Author's response to reviews

Title: The Site of Exposure May Influence the Time of Virus Appearance in the Blood and Virus-Specific Immune Responses in Primary SIVmac251 Infection of Rhesus Macaques

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PDF covering letter
Point by point reply to reviewers' comments:

1. All the references to the Mamu A*01 status in regards to CD4+ T cells depletion has been removed. In addition, we tried to tone down the potential for difference in CTL kinetics and replaced the text with the following:

**Virus-specific CD8+ T-cell response in the blood.** Virus-specific CD8+ T-cells were quantitated in the blood of both animals at days 0, 4, and 12 postinfection using 3 tetrameric Mamu-A*01 molecules complexed with the SIV$_{mac}$ Gag 181, Env 622, and Tat 28 peptides (T.M. Allen et al., submitted).

In the blood, virus-specific tetramer-binding CD3+ CD8+ T-cells appeared earlier in macaque 817, exposed to SIV$_{mac251}$ by the intrarectal route, than in 819 for all the 3 antigens studied (Figs. 1B and 1D).

2. We believe the reviewer is referring to the PE-Texas red conjugate that is produced by Pharmingen. This has a different emission wavelength and is seen by Facscalibur as FL2. The Texas red antibody that we used (Vector Laboratories) was recognized by the Facscalibur as FL3 and no significant (or uncompensatable) overlap could be seen. Therefore, it was used in conjunction with CD8PE. We are including the results from one of the preliminary stainings with this antibody as well as our results from staining of healthy colonic lamina propria cells. Cells have been gated through the CD8 gate so the percentages are percentage of CD62L+ cells of the CD8+ T cells. However, as this is not a crucial point in this manuscript, we do not have any remaining samples to repeat the staining and in order to expedite the publication, we decided to remove the CD62L staining all together.

3. Novocastra’s anti-CD62L antibody was used as, at the time the study was done, it was the only antibody that was reliably shown to label macaques cells as well as humans. The antibody was only available unconjugated, therefore, secondary antibody had to be used. To ensure that the secondary antibody did bind specifically, several tests were done including:
   - staining with the secondary antibody first, than with the primary antibody + anti-CD8 (in addition to this, staining with secondary antibody, than with anti-CD8 and staining with anti-CD8 than with secondary antibody was done)
   - staining with secondary antibody, than anti-CD62L than anti-CD8
   - staining with anti-CD62L than anti-CD8 only

The sentence in question has been removed since it was left by mistake.

4. We agree that it is possible that depletion of CD4+ T cells in tissues was not seen simply because it was too early. The observation regarding Mamu A*01 animals was also recorded in experiments from our laboratory. However, we agree that it may not be suitable to mention it in the context of this manuscript and the sentences referring to this were removed and in the discussion replaced by:
It is possible that both animals could have controlled viral replication or that the tissues were examined too early and that depletion would have occurred at a latter stage of the infection.

The staining with anti-CD4 PE was used in conjunction with anti-CD8 FITC and anti-CD3 PerCP and this was clarified in the method section.

It is also our observation from biopsies of healthy macaques that expression of CD62L is very low in intestinal tissues (0.72-9%). High CD62L expression was only found in the infected animals. May we remind the reviewer that high CD62L expression in infected macaques as determined by immunofluorescent staining was reported in a study that he co-authored published in Am. J. Pathology [Vajdy et al., 2000] (see Figure 2c and d). Animals in our study have been infected for only 12 days, however, whatever the mechanism of the increased expression of CD62L is, it may be similar in both studies. It is not possible that any other tissue was sampled rather than lamina propria as mucosal layer was separated from the submucosa prior to isolating the cells and, as described in Materials and Methods, histological analysis with H&E staining was done after removing the epithelial layer and prior to digestion.