Twin Study Indicates Loss of Interaction Between Microbiota and Mucosa of Patients With Ulcerative Colitis

PATRICIA LEPAGE,[*,‡] ROBERT HÄSLER,[*] MARTINA E. SPEHLMANN,[*] ATEEQUR REHMAN,[*] AIDA ZVIRBLIENE,[§] ALEXANDER BEGUN,[*] STEPHAN OTT,[*,‖] LIMAS KUPCINSKAS,[#] JOËL DORÉ,[‡] ANDREAS RAEDLER,[¶] and STEFAN SCHREIBER[*,‖]

[*]Institute of Clinical Molecular Biology and [‖]Department of Internal Medicine I, Christian-Albrechts University - Kiel, Kiel, Germany; [‡]INRA, UMR1319, MICALIS, Jouy-en-Josas, France; [§]Institute for Digestive Research and [#]Department of Gastroenterology, Lithuanian University of Health Sciences, 44307 Kaunas, Lithuania; and [¶]Department of Internal Medicine, Asklepios Westklinikum Hamburg, Hamburg, Germany

BACKGROUND & AIMS: Interactions between genetic and environmental factors are believed to be involved in onset and initiation of inflammatory bowel disease. We analyzed the interaction between gastrointestinal mucosal microbiota and host genes in twin pairs discordant for ulcerative colitis (UC) to study the functional interaction between microbiota and mucosal epithelium. **METHODS:** Biopsy were collected from sigmoid colon of UC patients and their healthy twins (discordant twin pairs) and from twins without UC. Microbiota profiles were determined from analysis of 16S ribosomal DNA libraries; messenger RNA profiles were determined by microarray analysis. **RESULTS:** Patients with UC had dysbiotic microbiota, characterized by less bacterial diversity and more Actinobacteria and Proteobacteria than that of their healthy siblings; healthy siblings from discordant twins had more bacteria from the Lachnospiraceae and Ruminococcaceae families than twins who were both healthy. In twins who were both healthy, 34 mucosal transcripts correlated with bacterial genera, whereas only 25 and 11 correlated with bacteria genera in healthy individuals and their twins with UC, respectively. Transcripts related to oxidative and immune responses were differentially expressed between patients with UC and their healthy twins. **CONCLUSIONS: The transcriptional profile of the mucosa appears to interact with the colonic microbiota; this interaction appears to be lost in colon of patients with UC. Bacterial functions, such as butyrate production, might affect mucosal gene expression. Patients with UC had different gene expression profiles and lower levels of biodiversity than their healthy twins, as well as unusual aerobic bacteria. Patients with UC had lower percentages of potentially protective bacterial species than their healthy twins.**

Keywords: Dysbiosis; Crohn Disease; Transcript; Inflammation; Microbiome.

Chronic inflammatory bowel diseases (IBDs), that is, Crohn's disease (CD) and ulcerative colitis (UC), are characterized by dysbiosis of the intestinal microbiota. Dysbiosis, an altered composition of the commensal bacterial populations, is discussed as a major factor in disease pathogenesis that interacts with genetic susceptibility and leads to the dysregulation of the immune response to bacterial antigens observed in IBD.[1–4] Loss of natural intestinal diversity and a shift of bacterial composition toward a more deleterious profile might reflect the net effect of environmental influences over the past decades leading to the dramatic increase in the incidence of IBD in the industrialized world.[5]

Familial aggregation suggests a genetic predisposition to IBD, with 5% to 20% of patients with IBD having a family history of the disease.[6] However, a lower monozygotic twin concordance rate for UC than for CD suggests a smaller contribution of genetic factors in UC.[7] Systematic studies have given insights into the genetic architecture of both IBDs. More than 40 disease genes and loci have been identified and point to cytokine-driven immune dysregulation (eg, the interleukin [IL]-23 pathway), innate immunity, autophagy, and other factors important for integrity of the intestinal epithelial cell.[8–13]

Genes involved in the IL-23 pathway (IL-23R, IL-12B, STAT3) and NKX2-3, DLG5 genes are associated with both CD and UC. Recently, 2 single nucleotide polymorphisms on the multidrug resistance 1 gene (MDR1) have been observed in association with UC.[14] Another single nucleotide polymorphism on the same gene, rs3789243, was found to be associated with pancolitis in patients with UC.[15] Very recently, a risk locus on IL17REL was also described in UC.[11]

In contrast, only a few systematic analyses are available that describe the gut microbiota dysbiosis in this disease. Using fingerprinting methods, several groups observed a decreased diversity in gut microbiota of patients with UC compared with healthy controls at both mucosal[16,17] and fecal levels.[18] Sokol et al applied in situ hybridization coupled to flow cytometry to analyze fecal microbiota of patients with IBD.[3] They highlighted a high percentage of uncommon bacteria in patients with UC, with 40% of total bacteria not detected by their probes designed to target major groups of gut commensal microbiota.

