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Background. *Clostridioides difficile* infections (CDI) result from antibiotic use and cause severe diarrhea (*C. difficile*-associated diarrhea, CDAD) which is life-threatening and costly. A specific probiotic containing *Lactobacillus acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 (Bio-K+) has demonstrated benefits in preventing CDI and has a strong inhibitory effect on the growth of several nosocomial *C. difficile* strains *in vitro*. Many Lactobacilli can inhibit CD growth though lactic acidification. Here, we have investigated novel acid-independent mechanisms by which these strains impair *C. difficile* virulence.

Methods. The hypervirulent strain *C. difficile* R20291 was co-cultured anaerobically with Bio-K+ probiotic strains in various media and glucose concentrations (5 g/L, 3 g/L, 0 g/L), for 24 hours at 37°C. Parameters such as Log CFU, pH, Toxin A and B, cell cytotoxicity were measured. Statistical comparisons using ANOVA one-way was performed in order to determine whether the groups were significantly different.

Results. At 5 g/L glucose, no *C. difficile* toxin was produced and co-culture with these lactobacilli resulted in potent acidification and growth inhibition. At 3 g/L glucose, *C. difficile* toxin production occurred and acidification by the lactobacilli resulted in growth inhibition as well as >99% reduced Toxin A and B production. In the absence of glucose and a starting pH of 7.0, TY broth, the lactobacilli did not acidify the medium and *C. difficile* growth was normal yet Toxin A and B production was partially reduced at, 20% and 41% lower. Toxin B from the supernatant of *C. difficile* grown in TY was cytotoxic to human fibroblast cells, but this was less cytotoxic when co-cultured with the Lactobacilli.

Conclusion. These results suggest that the combination of *L. acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 interferes with *C. difficile* pathogenesis through: 1) inhibition of *C. difficile* growth (via lactic acid secretion), 2) reduced toxin A/B synthesis and (3) toxin neutralization. These results might explain the strain specificity of Bio-K+ probiotic bacteria in potentially preventing *C. difficile*-associated diarrhea in antibiotic treated patients.

Table 1. *In vitro* model of *Clostridioides difficile* R20291 (CD) growth kinetic after 24h in co-culture with or without *Lactobacillus acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 (LB) in RCM (5g/L), BHI (3g/L) and TY (0 g/L) medium. Log_{10} (CFU/mL), pH and toxin A and B concentration (ng/CFU) were measured in all assays. All experiments were carried out in triplicate.

Culture conditions		Log (CFU/mL)		P	pH		P		Toxin A and B ng/CFU after 24h		%, P
		CD	CD+LB	CD vs CD+LB	CD	CD+LB	CD vs CD+LB		CD	CD+LB	CD vs CD+LB
5g /L	0	6.6± 0.2	6.8± 0.5	>0.05	6.4± 0.1	6.3± 0.1	>0.05	A	0	0	N/A, >0.05
	24	7.8± 0.0	0.0± 0.0	<0.05	5.5± 0.0	4.5± 0.1	<0.05	B	0	0	N/A, >0.05
	Δ	+1.2± 0.1	-6.8± 0.2	<0.05	-0.9± 0.1	-1.8± 0.2	<0.05		---	---	---
3 g/L	0	7.0± 0.1	7.0± 0.1	>0.05	6.9± 0.0	6.2± 0.3	<0.05	A	5	0	99%, <0.05
	24	6.6± 0.2	2.8±1.9	<0.05	5.6± 0.1	4.9± 0.1	<0.05	B	3	0	99%, <0.05
	Δ	-0.4± 0.2	-4±2	<0.05	-1.3± 0.1	-1.3± 0.2	>0.05		---	---	---
0 g/L	0	6.9± 0.7	6.9± 0.5	>0.05	7.0± 0.1	7.0± 0.1	>0.05	A	18	14	20%, <0.05
	24	7.7± 0.3	7.4± 0.1	>0.05	6.3± 0.0	6.6± 0.2	>0.05	B	30	18	41%, <0.05
	Δ	+0.8± 0.9	+0.5± 0.4	>0.05	-0.7± 0.1	-0.4± 0.3	<0.05		---	---	---

* indicates values that are significantly different from the monoculture of *C. difficile*, p-value ≤0.05.
% represents the % of reduction of toxin A and B compared to the monoculture of *C. difficile*

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2569. The Gut Microbiome and Acute Graft vs. Host Disease Risk in Hematopoietic Stem Cell Transplantation Recipients

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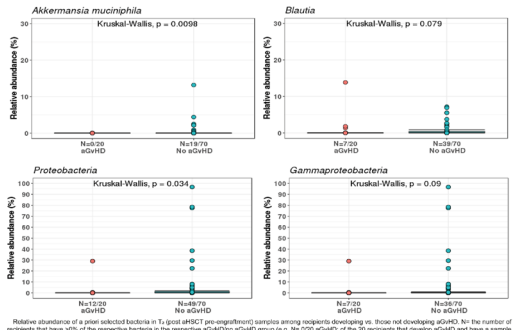
Background. 16S rRNA gene-based studies report that allogeneic hematopoietic stem cell transplant (aHSCT) recipients with low bacterial diversity and certain bacteria close to engraftment have increased risk of developing acute graft-vs.-host disease (aGvHD). Using shotgun metagenomic data, we aim to confirm and extend these observations in a larger cohort.

