GUT MICROBIOTA

Outrunning *Salmonella* – a role of endogenous *Enterobacteriaceae* in variable colonization resistance

The mammalian gut microbiota confers colonization resistance against pathogenic bacteria. Specific pathogen-free C57BL/6 mice from different vendors are variably resistant to oral non-typhoidal Salmonella infection. New work shows that differences in endogenous Enterobacteriaceae determine this phenotypic variability.

Siegfried Hapfelmeier-Balmer

The mouse model of non-typhoidal *Salmonella* infection is a prime example of the microbiota-related phenotypic variability of mouse models. As first demonstrated by studies in which antibiotics were administered to mice, an intact gut microbiota confers resistance against intestinal colonization of non-typhoidal *Salmonella* and subsequent intestinal and systemic infection. More recent work has established that *Salmonella* actively induces microbiota alterations to shape a favorable intestinal niche. Acute *Salmonella*-induced intestinal inflammation abolishes colonization resistance [2], by specifically generating electron acceptors for the anaerobic respiration of *Salmonella* while damaging the integrity of the competing anaerobic gut consortia [3]. However, *Salmonella* has first to reach a critical intestinal density to induce the acute inflammatory disease. Bäumler and colleagues have worked out previously that the early intestinal bloom of *Salmonella* is fueled by aerobic respiration, which is enhanced by virulence factor-induced microaerophilic conditions [4]. Recent experimental evidence supports that commensal facultative aerobic bacteria including *Enterobacteriaceae* have an important protective function by blooming under the same microaerophilic conditions and outcompeting *Salmonella* [5,6] (Fig. 1).

Velazquez and colleagues [7] now show that endogenous facultative aerobes also underlie the variation in susceptibility to *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) of unmanipulated specific pathogen-free (SPF) mice from different vendors. Through transplantation of SPF microbiotas into germ-free mice, they prove that gut microbiota rather than host genome variability between the different C57BL/6 substrains explains their variability in susceptibility to oral *Salmonella*. Using a combination of unbiased DNA sequencing-based microbiome profiling and selective culture techniques, the authors found a strong correlation between *Salmonella* resistance and the abundance of *Enterobacteriaceae.* The authors also confirmed causality, according to Koch's postulates, by showing that inoculation of *Salmonella*-susceptible mice with *Enterobacteriaceae* isolated from more highly colonization resistant mice conferred resistance to the susceptible mice. Finally, they carried out a similar experiment using the well-characterized probiotic *E. coli* strain Nissle. Wild-type *E. coli* Nissle, but not an isogenic mutant deficient for aerobic respiration under microaerophilic conditions, conferred resistance. Although the authors did not make isogenic mutants of murine *Enterobacteriaceae* isolated in the present study, this result strongly suggests that a similar metabolic mechanism underlies the effect of the relevant endogenous *Enterobacteriaceae*. Velazquez and colleagues focused their mechanistic studies on the *Enterobacteriaceae*; however, they found that the *Deferribacteriaceae* family was also positively associated with *Salmonella* resistance. Interestingly, a recent study by Stecher and colleagues independently showed that the Altered Schaedler Flora strain *Mucospirillum schaedleri*, a representative of the *Deferribacteriaceae*, contributes to protection from *S.* Typhimurium by competing for terminal electron receptors [8].

Outstanding questions remain. How generalizable are these findings with regards to other infectious diseases? Head-to-head comparisons of this model and other infection models, for example of the facultative aerobes *Citrobacter rodentium* and *Vibrio cholerae*, are currently lacking. How relevant is this mouse study for human biology or veterinary medicine? *E. coli* is commonly viewed as an important human, but not murine, commensal/symbiont, and hence translatability of *E. coli* work in the mouse model is sometimes questioned. Most well-studied *E. coli* strains (including Nissle) are of human origin, whereas murine isolates and their mouse-specific adaptations remain poorly characterized. A more general point is that the gut microbiota composition of laboratory mice is overall very different from feral mice, and has been made partly responsible for the phenotypic differences between human and laboratory mouse immunity and disease resistance [9,10]. Commonly used inbred laboratory mouse lines, such as C57BL/6 or BALB/C, were separated from free-living populations many decades ago, and since then have been maintained in fairly closed and artificial environments, with monotonous laboratory rodent diet, and with limited or no (in strict barrier facilities) input of environmental microbes, in particular of murine origin. Intestinal species diversity loss and transfer of human microbial contaminants (from animal caretaker personnel) might therefore be hallmarks of lab rodent microbiota evolution. Maintaining mice in barrier facilities of "optimal" hygiene status certainly helps to prevent infection of experimental animal stocks with known and unknown pathogens. However, the resulting loss of symbiotic species is usually not actively compensated.

