Reviewer’s report

Title: Anti-HIV-1 activity of cellulose acetate phthalate: Synergy with soluble CD4 and induction of "dead-end" gp41 six-helix bundles

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Reviewer: Dr Carl Wild

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Unable to decide on acceptance or rejection until the authors have responded to the compulsory revisions

The paper titled: Anti-HIV-1 activity of cellulose acetate phthalate: Synergy with soluble CD4 and induction of "dead-end" gp41 six-helix bundles by Neurath, et al. was reviewed. The major goals of the study involved characterizing the level of synergy between cellulose acetate phthalate (CAP) and sCD4 and determining the mechanism of action of CAP.

All requests for additional information or suggested revisions should be considered compulsory.

This reviewer has several questions concerning the experimental methods used in the manuscript.

1) Has the specificity of the NC-1 antibody for the HIV-1 BaL virus isolate been established? Since many of the experiments rely on the ability of this mAb to recognize gp41 structures from this virus isolate it is important to establish this point. There is no direct reference to HIV-1 BaL in original referenced report.

2) In the ELISA experiments why is chicken serum used in assays involving the BaL isolate while BSA is used in assays involving HIV-1 IIIB? Also, during the wash step why is a 1:50 dilution of an anti-p24 monoclonal antibody used for the experiments involving BaL and ice-cold PBS is used in the IIIB experiments?

In addition to these questions this reviewer has several suggestions for additional experimental controls that would add credibility to the results.

1) In figure 1, the binding of gp120 and gp120/CD4 complexes to immobilized CAP are determined by ELISA. A sCD4 control should be included in this experiment to demonstrate that the effect of sCD4 on gp120/CAP binding rather than the interaction of sCD4 with CAP accounts for the observed enhancement. It is also suggested that this experiment be carried out in a format utilizing an oligomeric-form of envelope.
2) As a general control it should be shown that CAP does not bind to or interact with the NC-1 antibody.

3) In figure 4 it would be of interest to see the data relating to the exposure of the six-helix bundle by sCD4 alone. Several investigators (including Authors on this report) have demonstrated the effect of this treatment on the exposure of cryptic gp41 epitopes. Comparison of this data with work by other investigators would help validate the method.

Although the paper addresses questions of significant interest to the HIV community this reviewer cannot recommend publication until several additional pieces of data have been generated. While the synergy data is sufficient to support the manuscripts conclusions, in the opinion of this reviewer, the data demonstrating that CAP initiates conformational changes in HIV envelope that lead to the formation of a six-helix bundle in gp41, while suggestive, is insufficient to support this conclusion. In particular, the experiments that involve lysis of viral particles followed by capture of six-helix bundle structures by immobilized antibodies present data that is inconsistent with previously published work. Specifically, the lack of binding by untreated, lysed viral particles is inconsistent with work cited by the authors (ref. 33) that demonstrates that treatment of viral envelope with lysis buffer results in the formation of the gp41 six-helix bundle. A simple immunoprecipitation experiment should resolve this issue In addition, it is suggested that if possible these experiments be carried out in a format other than the described ELISA. Other techniques, including several types of immuno-staining would be very useful in supporting the ELISA data. Until the discrepancy between observations in previous reports and this study and the issues outlined above are resolved this reviewer cannot recommend publication.

**Competing interests:**

None declared.