Linking microbiota composition with antimalarial antibody response

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Abstract

Microbiota composition recently arose as a factor correlating with malaria infection. Mandal *et al.* showed via cecal transplant and antibacterial treatment that the mouse microbiota modulates parasitemia by affecting spleen germinal centers where B-cells are matured. They further identified correlations between microbiota composition and malaria severity in Ugandan children.

In mammals, the gut microbiota shapes both mucosal and systemic immunity, impacting the outcome of infections, even those that take place distally from the gut. Malaria infections represent an interesting working model on this regard, as the causing *Plasmodium* parasites primarily infect the liver and blood of the mammalian host, without colonizing the gastrointestinal lumen. *Plasmodium* parasites are transmitted by *Anopheles* mosquitoes. After being introduced into the skin during a bite, parasites travel to the liver where they multiply inside hepatocytes. The new progeny released in the bloodstream will then cyclically infect and proliferate in red blood cells. Antibodies against *Plasmodium* parasites, which are produced by germinal centers in the spleen, are critical to control infection.

Plasmodium infection has recently been found to correlate with microbiota composition in mice [1,2]. These correlations were linked to the presence of specific bacteria [1] or to a gut microbiota-dependent production of *Plasmodium*-specific antibodies in germinal center immune cells [3]. To further investigate these associations, Mandal and collaborators took advantage of a mouse model (strain C57BL/6) purchased from two different vendors (Tac, for *Taconic Biosciences*, and CR, for *Charles River Laboratories*; [4]). These mice represent a unique system to study associations between gut microbiota composition and malaria infection, as they are characterized by a distinct response to *Plasmodium yoelii* 17XNL, with Tac mice efficiently restricting parasitemia and CR being more permissive [2]. Using cecal transplants, Mandal *et al.* showed that the CR enterotype (i.e. microbiota composition) "dominates" over the Tac one: after cecal transplants from CR to Tac, mice shift from a Tac to a CR enterotype, but after a transplant from Tac to CR, mice keep a CR-enterotype. Accordingly, Tac mice receiving CR caecum content exhibit higher parasitemia, but not vice versa (**Figure 1A**).

Mandal and colleagues then perturbed the microbiota by providing mice with five alternative antibiotic treatments. They observed a strong decrease in parasite burden when

48 treating CR susceptible mice with these antibiotics, which links the bacterial component of the gut
49 microbiota to *P. yoelii* infection. Using vancomycin, an antibiotic that does not cross the gut
50 barrier, at different time points prior to or after *Plasmodium* infection, they linked microbiota
51 perturbation with efficiency of parasite clearance. First, they found that antibiotic treatment
52 several weeks before infection reduced *P. yoelii* parasitemia in CR mice and protected animals
53 against experimental cerebral malaria upon subsequent *Plasmodium berghei* ANKA infection.
54 Second, treatment 7 days after infection was still able to significantly reduce *P. yoelii* infection
55 burden, indicating that microbiota perturbation can rapidly influence defense against infection.
56 Finally, to investigate connections between microbiota and immunity, the authors examined
57 germinal center reactions in CR mice treated with vancomycin. They found that vancomycin
58 treatment induced the production of CD4+ follicular helper T cells and germinal center B cells.
59 Disruption of germinal center functions mitigated the effect of antibiotic-treatment on immune
60 cells and on parasitemia (**Figure 1B**).

61 The authors also assessed correlations between malaria infection and microbiota
62 interactions in a small cohort of children in Uganda. They found different bacteria compositions in
63 the asymptomatic *Plasmodium falciparum*-infected patients, the infected with severe malarial
64 anemia and the non-infected children. They did not identify any bacteria that appear protective
65 against severity, but rather found that the frequency of specific bacteria, such as *Flavonifractor*
66 *plautii*, *Escherichia coli* and *Faecalibacterium prausnitzii* positively correlated with severe malaria.
67 This profiling allowed them to build a supervised machine learning model that predicted *P.*
68 *falciparum* status with 74% accuracy.

