Writing the discussion section

Adapted from: https://bit.ly/39zfpBW

Big summary/ importance statement for the science.

Scientific findings as extension of previous work.

Findings' scientific implications.

How this engineering will facilitate future research.

Forward-looking statements about the field as a whole.

These results show that the capacity to induce Tregs and modify their phenotype is a characteristic of more effector strains than was appreciated previously. Our findings concerning the role of human gut bacteria in shaping features of the gut mucosal immune system complement and extend the elegant work by Atarashi et al. (16). They used a single selective condition (chloroform treatment) to recover a group of 17 strains (all of which were described as members of the class Clostridia) from the human fecal microbiota of a single donor and showed that the consortium was capable of expanding the colonic regulatory T cell compartment in gnotobiotic mice. The fact that we found this effector activity among gut species belonging to other bacterial phyla suggests that distribution of this functional capacity may be beneficial in ensuring that this tolerogenic cell type is consistently and persistently maintained in different microbial community and host contexts. The approach we describe allows systematic follow-up analyses of the extent to which the Treg response is affected by factors such as age at colonization or by different diets that produce abrupt and substantial alterations in microbiota configurations (45–47). Despite identifying members of different human gut bacterial phyla that shape the Treg response, our study and that of Atarashi et al. revealed that intestinal short-chain fatty acid concentrations increased upon colonization. Given the substantial amount of data supporting a role for short-chain fatty acids in the induction of Tregs (42–44), this suggests a common pathway by which different microbes converge to modulate this facet of the host immune system. The genetic manipulability of some of the bacterial strains identified here, notably the Bacteroides, affords an opportunity to test this and other hypotheses, and advance our knowledge about the molecular underpinnings of microbiota-Treg crosstalk.

As the field of human microbial ecology research moves from observational studies to hypothesis-driven experiments designed to directly test the contributions of the microbiota and its components to health, there is a growing need to develop and transition to a modernized set of Koch's postulates (48) where the groups of microbes that modulate host phenotypic responses are identified along with the environmental factors (for example, dietary) necessary for the response to be fully manifest.

A summary/ importance of the engineering aspect of the work.

How it facilitates scientific research.

Limitations of the platform, show which questions would be unanswered.

How it will facilitate medical research.

We have developed a platform for systematically identifying microbe-host phenotype interactions in different (human) donor microbiota using gnotobiotic mice that can represent different host genetic features and different environmental conditions of interest. With the 17 strains in our culture collection, there were more than 100,000 possible combinations to search for effector strains. Using the mathematical and experimental strategies described, we only needed 100 combinations to identify multiple effector microbes for three very diverse biological responses (metabolic, adiposity, and Tregs). This represents a 1000-fold reduction in the search space compared to what would be required theoretically. By testing these 100 combinations of microbes in an out-of-the-isolator gnotobiotic caging system rather than in traditional flexible film isolators, we overcame what would have been an insurmountable practical barrier to performing these studies for most groups. Our entire study could have been completed with a single flexible film isolator to generate the required germ-free mice. This feature suggests that our overall approach should be accessible to many investigators because animal facilities with small numbers of gnotobiotic isolators already exist in numerous universities.

Although identifying effector strains represents a critical first step in mechanistic analyses of how the gut microbiota affects various facets of host biology, once such strains are identified, much additional work needs to be done. For example, numerous other important components of the intestinal immune system may also be affected by colonization with the strains we identified, including B cell class switching to IgA, macrophage/dendritic cell effector or migratory properties, and γδ T cell function. Another important goal is to identify the effector molecules produced by the identified effector strains and the host signaling pathways through which these molecules act. Using gnotobiotic mice genetically deficient in various components of the immune system (such as Toll-like receptors or inflammasomes) and effector strains that are genetically manipulated (for example, through whole-genome transposon mutagenesis) represent ways for pursuing this goal. Although additional elements of these mechanistic analyses will be dependent on the biological processes being interrogated, in principle this platform can be applied to any microbiota-associated phenotype. Finally, our approach has therapeutic implications because it represents an enabling system for identifying and characterizing next-generation probiotics or combinations of pre- and probiotics (synbiotics).

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