Gut Immune Maturation Depends on Colonization with a Host-Specific Microbiota

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Markus Geuking
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Introduction

• The human microbiota is similar to the microbiotas of other mammals at the phylum level but distinct at the species and strain levels.

• Germ-free mice have developmental defects including abnormal nutrient absorption and altered intestinal morphology and motility and the gut microbiota is critical for intestinal immune maturation.

• GF animals have smaller PP, fewer plasma cells, fewer IEL, impaired antimicrobial peptide and IgA secretion, and other immunologic deficiencies.

• Many deficiencies are corrected by recolonization with a mouse commensal microbiota.

• It remains unclear whether health-associated development depends on specific bacterial species exclusive to the host.

• Is mammalian immune maturation dependent on the mere presence of bacteria, or is a host-specific microbiota required?

• Colonize GF Swiss Webster mice at birth with a mouse gut microbiota (MMb) or a human gut microbiota (HMb).
Colonization strategy

GF Swiss Webster (SW) mice underwent oral gavage with pooled fecal specimens from two healthy humans or with fecal/cecal contents from specific pathogen-free (SPF) SW mice.

The two groups of recipient mice were then maintained in separate gnotobiotic isolators.

Both MMb and HMb offspring had a smaller cecum than GF mice, whose cecum is abnormally large.
MMb and HMb Mouse Gut Microbiotas Are Similar in Major Bacterial Phyla Abundance with Differences at the OTU Level

Figure 1. MMb and HMb Mouse Gut Microbiotas Are Similar in Major Bacterial Phyla Abundance with Differences at the OTU Level

(A) Schematic of colonization model (see text for details) is illustrated. Blue and red arrowheads indicate fecal pellet collection for bacterial 16S rDNA sequencing. Offspring were sacrificed for immune system analysis.

Similar phyla

Only small overlap for Operational Taxonomic Units
T Cell Proliferation Plays a Role in Expansion of Small Intestinal T Cells and Depends on a Host-Specific Microbiota

Recruitment of CD4+ and CD8αβ+ T cells to the mucosa reportedly is initiated through antigen uptake by antigen-presenting cells (APCs) in intestinal tissue followed by further priming and activation of naive lymphocytes by antigen-loaded APCs in secondary lymphoid organs such as PPs and MLNs. Primed lymphocytes expressing α4β7 and CCR9 in secondary gut lymphoid organs enter the bloodstream and ultimately exit into gut tissue through vessels in the small intestinal LP where MAdCAM and CCL25—ligands of α4β7 and CCR9, respectively—are expressed (Mowat, 2003). Because we found low numbers of T cells in both small intestinal tissue (IEL compartment, LP) and secondary lymphoid organs (PPs, MLNs) of...

How about Colon?

MMb Mice Have More Small Intestinal T Cells Than Do HMb Mice

(A) LP - CD4 T cell

(B) IEL (αβ TCR)

(C) MMb

(D) HMb

(E) GF

Blue: DAPI
Green: CD3

IgA induction?
The MMb Expands T Cell Populations in Small Intestinal Tissue and Secondary Gut Lymphoid Organs

(A–C) PP number (A) and average PP size (B) per small intestine were compared. PPs were mashed, stained for CD3, and subjected to flow cytometry (C). See also Figures S3A and S3B.

(D and E) Total T cell numbers in MLNs (D) and spleen (E) are shown. See also Figures S3C–S3G.

(F) GF mice (3–4 weeks old) were orally gavaged with the original mouse (M) or human (H) inoculum or with feces pooled from ten additional human donors (H10 inoculum). T cell numbers were measured after 4 weeks of colonization.

(G) GF mice were orally gavaged with Sprague-Dawley rat feces and bred in vinyl isolators to obtain RMb offspring. T cell numbers in age-matched MMb, HMb, and RMb offspring were compared.

*p < 0.05, **p < 0.01, ***p < 0.001. NS, not significant.
When we compared CD4^+ T cells in PPs of HMb and MMb mice, we found a lower frequency of effector/memory cells (CD44^{hi}CD62L^{lo}) but a higher frequency of naive T cells (CD44^{lo}CD62L^{hi}) in HMb mice. Analysis of CD8^+ T cells in PPs yielded similar results (Figures 4A and 4B). T cell activation in secondary lymphoid organs can also be assessed by

Induction of T Cell Proliferation in Secondary Gut Lymphoid Organs

(A) Representative flow cytometry plots of CD44^{hi}CD62L^{lo} (effector/memory) and CD44^{lo}CD62L^{hi} (naive) expression on CD3^+CD4^+ T cells in PPs of MMb and HMb offspring are presented. Numbers indicate cell percentages in the quadrant.

