METABOLIC RESEARCH ALLIANCE
Project Proposal

Analysis of the Effects of the Microbiome on Immune Responses in Humanized Mice

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BACKGROUND

The human immune system is modulated by the microbiome. Several lines of investigation have described relationships between increases in common diseases such as allergies, autoimmune illnesses, and even obesity and type 2 diabetes to changes in the microbiome. These have increased in frequency in Western countries. Moreover, microbiome-related infections have become increasingly common, such as colitis from *Clostridium difficile*. In addition to serving as accelerators in the pathogenesis of these afflictions, the microbiome also can modify responses to biologics that are commonly used to treat patients. For example, recent studies have shown that in patients with cancer, responses to two commonly used immune therapeutics, anti-CTLA-4 monoclonal antibody (mAb) and anti-PD-L1 mAb are associated with the presence or absence of certain *Bacteroides* and *Bifidobacterium* species (Vetizou et al Science 2015, Sivan et al, Science 2015), and possibly mediated via surface polysaccharides of the bacteria.

There has been an explosion of technologies that can be used to characterize the components of the microbiome. This includes not only enumeration of bacteria taxa, but also viruses (the virome), and fungi and other eukaryotic microbes. Gene expression analysis (meta-transcriptomics) can be performed on microbiome communities and genes and pathways can also be enumerated. Methods to characterize the microbial components have been refined and applied to studies of human populations. For example, the disparate rates of Type 1 diabetes between three genetically similar populations (Finland and Estonia, with high or increasing rates of T1D; and Russian Karelia, where Type 1 diabetes and allergies are rare) was ascribed to differences in immune stimulation in infants by the lipopolysaccharide of *Escherichia coli*, a bacteria found in the human gut microbiome (Vatanen et al Cell 2016).

However, it remains a challenge to determine whether the associations that are found between the microbiome and biologic behavior are causative or correlative. Furthermore, it is difficult to identify the mechanisms that can account for the changes. Development of model systems in which these relationships can be studied are important for understanding the significance of the microbiome and in using the discoveries to modify human immune responses. In this proposal, we plan to use model systems to understand the relationships between human immune responses and the microbiome. Our long term goal is to use this

information to identify individuals who are most likely to respond to biologics or those who are at the highest risk for adverse events that may be avoided. Our studies will also have bearing in other clinical settings including spontaneous autoimmunity.

OVERVIEW OF METHODS AND GENERAL EXPERIMENTAL PLANS

Our studies utilize humanized mice so that we can study the effects of the microbiome and biologics that are in clinical use on human immune cells. We have utilized immune deficient NOD/scidγc-/- mice (NSG) mice, that are commercially available from Jackson Laboratories, and are reconstituted with human fetal liver-derived CD34+ stem cells at 48 hours of life. These mice develop human immune cells that can mount productive immune responses including xenograft rejection and others.

We have used these mice to study the effects of human biologics on immune responses. We found that humanized mice that were treated with anti-CTLA-4 mAb developed hepatitis, adrenalitis, sialitis, and anti-nuclear antibodies. The induction of autoimmunity involved activation of T cells and activation of antigen presenting cells. We also studied the effects of anti-CD3 mAb, currently in clinical trials for treatment of Type 1 diabetes and found that the mAb caused migration of the cells to the gut wall where they developed into cells with regulatory features such as production of IL-10.

The humanized mice not only allow us to examine cells at sites that are inaccessible in patients, but they also allow us to determine the cause/effect relationships since we can modify cell migration, activation, the microbiome among other factors. For example, we have found that manipulation of the microbiome in humanized mice, with antibiotics (abx) that are in clinical use, can modify the responses to biologics.

We aim to identify specific microbes that are involved in immune processes. We have done this for other mouse models (microbiome protection against *Clostridium difficile* infection; microbiome protection against experimental autoimmune encephalomyelitis, a model for multiple sclerosis) and succeeded in isolating individual taxa that were bioactive. For these examples, it appears that multiple species are needed to see the effects. In addition we have built a collection of purified bacteria isolated from human samples that is a resource as a source of organisms for testing.

