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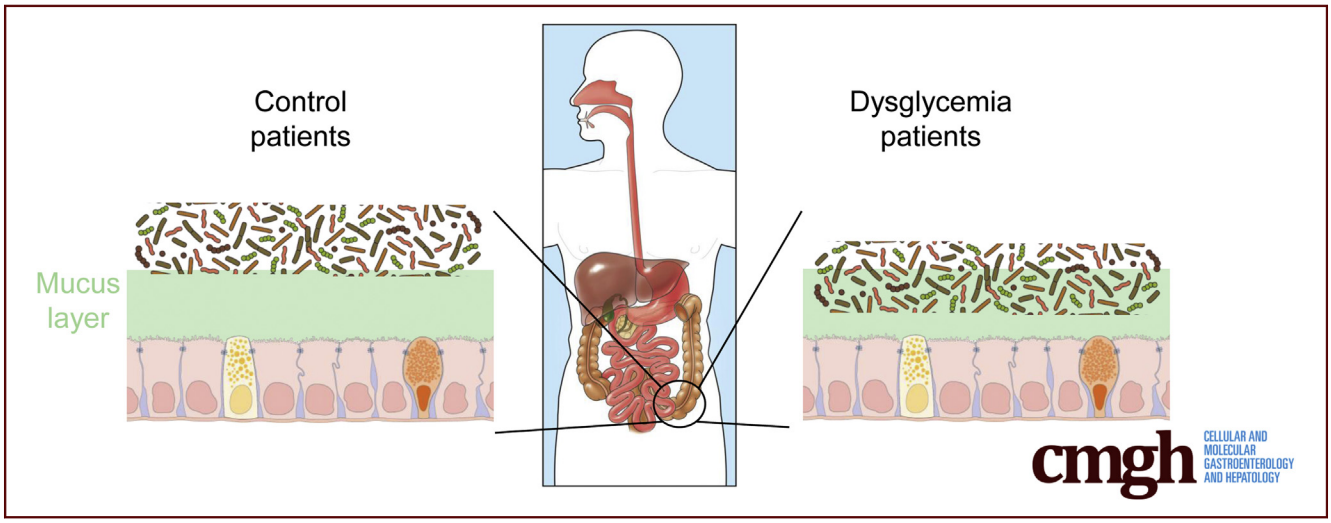
ORIGINAL RESEARCH

Colonic Microbiota Encroachment Correlates With Dysglycemia in Humans

Benoit Chassaing,[1] Shreya M. Raja,[2,3] James D. Lewis,[4] Shanthi Srinivasan,[2,3] and Andrew T. Gewirtz[1,2]

[1]Center for Inflammation, Immunity and Infection, Institute for Biomedical Sciences, Georgia State University, Atlanta, Georgia; [2]Digestive Diseases Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia; [3]Atlanta VA Medical Center, Decatur, Georgia; [4]Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania



SUMMARY

We previously reported that, in multiple murine models of low-grade intestinal inflammation, development of metabolic syndrome correlates with encroachment of bacteria into the normally sterile inner colonic mucus layer. Here, we report that microbiota encroachment is also a feature of metabolic disease, particularly insulin resistance–associated dysglycemia, in humans.

BACKGROUND AND AIMS: Mucoid structures that coat the epithelium play an essential role in keeping the intestinal microbiota at a safe distance from host cells. Encroachment of bacteria into the normally almost-sterile inner mucus layer has been observed in inflammatory bowel disease and in mouse models of colitis. Moreover, such microbiota encroachment has also been observed in mouse models of metabolic syndrome, which are associated low-grade intestinal inflammation. Hence, we investigated if microbiota encroachment might correlate with indices of metabolic syndrome in humans.

METHODS: Confocal microscopy was used to measure bacterial-epithelial distance of the closest bacteria per high-powered field in colonic biopsies of all willing participants undergoing cancer screening colonoscopies.

RESULTS: We observed that, among all subjects, bacterial-epithelial distance was inversely correlated with body mass index, fasting glucose levels, and hemoglobin A_{1C}. However, this correlation was driven by dysglycemic subjects, irrespective of body mass index, whereas the difference in bacterial-epithelial distance between obese and nonobese subjects was eliminated by removal of dysglycemic subjects.

CONCLUSIONS: We conclude that microbiota encroachment is a feature of insulin resistance–associated dysglycemia in humans. *(Cell Mol Gastroenterol Hepatol 2017;4:205–221; http://dx.doi.org/10.1016/j.jcmgh.2017.04.001)*

Keywords: Metabolic Syndrome; Mucus Layer; Microbiota.

See editorial on page 324.

Abbreviations used in this paper: **BMI, body mass index; HPF, high-powered field; IBD, inflammatory bowel disease; PBS, phosphate-buffered saline; TLR, Toll-like receptor.**

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The intestinal tract is inhabited by a large diverse community of bacteria collectively referred to as gut microbiota. When stably maintained, at an appropriately safe distance from epithelial cells, gut microbiota provides a benefit to the host, especially in terms of energy harvest and promotion of immune development.[1] However, disturbance of the microbiota-host relationship can drive chronic gut inflammation, including Crohn's disease and ulcerative colitis, collectively referred to as inflammatory bowel disease (IBD).[2,3] Accordingly, patients with IBD, and persons deemed to be at elevated risk for IBD development, exhibit alterations in gut microbiota composition and, moreover, exhibit altered bacteria localization.[4,5] Specifically, IBD-prone individuals frequently display gut bacteria close to, and/or in direct contact with, the epithelium, often accompanied by a thinner or disorganized mucus layer, whereas in control subjects the dense inner layer of mucus rarely exhibits bacteria.[6,7] Such encroaching bacteria are thought to play a role in triggering the activation of the mucosal immune system that characterizes IBD.