(B) Combined data for PP CD3^+CD4^+ and CD3^+CD8^+ cells (n = 7) are illustrated.

(C) Mice injected with BrdU were sacrificed 2 hr later. CD3^+T cells were stained with FITC-conjugated antibody to BrdU for detection of proliferating cells. See also Figures S4A–S4C.

*p < 0.05, **p < 0.01, ***p < 0.001. NS, not significant.

Mice injected with BrdU were sacrificed 2 hours later.
Distinct Gene Expression Profile in Small Intestinal T Cells from HMb Mice

A

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<th>SPL</th>
<th>MLN</th>
<th>LP</th>
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<tbody>
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<td>HMb</td>
<td>8</td>
<td>3</td>
<td>52</td>
</tr>
<tr>
<td>MMb</td>
<td>12</td>
<td>27</td>
<td>241</td>
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Figure 5. Distinct Gene Expression Profile in Small Intestinal T Cells from HMb Mice

- Strong induction of T cells sorted from spleen (SPL), MLNs, and small intestinal LP. Data are mean values from three to five independent experiments. Numbers indicate genes showing 2-fold difference in expression between groups; red numbers indicate overexpression and blue numbers underexpression.

- SFB lack virulence factors and may depend largely on the host for amino acids and essential nutrients.

- Heatmap (right) shows differentially expressed genes in CD4+ T cells from small intestinal LP of GF mice versus MMb mice (y axis) and MMb mice (x axis) (left). A heatmap (right) shows differentially expressed genes in CD4+ T cells from small intestinal LP of GF mice versus MMb mice (y axis) and MMb mice (x axis) (left).

- Genes for IFN-γ (Ifng), IL-10 (Il10), IL-17F (Il17f), IL-22 (Il22), and IL-21 (Il21) were detected with qPCR.

- In an SFB-specific qPCR assay for 16S rDNA probe (SFB probe), we found that all HMb mice (and the HMb inoculum) were negative for SFB (lack of SFB DNA). In contrast, all MMb mice (and the MMb inoculum) carried SFB DNA. In an SFB-specific qPCR assay for 16S rDNA probe (SFB probe), we found that all HMb mice (and the HMb inoculum) were negative for SFB (lack of SFB DNA). In contrast, all MMb mice (and the MMb inoculum) carried SFB DNA.

- IFN-γ (Ifng), IL-10 (Il10), IL-17F (Il17f), IL-22 (Il22), and IL-21 (Il21) were detected with qPCR.

- Figure S5F. *p < 0.05, MMb versus GF; **p < 0.05, HMb versus GF; ***p < 0.05, HMb versus MMb.

- This figure shows the distinct gene expression profile in small intestinal T cells from HMb mice.
Figure 6. SFB Play a Role in Rescuing Intestinal T Cell Numbers and Exhibit Host Specificity

(A) Abundance of SFB in MMb, HMb, and SFB-monocolonized mice, measured as SFB-specific 16S rDNA copy numbers by qPCR analysis of fecal pellets, is shown. Inset values indicate number of SFB 16S rDNA copies/ml in inocula. ND, not detected.

(B and C) Absolute T cell numbers in IEL (CD3^+^CD103^+^TCR^b^) and PP (CD3^+^CD4^+^and CD3^+^CD8^+^) compartments of MMb, SFB-monocolonized, and GF mice (B) and HMb mice cohoused with MMb or SFB-monocolonized mice for 4 weeks (C) are presented. In (C), as a negative control, HMb mice were cohoused with HMb mice. See also Figures S6A–S6E.

A) Abundance of SFB measured as SFB-specific 16S rDNA copy numbers by qPCR analysis of fecal pellets.

B) MMb, SFB-monocolonized, and GF mice

C) HMb mice cohoused with MMb or SFB-monocolonized mice for 4 weeks
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Segmented Filamentous Bacteria Only Partially Expand Mouse Intestinal T Cell Numbers

Peyer’s patches

D

E

CD4+RORgt+ T cell #

CD4+Foxp3+ T cell #

1.0x10^6

1.0x10^5

1.0x10^4

1.0x10^3

1000

2000

3000

4000

5000

6000

7000

8000

9000

10000

MMe

SFB

GF

HMb

RORgt+

Foxp3+

***

**

NS

**

NS

NS

Treg phenotype?
MMb Confers Better Protection against Salmonella enterica Serovar Typhimurium Than HMb

(A) Mice colonized with different microbiotas were orally gavaged with Salmonella; the Salmonella load in fecal pellets was measured daily. Mice were sacrificed on day 4 after infection, and the Salmonella load in the spleen was measured. ***p < 0.001.

(B) Cecal sections were stained with hematoxylin and eosin, and disease was scored. ***p < 0.001.