PROPOSED STUDIES

In the proposed studies, we plan to study the effects of the microbiome on human biologics that are used commonly such as the anti-cancer agents, anti-CTLA-4 and anti-PD-L1 mAbs. As studies from patients with cancer, treated with anti-CTLA-4 mAb have identified a relationship between the microbiome and responses to the therapy, we will begin by determining which components of the microbiome are responsible for biologic effects in the humanized mice.

Our studies entail an evaluation of the host/immune system interaction as dictated by the host microbiome. The studies involve a close collaboration between investigators at Yale and the Jackson Laboratory. The analyses will focus on identifying agents that can modify human immune responses in vivo with confirmation using ex vivo culture systems and reconstitution of mice with select organisms. The general plan for study will involve:

1) Preparation of humanized mice. We will begin with NSG mice reconstituted with human CD34+ cells as we have done in the past. In future studies, we plan to expand this model system to utilize other host

mice that have been produced by Dr. Richard Flavell at Yale, which have an even more robust reconstitution of mucosal immune cells.

2) Treatment with biologics and analysis of the effects of changing the microbiome on the immune responses. We have used cocktails of broad spectrum abx to modify the microbiome, but individual abx can be used to affect subsets of bacteria. In addition, we have used dietary and fecal transplant by co-housing as other methods to introduce alternative microbiomes. In each case, the relationship between the host microbes and immune responses will be analyzed.
3) When an altered immune response is observed, we will perform a detailed analysis of the microbiome of the two classes of animals and identify specific bacterial taxa as candidates for causative agents. These bacteria can be purified for studies described below, as we have done before, or the same species in our microbiome strain collection can be tested.
4) Studies of the effects of microbes on human immune cells ex vivo. Human immune cells are modified by the microbiome through the production of ligands by the microbes that interact with innate immune receptors and through effects of the microbiome on immune cell survival and function. Our studies above in #2 will be followed by studies of the direct effects of the microbiome on human immune cells ex vivo.
5) Modification of the host to create models of human disease. We plan to modify the immune deficient mice to improve our humanized models to study human disease. For example, despite our ability to induce autoimmunity with anti-CTLA-4 mAb, we have been unable to induce it with anti-PD-1 antibodies despite the clinical observations of increasing rates of autoimmune diabetes in patients with cancers treated with these checkpoint inhibitors. We believe that the reason for the failure of the murine models to recapitulate the human disease is the failure of cross reactivity between the ligands on murine cells (e.g. PD-L1) and the human receptors (e.g. PD-1). We will therefore create immune deficient mice with human costimulatory ligands that can be used to study human immune responses to tissues expressing the human ligands.

IN SUMMARY

These studies will use cutting edge technologies, established at institutions in the State of Connecticut, to identify causal relationships between the microbiome and human immune responses. The findings from these studies are likely to suggest future clinical trials to test these findings in patients with autoimmune diseases such as Type 1 diabetes, metabolic diseases including Type 2 diabetes and the metabolic syndrome, as well as cancers, and other common medical conditions, and to develop new therapies to treat them. The studies may also identify individuals who are most likely to respond to immune therapies, which, if confirmed in clinical trials, may allow clinicians to identify those individuals most likely to respond to therapies. This would result in reduced risk and expense and improved clinical outcomes. This technology and approach offers a new opportunity for commercialization. We anticipate that the results will facilitate the development of new assays that can be used to “personalize” approaches to treatment.

In addition to the potential “spin-offs” of the discoveries, the investigations will employ between 8-10 individuals into the biotechnology field. The range of new hires will include new immunologists, microbiologists, bioinformaticians, and highly skilled technicians, as well as individuals who are directly involved in the care of laboratory animals. Thus, there is both immediate and long term gains to be realized through these investigations.

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