LETTER TO THE EDITOR

THE FINGERPRINT OF THE HUMAN GASTROINTESTINAL TRACT MICROBIOTA: A HYPOTHESIS OF MOLECULAR MAPPING

G. TOMASELLO[1-3-4], M. MAZZOLA[6], A. JURJUS[2] F. CAPPELLO[1,4], F. CARINI[4], P. DAMIANI[3], A. GERGES GEAGEA[2], MN. ZEENNY[2] and A. LEONE[5]

[1]Euro-Mediterranean Institute of Science and Technology (IEMEST), Italy; [2]Department of Anatomy, Cell Biology and Physiology, American University of Beirut, Beirut, Lebanon; [3]Gastroenterology and Endoscopy Unit, Fondazione Istituto S. Raffaele-G. Giglio, Cefalù, Italy; [4]AOUP "P Giaccone", School of Medicine, University of Palermo, Italy; [5]Department of Experimental Biomedicine and Clinical Neurosciences, Section of Histology, School of Medicine and Surgery, University of Palermo, Italy; [6]Department of Experimental Biomedicine and Clinical Neurosciences, Section of Human Anatomy, School of Medicine and Surgery, University of Palermo, Italy

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The precise etiology of Inflammatory Bowel Disease (IDB) remains unclear and several factors are believed to play a role in its development and progression, including the composition of microbial communities resident in the gastrointestinal tract. Human intestinal microbiota are extensive with at least 15,000-36,000 bacterial species. However, thanks to the new development in sequencing and molecular taxonomic methodologies, our understanding of the microbiota population composition, dynamics, and ecology has greatly increased. Intestinal microbiota play a critical role in the maintenance of the host intestinal barrier homeostasis, while dysbiosis, which involves reduction in the microbiome diversity, can lead to progression of inflammatory disorders, such as IBD and colorectal cancer. It is hypothesized that fingerprinting characterization of the microbiota community composition is the first step in the study of this complex bacterial ecosystem and a crucial step in the targeted therapy. Molecular fingerprinting of human gastrointestinal tract microbiota could be performed by different techniques including the semi quantitation, 16SrRNA, the DNA- microarray as well as other relatively new methods which were developed to study many complex bacterial ecosystems. These techniques provide individual data and profiles, using fast and sensitive tools for the high taxonomic level fingerprint of the human intestinal microbiota and provide estimation of the relative presence of the microbial target groups within each individual. Such personalized information serves as a remarkable and unprecedented opportunity to improve targeted medical treatment and probably develop strategies to prevent disease.

To the Editor,

The human normal flora, or microbiota, is extensive, both in its absolute quantitative mass and in its qualitative diversity. The human intestinal tract hosts about 100 trillion microorganisms with at least 15,000-36,000 bacterial species. The microbiome is

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Mailing address:
Dr. Angelo Leone,
BioNec, Section of Histology and Embriology,
School of Medicine, University of Palermo,
Via del Vesrpo, 129 - 90127 Palermo, Italy
Tel.: +39 0916553581
e-mail: angelo.leone@unipa.it

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now considered to be a functional organ involved with multiple physiological functions. On the other hand, many pathological conditions arise in dysbiosis, when the microbiome is drastically altered. IBD is one of those conditions which clearly involves a breakdown in the healthy interactions between the host and the microbiome. A dramatic reduction occurs in the phylum Firmicutes and concomitant increase in Proteobacteria (1).

The vast majority of bacteria in the human intestinal microbiota (>99%) belongs to the six bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia. However, the two dominant divisions are Fermicutes (65%) and Bacteroidetes (25%) of total microbiota. The intestinal microbiota of each individual have a specific complement of hundreds of genera and thousands of bacterial species (1). Conventional microbiological techniques fail to give a detailed inventory of the normal microbiota, but the development of recent high-throughput sequencing and high level molecular taxonomic methodologies have greatly increased our understanding of the microbiota population composition, dynamics, and ecology (2).

The composition of intestinal flora is remarkably stable at different anatomic locations along the gut, but absolute numbers vary greatly, ranging from 10^{11} cells/g in the ascending colon to 10^{7}-10^{8} in the distal ileum, and 10^{2} to 10^{3} in the proximal ileum and jejunum (1, 3).

It is also well documented that intestinal microbiota play a critical role in the maintenance of host intestinal barrier homeostasis by exerting beneficial effects on diverse physiological intestinal functions while dysbiosis can lead to progression of inflammatory disorders such as inflammatory bowel disease (IBD) and colorectal cancer (CRC) (4, 5).