(C) MMb, HMb, SFB, and GF bacterial populations were visualized by Gram staining.
Figure S7. An Overview of the Regulation of the Mouse Small Intestinal Immune System by MMb versus HMb, Related to Table S3

Microbiota: In both MMb and HMb mice, Bacteroidetes were the most dominant phyla followed by Firmicutes, and Proteobacteria in the gut. Within the Bacteroidetes phyla, 34% of OTU (or bacterial species) were shared by both MMb and HMb. Among the OTUs within the Firmicutes, only 1.5% were shared by both MMb and HMb. SFB—a commensal bacterium and member of the Firmicutes—was detected in MMb, but not HMb—exemplifying host specificity within the Firmicutes phyla.

PP, MLN: Compared to MMb mice, HMb mice PP/MLN contains lower absolute numbers of DCs and T cells. The lower rate of T cell proliferation observed in HMb mice could contribute to the lower absolute number of small intestinal T cells in HMb mice. In both MMb and HMb mice, proliferating T cells in PP/MLN upregulate the intestinal homing receptors $\alpha_4\beta_7$ and CCR9.

LP: Proliferating T cells in the PP/MLN empty into the thoracic duct, enter the bloodstream, and exit into the gut mucosa through intestinal venules in the small intestinal LP where MAdCAM and CCL25—ligands of $\alpha_4\beta_7$ and CCR9, respectively—are expressed. No differences were observed in the expression of MAdCAM and CCL25 between MMb and HMb mice. Due to low numbers of proliferating T cells originating from the HMb mice PP/MLN, the HMb mice small intestinal LP have lower numbers of T cells compared to MMb mice LP. A lower number of secretory IgA$^+$ cells were observed in the LP of HMb mice compared to the same compartment in MMb mice.

Epithelium: Low number of T cells in the small intestinal LP of HMb mice can lead to lower numbers of IELs embedded in the HMb mice epithelium, compared to the MMb epithelium. MMb mice epithelial cells, but not HMb epithelial cells, upregulate RegIII$^g$—an antimicrobial peptide. The expression of chemokines CCL20, CCL28, and CXCL9 are induced by MMb, but not HMb.
Discussion

• MMb and HMb mouse gut microbiotas are similar in relative abundances of the major bacterial phyla but have substantial differences at the OTU level, particularly among Firmicutes.

• Colonization with HMb results in an immature adaptive and innate intestinal immune system, most notably in the small intestine.

• The lack of difference between MMb and HMb mice in large intestinal LP CD3+ and abTCR IEL numbers suggests that the microbiota regulates the small and large intestinal immune compartments via distinct mechanisms.

• The absence of the “right” gut microbes may conceivably shift the balance toward disease in individuals genetically predisposed to autoimmune diseases.
LETTER

Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/− mice

Suzanne Devkota¹, Yunwei Wang¹, Mark W. Musch¹, Vanessa Leone¹, Hannah Fehlner-Peach¹, Anuradha Nadimpalli¹, Dionysios A. Antonopoulos², Bana Jabri¹ & Eugene B. Chang¹

Markus Geuking
July 6th, 2012
Introduction

• IBD and other immune-related human disorders are relatively ‘new’ diseases in that their incidence has increased considerably over the past half century, matching developments in cultural westernization.

• The rapidity of these developments are probably not caused by genetic drift, but by exposure to non-genetic factors introduced through changes in the diet and lifestyle of genetically susceptible individuals, triggering aberrant host responses that lead to IBD.

• Are certain dietary fats present in Western diets capable of precipitating colonic inflammation through their actions on the enteric microbiota of genetically susceptible hosts?

• The Deltaproteobacteria, *Bilophila wadsworthia*, is a sulphite-reducing, immunogenic microbe that is difficult to detect in healthy individuals, but emerges under pathological conditions such as appendicitis and other intestinal inflammatory disorders.
High-fat diets decrease the richness and diversity of intestinal microbiota in wild type C57BL/6 mice.

Supplementary Figure 1. High-fat diets decrease the richness and diversity of intestinal microbiota in wild type C57BL/6 mice. C57BL/6 mice were fed a low-fat (LF), saturated milk fat (MF) or polyunsaturated safflower oil (PUFA) diet for 24 days. The rarefaction curve is based on 454-based DNA sequencing of 16S rRNA gene libraries from cecal contents and depicts the number of unique operational taxonomic units (OTUs) binned per sample with each line representing sequences derived from one mouse. The slope of the line is directly related to the number of unique OTUs (i.e. increased diversity) in a sample.
Saturated MF-induced colitis is associated with bloom of *B. wadsworthia* in Il10-/- mice

**a** On diet for 24 weeks

<table>
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<tr>
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<th>LF</th>
<th>PUFA</th>
<th>MF</th>
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<tr>
<td>Firmicutes</td>
<td>12% ± 4%</td>
<td>44% ± 4%*</td>
<td>52% ± 5%*</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>88% ± 5%</td>
<td>56% ± 4%*</td>
<td>5% ± 1%†</td>
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<tr>
<td>Deltaproteobacteria (<em>B. wadsworthia</em>)</td>
<td>6% ± 1%†</td>
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**b** Incidence of colitis (%)

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<td>24</td>
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<td>60</td>
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**c** Hematoxylin and eosin staining of distal colon (bottom). Scale bars, 400 µm.

