Reviewer’s report

Title: Characterization of gut microbiota in children with pulmonary tuberculosis

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Reviewer: Ronan O'Toole

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In this manuscript, Zhu and colleagues present data on the gut microbiome of patients with pulmonary tuberculosis (PTB) with respect to healthy controls. The manuscript contains a number of areas of concern which include the following:

1. The study group age ranges from 0.2 to 15.5 years for PTB patients and from 0.6 to 16.0 years for healthy controls. The WHO uses an age threshold of <15 years in its epidemiological analysis of childhood TB. In terms of WHO treatment guidelines, a child refers to the 0-10-year age group, within which infant refers to the <1 year age, and adolescent refers to the 10-18-year age group. The wide range ages used in this study for both groups across which a relatively small number of subjects (n=18) is spread creates the possibility of over- or under-representation of an age subset within one or both of the subject groups in the microbiome analysis. More precise information on the individual ages contained in the two subject groups, and a more refined analysis of age group subsets, is required. The latter may necessitate a larger patient cohort to be sampled in order to provide reliable data on any differences in microbiota structure or content.

2. The manuscript overstates its findings in places. For example,

"Our finding implicated the critical role of the gut microbiota and gut-lung axis in the development of PTB and provides a theoretical basis for the treatment of PTB through gut microbiota intervention." No experimental data are provided in this study to support the critical role of the gut-lung axis in the development of PTB, or the use of gut microbiota intervention in PTB treatment.

3. The manuscript refers to the study by Namasivayam et al (Longitudinal profiling reveals a persistent intestinal dysbiosis triggered by conventional anti-tuberculosis therapy. Microbiome 2017, 5(1):71) i.e. "In addition, Namasivayam et al found that compared with healthy controls, the diversity of gut microbiota in a murine model of TB presented a slight but significant reduction in the 12 weeks after infection[21]."

It is important to refer to the specific findings by Namasivayam et al i.e.
"To determine the changes in the intestinal microbiota due to Mtb infection in this particular experimental setting, we first performed a longitudinal comparison of the microbiota of the mice from the untreated naïve and TB groups. When data from all of the time points were pooled, we did not observe a statistically significant change in diversity resulting from Mtb infection as assessed by Chao1 and Shannon indices (Additional file 2: Figure S2a) which measure the total number of operational taxonomic units (OTUs) and, in case of the Shannon index, the richness, abundance, and evenness of the OTU distribution. However, a slight but significant decrease in diversity was evident at W12 of infection (Fig. 1a). We then used the phylogeny based UniFrac method to compare the bacterial communities in the naïve versus TB animals (Fig. 1b). Although unweighted UniFrac analyses, which cluster the data based on presence or absence of OTUs, clustered the naïve and TB samples separately (p < 0.001), the clustering driven by Mtb infection was not statistically significant based on weighted UniFrac distances (p = 0.203) that also take into account the relative abundance of the OTUs. We next compared the composition of the microbiome to identify bacterial taxa that differ between the two groups. In agreement with Winglee et al. [54], we observed trends of differential abundance in members of the order Clostridiales of phylum Firmicutes and certain members of phyla Bacteroidetes and Tenericutes between the two groups (Fig. 1c, Additional file 3: Figure S3). Nevertheless, none of these differences, except genus Alkaliphilus that was increased in naïve mice, remained significant over the entire duration of the experiment. Together, these findings involving our specific infection and animal housing conditions and one inbred host genetic background, while distinct in detail from the previously published data, confirm that Mtb exposure by itself causes only minor changes in the composition of the murine intestinal flora."

The Namasivayam et al study found very limited changes in M. tuberculosis infected mice versus naïve controls and most were not significant. The main findings of the Namasivayam et al study were that administration of anti-TB drugs isoniazid (H), rifampin (R), and pyrazinamide (Z) was significantly associated with an immediate decrease in the Shannon and Chao1 microbial diversity indices in the first two weeks of therapy. They reported changes in the abundance of specific components such as members of the order Clostridiales which were maintained for at least 3 months after cessation of HRZ treatment.

Unfortunately, a major drawback of the submitted manuscript by Zhu and colleagues is that no data are provided on samples obtained for gut microbiota analysis following commencement of TB treatment (the Zhu et al., manuscript states that "none of the enrolled participants including the PTB group and healthy controls had received probiotics, prebiotics or antibiotics within one month before admission" and that "Stool samples were collected from all participants on the day of their admission"). The provision of additional data post commencement of TB treatment would have been insightful with regard to any effect of TB drugs on the gut microbiome of children.
4. The manuscript requires thorough proof reading as it contains multiple typographical errors e.g.

"Tuberculosis (TB) is one of the most common infectious disease in the word"

"caused by mycobacterium tuberculosis" - should be Mycobacterium tuberculosis

"interventions needs to be addressed"

"the critical role of the gut microbiota in pulmonary infectious disease has also been increasing recognized"

There is an overuse of the term "What's more" i.e.

"What's more, the PTB patients presented a significant reduction of beneficial bacteria"

"What's more, the growing epidemics of drug-resistant TB is a continuing threat"

"What's more, in the present study none of the enrolled participants"

"What's more, a prospective exploration performed by Luo et al"

"What's more, Sabino et al reported that the microbiota of patients with primary sclerosing cholangitis"

"What's more, SCFAs can regulate the proliferation of colonic epithelial cells and enhance the permeability of the intestinal mucosa"

Overuse of "great" and "many" e.g. "A great amount of evidence suggests that the gut microbiota exerts many beneficial effects on humans through the involvement of many significant physiological processes"

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
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