Studies in mice suggest that alteration of the host-microbiota can also result in more mild forms of inflammation characterized by modest elevations in proinflammatory gene expression that associate with metabolic syndrome. For example, loss of genes involved in innate immune-mediated recognition of bacteria, such as Toll-like receptor (TLR) 5, TLR2, and NLRP6, resulted in alterations in microbiota composition, low-grade inflammation, and a metabolic syndrome–like phenotype that could be transferred via fecal transplant indicating a central role for the microbiota in these mouse models.[8–11] Such alterations in microbiota result in microbiota encroachment that can be envisaged to play a role in driving elevated proinflammatory gene expression.[12] Microbiota encroachment, low-grade inflammation, and metabolic syndrome could be induced, in wild-type mice, by administration of dietary emulsifiers leading to the suggestion that this ubiquitous class of food additives might be a contributor to the post-mid-20th-century increased incidence of metabolic syndrome.[13] However, whether microbiota encroachment might be a feature of metabolic syndrome in humans has not been investigated and, hence, was the focus of this study.

Methods

Human Subjects

Subjects were enrolled at the Veteran's Administration Hospital (Atlanta, GA), following their provision of informed consent using procedures approved by the institutional review board, in consecutive fashion from August 1, 2013, to December 31, 2015 (Table 1). Inclusion criteria were subjects undergoing colonoscopy for screening for colon cancer who were at least 21 years of age and had no major health problems besides diabetes. Exclusion criteria were greater than 75 years of age, history of IBD, having a history of systemic neurologic or muscular disorder (eg, Parkinson disease, multiple sclerosis, or Alzheimer disease), or having significant comorbid conditions (eg, chronic liver disease or malignancy) or laboratory abnormalities that preclude colonoscopy. Additionally, patients with history of recent significant gastrointestinal bleeding were excluded from the study. A history, focusing on history of diabetes and gastrointestinal complaints including any prior trials of medications for these complaints, was obtained by review of the medical record database and interview by a study associate. A limited review of the patient medical record was conducted to determine control of diabetes as shown by glycosylated hemoglobin and fasted serum glucose levels. During the colonoscopy procedure 2 mucosal biopsies were taken in the left colon approximately 40 cm from the anus using regular forceps. The biopsies were immediately placed in Carnoy fixative and analyzed by confocal microscopy as described later.

Localization of Bacteria and Quantitation of Bacterial-Epithelial Distance by Fluorescent In Situ Hybridization/Confocal Microscopy

Mucus immunostaining was paired with fluorescent *in situ* hybridization, as previously described,[14] to analyze bacteria localization at the surface of the intestinal mucosa. Briefly, colonic tissues (proximal colon, 2 cm from the cecum) containing fecal material were placed in methanol-Carnoy fixative solution (60% methanol, 30% chloroform, 10% glacial acetic acid) for a minimum of 3 hours at room temperature. Tissue were then washed in methanol 2 × 30 minutes, ethanol 2 × 15 minutes, ethanol/xylene (1:1) 15 minutes, and xylene 2 × 15 minutes, followed by embedding in paraffin with a vertical orientation. Sections of 5 μm were performed and dewaxed by preheating at 60°C for 10 minutes, followed by xylene 60°C for 10 minutes, xylene for 10 minutes, and 99.5% ethanol for 10 minutes. Hybridization step was performed at 50°C overnight with EUB338 probe (5'-GCTGCCTCCCGTAGGAGT-3', with a 5' labeling using Alexa 647) diluted to a final concentration of 10 μg/mL in hybridization buffer (20 mM Tris–HCl, pH 7.4, 0.9 M NaCl, 0.1% sodium dodecyl sulfate, 20% formamide). After washing 10 minutes in wash buffer (20 mM Tris–HCl, pH 7.4, 0.9 M NaCl) and 3 × 10 minutes in phosphate-buffered saline (PBS), PAP pen (Sigma, St. Louis, MO) was used to mark around the section and block solution (5% fetal bovine serum in PBS) was added for 30 minutes at 4°C. Mucin-2 primary antibody (rabbit H-300, Santa Cruz Biotechnology, Dallas, TX) was diluted 1:1500 in block solution and applied overnight at 4°C. After washing 3 × 10 minutes in PBS, block solution containing antirabbit Alexa 488 secondary antibody diluted 1:1500, phalloidin-tetramethylrhodamine B isothiocyanate (Sigma) at 1 μg/mL and Hoechst 33258 (Sigma) at 10 μg/mL was applied to the section for 2 hours. After washing 3 × 10 minutes in PBS slides were mounted using Prolong antifade mounting media (Life Technologies, Carlsbad, CA). Observations of bacterial localization and quantitation of bacterial-epithelial distance were performed in a blinded manner by the first author (B.C.) via confocal microscopy. Instrument software was used to determine the distance between bacteria and epithelial cell monolayer. For each subject, 5 high-powered fields (HPF) were arbitrarily selected with the following inclusion criteria: (1) the presence of stained bacteria, (2)