Garrett et al showed a link between the expression pattern of host genes and gut bacterial communities in a

Abbreviations used in this paper: FDR, false discovery rate; IL, interleukin; PCA, principal component analysis; rDNA, ribosomal DNA.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.04.011

TRUC (Tbet$^{-/-}$, Rag$^{-/-}$, Ulcerative Colitis) mouse model of colitis.[19] TRUC mice lack the T-bet transcription factor, which is a negative regulator of the transcription of tumor necrosis factor α. These TRUC mice spontaneously develop a colitis that resembles UC. This colitis is vertically transmissible to progeny through their intestinal microbiota. Moreover, they showed that healthy wild-type mice that were fostered by TRUC mice developed colitis, which strongly supports the hypothesis of the involvement of environmental factors, such as the gut microbiota, in the onset of UC.

Analysis of human twin pairs provides the unique opportunity to discriminate between the contribution of genetic and environmental factors to phenotypic variance. We have chosen UC because the phenotype is mimicked in animal models that were investigated for the interaction between epithelial genomics and gut microbiota and to reach a higher homogeneity in the clinical phenotype. The present study uses systematic molecular technologies to describe gut dysbiosis in UC at both mucosal microbiota and host level. It also provides insight into the genetic determination of dysbiosis compared with healthy twins.

Patients and Methods

Biopsy specimens were sampled from the sigmoid colon of 62 volunteers: 11 healthy dizygotic Lithuanian twin pairs (Hli - aD/bD), 7 healthy monozygotic Lithuanian twin pairs (Hli - aM/bM), 8 monozygotic German twin pairs discordant for UC (Hu/UC), and 10 healthy unrelated German volunteers as a control cohort (Hger). Clinical data of the UC discordant twins are shown in Table 1. All twins recruited for this study were tested for monozygosity or dizygosity as previously reported.[20,21] Female to male ratio was 1.3:1 (Supplementary Table 1).

Mucosal Microbiota Composition and Diversity Assessment

Following total DNA extraction from biopsy specimens, 16S ribosomal DNA (rDNA) amplification, and cloning as previously described,[1] 53 clone libraries were sequenced (ABI PRISM; Applied Biosystems, Foster City, CA) containing 144 clones each (except the pooled German control cohort: 840 clones). After chimeras check (Mallard software, http://www.bioinformatics-toolkit.org/Mallard, Cardiff, UK), sequences were analyzed with the RapidOTU pipeline[22] (CLUSTALW alignment, DNAdist program, DOTUR software, http://www.bio.umass.edu/micro/schloss/software/dotur.html). For phylogenetic affiliation, sequences were subjected to National Center Biotechnology Information blast analyses and the Seqmatch and Classifier programs at RDPII[23] (Ribosomal Database Project, release 9.58). Bacterial phylotypes (or operational taxonomic units) were defined as groups of sequences sharing at least 98% of similarity and phylogenetically affiliated to their closest relative bacterial species. The 2145 operational taxonomic unit representative sequences have been submitted to GenBank database under the accession numbers HM805116 to HM807260.

Estimates of phylotypes richness and similarity indices were calculated according to the bias-corrected Chao1 estimator and Sørensen similarity index, respectively. Phylotypes richness is a measure of biodiversity and consists of a count of the number of different species (or phylotypes) in a given ecosystem. High species richness for a given ecosystem means a high level of redundancy in its function, which further denotes the ability of the ecosystem to withstand natural disturbances. Principal component and clustering analyses were performed to map each individual's microbiota based on their overall bacterial species composition and to assess similarities between individual's microbiota and further define relevant clusters, respectively (R packages ade4 and pvclust). Principal component analyses with the different clinical factors as instrumental variables (interclass principal component analyses) were computed and statistically assessed by a Monte Carlo rank test to observe the net effect of the different factors on the scattering of the microbiota of

Table 1. Clinical Data for UC Discordant Twin Pairs

ID	Clinical status	Gender	Age (y)	Smoking Status	CAI	Duration of the disease	Location	Treatment	Surgery
Hu1	H	M	52	NS	na				
UC1	UC	M	52	NS	4	15	Pancolitis	5-ASA/Steroids	0
Hu3	H	F	26	NS	na				
UC3	UC	F	26	NS	2	5	Left-sided	5-ASA	0
Hu4	H	M	42	S	na				
UC4	UC	M	42	NS	3	14	None	na	1
Hu5	H	F	18	NS	na				
UC5	UC	F	18	NS	3	3	Proctitis	5-ASA	0
Hu6	H	F	21	NS	na				
UC6	UC	F	21	NS	7	4	Pancolitis	5-ASA/Azathioprin	0
Hu7	H	M	23	NS	na				
UC7	UC	M	23	NS	7	4	Left-sided	5-ASA/Steroids	0
Hu8	H	F	49	NS	na				
UC8	UC	F	49	NS	6	6	Left-sided	5-ASA/Azathioprin	0
Hu9	H	M	21	NS	na				
UC9	UC	M	21	NS	7	5	Left-sided	5-ASA/Steroids	0

CAI, Colitis Activity Index; na, non applicable; Hu, Healthy sibling; UC, ulcerative colitis sibling; NS, Never smoked; S, Smoker; ASA, mesalamine.