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Gerardo M. Nava
Washington University School of Medicine in St. Louis

Thaddeus S. Stappenbeck
Washington University School of Medicine in St. Louis

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Diversity of the autochthonous colonic microbiota

Gerardo M. Nava and Thaddeus S. Stappenbeck*
Department of Pathology and Immunology; Washington University School of Medicine; St. Louis, MO USA

Alongstanding hypothesis in intestinal microbial ecology is that autochthonous microbes (resident) play a role that is distinct from allochthonous microbes (transient microbes in the fecal stream). A challenge has been to identify this pool of microbes. We used laser capture microdissection to collect microbes from the mouse ascending colon. This area contains transverse folds that mimic human intestinal folds and contains a distinct population of intestinal microbes that is associated with the mucosa. Our analysis of bacterial 16S rRNA genes showed that this area was enriched for Lachnospiraceae and Ruminococcaceae. In this addendum, we further compare this community to studies of mucosa-associated microbes in humans. This analysis reveals common phylogenetic groups of bacteria that are present in both mouse and human. However, we found microorganisms at the genus and species levels including Faecalibacterium prausnitzii which appears to be specific for humans. We propose that this examination of the mucosa-associated microbes in wild type and genetically modified mice will be a valuable component to define host microbial interactions that are essential for homeostasis.

Intestinal Microbiota and Host Homeostasis

The intestinal microbiota plays a pivotal role in local and systemic host physiology. Studies in germ-free mice demonstrated that intestinal colonization by commensal bacteria contributes to the development of adaptive lymphoid tissue, innate immune responses2-4 and intestinal angiogenesis.5 Also, at the level of the whole organism, the intestinal microbiota plays an active role in host’s energy harvesting and storage6-8 as well as impacts the pathogenesis of autoimmune9 and metabolic diseases.10,11 In contrast, our understanding of the biological basis controlling selection, colonization, persistence and function of intestinal microbes remains limited.

Specialized Microbial Niches in the Intestinal Mucosa

One strategy to discern factors that drive the selection, diversity and function of microbial populations is the study of their spatial distribution. Studies in soil,12 hypersaline mats13 and sewage biofilms14 have shown that microbial communities are spatially organized in predictable patterns determined by energy source gradients (reviewed in ref. 12–14). However, the application of the techniques used in these environmental studies is challenging for the intestinal microbiota due to the difficulties to collect samples without disturbing the structure of the microbial populations and their nearby environment.

In the intestine, the spatial distribution of intestinal microbes occurs along two basic axes, (1) longitudinally from proximal to distal and (2) radially from the central lumen to the mucosal surface.15 Many studies used culture-independent methods (e.g., PCR-based techniques) to document the microbial density and composition in different regions along the length of the intestine. These comprehensive analyses of microbial populations showed alterations in the diversity and density of bacterial 16S rRNA genes
in ileum, cecum, proximal, distal colon
and feces of healthy humans and mice.\textsuperscript{16-20} However, comprehensive studies of the
spatial organization of microbes across
the radial axis of the intestine are scant.

The spatial organization of the intes-
tinal microbiota is of interest as it implies
the determination of autochthonous (resi-
dent) versus allochthonous (transient)
microbes. Resident microbes have been
proposed to be closely associated with
the intestinal mucosa perhaps in associa-
tion with a portion of the mucus that
overlies the intestinal epithelium, while
more transient microbes are thought to be
located in the central lumen as part the
fecal stream.\textsuperscript{21} The anatomy of the intes-
tinal mucosa contains important local
variations which may provide a niche for
autochthonous microbes. In humans, the
small intestine and proximal colon con-
tain complete circular or semi-circular
folds (also known as \textit{plicae circularis} or
\textit{plicae semilunaris}, respectively) that pro-
ject approximately 1–2 mm into the intes-
tinal lumen and are perpendicular to the
direction of the fecal stream. Their func-
tion is unproven, though these structures
have been proposed a mechanism to slow
transit time.\textsuperscript{22}

As the acquisition of microbes from
this anatomic location in humans is dif-
ficult without manipulation of the lum-
inal contents, we examined a portion of
the mouse intestine that contains similar
mucosal anatomy. The mouse proximal
colon (also known as the ascending colon)
contains transverse folds that project \(\approx 1\)
millimeter into the lumen and are ori-
ented in a direction perpendicular to the
fecal stream. This mucosa niche provides
an excellent biological system to examine
the interaction between host and micro-
bial communities.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image1}
\caption{Model of the interaction of host and microbiota in the intestine. The epithelial monolayer produces mucus as well as antimicrobial peptides and proteins that form electrostatic interactions in the intestinal lumen. This forms a specialized niche where autochthonous (resident) microbes appear to reside. Autochthonous microbes (1) are comprised of compact interlacing layers of predominately large, fusiform-shaped bacteria that appeared in close apposition to the apical surface of the colonic epithelium. These microbial communities are distinct, both morphologically and phy-
logenetically, as compared to allochthonous (transient) microbes (2) that are located in the central lumen (digesta) as part the fecal stream. Allochthon-
ous communities include rod- and coccoid-shaped bacteria associated with undigested food particles.}
\end{figure}

In mammals, the radial organization of intestinal microbes has been a long-
standing interest. Morphological exami-
nation of the mouse and rat intestine
showed that microbes characterized by
long spiral rod morphology colonize at
high density in select mucosal associated
areas. Such niches are in close proxim-
ity to the columnar epithelium that lines
the intestine whereas coccus-shaped
microbes are more prevalent to low den-
sity areas, such as the central intestinal
lumen.\textsuperscript{23-25} These morphologic patterns
of spatial structure and niche adaptation
by the colonic microbiota were initially
proposed in a series of studies by Savage
and Dubos.\textsuperscript{26-29} Their observations of
To collect microbes in these distinctive locations and perform a comprehensive characterization of microbial communities. Our goal was to identify the resident microbes discovered by Dubos and colleagues more than four decades ago.