69 This work sheds new light into the interplay between microbiota and immune system. It
70 demonstrates that bacteria, rather than fungal, eukaryotic, or viral components of the microbiota,
71 are responsible for the observed effects on parasitemia in mice, as antibacterial treatment fully

recapitulates the impact of cecal transplant. By providing an intriguing example of antibiotic treatment strengthening the immune system, this study may have important implications in humans, especially in malaria-endemic regions where antibiotics are often prescribed to treat non-specific febrile symptoms or used as self-medication [5,6]. An association between malaria infection and microbiota composition in humans has been described in few independent studies [1,7]. Such correlations may be explained both by an influence of specific bacteria on host immunity affecting malaria infection [1], as highlighted in Mandal *et al.*'s study, and by the alteration of host physiology by *Plasmodium*, promoting colonization by specific bacteria [8,9].

On an experimental perspective, this work further highlights that besides host and parasite genetics, the host microbiota can strongly influence the outcome of malaria infections in the laboratory. Microbiota, which is directly related to host diet and nutrition, should be monitored as an additional experimental variable to achieve reproducible and solid results across replicates and laboratories [10]. Reproducibility could be improved by using experimental models with a known microbiota composition, using for instance germ-free mice recolonized with specific microbiota communities.

Our understanding of interactions between gut microbiota and malaria severity is still preliminary and further studies are needed to understand the mechanisms behind these results, to identify bacterial species responsible for these observations and to assess whether these results could be recapitulated in other mouse models or with other parasites. While we are far from discovering the "magic pill" that will make us resistant to infectious diseases, such advances in the study of microbiota-immune system crosstalk may eventually lead to the development of probiotics as living therapeutics for improving human health, even when dealing with pathogens that never infect the gut. This also opens perspectives to personalized medicine, which considers each patient's identity in terms of genome, proteome and metabolism, and which may also

include the microbiota composition as an important parameter to monitor and to link to specific medical conditions.

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Declaration of Interests

The authors declare no competing interests.

References

1 Yilmaz, B. *et al.* (2014) Gut Microbiota Elicits a Protective Immune Response against Malaria Transmission. *Cell* 159, 1277–1289

2 Villarino, N.F. *et al.* (2016) Composition of the gut microbiota modulates the severity of malaria. *Proc National Acad Sci* 113, 2235–2240

3 Waide, M.L. *et al.* (2020) Gut Microbiota Composition Modulates the Magnitude and Quality of Germinal Centers during Plasmodium Infections. *Cell Reports* 33, 108503

4 Mandal, R.K. *et al.* (2021) Dynamic modulation of spleen germinal center reactions by gut bacteria during Plasmodium infection. *Cell Reports* 35, 109094

5 Griensven, J. van *et al.* (2020) Point of care biomarkers to guide antibiotic prescription for acute febrile illness in sub-Saharan Africa: promises and caveats. *Open Forum Infect Dis* 7, ofaa260-

6 Yeika, E.V. *et al.* (2021) Comparative assessment of the prevalence, practices and factors associated with self-medication with antibiotics in Africa. *Trop Med Int Health* DOI: 10.1111/tmi.13600