Methods. Adult aHSCT recipients with stool samples collected days −30 to +100 relative to aHSCT, but prior to aGvHD, were included. One sample was selected per patient and time point: pre-aHSCT (T_1), post-aHSCT pre-engraftment (T_2). Complete ascertainment of aGvHD (grades ≥ 2) until day +100 was performed. Bacterial microbiome factors (α-diversity, gene richness and 16 *a priori* bacteria) and clinical factors were tested for associations with aGvHD across $T_{1:2}$ in univariable models. Significant factors ($P < 0.1$) were included in a multivariable model.

Results. Of 147 aHSCT recipients, 35 developed aGvHD a median of 35 days (IQR 24–51) post-aHSCT. We found that higher gene richness was significantly associated with lower aGvHD risk in T_2, but not T_1, samples (OR 0.65 (95% CI 0.42–1.00), $P = 0.04$ vs. OR 1.14 (95% CI 0.67–1.94), $P = 0.64$ per doubling of unique genes). A decreased abundance of *Akkermansia muciniphila*, *Proteobacteria*, *Blautia* and *Gammaproteobacteria* was observed in those that developed aGvHD, again in T_2 samples only (Figure 1). Among clinical factors, donor sex, donor/recipient (related/unrelated) and conditioning regimen (adjusted OR = 0.34 for non-myeloablative vs myeloablative (95% CI 0.15–0.77)) were significantly associated with aGvHD. Conditioning regimen was also strongly associated with microbiome changes; myeloablative recipients had lower gene richness and differences in bacterial abundance, including decreased abundance of aforementioned bacteria, compared with non-myeloablative recipients at T_2 (Figures 2and 3).

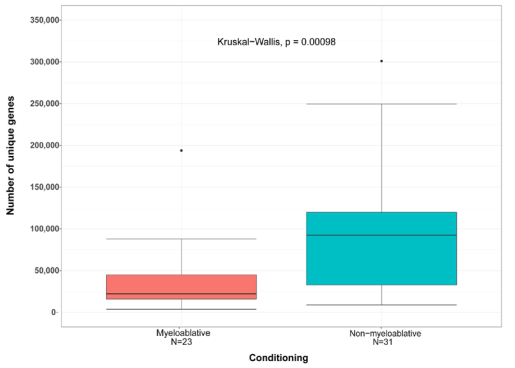
Conclusion. Post-aHSCT pre-engraftment was a crucial timepoint where microbial changes, including lower gene richness and abundance of certain bacteria, were associated with development of aGvHD. Myeloablative regimes were also associated with both aGvHD and microbiome changes, suggesting that intense conditioning may affect aGvHD risk through perturbation of the gut microbiome.

Figure 1: Relative Abundance of A Priori Bacteria Significantly Associated with aGvHD at Timepoint T_2



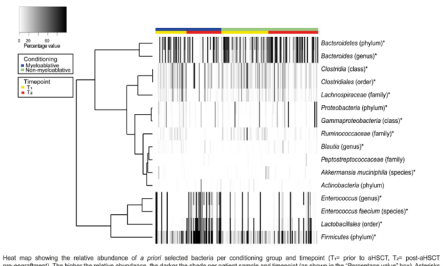
Relative abundance of a priori selected bacteria in T_2 (post aHSCT pre-engraftment) samples among recipients developing vs. those not developing aGvHD. N= the number of recipients that have >0% of the respective bacteria in the respective aGvHD/no aGvHD group (e.g. N= 0/20 aGvHD: of the 20 recipients that develop aGvHD and have a sample at timepoint T_2, none have >0% of the bacteria *Akkermansia muciniphila*). Other tested *a priori* bacteria include: *Firmicutes, Enterococcus, Lactobacillales, Enterococcus faecium, Clostridia, Clostridiales, Ruminococcaceae, Lachnospiraceae, Peptostreptococcaceae, Bacteriodes, Bacteriodetes* and *Actinobacteria*, of which none were found to be significant ($p<0.1$).

Figure 2: Gene Richness at Timepoint T_2 in Myeloablative vs. Non-myeloablative aHSCT Recipients



Gene richness at timepoint T_2 (post-aHSCT pre-engraftment) in myeloablative vs. non-myeloablative aHSCT recipients. Due to a higher read number cut-off for our gene richness analyses (min. 4*10^6 reads vs. min 1*10^6 reads) there are fewer samples included than for other analyses.

Figure 3: Heat Map of Relative Abundancies of *A Priori* Bacteria per Conditioning Group and Timepoint



Heat map showing the relative abundance of *a priori* selected bacteria per conditioning group and timepoint (T_1= prior to aHSCT, T_2= post-aHSCT pre-engraftment). The higher the relative abundance, the darker the shade per patient sample and timepoint (as shown in the "Percentage value" box). Asterisks (*) show if there was a significant difference ($p<0.05$) in the relative abundance of the respective bacteria between myeloablative and non-myeloablative T_2 samples (based on Kruskal–Wallis testing).

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