The work of Velazquez and colleagues has two additional implications in biomedical research areas that can be affected by microbiota-related phenotypic variability. First, microbiota variability in lowly abundant taxa might underlie phenotypic variability and escape detection by microbiota compositional analyses of limited depth or inappropriate design. Second, the study of Velazquez *et al.* exemplifies that it can be difficult to clarify whether host genome or microbiome contribute mainly to a biological phenotype: as shown, simple co-housing of adult animal cohorts is often ineffective at equalizing the gut consortia, whose ontogeny is subject to early life ecological successions. The current gold standards are fecal microbiota transplantation into germ-free mice or embryo transfer derivation of a mouse line. Littermate control breeding and litter swap experiments in which the animal lines to be compared are nursed by the same parents are less rigorous approaches to equalize microbiota. The growing number of biological functions and diseases shown to be influenced by the microbiota underscores that continued efforts are needed to better standardize not only the genomes, but also the microbiomes of experimental animals to improve reproducibility in biomedical research. The control of host genome variability is already standard today, as most researchers rely on isogenic inbred rodent lines; the standardization of microbiome studies, by contrast, is still in its infancy. Gnotobiotic animals, generated from germ-free animals by colonization with defined bacterial species, are a powerful tool to control the microbiota [5]. However, besides facing the issues of costs and infrastructure availability, we are still far from having available gnotobiotic models that fully reproduce the functional complexity of natural microbiomes.

Siegfried Hapfelmeier-Balmer
Institute for Infectious Diseases, University of Bern, Bern, Switzerland
e-mail: siegfried.hapfelmeier@ifik.unibe.ch

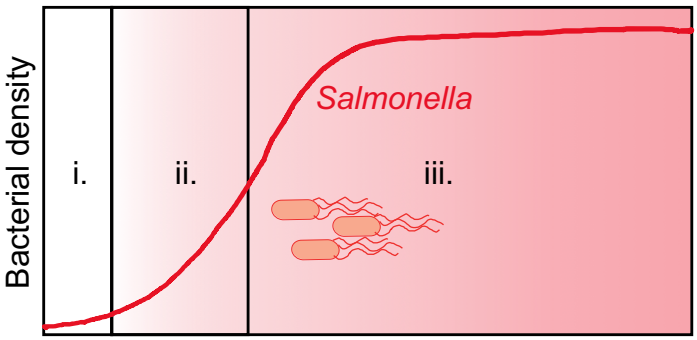
Fig 1| . Protective Enterobacteriaceae bloom. a, Microbiota lacking Enterobacteriaceae. *Salmonella* overgrowth (red curve) in the progressively inflamed gut is fueled by microaerophilic and anaerobic respiration. **b**, Microbiota containing Enterobacteriaceae. A bloom of endogenous commensal Enterobacteriaceae (green curve), also driven by microaerophilic respiration, might outcompete *Salmonella* (red curve).

References

1. Barthel, M. et al. *Infect. Immun*. **71**, 2839–2858 (2003).
2. Stecher, B. et al. *PLoS Biol.* **5**, 2177–2189 (2007).
3. Rivera-Chávez, F. & Bäumler, A. J. *Annu. Rev. Microbiol*. **69**, 31–48 (2015).
4. Rivera-Chávez, F. et al. *Cell Host Microbe* **19**, 443–454 (2016).
5. Brugiroux, S. et al. *Nat. Microbiol.* **2**, 16215 (2016).
6. Litvak, Y. et al. *Cell Host Microbe* **25**, 128–139.e5 (2019).
7. Velazquez, E. M. et al. *Nat. Microbiol.* **4**, 1057–1064 (2019).
8. Herp, S. et al. *Cell Host Microbe* **25**, 681–694.e8 (2019).
9. Beura, L. K. et al. *Nature* **532**, 512–516 (2016).
10. Rosshart, S. P. et al. *Cell* **171**, 1015–1028 (2017).

Microbiota lacking *Enterobacteriaceae:*

i. Anaerobe phase (fueled by what? No inflammation yet)
ii. Microaerobic respiratory phase (mild inflammation)
iii. Anaerobic respiratory phase (acute inflammation)



Microbiota containing *Enterobacteriaceae:*

i. Anaerobe phase (limited by what?)
ii. Microaerobic respiratory phase (mildly inflamed) and Outcompetition by *Enterobacteriaceae*

i. Anaerobe phase (baseline colonization)
ii. Microaerobic respiratory phase (protective bloom)
iii. Recovery of anaerobiosis and return to homeostasis