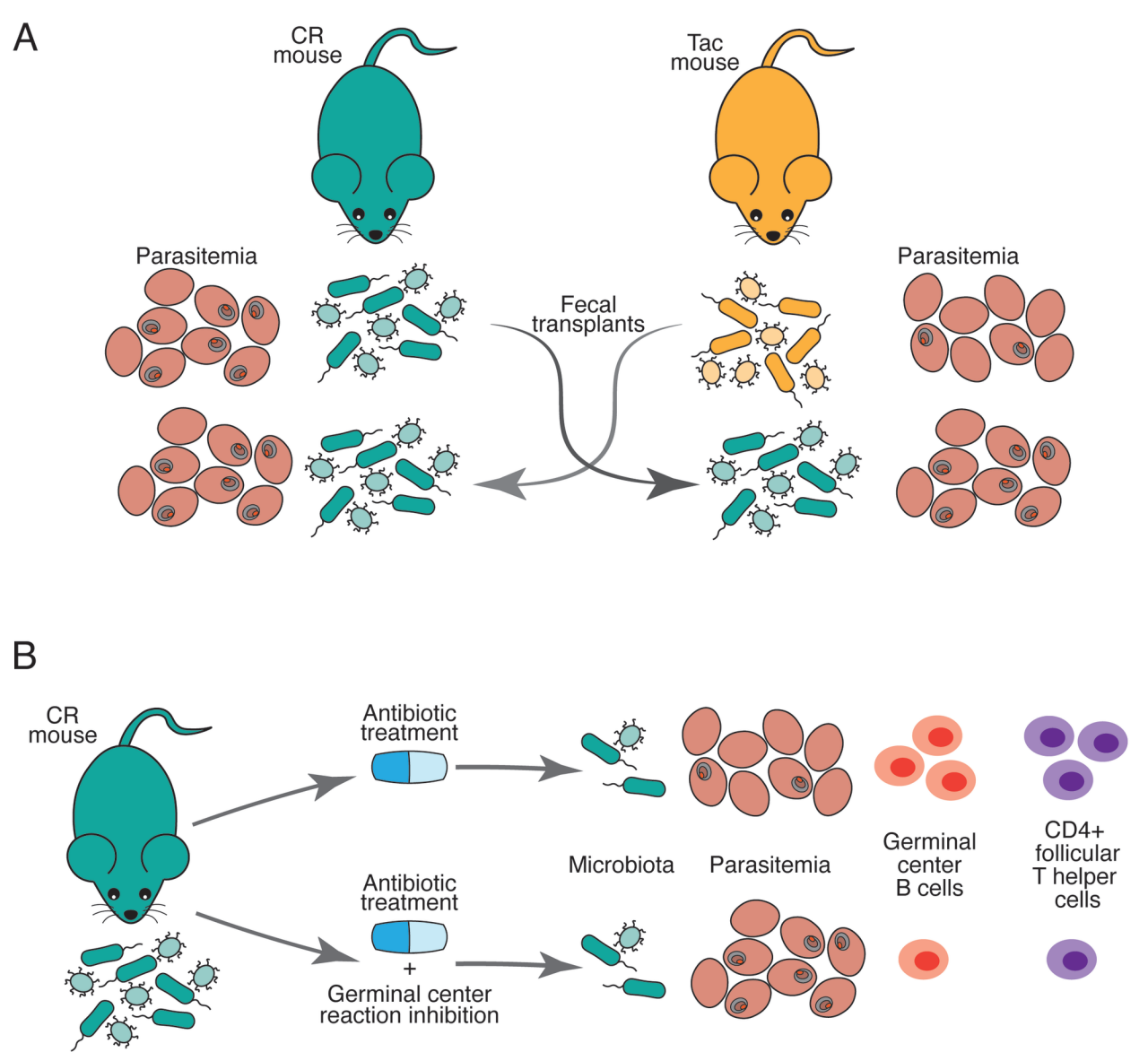
7 Yooseph, S. *et al.* (2015) Stool microbiota composition is associated with the prospective risk of Plasmodium falciparum infection. *Bmc Genomics* 16, 631

8 Walker, G.T. *et al.* (2021) Malaria parasite infection compromises colonization resistance to an enteric pathogen by reducing gastric acidity. *Sci Adv* 7, eabd6232

9 Mukherjee, D. *et al.* (2020) Microbiota, a Third Player in the Host–Plasmodium Affair. *Trends Parasitol* 36, 11–18

10 Mandal, R.K. *et al.* (2020) Temporospatial shifts within commercial laboratory mouse gut microbiota impact experimental reproducibility. *Bmc Biol* 18, 83

Figure 1. The gut bacterial microbiota modulates spleen germinal center reactions during *Plasmodium yoelii* infections. (**A**) Gut microbiota composition differs in mice purchased from two distinct vendors (CR, Charles Rivers Laboratories; Tac, Taconic Biosciences) and correlates with different ability to control parasitemia during *P. yoelii* infection (CR: susceptible to hyperparasitemia; Tac: resistant to hyperparasitemia). Fecal transplants can induce the CR phenotype in Tac mice but not vice versa. (**B**) An antibacterial treatment reduces parasite burden and induces the production of germinal center B cells and CD4+ follicular helper T cells. Disruption of germinal center functions mitigates the effect of antibiotic treatment on immune cells and on parasitemia.


A
CR mouse
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Parasitemia
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CR mouse
Antibiotic treatment
Microbiota
Parasitemia
Germinal center B cells
CD4+ follicular T helper cells
Antibiotic treatment
+
Germinal center reaction inhibition