Figures and figure supplements

Computer-guided design of optimal microbial consortia for immune system modulation

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Figure 1. Conceptual figure. A microbiome–immune system mathematical model describing the activation of regulatory T-cells (T\(_{\text{reg}}\)) in response to colonization profiles of T\(_{\text{reg}}\)-stimulating Clostridia strains is the central element of this work. It consists of a previously derived microbiome ecological model that describes the short and long-term temporal dynamics of Clostridia strains in germ-free mice (Bucci et al., 2016) and is supplemented by a microbiome–T\(_{\text{reg}}\) model of CD4\(^{+}\)FOXP3\(^{+}\) T\(_{\text{reg}}\) activation in response to long-term compositions in the microbiome. The individual contribution of each strain to the measured T\(_{\text{reg}}\) activation is inferred using long-term colonization data from mouse experiments with subsets of these strains and corresponding measurements of T\(_{\text{reg}}\) induction. The T\(_{\text{reg}}\)-induction score, TrIS, which accounts for ecological stability and immune activation, assigns a score to every possible strain combination and thereby identifies candidate probiotic consortia for experimental validation.

DOI: https://doi.org/10.7554/eLife.30916.003
**Figure 2.** Data used for inference of the CD4^+^FOXP3^+^ T<sub>reg</sub> induction parameters. (A) To infer the strain-resolved CD4^+^FOXP3^+^ T<sub>reg</sub> induction parameters CD4^+^FOXP3^+^ T<sub>reg</sub> abundance measurements and corresponding microbiome colonization data are used. CD4^+^FOXP3^+^ T<sub>reg</sub> data of single strains originates from previously published measurements from Atarashi et al. (2013). Because in Atarashi et al. (2013) microbial mono-colonization levels were not measured, a previously published predictive model (Bucci et al., 2016) was used to simulate these experiments. In addition, newly generated CD4^+^FOXP3^+^ T<sub>reg</sub> and colonization mouse stool data from three 11-strain combinations was included into the analysis (see also Figure 3). The 11-strain combinations were chosen according to results of the ‘keystoneness’ analysis previously described in Bucci et al. (2016). The microbiome compositions of these three 11-strain combinations were estimated by strain-specific qPCR. (B) The resulting CD4^+^FOXP3^+^ T<sub>reg</sub> induction parameters quantify the contribution of each individual strain to T<sub>reg</sub> induction. (Coefficients are scaled by the microbial mono-colonization concentrations for display reasons.)

DOI: https://doi.org/10.7554/eLife.30916.004
Figure 3. Validation of microbiome model describing the dynamics of Clostridia strains in germ-free mice. (A) The model-predicted dynamics of three 11-strain combinations from the 13 Clostridia strains described in Bucci et al. (2016) (areas) are compared to measured data from germ-free mice (stacked bars) and each panel corresponds to an individual mouse (Figure 2A). Predictions were obtained by numerically integrating the corresponding generalized Lotka–Volterra equations with parameters from Bucci et al. (2016) using only each mouse’s initial microbial composition. In timelines/mouse 1–5, Strain 15 is absent referring to 11-strain set I (five biological replicates); in timelines 6–9, Strain 4 is absent (11-strain set II; four biological replicates), and in timelines 10–14, Strain 29 is absent (11-strain set III; five biological replicates). Densities are computed as averages of three technical replicates. The number of mice used in each condition was chosen consistently with previous experimental work (Atarashi et al., 2013; Bucci et al., 2016) and combined with extensive in silico testing of the inference error as a function of sampling frequency and noise levels (Bucci et al., 2016). (B) Spearman’s rank correlation coefficient between observed and predicted data was computed at different time points. All displayed coefficients have a p-value of less than $10^{-16}$. DOI: https://doi.org/10.7554/eLife.30916.006
Figure 4. Identification and experimental validation of model-predicted $T_{reg}$-stimulating consortia. (A) Predicted $T_{reg}$ Induction Score (TrIS) in germ-free mice as a function of probiotic consortium size. Each data point represents one of the $2^N-1$ possible non-trivial consortia of size $N$. The five highest
and lowest TrIS consortia of size seven are highlighted by violet and cyan filled dots, respectively, as well as, the 4-strain consortia used for experimental validation in subpanel E. (B) Distribution of the TrIS plotted against the total population density of each model-predicted consortium. This analysis shows a strong correlation (Spearman’s Rank Correlation with coefficient 0.88 and p<0.05) between TrIS and total microbial density. The color of each dot represents the abundance of Strain 27, which is predicted to strongly colonize in high-TrIS subsets. (C) Heatmap of consortium membership of the five highest and lowest TrIS-consortia of size seven and their predicted $T_{\text{reg}}$ induction and estimated butyrate production using data from Atarashi et al. (2013). (D) Estimation of potential SCFA output based on single-strain in vivo metabolic profiling from Atarashi et al. (2013). The five highest TrIS consortia of size seven (from 4C) are predicted to have a significantly higher SCFA output relative to the five lowest ones. (E) Five 4-strain consortia were used for experimental validation: the two highest-ranked consortia (H1 and H2, respectively), the lowest-ranked consortium (L) and two intermediates (M1, M2). M1 and M2 were included because of interest in other disease areas. The experimentally introduced strains are listed next to each bar/consortium. Strains with numbers in black were detected by 16S rRNA sequencing in mouse stool samples, strains with numbers in gray were introduced but failed to colonize. A Pearson’s correlation of 0.97 between the predicted TrIS and the average of each consortium’s measured $CD4^+FOX3^+T_{\text{reg}}$ percentage proves the ability of the TrIS-based selection to correctly recover the experimentally observed $T_{\text{reg}}$ induction. (Pearson’s correlation of all points has a value of 0.54.) Importantly, the H2 consortium displays an average increase in immune activity of 107% relative to the average germ-free mouse control. Eight biological replicates were used for GF, H1 and H2, five biological replicates were used for M1, M2 and three biological replicates were used for L. Replication and design of the validation experiment for $T_{\text{reg}}$ induction assessment is consistent with work from us and others (Atarashi et al., 2013; Narushima et al., 2014).

DOI: https://doi.org/10.7554/eLife.30916.007
Figure 4—figure supplement 1. TrIS versus estimated mean population density. In contrast to Figure 4B, which highlights only the abundance of Strain 27 in each consortium, the predicted total population density of the microbial consortia is compared to the TrIS while the color in each subpanel represents the corresponding strain’s abundance.

DOI: https://doi.org/10.7554/eLife.30916.008
**Figure 4—figure supplement 2.** Metabolic profiling of three selected microbial consortia and comparison of predicted and measured metabolic profiles of three selected microbial consortia. (A) Metabolic profiling results of fecal content of selected consortia with respect to acetic acid. (B) Comparison of predicted mean germ-free normalized acetate concentrations based on single-strain measurements in caecal content from (Atarashi et al., 2013) with the observed germ-free mean normalized acetic acid levels in mouse stool from (A).

DOI: https://doi.org/10.7554/eLife.30916.009