MICROBIOME SHIFTS WITH 6-THIOGUANINE PROVIDE INSIGHTS ABOUT IBD AND ITS TREATMENT

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Introduction

The microbiome is a major determinant of colonic health. Shifts in the microbiome community (dysbiosis) have been reported in IBD but interpretation of dysbiosis is fraught by confounding factors that include a large inter-individual variation due to many factors including the genetics of the host glycobiome and mucosal immune system, stochastic microbiota founder effects and exogenous substrate.

Aim & Methods

To characterise the caecal mucosa-associated microbiome of contemporaneous regular chow-fed 5-7 week wild type and Muc2 Winnie -/- C57Bl/6 mice inbred mice strains derived from the same animal facility and housed in the same cages for each test condition : vehicle gavage, daily 6-thioguanine (6TG) gavage 0.2-0.5 mg/kg over 14 days. DNA was extracted from faeces (F) at D0 and D14 and caecal mucosa (CM) and contents (CC) at D14. The resident microbiota in the samples was analysed employing a bar-coded pyrosequencing approach targeting Bacterial 16S rRNA genes.

Results

The faecal microbiome was not stable over time in juvenile mice given vehicle only. 6TG altered the CM microbiome in wild type C57Bl/6 mice towards less *Bacteroidetes* and increased *Firmicutes* (mainly *Lachnospiraceae*, and *Erysipelotrichaceae*) (Figures 1 and 2). 6TG did not significantly alter the Muc2 Winnie -/- CM microbiome, which had a reduced proportion of *Firmicutes* and an increased proportion of all *Bacteroidetes* groups.

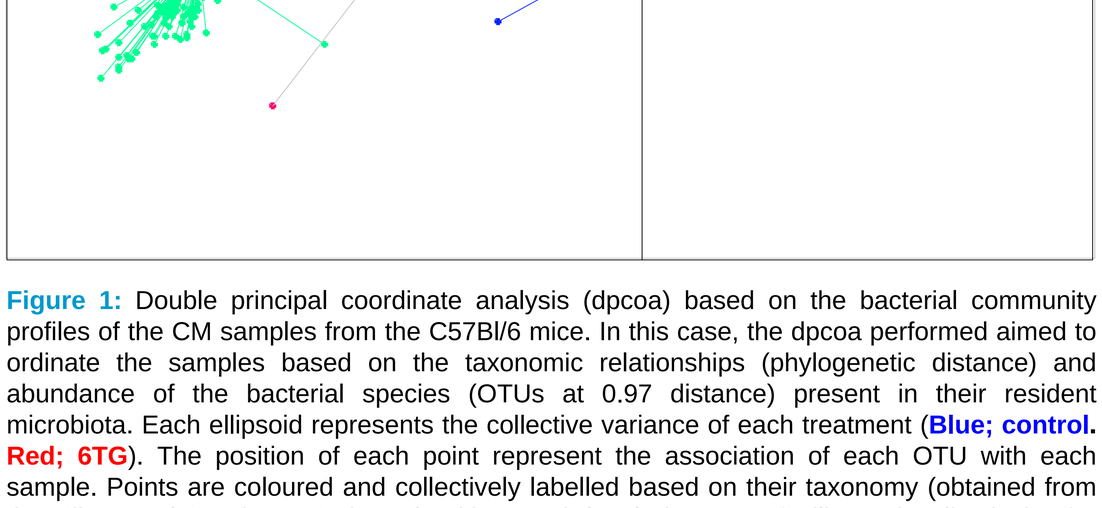

Figure 1: Double principal coordinate analysis (dpcoa) based on the bacterial community profiles of the CM samples from the C57Bl/6 mice. In this case, the dpcoa performed aimed to ordinate the samples based on the taxonomic relationships (phylogenetic distance) and abundance of the bacterial species (OTUs at 0.97 distance) present in their resident microbiota. Each ellipsoid represents the collective variance of each treatment (**Blue; control. Red; 6TG**). The position of each point represent the association of each OTU with each sample. Points are coloured and collectively labelled based on their taxonomy (obtained from the Ribosomal Database Project algorithm, rank level chosen to facilitate visualization). The analysis indicates a strong partitioning of the samples based on treatment type driven by the relative abundances of *Bacteroidetes* (but possibly not *Rikenellaceae* and *Prevotellaceae* within this clade) and several groups within the *Firmicutes*. A subsequent between-group analysis coupled with a Montecarlo permutation test confirmed that treatment type had a significant effect on the microbiota profiles ($p < 0.02$), and explained 34% of the total variance in the dataset.

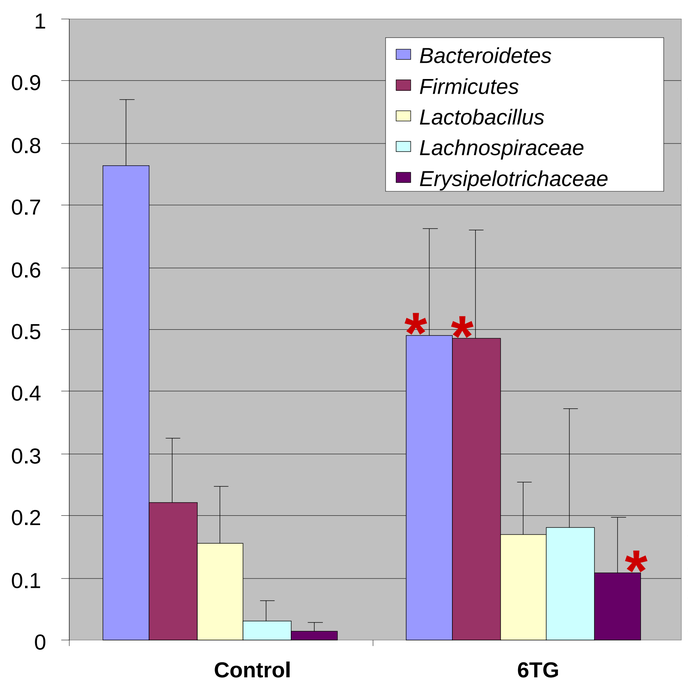

Figure 2: Based on the results derived from the dpcoa exploring variation across the CM samples from wild type C57Bl/6 mice (Figure 1), relative abundances of chosen phylogenetic groups across these samples were recovered. Observed differences between the samples originating from the control and 6TG treatments were further analyzed using a statistical test (wilcoxon test, α=0.05, FDR). The results are concordant with the dpcoa and show that treatment with 6TG produced a significant (*) decrease in the relative abundance of members of the *Bacteroidetes* with a concomitant significant increase in the proportion of *Firmicutes* related sequences and more specifically in sequences related to the *Erysipelotrichaceae* family.

Conclusion

There is large variation in the caecal mucosal and faecal microbiomes even in inbred strains of contemporaneous juvenile C57Bl/6 mice littermates. 6TG failed to shift the microbiome of Muc2 Winnie -/- suggesting that the epithelial defect is more important in determining the dysbiosis. 6TG shifted the caecal mucosal microbiome in wildtype mice. A recent high throughput sequence analysis of the mucosal microbiome of IBD patients has shown "*Firmicutes* were reduced in IBD samples and there were concurrent increases in *Bacteroidetes*"*. This together with our study of a thiopurine effect on the CM microbiome of inbred mice in a controlled environment, suggests that 6TG–antibioisis may contribute to Rx efficacy.

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References

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