Clinical and metagenomic investigations into the etiology and treatment of chronic enterocolitis in rhesus macaques

2013 ACLAM Forum

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Chronic enterocolitis (CE)

• CE is a common cause of morbidity in captive rhesus macaque (*Macaca mulatta*) populations

• CE is generally recognized as a prolonged and/or recurrent diarrhea in macaques that is limited in scope to a small percentage of colony individuals

• CE has been diagnosed in 4.8% of the ~1100 animals at our facilities

• To date, CE lacks a definitive etiology
Fecal bacteriotherapy (FBT)

• FBT is a clinical procedure in which feces from a healthy donor is made into a suspension and deposited into the gastrointestinal tract of a diseased individual in efforts to ‘reshape’ the microbiome.

• This procedure has been reported to be highly effective at ameliorating a variety of, otherwise nonresponsive, bacterial and idiopathic causes of chronic human enteritis.
Metagenomic analysis (MA)

• MA is an assay by which the 16s rRNA acquired from feces can be qualitatively and quantitatively assessed to identify the bacterial populations comprising the intestinal microbiome

• The greatest asset of MA is that it identifies the numbers and ratios of bacterial types within the intestinal tract and not just whether one (or more) particular species of potential pathogenic bacteria is present or not
Contemporary thoughts on the etiology of chronic intestinal disease

- Some chronic intestinal disease of humans (e.g. Chrohn’s disease, IBD) have been identified to have decreased or altered intestinal bacterial microbiomes as compared to healthy people.

- Research suggests that macaque CE may also be in part due to imbalances within the microbiome of the gastrointestinal tract.
Female animals affected with chronic enterocolitis (CE) identified

Day 1: Feces collected from CE monkey for #1 metagenomic analysis and microbial culture prior to treatment

Day 1-14: CE animals hospitalized with dietary modification and antibiotic therapy as is the current standard-of-care

Day 15: Feces collected from CE monkey for #2 metagenomic analysis and microbial culture post treatment

Day 15: Feces from “healthy” female cagemate to each individual CE monkey is collected and used to make a fecal bacteriotherapy (FBT) slurry and 25 ml of the slurry is passed into duodenum of the CE monkey via endoscope

Day 15: Feces is also collected from fecal-donor monkey for metagenomic analysis and microbial culture

Day 18: Feces collected from CE monkey for #3 metagenomic analysis and microbial culture post-FBT treatment

Day 105 or as the CE animal represents with diarrhea: Feces collected from CE monkey for #4 metagenomic analysis and microbial culture post-FBT treatment

Following collection of the #4 fecal sample, all FBT CE monkeys that represented with diarrhea prior to Day 105 are retired from the study. Those monkeys that did not represent with diarrhea by Day 105 are monitored until diarrhea recurrence.
Female animals affected with chronic enterocolitis (CE) identified

**Day 1:** Feces collected from **CE monkey** for #1 metagenomic analysis and microbial culture prior to treatment

**Day 1-14:** CE animals hospitalized with dietary modification and antibiotic therapy as is the current standard-of-care

**Day 15:** Feces collected from **CE monkey** for #2 metagenomic analysis and microbial culture post treatment

**Day 15:** 25 ml of **0.9% saline** (rather than donor monkey feces) is passed into duodenum of the CE monkey via endoscope

**Day 18:** Feces collected from **CE monkey** for #3 metagenomic analysis and microbial culture post-saline treatment

**Day 105 or as the CE animal represents with diarrhea:** Feces collected from **CE monkey** for #4 metagenomic analysis and microbial culture post-saline-treatment

Following collection of the #4 fecal sample, all **CE monkeys** that relapses with diarrhea prior to Day 105 are **re-entered** back into the study and are treated with dietary modification and antibiotics for 14 days and then FBT, identical to those of **Group 1**. Monkeys that make it to Day 105 without recurrence are not retreated with FBT but are monitored for diarrhea recurrence
Assessing clinical efficacy of FBT in resolving diarrhea in CE monkeys

• Recurrence rates of diarrhea in Group 1 (FBT) monkeys compared to their own historical diarrhea recurrence rates (within-group comparison)

• Recurrence rates of diarrhea in Group 1 monkeys compared to the recurrence rates of those in the saline-treated Group 2 (between-group comparison)

• Recurrence rates of diarrhea in FBT-Group 2 monkeys compared to their own recurrence rates when treated with saline and also compared back to their own historical diarrhea recurrence rates (2 separate within-group comparisons).
Clinical study progress to date

• 17 animals have undergone 16 treatments
• 6 animals were treated with saline
  – 4 of these animals represented with diarrhea and were re-entered into the study for FBT treatment
• 14 animals were treated with FBT
  – 5 of these animals represented have completed the study
Findings as to Clinical Utility of FBT

- Two of 5 FBT-animals had shorter diarrhea recurrence rates than their individual historical recurrence rate while the other 3 animals had longer recurrence rates.
- On average, the recurrence rate of all 5 retired FBT-animals was 96.6 days historically and was 108.2 days post-FBT treatment.
  - If only those 3 animals that responded positively to FBT are calculated, the average recurrence rate of diarrhea was 87.5 days historically and 189.9 days post FBT treatment.
Assessing microbiome variations between CE and healthy monkeys

*Group comparisons*

- Compare the numbers of bacterial genera in CE monkeys to those of donor monkeys
- Compare the ratios of bacterial phyla in CE monkeys to those of donor monkeys
- Identify numbers of potential pathogenic bacteria genera in CE monkeys
Assessing efficacy of FBT in altering the recipient microbiome

*Individual animal comparisons*

- Compare microbiome of the individual CE monkey prior to treatment to donor monkey
- Compare microbiome of the individual CE monkey after FBT-treatment to donor monkey
- Compare microbiome of the individual CE monkey at Day 105 (or after diarrhea recurrence) to donor monkey
- If post-FBT there is clinical improvement in CE and a change in microbiome toward the donor this would suggest alterations in microbiome are responsible for the success
- If post-FBT there is clinical improvement in CE and NO change in microbiome toward the donor this would suggest other physiological changes are responsible for the success
Microbiome-related progress to date

- Fecal samples are currently being processed in batches of 96 samples/run in efforts to reduce inter-assay variability and reduce costs
- 14 animals have had their fecal samples submitted to date
  - 10 CE animals
    - Presentation with diarrhea
    - Post-antibiotic treatment
    - Post-FBT or saline treatment
    - At Day 105 or when represented with diarrhea
  - 4 donor animals
Affected and Not Affected Animals

![Graph showing the comparison of Average Shannon Index between Affected and Not Affected animals as a function of Sequence per Sample.]
Phylum Level Bar Chart
(data presented here is not from this study)
Work to be completed

• Clinical trails and case follow-up
• Complete metabolic analysis
• Metagenomic comparisons of the colon microbiome in rhesus macaques of healthy, CE and colon cancer in efforts to identify any role CE may play in carcinogenesis
Funding for this project was generously provided through a 2012 grant from the

**ACLAM Foundation**

with additional support from

The Keeling Center for Comparative Medicine and Research, The University of Texas, MD Anderson Cancer Center

and the

The Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine
Acknowledgements and Gratitude

Colleagues and technical support

Bruce Bernacky        Luis Velez
Patrick Hanley        Larry Williams
Joe Petrosino         Jeff Rogers
Chris Abee            Luke Segura

The husbandry and clinical staff of the rhesus colony at the KCCMR, University of Texas, MDACC

ACLAM Foundation Grant Committee
Thank you!

Questions?