From: **Ambrose, Zandrea** zaa4@pitt.edu
Subject: Re: We'll be sending you data on the R21 project
Date: February 26, 2019 at 8:47 AM
To: Pleuni Pennings pspennings@gmail.com
Cc: Kaho Tisthammer ktisthammer@gmail.com

ZA

Hi Pleuni,

I think you are probably in Israel right now? I hope you are having a good time!

When you return, it would be great if you could look at the sequences that Chris sent for the R21 study. He's working on some more to wrap up this study. We will have SIV only animals and SIV-Mtb co-infected animals. I think the co-infected animals include animals that had their latent TB reactivated and other that did not have reactivation. We should have at least 2 plasma samples at an early (week 1 or 2) and a late (week 8-10) time point after infection. In addition, they have given us lymph nodes and lung granulomas from the last time point to compare with the blood.

In regards to the sequences that Kaho previously analyzed for the R01 study, I've been thinking about the low diversity that was observed. It would be helpful for you to look at the diversity of the SIVB670 virus stock used to infect the animals. I don't think you did that? Or I don't have that information.

I've looked up some papers on diversity of SIV env in acute infection by others and the nucleotide diversity you see is definitely low (see summary on attached spreadsheet). A couple of points:

1. Most studies have looked at this in the context of transmission and how many viruses are transmitted, but several look at later time points. Early after transmission, most animals (and people) are infected with 1 or a few viruses and diversity is quite low. Diversity usually increases over time (10-fold over 16-20 weeks). Plasma viremia levels did not correlate with changes in diversity over time.
2. Most of these studies used the old SGS assay and sequenced a much larger region than we are, so only analyzed <40 sequences per time point/animal. But I don't think that would account for differences between their results and ours.
3. Interestingly, the first study (by my friend Welkin who trained with John Coffin) showed that the V1 region is the most diverse in env (which is what we're sequencing), but this is SIVmac251 in rhesus.
4. Although this is a limited list of papers, the only instance I saw a lack of diversity between peak viremia and later time points was in a study when some animals got infected during 30 weeks of PrEP and obtained drug resistance (M184V). These animals had low diversity for 18-26 weeks post-infection during suboptimal PrEP, which the authors said was likely due to partial suppression of replication by the treatment.

I would expect that a lack of diversity would occur if replication was poor, which is not the case with SIVB670 in these monkeys (but is the case for SIVmac251). Another reason diversity would remain low is selective pressure. I'm assuming a lack of selective pressure should keep the mutation rate constant? There is evidence that this species of macaque has a limited repertoire of MHC alleles (i.e. somewhat inbred population). Or purifying selection could explain this, like in #4 above, right? Just trying to figure out what the data mean while we're generating more sequences.

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Thanks!

Zandrea

From: Pleuni Pennings <pspennings@gmail.com>
Date: Thursday, February 21, 2019 at 12:43 PM
To: "Ambrose, Zandrea" <zaa4@pitt.edu>
Cc: Kaho Tisthammer <ktisthammer@gmail.com>, Christopher Kline <cjk14@pitt.edu>
Subject: Re: We'll be sending you data on the R21 project

Awesome!

> On Feb 21, 2019, at 7:37 AM, Ambrose, Zandrea <zaa4@pitt.edu> wrote:
>
> Hi Pleuni and Kaho,
>
> We should have MiSeq sequences (no PrimerID) from plasma samples from the R21 study over the weekend for you. Chris will get those to you via BaseSpace early Monday. He plans to have a second run for those animals that will include a few more plasma samples and some tissues.
>
> And then we'll get back to the B670 animals (R01 study) with PrimerID – we're meeting with Ling about which time points to run post-Mtb, which will be exciting since virus replication decreases at 3-7 weeks post-challenge for most of the animals (see below).
>
> Thanks!
>
> Zandrea
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> <image001.png>