Catherine Daniel

Gut Bioengineered Models to Study Host-microbiome Direct Interactions

Introduction

The gastrointestinal tract is the main ecological niche in which *Lactobacillus* strains provide health benefits in mammals. There is currently a need to better characterize host-microbe interactions in space and time by tracking these bacteria. To do so, we have set up new bio-imaging tools for directly tracing the spatio-temporal dynamics of lactic acid bacteria at the host intestinal interface and followed two strategies based on (i) bioluminescence-fluorescence live-imaging in mice and (ii) live imaging in advanced biomimetic human gut-on-chip model.

Methods

First, we engineered different luciferase-expressing probiotic strains and demonstrated that the click-beetle red luciferase is the best performing system for noninvasive *in vivo* bioluminescence imaging in healthy anesthetized mice. Then, we developed fluorescent *Lactobacillus* strains and combined noninvasive whole-body imaging with *ex vivo* fluorescence confocal microscopy imaging to monitor the impact of intestinal inflammation on the persistence of orally administered *Lactobacillus* in healthy and inflamed mouse colons.

Results

In healthy mice, we were able to demonstrate differences in gut persistence between various strains after oral administration and we could follow the precise gut localization of the strains over a week. We showed that *in vivo* bioluminescence imaging is more efficient than fluorescence imaging to visualize probiotic strains. However, only fluorescent microorganisms can be directly detected in the intestine of mice by intravital microscopy after oral administration. We showed that an anti-inflammatory *Lactobacillus*, orally administered strain, persists for longer and at higher counts in the inflamed colon than in the healthy colon. Extended orthogonal view projections enabled us to localize individual *Lactobacillus* in sites that differed for healthy *versus* inflamed guts. In healthy colons, orally administered bacteria were localized in the lumen (in close contact with commensal bacteria) and sometimes in the crypts (albeit very rarely in contact with intestinal cells). The bacteria were observed within and outside the mucus layer. In contrast, in more intensely inflamed areas (i.e. where the colon had undergone structural damage), the *Lactobacillus* strains were in direct contact with damaged epithelial cells. Finally, we recently started to investigate the interactions of *Lactobacilli* at the interface of a 3D human intestinal epithelium reconstituted in an organ-on-chip system. Using this novel model, we introduced a low inoculum of fluorescent *Lactobacillus* strains within the intestinal lumen under low shear stress and regular aerobic conditions. Using spinning-disc microscopy, we observed over a 48-hour period that these bacteria stably populate the biomimetic intestine without major morphological damage.

Discussion

Overall, our ability for monitoring microbial spatio-temporal dynamics at tissue levels using *in vivo* and *ex vivo* models allows us to better characterize host-microbiome interactions in the gut.