observations of *F.nucleatum* colonization of the gut, and provided some intriguing results. I attach two abstracts for poster presentations that have been given by my graduate student, Jaclyn Strauss, within the last few months:

### **1) Contribution of the oral pathogen *Fusobacterium nucleatum* to the aetiology of inflammatory bowel disease**

### **2) Characterization of *Fusobacterium nucleatum* isolated from human intestinal biopsies – are strains from Inflammatory Bowel Disease patients more virulent?**

The first of these gave details of the results of our blinded study looking at *F.nucleatum* colonization of biopsy specimens taken from IBD patients and healthy controls. In total, 59 patients were recruited into our study and from these patients we were able to isolate 17 *F.nucleatum* strains. 12 of these isolates came from patients with GI disease (microscopic colitis, IBS or IBD), and 9 isolates were from IBD patients. This correlation between *F.nucleatum* recovery and IBD was proven to be statistically significant, and encouraged us to look at the strains in further detail. We were able to separate the isolates into groups according to their similarities in a key taxonomic marker gene. Interestingly, many of our gut isolates from patients with GI disease fell into one group, and this correlation is one that we are currently trying to elucidate further.

The second study presented showed the results of our initial attempts to look at the pathobiology of the isolates. A comparative study such as this has never been previously attempted with *F.nucleatum* because of the wide differences between isolates in terms of growth phase and immunogenicity. A particular effort was invested into determining growth curves for the different strains, as well as raising antibodies against them, a task complicated by the fact that antibodies to one strain do not tend to work against other strains, even though they are in the same species. We persevered, however, and were rewarded by some very intriguing data that suggested that the *F.nucleatum* strains isolated from IBD patients were significantly more invasive in a tissue culture cell model than isolates recovered from control (healthy) patients. Not only that, but our more invasive isolates were found, in general, to secrete much larger amounts of the autoinducer molecule AI-2, a molecule which has been proposed to be a universal signal for interspecies communication and has been shown to control gene expression for numerous virulence factors in a number of important enteric pathogens. This is particularly interesting because anecdotal evidence suggests that *F.nucleatum* isolates in general do not tend to produce much AI-2. Jaclyn won the student poster competition at the Anaerobe Society of the Americas congress 2008 with the presentation of poster 2, which stands testament both to her abilities as a student, as well as to the interest that this work is generating in the field.

In summary this has been a very exciting year and we look forward to more successes in the coming year. Through a collaboration with the Broad Institute of MIT & Harvard, we are working to sequence the genomes of most of the *F.nucleatum* isolates obtained from our gut biopsy samples, and we are currently halfway to our goal. This will allow us to initiate comparative genomics of around 20 strains, representing the largest single study of this type so far attempted, and will help us to answer questions surrounding the differences between isolates from IBD patients, and those from healthy controls.

Poster abstract 1:

**Contribution of the oral pathogen *Fusobacterium nucleatum* to the aetiology of inflammatory bowel disease**

Inflammatory Bowel Disease (IBD) is an umbrella term used to describe a group of chronic, relapsing/remitting disorders of the gastrointestinal tract. While the precise aetiology of IBD is unknown, dysbiosis, an imbalance between beneficial and harmful bacterial species in the gut, is a leading hypothesis for the role of bacteria in IBD. Thus the search for potentially pathogenic microbial residents of the GI tract is a current research focus.

*Fusobacteria* are anaerobic, Gram-negative members of the human microflora, including the GI tract, and *Fusobacterium varium* has been previously demonstrated to play a role in the exacerbation of ulcerative colitis, a type of IBD. We were interested to determine whether the related species, *F.nucleatum*, a known invasive and pro-inflammatory pathogen in the human mouth and which is thought to be an occasional resident of the human gut, might also be associated with IBD pathogenesis.

Using selective media under strict anaerobic conditions, we recovered *Fusobacterium* spp. from human intestinal biopsy samples taken from IBD patients or control healthy patients undergoing colon cancer screening. There was a significant correlation between IBD and recovery of *F.nucleatum*, with *F.nucleatum* being recovered from 43% of IBD patients compared to 16% of healthy controls. Given that *F.nucleatum* represents a highly heterogenic species and certain *F.nucleatum* subspecies are thought to be more pathogenic than others, we were curious to determine the heterogeneity within our gut isolates. Using phylogenetic analysis, we determined that our *F.nucleatum* isolates could be clustered into distinct groups based on genetic differences within 16S rRNA and *rpoB* gene sequences. Although these differences were not predictive of phenotype, as assayed by a variety of biochemical tests, 7 of 11 gut biopsy isolates in one group were associated with IBD. Together our data indicates that *F.nucleatum* represents a potential pathogenic factor in cases of IBD.

Poster Abstract 2:

**Characterization of *Fusobacterium nucleatum* isolated from human intestinal biopsies – are strains from Inflammatory Bowel Disease patients more virulent?**

Inflammatory Bowel Disease (IBD) is an umbrella term used to describe a group of chronic, relapsing/remitting disorders of the gastrointestinal tract. While the precise aetiology of IBD is unknown, dysbiosis, an imbalance between beneficial and harmful bacterial species in the gut, is a leading hypothesis for the role of bacteria in IBD. Thus the search for potentially pathogenic microbial residents of the GI tract is a current research focus.

The Gram-negative human gut resident *Fusobacterium varium* has been previously demonstrated to play a role in the exacerbation of ulcerative colitis, a type of IBD. We were interested to determine whether the related species, *F. nucleatum*, which is a known invasive and pro-inflammatory pathogen in the human mouth and is thought to be an occasional resident of the human gut, might also be associated with IBD pathogenesis. Using selective media under strict anaerobic conditions, we isolated *F. nucleatum* from human intestinal biopsy samples taken from IBD patients or control healthy patients undergoing colon cancer screening, and characterized them phenotypically, using a variety of biochemical and morphological markers. Invasion of cultured Caco-2 cells by our *F.nucleatum* intestinal isolates was characterized with immunofluorescence microscopy. Additionally, we used a high-throughput screening assay to measure isolate production of autoinducer-2 (AI-2), a molecule which has been proposed to be a universal signal for interspecies communication and has been shown to control gene expression for numerous virulence factors in a number of important enteric pathogens. We found that *F.nucleatum* could be isolated from IBD patients at a significantly greater frequency than from control patients, and that IBD-associated strains were often more invasive than isolates retrieved from healthy persons. AI-2 production was highly variable among the different *F.nucleatum* isolates, however more invasive strains tended to produce greater amounts of this key auto-inducer molecule. This correlation between invasiveness and AI-2 production is intriguing and studies are underway to further elucidate the relationship between AI-2 production and virulence of the *F.nucleatum* isolates. In summary, our data indicate that *F.nucleatum* could potentially play a key role as a pathogenic factor in cases of IBD.