Humoral Immunity in the Gut Selectively Targets Phenotypically Virulent Attaching-and-Effacing Bacteria for Intraluminal Elimination

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Pathogenicity

> nutrients
> exploit the host machinery
> circumvent host defense

Colonization
Invasion

Expression of bacterial virulence?
Background

EHEC
EPEC
C. rodentium

Kaper et al, 2004
Deng et al, 2001
Aim
- how is the elimination of LEE virulence mediated?

Background

*C. rodentium*
- TLR signaling clearance + damage reduction
- IL-22 prevention of systemic spreading
- humoral immunity clearance + limitation of systemic spread

Kamada et al, 2012
Adaptive immunity is required for the clearance of C. rodentium

Aim To address the role of the adaptive immunity in C. rodentium infection

Figure 1
Adaptive immunity is required for LEE virulence downregulation during C. rodentium infection

Aim: To assess whether the adaptive immunity regulates LEE virulence
Adaptive immunity is required for the clearance of the C. rodentium

Figure 2

Histological analysis of the distal colon of SPF and GF WT or Rag1−/− mice infected with C. rodentium on day 14 post-infection. Arrows denote marked submucosal edema and infiltration of acute inflammatory cells (magnification 250X). Arrow heads in high power fields (magnification 1000X) show bacterial colonies that had invaded the mucosa.

Figure S1. Kamada N. et al.

Figure S1
LEE virulence is required for the demise of the \textit{Rag1}^{-/-} mice

![Figure 2](image1.png)

**Figure 2**

![Figure 2A](image2.png)

**Figure 2A**

![Figure S2](image3.png)

**Figure S2**
The presence of commensals is critical for the elimination of C. rodentium.
Use of a QM-mouse to avoid the production of specific antibodies

**Figure S3**

Pathogen-specific antibodies are found in the intestinal lumen of *C. rodentium*-infected mice. GF WT mice were infected orally with $1 \times 10^9$ cfu of *C. rodentium*. Production of total IgG, IgM, and IgA against *C. rodentium* in the luminal content of GF mice before (d0) and after (day 12 and 22) oral infection with *C. rodentium* was analyzed. Dots represent individual mice. ***, p<0.001; N.S., not significant by Dunnett's test.**

**Figure 3**

Use of a QM-mouse to avoid the production of specific antibodies.
Pathogen-specific IgG is required for downregulation of LEE expression.
Pathogen-specific IgG selectively bind virulent bacteria

Fig. S4, related to Fig. 4.

C. rodentium infection induces Ler-regulated virulence factor-specific IgG.

A, GF WT mice were infected with GFP-expressing C. rodentium. Cecal bacteria were harvested at indicated days post-infection and binding of IgG was analyzed by flow cytometry. Results are representative of 3 experiments.

B, Purified IgG from the sera of C. rodentium-infected mice (day 42 post-infection), or control IgG was added to the reporter ler-lux C. rodentium strain in DMEM medium and expression of ler overtime was determined by luminescence (left panel). RLU, relative light unit. Bacteria grown in DMEM (positive control) or LB medium (negative control) are shown for comparison. Bacterial growth in DMEM or LB medium was assessed in parallel (right panel). Data are representative of 2 independent experiments in triplicate cultures (mean ± SD).

C, Bacterial lysates of WT and ler mutant C. rodentium were loaded with SDS-PAGE. Serum or luminal content were obtained from naïve (d0) and C. rodentium-infected (d21) SPF mice, and used as primary antibodies. C. rodentium-specific IgG was detected by anti-mouse IgG secondary Ab. Results are representative of 2 independent experiments.

D, Purified intimin protein was loaded with SDS-PAGE (left), and blotted with luminal content obtained from naïve (d0) and C. rodentium-infected (d21) GF mice. C. rodentium-specific IgG was detected by anti-mouse IgG secondary Ab. Results are representative of 2 independent experiments.
Specific IgG are produced in response to the virulence factors of C. rodentium. Fig. S4, related to Fig. 4.

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There are 2 phenotypically different C. rodentium populations

Figure 5
Neutrophils are required for the elimination of phenotypically virulent C. rodentium

**Figure S5, related to Figure 6.** Impaired eradication of C. rodentium in LysM Cre Mcl1 fl/fl chimeric mice.

A, GF WT mice were infected with C. rodentium. At day 7 post infection, cecal tissues were harvested and fixed with Carnoy's to preserve mucus layer. Luminal neutrophils were assessed by H&E staining. Inset denotes intraluminal neutrophil surrounded by C. rodentium. 

B, Confirmation of neutrophils depletion in LysM Cre Mcl1 fl/fl chimeric mice. Cells were isolated from the peripheral blood of LysM Cre Mcl1 wt/wt or LysM Cre Mcl1 fl/fl chimeric mice. Isolated cells were stained with CD45, CD11b, Ly6G and DAPI, and analyzed by flow cytometry. Percentage of neutrophils (CD45+ CD11b+ Ly6G hi) and monocytes (CD45+ CD11b+ Ly6G lo) is indicated. Data are representative of 4 individual mice.

C, LysM Cre Mcl1 wt/wt or LysM Cre Mcl1 fl/fl chimeric mice (n= 12; Mcl1 wt/wt, n=9; Mcl1 fl/fl) were infected orally with 1x10^9 cfu of C. rodentium and pathogen load in feces was determined over the indicated time. Data points are given as median. † denotes bacterial loads could not be determined beyond this time due to mouse lethality.

D, LysM Cre Mcl1 wt/wt or LysM Cre Mcl1 fl/fl chimeric mice were infected orally with 1x10^9 cfu of C. rodentium. Production of C. rodentium-specific IgG in the serum and the luminal content of GF mice was analyzed before (d0) and after (day 7) infection. Dots represent individual mice. *, p<0.05; **, p<0.01; N.S., not significant by Dunn's test.