A well balanced gut microbiota promotes the health of colocytes through the production of a variety of important compounds and the correct modulation of the immune system (2).

Along this line, probiotics (PBs) have been used, and some studies have suggested three mechanisms for their action in IBD therapy they may: i) block the pathogenic bacterial effect and compete with pathogenic toxins for adherence to the intestinal epithelium; ii) enhance innate immunity and modulate inflammation through their regulated signaling pathways via their interaction with Toll-like receptors, and iii) enhance intestinal barrier function and thus regulate intestinal epithelial homeostasis. Moreover, PBs modulate different host cell signaling pathways such as Akt, mitogen-activated protein kinases and nuclear-kappa β that could potentially mediate intestinal epithelial functions (6).

Some bacteria, such as *Lactobacillus delbruekii* and *Lactobacillus fermentum* have been documented to ameliorate the inflammatory background. In fact, clinical studies on ulcerative colitis patients showed significant amelioration of the inflammatory state by decreasing the colonic concentrations of multiple pro-inflammatory mediators and reducing myeloperoxidase activity compared to controls. Furthermore, re-establishment of microbial homeostasis and manipulation of microbiota in IBD treatments, through fecal microbiota transplantation of selective bacteria, have been practiced and are gaining great interest (7).

Based on this background and the authors experimental and clinical experiences, we propose the following working hypothesis: the characterization of the microbiota community composition is the first step in the study of a complex bacterial ecosystem. Fingerprinting of IBD patients' microbiota and its mapping, using various molecular techniques, could serve as a very crucial and essential step in the targeted therapies of IBDs. The metagenomic sequencing data provide new insight into the importance of the microbial network and its interaction with the host genome, which could be therapeutically important, as early targeted intervention can restore normal flora (8).

MATERIALS AND METHODS

Gut microbiota have been extensively investigated by anaerobic culture techniques, but it is now well known that culture-based methods provide only an incomplete picture of the overall diversity of microbiota (9).

However, further techniques have been successively developed to explore microbial biodiversity. In fact, the

composition of the adult intestinal microbiota has been determined in 3 large scale 16SrRNA sequence surveys. Other methods, such as the High Taxonomic Fingerprint (HTF-Microbi.Array) and the Ligase Detection Reaction-Universal Array (LDR-UA) approach have been recently extensively used. The HTF Microbi-Array enables specific detection and approximate relative quantification of 16S rRNA from 30 phylogenetically-related groups of human intestinal microbiota. This technique was applied to adult human subjects with high reproducibility of the high level taxonomic microbiota fingerprint for each individual (8, 10-12). Other techniques are also being employed such as:

i. Denaturing/temperature gradient gel electrophoresis (D/TGGE) which is used to separate proteins or nucleic acids (DNA and RNA) across a temperature gradient. TGGE is based on the principles that the structure of proteins and DNA changes with temperature and that these changes can be characterized by their movement through a gel. It is often associated with Polymerase Chain Reaction (PCR) analysis.

ii. The strand conformation polymorphism (SSCP) analysis is a simple and sensitive technique for mutation detection and genotyping. The principle of SSCP analysis is based on the fact that single stranded DNA has a defined conformation. Altered conformation due to a single base change in the sequence can cause Single-Stranded DNA to migrate differently under a non-denaturing electrophoresis conditions.

iii. Fluorescent *in situ* hybridization (FISH) is a cytogenetic technique which uses fluorescent probes binding parts of the chromosomes to show a high degree of sequence complementarity. This technique provides a novel way for researchers to visualize and map the genetic material in an individual cell or bacteria, including specific genes or portions of genes.

iv. Terminal restriction fragment length polymorphism (T-RFLP) analysis is used to study complex microbial communities based on variation in the 16SrRNA gene. This technique can be used to examine microbial community structure and community dynamics in response to changes in different environmental parameters or to study bacterial populations in natural habitats. It has been applied to the study of complex microbial communities in diverse environments, such as soil, marine, oral bacterial flora in saliva in healthy subjects versus patients. RFLP is culture independent, rapid, sensitive and reproducible without the need for any genomic sequence information.

v. Real time polymerase chain reaction (PCR) is a powerful method for amplyfing particular segments of DNA, distinct from cloning and propagation within the host cell; this technique uses the oligonucleotide as a primer and elongates its 3' end to generate an extended region of double stranded DNA.

vi. Human Intestinal Tract Chip (HIT chip) is a 16SrRNA gene-based diversity microarray which was developed and validated for the characterisation of human gut microbiota. It is an oligonucleotide microarray commonly used for phylogenetic profiling (13, 14).

