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Commentary

Inventory of a reservoir : friends & foes

There has been a dramatic rise in the incidence of inflammatory bowel disease (IBD) which comprises ulcerative colitis (UC) and Crohn's disease (CD) both in western world as well as developing countries, at places doubling every decade[1-4]. This suggests that environmental factors could be the culprit for immediate ascent of disease incidence. IBD is a multifactorial disease where genetically predisposed individuals develop aberrant innate and adaptive immune responses possibly to commensal bacteria. Genome-wide association studies (GWAS) have shown 163 loci predisposing for IBD and these are enriched for pathways that integrate modulation of intestinal homeostasis with environmental factors[5]. One such factor, which has become the cynosure of attention has been the gut microbiome. Gut microbiome in both health and disease is currently under intense investigation worldwide by scientists and clinicians with diverse expertise and interests.

Another breakthrough over the last decade has been the remarkable advances in our ability to decipher gut microbiome. Studies restricted to cultivable bacteria have been replaced by metagenomic studies which use shotgun-sequencing techniques, which include both DNA-focused metagenomic and RNA-focused metatranscriptome analyses[6]. These high throughput methods are especially useful in integrating microbial diversity and composition with its function and ability to influence gut immune system. Initial efforts were based around the fact that portions of the gene encoding the small subunit 16S ribosomal RNA (rRNA) were highly conserved among bacteria. Methods for analyzing 16S sequencing data from the human microbiome and other environments are now well developed. Hence, a great deal of information on gut microbiome in IBD has emerged from earlier studies based on low resolution surveys of the microbial community to high resolution description using next generation sequencing[6].

A broad pattern which seems to have emerged following interrogation of the gut microbiome in IBD population has been along the following lines[5]:

(I) Alpha diversity is a measure of species richness or a measure of the total number of species and it has been observed that alpha diversity has been reduced in CD patients. *(i)* Predominant reduction has been in *Firmicutes* phylum, *(ii)* inflamed tissue has demonstrated reduced biodiversity as compared to non affected tissue in the same patient.

Taxonomic shifts may result in reduction of microbiome, which are beneficial and protective against IBD. The microbiome protective mechanisms include prevention of colonization by pathogenic bacteria by niche occupation or by dampening virulence related gene expression, down gradation of intestinal inflammation by expansion of T regulatory cells and consequent abundance of interleukin-10 (IL-10) or production of short chain fatty acids like acetate, propionate and butyrate which direct tolerogenic colonic immune responses. Predominant changes include *(i)* decrease in *Bacterioides* (Bacteroidetes phylum) and *Firmicutes (ii)* decrease in *Clostridium, Ruminococcaceae, Bifidobacterium, Latobacillus* (*Firmicutes* phylum), and *(iii)* decrease in *Faecalibacterium prausnitzii* in both CD and UC[5,7]. On the contrary, there are microbial populations which may be enriched and predispose to IBD, these observations include *(i)* increase in *Enterobactericeae* particularly adherent invasive *Escherichia coli* in both UC as well as CD, *(ii)* increase in *Gammaproteobacteria*, and *(iii)* increase in *Fusobacterium* which are adhesive and invasive seen mostly in UC[5,7].

(II) The next important information is functional component of microbiome or also termed as functional composition. Information on functional composition is provided by the next generation sequences rather than low throughput 16SrRNA studies. While bacterial diversity may change in an individual with time, functional composition remains steady hence highlighting the possible potential of functional composition in defining a disease as compared to taxonomic identification only[6]. The composition

changes which have been noted in IBD patients include *(i)* decrease in short chain fatty acids, *(ii)* decrease in amino acid production, *(iii)* increase in sulphate reducing bacteria like *Bilophila wadsworthia*, and *(iv)* increase in oxidative stress[5,7]. These shifts in bacterial complexity, diversity and composition constitute what is termed as dysbiosis, which may be responsible for a shift in the homeostatic healthy flora to a pro-inflammatory microbiome , which can later predispose to intestinal inflammation.

