Author's response to reviews

Title: Impact of antigen specificity on CD4+ T cell activation in chronic HIV-1 infection

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Author's response to reviews: see over
To:
BMC Infectious Diseases
Dr Mirko Paiardini

Zurich, February 1st 2013

Re: "Impact of antigen specificity on CD4+ T cell activation in chronic HIV-1 infection"

Dear Dr Paiardini,

Thank you for the review of our manuscript. We have amended the manuscript according to the reviewer’s comments and present here the final version.

Reviewer 1 (Victor Appay) suggested only that our supplementary figure could be left out. We felt that since the figure is only supplementary, it should remain, as it clearly demonstrates the integrity of our assay. This figure is now Supplementary Figure 2.

Reviewer 2 (Nichole Klatt) made several suggestions for changes and these are addressed here;

Major Compulsory Revisions:

1. The reviewer correctly states that reports have found peripheral CD4+ T cells to express the integrin β7. However, in our text, we clearly state that ‘we did not find integrin β7 expression on HLA-DR and CD38 co-expressing cells’. We did find low levels of β7 expression on total CD4+ T cells, but not on those expressing the activation markers HLA-DR and CD38. Our staining was all done using FMO controls as outlined in the methods section. We have prepared an additional supplementary figure (Supplementary Figure 1) showing the gating for PD-1 and β7 on CD4+ T cells.

2. The reviewer asks whether we assessed PD-1 expression on antigen-specific cells. Due to the restriction on the number of fluorescence markers that could be analysed...
simultaneously, we were not able to assess PD-1 expression at the same time as cytokine production. Our analysis of PD-1 expression was done on an additional PBMC sample from a subset of our HIV+ and HIV- donors, where we compared PD-1 expression levels on activated (CD4+CD45RA-CD38+HLA-DR+) and bulk memory (CD4+CD45RA-) cells. We feel that this is a robust comparison.

3. The reviewer requested data or references supporting our statement regarding the hierarchy of responses we observed. We have toned down this comment and added two references so that the sentence ‘This ‘hierarchy’ correlates well with the level of persistence or the likelihood of reactivation/re-encounter of the respective virus/antigen.’ now reads ‘This ‘hierarchy may correlate with the level of persistence or the likelihood of reactivation/re-encounter of the respective virus/antigen, although this has not been formally addressed but may be inferred from the differentiation profile of CD8+ T cells specific for these viruses/antigens (van Lier, 2003; Derhovanessian, 2011).’

Minor Compulsory Revisions:

1. We have modified our sentence regarding antigen encounter and activation levels so that it now states ‘The activation level of the CD4+CD45RA- population was significantly higher than the CD4+CD45RA+ population (p < 0.01), suggesting that antigen encounter may influence activation levels in HIV+ individuals’ instead of ‘highlighting the influence of antigen encounter on activation levels’, allowing for the observation that memory cells may be more prone to bystander activation as noted by the reviewer.

2. The reviewer has requested that we re-label our graphs to refer to ‘Naïve’ instead of RA+ and ‘Memory/Effector’ instead of RA-. We had deliberately selected these labels because the RA+ population, while predominantly naïve, may also include terminally differentiated cells and we felt it was more correct to refer to the CD45RA status rather than memory subsets.
We would like to thank the editors to give us the opportunity to resubmit a revised version of our manuscript which we hope is now acceptable for publication in *BMC Infectious Diseases*.

Yours sincerely,

Annette