**d** IL-10-/- P < 0.0001

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<tr>
<th></th>
<th>LF</th>
<th>PUFA</th>
<th>MF</th>
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<tbody>
<tr>
<td>IFN-γ</td>
<td>10</td>
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<td>1</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>15</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>20</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>IL-6</td>
<td>20</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>IL-23</td>
<td>20</td>
<td>15</td>
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**e** Inflammatory mucosal cytokine levels in the colon.

**f** qPCR for dissimilatory sulphite reductase A gene.

**g** Relative fold change (dsrA).

**LF:** low fat, **MF:** saturated milk fat, **PUFA:** polyunsaturated safflower oil

These observations suggest that the bloom of sulphite-reducing Deltaproteobacteria, particularly *B. wadsworthia*, is associated with colitis in hosts that are genetically susceptible (or have compromised mucosal barrier function (DSS model, not shown).
**B. wadsworthia** mono-association of GF IL-10−/− (5 weeks)

Mono-association of germ-free IL-10−/− mice with **B. wadsworthia** that were consuming either LF, PUFA or MF.

Polyclonal activator of all T cells?

IL-17A?

**B. wadsworthia**

vs

**L. murinus**
B. wadsworthia flourishes in the presence of taurine-conjugated (TC, taurocholic acid) bile acid (a property from which it got its name), a rich source of organic sulphur, which is used as the terminal electron acceptor of the electron transport chain resulting in the formation of H2S as a by-product.

LF: low fat, MF: saturated milk fat, PUFA: polyunsaturated safflower oil

**Induction of TC by MF promotes bloom of B. wadsworthia (all in SPF IL-10^{-/-})**

**Figure 3**

TC or GC daily for 3 weeks while on LF diet

**A gene unique to sulphite-reducing bacteria of which B. wadsworthia** encoding gene from caecal-derived DNA using universal primers, merase chain reaction (qPCR) of the dissimilatory sulphite reductase (dsrA) gene. (a) Percentage taurorocholate (TC) of total bile. Diet-derived bile. (b) B. wadsworthia growth curve. (c) Glycocholic acid and Taurocholic acid. (d) Relative fold change in the abundance of cecal contents. (e) Colitis score. (f) Representative histological sections of the colon at 5x magnification stained with haematoxylin and eosin. (g) Percentage of CD4+ cells producing IFN-γ, and number of IFN-γ-producing CD4+ T cells. (h) Percentage of CD4+ T cells producing IL-17, and number of IL-17-producing CD4+ T cells. (i) Percentage of CD4+ T cells producing IL-23, and number of IL-23-producing CD4+ T cells. (j) Percentage of CD4+ T cells producing IL-6, and number of IL-6-producing CD4+ T cells. (k) Percentage of CD4+ T cells producing IL-2, and number of IL-2-producing CD4+ T cells.
Mono-association with B. wadsworthia in GF Il10⁻/⁻ mice is successful only if accompanied by TC gavage
Discussion

• Bile formation is unique to vertebrates, providing the host with the ability to digest and utilize a far greater variety of dietary substrates.

• The dependence of *B. wadsworthia* on diet-induced taurocholic acid might be representative of how certain gut microbes use bile to their advantage.

• Bile also has potent antimicrobial properties that can contribute to the selection or exclusion of many potential gut microbiota.

• Several intestinal pathogens, including protozoa such as *Giardia, Microsporidia* and *Cryptosporidium*, and bacteria such as *B. wadsworthia, H. hepaticus* and *Listeria monocytogenes*, are not only bile-resistant, but highly favoured in the presence of bile.

• This may be due to suppression of symbiotic, commensal microorganisms, allowing pathobionts and pathogens an opportunity to establish a niche in the intestine.

• Once established, the by-products of these bacteria, whether H2S or secondary bile acids, can serve as gut mucosal ‘barrier breakers’, allowing for increased immune-cell infiltration and thus acting synergistically with the bacterial antigen-specific immune response to induce tissue damage.

• In genetically susceptible hosts, this development has the capacity to tip a compensated state of immune balance in favour of chronic disease.