It is interesting and important for us to improve our understanding about dysboisis of bacterial subgroups in IBD patients from diverse geographical regions. The study by Kabeerdoss and colleagues[8] in this issue showed that *Bacteroides* and *Lactobacillus* abundance was greater in UC patients compared with controls or CD. *Escherichia coli* abundance was increased in UC compared with controls. *Clostridium coccoides* group and *C. leptum* group abundances were reduced in CD compared with controls. Microbial population did not differ between diseased and adjacent normal mucosa, or between untreated patients and those already on medical treatment. The *Firmicutes* to *Bacteroidetes* ratio was significantly decreased in both UC and CD compared with controls, indicative of a dysbiosis in both conditions[8]. In another study done in Vellore by the same investigators[9], faecal samples of IBD patients and controls were examined for the abundance and diversity of *C. leptum* group by targeting 16S rRNA gene sequences. Quantitative PCR was used to quantitate *C. leptum* group and its most prominent constituent, *F. prausnitzii*. Total numbers of *C. leptum* group bacteria and *F. prausnitzii* were reduced in both CD and UC compared with controls. Disease activity did not influence numbers of *C. leptum* or *F. prausnitzii* in patients with CD or UC[9]. In one of the two studies from Delhi[10,11], faecal samples from UC patients and controls were subjected to fluorescent *in situ* hybridization in combination with flow cytometry to enumerate the *clostridium* cluster population targeted by 16S rRNA gene probe. This was further validated by qPCR, and gas chromatography was also done to evaluate the changes in concentration of major short chain fatty acids (SCFA). A decrease of predominant butyrate producers of clostridial clusters was observed which correlated with the reduced SCFA levels in active UC patients. This was further confirmed by the restoration in the population of some butyrate producers with simultaneous increase in the level of SCFA in UC patients in remission[10]. Another study from north India included 84 patients (72 with UC and 12 with CD) and 65 controls and looked at mucosa-associated bacterial flora by real-time analysis using 16S rRNA-based genus-specific primers[11]. The *Bacteroides* group was abundant in healthy samples; however, there were significant drops in its concentrations in UC as well as CD patients. Significant decreases in the populations of *Lactobacillus*, *Ruminococcus*, and *Bifidobacterium* in both UC and CD patients were observed[11]. This supported earlier observations proving the hypothesis that loss of commensal organisms profoundly modifies gut mucosal homoeostasis through loss of essential micronutrients (short chain fatty acids) and redox potential. This study also recorded increases in the populations of two subdominant inhabitants, the methanogenic bacterium *Methanobrevibacter* and sulfate-reducing bacteria for UC as well as CD patients, compared with the levels for the controls[11]. These four Indian studies have reinforced the presence of dysbiosis in IBD both in south Indian as well as north Indian IBD population[8-11], however, these studies suffer from certain limitations such as lack of statistical power and non inclusion of treatment naïve patients.

Although 16S sequencing is the most widely used plateform for studies of the gut microbiome because of its low cost, it has several evident limitations. Its exactitude depends on whether the observed proportions of 16S gene sequences reflect the proportion of bacteria in the sample, but the 16S gene is subject to PCR primer and amplification bias as well as copy number variation. 16S sequencing generates information on overall microbiome diversity but it does not provide information about the microbial genome members or microbiome function[6]. This particular handicap has been somewhat addressed in recent years. For niches like intestine where information on bacterial communities and reference genomes is available, it is possible to infer an approximate metagenome using methods such as PICRUSt[12]. This technology couples functions of gene products encoded by the most closely related sequenced genomes with observed taxonomic profiles to produce a functional profile. Most importantly, 16S sequencing identifies only bacterial components of a community, not other intestinal residents groups like arachaea, fungi and viruses. Metagenome or metatranscriptome sequencing, also referred to as shotgun sequencing, DNA-seq, or RNA-seq, is the process of sequencing the entire nucleotide pool isolated from a culture-independent sample and hence it will include fungal genomes as well as viromes. Whole metagenome sequencing eliminates the danger of missing whole kingdoms or bacterial clades as a result of PCR primer bias.

A recent study addressed most of these limitations and in addition is the largest IBD-related microbiome study to date[13]. The multicenter study included new-onset CD paediatric cohort. The strengths of this study lay in the sampling prior to treatment, the size of the cohort, and the concurrent sampling of different sites, including multiple mucosal tissue sites, and the luminal content as stool samples. An axis defined by an increased abundance in bacteria which includes *Enterobacteriaceae*, *Pasteurellacaea, Veillonellaceae*, and *Fusobacteriaceae*, and decreased abundance in *Erysipelotrichales, Bacteroidales*, and *Clostridiales* correlated strongly with the disease status[13].

These studies have essentially provided proof of concept for microbial dysbiosis across different geographical regions and ethnicities. The exciting part is that these provide a roadmap for therapeutic manipulation of human intestinal microbiome and one such modality can be faecal microbiota transplantation (FMT). FMT is also known as intestinal microbiota transfer or faecal bacteriotherapy. FMT comprises the administration of a faecal solution from a donor into the intestinal tract of a recipient. To date, most clinical experience has focused on the use of FMT in patients with relapsing *Clostridium difficele* infection. FMT has shown efficacy in randomized controlled trials for therapy of recurrent *C. difficele* diarrhoea[14]. It has been shown to be beneficial in case series of patients with inflammatory bowel disease, irritable bowel syndrome, idiopathic thrombocytopenic purpura, multiple sclerosis, chronic fatigue syndrome, insulin resistance state and type 2 diabetes mellitus[14]. It has potential for application in treatment of obesity as well as nonalcoholic steatohepatitis. Restoration of physiological balance of intestinal microbiota presents a novel cost-effective interventional modality for treating diseases of public health importance ranging from IBD to metabolic as well as neurological autoimmune diseases. This is probably where the road which started with enumeration of gut members and then identifed friends and foes in health and disease amongst them may wind up.

Vineet Ahuja
Department of Gastroenterology
All India Institute of Medical Sciences,
Ansari Nagar, New Delhi 110 029, India
vins_ahuja@hotmail.com

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