**Figure S5.** Kamada N. et al.
Discussion

• Adaptive immune system is necessary for the survival of the animal in absence of other competing bacteria
• The presence of virulent and avirulent subpopulations contributes to the virulence stability
• LEE virulence is microbiota independent but its activation mechanism is not known
• IgG are generated against LEE virulence factors but not against other avirulent surface antigens: the mechanisms are not known
• Intimin is a highly antigenic protein
• The way of IgG to the lumen is not known
• Other component from the adaptive immune system may be critical for the outcome
The Bamboo-Eating Giant Panda Harbors a Carnivore-Like Gut Microbiota, with Excessive Seasonal Variations

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Background

- belong to the family of the Ursidae
- started to eat bamboo 7 million years ago (MYA)
- exclusively bamboo-eating species 2-2.4 MYA

- genetic: pseudogenization of umami taste receptor gene
- anatomic: powerful jaws and teeth, enlarged pseudothumb
  But harbor a carnivore-like gut!

symbiotic gut microbes?
<table>
<thead>
<tr>
<th>Herbivores bacteria</th>
<th>Carnivores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroidales, Clostridiales, Fibrobacterales, Spirochaetales</td>
<td>Enterobacteriaceae, Enterococcus</td>
</tr>
</tbody>
</table>
## Study design

| Sampling       | 121 fecal samples from giant pandas  
|               | 24 adults, 16 juveniles, 5 unweaned cubs  
|               | 12 males, 21 females completed the whole trial |
| Timecourse     | 3 seasons: Spring (T1), Summer (T2), late autumn (T3) |
| Sequencing     | barcoded pyrosequencing of V3 region from 16S rRNA genes |
| Dataset        | 92’819 usable reads corresponding to 781 OTUs |
Technical features of the dataset

Figure S1

A. OTU-level α-diversity of the giant panda population. (A to D) Raftation curves of T1 (A), T2 (B), T3 (C) and cub samples (D), respectively. (E to H) Shannon diversity index curves of T1 (E), T2 (F), T3 (G) and cub samples (H), respectively.

Legend:
- Pink: Adults
- Blue: Juveniles
- Green: Cubs
Overall gut microbiota structure in giant pandas

Figure 1
Seasonal and age-dependent variation of the alpha diversity

**Figure 2**

A: Boxplot showing the number of observed OTUs for different age groups (Adults, Juveniles, Cubs) across three time points (T1, T2, T3). Significance levels are indicated by asterisks (*** for p < 0.001, * for p < 0.05).

B: Boxplot showing Shannon diversity indices for the same age groups across the same time points. Significance levels are indicated by asterisks (** for p < 0.01, * for p < 0.05).
Interseasonal variation in species abundance

Figure S2 Abundance variations in the ten dominant genera (>1% of total sequences), Escherichia/Shigella (A), Klebsiella (B), Streptococcus (C), Lactococcus (D), Lactobacillus (E), Weissella (F), Enterococcus (G), Clostridium sensu stricto (H), Clostridium XI (I), and Turicibacter (J) across seasons, compared by paired sample Wilcoxon signed rank test. *P < 0.05, **P < 0.01, ***P < 0.001 (with Bonferroni post-hoc test). Only the 33 individuals that were sampled in all three seasons are included. See Figure 2 for definition of box and whisker plot.
Inter- and intraindividual variation

A

B

Figure 3
Comparison of gut microbiota structure of pandas with other mammals

Studies
- This study
  - 8 captives, 8 wild giant pandas
  - 3 other data sets with mammals

Animals
- 128 animals, 57 species from 13 different taxonomic orders

Sequences
- 229’288 sequences

Figure S5

The gut microbiota of the captive and wild giant pandas ($n=137$) had lower number of observed OTUs (A) and Shannon diversity indices (B) compared with other herbivores ($n=66$), omnivores ($n=26$) and carnivores ($n=16$) by Mann-Whitney test (with Bonferroni post hoc test). See Figure 2 for definition of box and whisker plot. NS (not significant; $P>0.05$).
Comparison of gut microbiota structure of pandas with other mammals

Figure 4

A: Weighted UniFrac

B: Unweighted UniFrac
Comparison of gut microbiota structure of pandas with other mammals: new samples

Figure S7

**Weighted UniFrac**

- PCoA 1 (25.64%)
- PCoA 2 (13.67%)

**Unweighted UniFrac**

- PCoA 1 (10.57%)
- PCoA 2 (8.12%)

- Giant panda (this study)
- Newly sampled giant panda
- Newly sampled herbivores
- Newly sampled omnivores
- Newly sampled carnivores
- Black bear
- Golden snub-nosed monkey
- Hoolock gibbon
- Blue peafowl
- Chimpanzee
- Siberian tiger
- Hoolock gibbon
- Blue peafowl

**Comparison of gut microbiota structure of pandas with other mammals: new samples.**

Newly sampled herbivores
Newly sampled omnivores
Newly sampled carnivores
Newly sampled giant panda
- Blue peafowl
- Hoolock gibbon
- Chimpanzee
- Black bear
- Golden snub-nosed monkey
- Siberian tiger

**PCoA** score plots based on the weighted and unweighted UniFrac distances are shown.
Phylotypes involved in the deviation of panda gut microbiota from the non-panda herbivores

Figure 5
Identification of putatively cellulolytic bacteria

Figure S8
- No adaptation of the microbiota to the lifestyle, and this over 2 Mio years
- Low microbial diversity <-> fragile lifestyle?
- intra- >> interindividual variation reflects the stability of the ecosystem
- Physiological mechanisms remain to be explained