We established methodologies to examine these mucosa-associated microbial communities and compare their spatial distribution across the radial axis. To achieve these objectives, we adapted the technology of laser capture microdissection (LCM) to procure intestinal microbes from defined locations within the proximal colonic mucosa. We recently reported in reference 30, morphologically comparable microbial structures in the interfold regions of the colonic mucosa of mice. These microbial communities were comprised of compact interlacing layers of predominately large, fusiform-shaped bacteria (size >5–10 μm) that appeared in close apposition to the apical surface of the colonic epithelium. Conversely, the central lumen (digesta) contained rod- and coccoid-shaped bacteria (size 1–2 μm) associated with undigested food particles (Fig. 1). Our contribution was to provide a method to collect microbes in these distinctive locations and perform a comprehensive characterization of microbial communities. Our goal was to identify the resident microbes discovered by Dubos and colleagues more than four decades ago.

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Figure 2. Mucosa-associated autochthonous microbes in the human and mouse colon are enriched for Lachnospiraceae and Ruminococcaceae families. We compared diversity at the family level. Each chart represents the taxonomic composition. Sequences were previously obtained in another study from the ascending-, transverse-, descending-colon and rectum biopsies of healthy humans. This data was extracted from a comprehensive molecular analysis of almost full-length 16S rRNA gene sequences. Pyrosequencing analysis was used to examine microbial diversity between interfold (29,560 reads) and digesta (38,120 reads) regions from the colon of wild-type mice. Lachnospiraceae and Ruminococcaceae are indicated by arrows and outlined with a dotted line to highlight these families. Noteworthy, the enrichment of both Lachnospiraceae and Ruminococcaceae families was also observed in mucosal biopsies obtained from healthy humans who had undergone bowel preparation. These data indicate that detection of these bacterial families can be accomplished regardless the methodology for sample acquisition. Unclassified Bacteroidetes and Firmicutes correspond to sequences not classifiable at family level (as of December 2010). Both data sets were classified using the Classifier version 2.2 at the Ribosomal Database Project.
such as Vibrionaceae have exploited this genomic plasticity to live as pathogens, symbionts, free-living forms and extremophile microorganisms. The challenge now is to develop a system to characterize members of the autochthonous microbiota to gain knowledge of different mechanisms involved in niche diversification. One potential strategy is to examine the spatial organization of the intestinal microbiota in different model organisms and perform comparative analysis, including microbial surveys obtained from intestinal biopsies from humans. The outcome of these analyses will facilitate the selection of bacterial targets for isolation and whole genome analysis.

**New Challenges and New Horizons in the Study of Intestinal Microbiota**

Based on the initial studies by Savage, Dubos and colleagues in the 70's and 80's and recent advances in microbial ecology techniques, our working hypothesis is that members of the autochthonous microbiota, (e.g., Lachnospiraceae and Ruminococcaceae) have undergone significant genome adaptations to facilitate niche specialization. This type of diversification may involve gain and lose of regulatory genes, interspecies horizontal transfer, gene mutations and genome reduction. Bacterial families such as Vibrionaceae have exploited this genomic plasticity to live as pathogens, symbionts, free-living forms and extremophile microorganisms.

The challenge now is to develop a system to characterize members of the autochthonous microbiota to gain knowledge of different mechanisms involved in niche diversification. One potential strategy is to examine the spatial organization of the intestinal microbiota in different model organisms and perform comparative analysis, including microbial surveys obtained from intestinal biopsies from humans. The outcome of these analyses will facilitate the selection of bacterial targets for isolation and whole genome analysis.
our new data,35 we hypothesize that these autochthonous communities, including Lachnospiraceae and Ruminococcaceae, evolved special mechanisms to survive in a hazardous niche that contain high concentrations of endogenous antimicrobial peptides (for a review see refs. 33 and 34) but at the same time remain innocuous to the host. To further support the idea that autochthonous microbes have evolved symbiotic relationships with their host, we have performed additional analysis comparing the diversity of colonic mucosa-associated bacteria in human and mice from our study and the published work of other investigators.37 We found that the mucosa associated surfaces of the colon of mice and humans is predominantly colonized by similar phylogenetic core of Lachnospiraceae and Ruminococcaceae genera: Butyricicoccus, Ruminococcus, Oscillibacter, Anaerotruncus, Coprococcus, Robisoniella, Dorea, Anaerostipes, Roseburia and Blautia (Fig. 3).

More importantly, comparisons of microbial diversity between human and mice uncovered indications of species-specificity. For example, genera Faecalibacterium, Subdoligranulum and Actibacillus were only observed in the human colon whereas genera Marvinbryantia and Butyricicoccus were only found in the colonic mucosa of mice (Fig. 3). Based on these observations, we conducted a systemic search using one of the most comprehensive, well annotated and frequently updated 16S rRNA gene database.35 We found that of the 6,429 sequences of Faecalibacterium archived in the database (as of December, 2010), only seven have been obtained from mice, a humanized gnotobiotic mouse model.36 Likewise, of the 319 sequences of Marvinbryantia archived in the database (as of December, 2010), none correspond to human intestinal samples. Interestingly, one of these genera, Faecalibacterium, was implicated in a human intestinal disease; a decrease in Faecalibacterium prausnitzii has been observed repeatedly in the ileal mucosa associated alterations in immune cell homeostasis. Inflamm Bowel Dis 2009; 15:148-58.


References