RESULTS

Thanks to such advances of the 16S ribosomal RNA genomic sequencing, a decrease in Bacteroides and Firmicutes numbers in the colon and an increase in Enterobacteriaceae, such as adherent-invasive *E. coli* and other Proteobacteria, have been detected in Crohn's disease (5, 12).

Moreover, the HIT Chip technology, which is a 16SrRNA gene based diversity for phylogenetic profiling of human intestinal tract communities (15), and the pyrosequencing have been developed to serve as a more economic sequencing approach than high-coverage amplicon sequencing, to study many complex bacterial ecosystems.

The data emanating from many techniques intersect, and could sometimes be complementary in determining microbial community profiling. Using the HIT Chip hybridization method has shown strong concordance with the pyrosequencing-based profiling data and sequencing of 16S rRNA gene clone libraries (4, 16).

In brief, microbiota fingerprinting in IBDs is a feasible and useful cost effective approach that could lead to a targeted therapy or even prevention through supplying missing bacterial species through probiotics or other.

DISCUSSION

It is well established that inflammatory pathologies affecting the gastrointestinal tract are narrowly correlated to dismicrobism (2).

However, the question frequently asked for more than a decade is whether dysbiosis is a cause of IBDs or just a secondary phenomenon. In this context, could a molecular mapping or fingerprinting of intestinal microbiota provide convincing evidence regarding its role in causing, maintaining and/or determining the severity of IBDs?

Earlier studies, whether experimental in animals or clinical in patients, are in support of such an approach. Saitoh et al. in 2002, as well as others, performed mucosal bacterial isolation in IBD patients and showed increased concentrations of *Bacteroides vulgatus* and *Enterobacteriacea*e, especially *E.coli*, and decreased concentrations of Bifidobacteria species (17).

In addition, Bull et al. in 2003 demonstrated, in mucosal specimens of CD patients, a highly decreased amount of *Faecalibacterium prausnitzii*, thought to predict a high risk for early reactivation of CD (18).

However, data also proved that administration of *Lactobacillus casei* and *Bifidobacterium lactis* in mice with TNBS induced colitis, resulting in a significant reduction of inflammation in the colonic mucosa (19). Thus, they reversed malignant changes and exerted a potential role in colorectal cancer management and prevention.

In addition, IBD treatments based on microbiota manipulation are gaining great interest in clinical practice. In this context, IBD management is being carried out by fecal microbiota transplantation from a healthy donor to IBD patients; by infusion of liquid stool suspension, re-establishment of microbial homeostasis has been demonstrated by significant increases in bacteriodetes and clostridium as well as decreases in proteobacteria according to healthy donor profiles. Such profiles are best determined by fingerprinting. More than 100 trillion microbes, which we host in the human microbiota to a great extent, can identify us as individuals much like fingerprints. Using publicly available data produced through the Human Microbiome Project (HMP), researchers examined microbes from 242 individuals over a 9-month period. Then they applied an adapted version of a classic computer science algorithm to scan the microbial genetic code and search through different sequence features for patterns that are unique to each individual. To do so, they combined stable and distinguishing sequence features from the individual's initial microbiome samples and compared them both to samples from the same individuals collected at follow-up visits and samples collected from control group individuals. Researchers found that the "codes" were unique among hundreds of participants and that also a number of them (more than 80%) remained stable over the sampling period of one year. In some ways, it could be said that they were able to identify "microbiome fingerprints". In brief, there are many aspects that are the same for many people but the existing differences are enough to characterise each individual.

Nowadays, it is possible to perform a comprehensive microbiota assessment of an individual, including DNA and RNA sequence, and at least some characterization of proteome, microbiome and epigenome. It has become abundantly clear that each of us actually has a one-of-a-kind biological content. Analysing the large volume of microbial gut, based on the whole genome sequencing (WGS) and preparing high quality representative RNAs for sequencing to generate metatranscriptome along with the establishment of the gut microbial interaction network model, is a major challenge (12).

Well beyond the allure of the matchless fingerprint or snowflake concept, these singular individual data and information set up a remarkable and unprecedented opportunity to improve medical treatment and develop preventive strategies for disease (13).

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