Author's response to reviews

Title: Infection of cells expressing CXCR4 mutants lacking N-glycosylation at the N-terminal extracellular domain is enhanced for R5X4-dualtropic human immunodeficiency virus type-1.

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PDF covering letter
The Editor,
BMC Infectious Diseases

Re: Revision of the manuscript: Infection of cells expressing CXCR4.......; Thordsen et al.

1.) **Comments:**
Now, our submission conforms to the BMC Medicine journals checklist.

2.) **Point by point response.**

2.1) **Response to the comments of reviewer: Ulf Dittmer**

**Comment 1:**
Since the use of viruses only differing in the V3 loop of gp120 is an important issue to interpret the results we have left the figure unchanged. In addition, the construction of NL-991 and NL-952 is not published yet and we believe it would be helpful for the reader to have the information provided by the figure #1.

**Comment 2:**
We have now clarified that issue, the comparison of the permissiveness of cell lines for the viruses used, in the text of the discussion section.

Comparison of permissiveness of cell lines for different viruses:
Permissiveness of the cell lines was compared for infection by three different viruses. NL4-3 produces 100% infection on day three and NL-991 was negative and therefore set to 0% infection on day three. These rates of permissiveness are seen by using an equivalent of 0.5 ng p24 for each of the viruses. As stated in the text, we have not shown infection kinetics of NL4-3 using 0.5 ng since infection with such a high amount of NL4-3 virus leads to 100% infection on day two. As shown in the paper, a better NL4-3 kinetic was observed by using only a tenth (0,05 ng) of the amount of p24 that was used for NL-991 and NL-952. Thus, infection rates on day three must be compared to 0.5 ng p24 inoculums for the three different viruses. Using 0.5 ng inoculums, NL4-3 showed 100% infection on all CXCR4 expressing cell lines. On the same cell lines 0.5 ng p24 of the NL-991 virus produced no infection, however NL-952 does. Since infection rates for NL-952 were between NL4-3 and NL-911, the p24 values obtained for NL-952 were 50% for all the CXCR4-g1 cell lines and 20% and 10% for the CXCR4-g2 cell lines and CXCR4 wt.

Comparison of permissiveness of cell lines for NL-952 infection:
In addition to this comparison between NL4-3, NL-991 and NL-952, the permissiveness of the cell lines with or without g1 of CXCR4 can also be compared for NL-952 only. Setting the NL-952 infection values for infection of the CXCR4 wt cell line to 100% the values for cells with mutated CXCR4 are 300% higher. This second comparison is a comparison for NL-952 infection only.

**Comment 3, 4 and 5:**
No changes made. To our understanding there were no specific comments, which would require a change in the manuscript. We think that any shortening of the sections might lessen the clarity of the paper; especially the section about our hypothesis of the action of CXCR4 glycans on viral infection should be unchanged.

**Comment 6:**
We have included a little graphical symbol legend to Fig 6. This will help to make the figure more understandable without going into the text of the figure legend.
2.2) **Response to the comments of reviewer: Jacqueline Reeves**

**Comment 1:**
Results on this issue will be part of a paper, currently in press in Virology (Polzer et al., 2002).

**Comment 2:**
The formula that converts OD values into p24 ng is now corrected and allows an easy comparison of OD values into p24 ng values.

**Comment 3:**
Changes were made in the manuscript to clarify this issue, See also our comments to the remarks of reviewer U.D, comment 2.

**Comments 4 and 5:**
Both comments, in our eyes, refer to the same subject. We have used viruses based on NL4-3 only differing in the V3 loop. This is one of the main experimental advantages of our study. The effects on CXCR4 infection can be interpreted just because of the differences in V3 loop dependent tropism and CXCR4 glycosylation. No other region of HIV differs and therefore can influence CXCR4 interaction in a “non-V3 loop” manner. That’s why we have focused on the NL 4-3 V3 chimeras. Based on this CXCR4 and also V3-specific experimental design we have observed a clear cut between CXCR4 infection of mutants all lacking g1 and mutant all containing g1.

Such a result is not seen in the paper of Picard et al. (Virology 1997). Although the authors have used 2 X4 and 2 R5X4 viruses for infection in their studies they observed no clear cut for CXCR4-g1 mutants. Infections for CXCR4-g1 (LG1) were lower, for CXCR4-g2 (LG2) higher and for CXCR4-g1g2 (LG1G2) rates were about wt. This means, their infection rates on CXCR4-g1+g2 were different to the rates for CXCR4-g1-g2. Since the paper is focused on e1 (first extra cellular domain of CXCR4) deletions, no kinetics for CXCR4 mutants were shown and the g1 glycosylation site was mutated by an N>I exchange we have not discussed our results in the light of these experiments.

In contrast to Brelot et al. we have used cell lines selected for CXCR4 expression, showing stably expressing CXCR4 levels as shown in our figure 2. In our eyes it is not clear if the infection rates shown by Brelot et al. (JV, 1997) can be compared to ours since they have infected cells 24h after CXCR4 transfection. CXCR4 transfection efficiency might vary between each of their experiments and the inoculums of HIV used for infection vary between 10 and 30 ng p24. Based on these arguments we have not discussed these two papers.

**Comments 6 to 9:**
The little errors found were corrected and the text suggestions made by the reviewer were considered in the new version of the manuscript.

We hope that we have now addressed all the reviewer’s remarks and would greatly appreciate hearing from you soon about the acceptance of the paper.

Sincerely yours

Michael Schreiber