Reviewer's report

Title: Raltegravir decreases inflammatory signaling in brain macrophages in human immunodeficiency virus infection in vitro

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Reviewer: Kenneth Witwer

Reviewer's report:

This is an interesting study by a productive research team on the effects of raltegravir on cells of importance to HIV-associated central nervous system disease. The authors are aware of and describe limitations to the work.

Major compulsory revisions

1. Methods description of supernatant removal should define "periodically" (p. 4—figure 2 suggests it's every 48 hours, at 1, 3, 5, 7, and 9 days). Any regular changes of media, which would seem to be needed over a 9-day experiment, should be indicated. The statistical analysis section on cytokine production re-written to indicate how medium volume changes were dealt with, if applicable.

2. The beginning of the Results and Discussion section should be re-cast to include more information and for better flow. Here are some suggestions and questions. a) Experimental set-up should be introduced immediately, not parenthetically lower in the paragraph: specifically, that four conditions were explored including RAL alone (this should also be done more clearly in methods); b) Briefly justify RAL dosage used, HIV infection parameters; c) The methods indicate that p24 supernatants were taken at multiple time points, but only 1 and 9 days are shown in Figure 1. Although it is appreciated that this figure is not the central point of the paper, brief discussion of the selection of these time points from among the others available would be useful, or at least a change of "over 9 days" to "at 9 days" or similar (if we don't get to see the "over"). d) Top of p. 7: Whether significant or not, "highest production" of all detected cytokines was in the presence of RAL alone; please revise to indicate this. e) There is some concern that so few cytokines were detected. Were others at or near the limit of detection in one or more conditions? Were these three simply above the limit of detection in all conditions and all replicates?

3. An introduction to the paragraph on tubulin and GFAP would be helpful to the reader—why these proteins were measured and how this related to the previous work.

4. In the discussion, p. 8, "RAL caused a significant reduction in the rate of production of these three cytokines" is incorrect. It should read something like, "RAL on its own caused an increase in the production of cytokines, but resulted in a significant decrease when administered with HIV."

5. It is remarkable that RAL and HIV separately induced cytokine production, but in combination, there was inhibition below the control level. This would seem to
merit more discussion, especially because the phenomenon could be interpreted
to indicate that RAL could be pro-inflammatory without a certain level of HIV
presence/replication.

6. Did the authors consider treating neuronal or other cells with supernatant
(and/or virus-depleted supt) from the macrophage experiments? If so, the results
would be an instructive addition to the manuscript. If not, this experiment could
be mentioned in the discussion as a future direction.

Minor essential revisions

Table 2: Is the control effect not expected to be 0?

Figure 1: indicate stats, if any. Indicate whether ND means not done or not
detected (presumably the latter).

Figure 2: y-axis label (units) missing, at least from the reviewer's copy

Second sentence of intro could be altered to "The principal targets of productive
HIV infection" as astrocytes are also targets and outnumber other cell types (but
generally do not produce replication-competent virus).

For accuracy, top of p. 3: "..3' end of _DNA in the_ HIV pre-integration
complex..."

bottom of p. 3, Materials and Methods: "in a selection medium" or "in selection
media"

top of p. 6, Methods: Clarify discrepancy between "7-plex pro-inflammatory
cytokine quantitation kit" and 10 cytokines measured (10-plex).

Abstract and beginning of Results and Discussion: no comma after "20 nM"
"Dunnett's" not "Dunnet's" (test)--this test is also not mentioned in the stats
methods section

p. 8: Rewrite IL-8/CSF sentence for clarity.

p. 8, bottom: latency sentence is unclear. Is IL-10 "leading to generation" or
rather suppressing replication and helping the incipient reservoir avoid detection,
or something else?

Level of interest: An article whose findings are important to those with closely
related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests