Reviewer's report

Title: Protective efficacy of Toxoplasma gondii calcium-dependent protein kinase 1 (TgCDPK1) adjuvated with recombinant IL-15 and IL-21 against experimental toxoplasmosis in mice

Version: 1 Date: 12 June 2014

Reviewer: Jason S. Stumhofer

Reviewer's report:

Discretionary Revisions:
1. I am unfamiliar with Kunming mice. A brief description of these mice and why they were chosen for use in the experiments could be provided.
2. Authors mention in discussion that CDPK1 is conserved between different strains of T. gondii, but is it expressed in the bradyzoite as well as the tachyzoite stage?

Minor Revisions:
1. One of the headings in the Methods section is “Expression of pVAX-IF2a plasmid in vitro” however, this is not the protein used for the vaccination studies in this manuscript. This needs to be corrected.
2. Authors state (page 6) that they vaccinated intramuscularly twice at 2-week intervals, but then list three time points. Clarify is it two or three immunizations?
3. In reference to Table I the authors mention there was not any significant difference between three control groups, but only two control groups are found in the table.
4. For figure 2 which control group is shown in the figure? They mention three control groups were used in this experiment.
5. Authors could draw upon more primary source material and not almost exclusively reference reviews, with the exception of their own work, throughout the paper.
6. Table I needs clarity. Do the different superscript letters refer to a difference in significance compared to the PBS control or between other groups?
7. In figure 1 the authors indicate there is a significant difference in IgG in the serum after pVAX/CDPK1 and pVAX/IL-21/IL-15 vaccination at 2 and 4 weeks after initiation of vaccination; however, looking at the data I don’t see how this is possible. Are the authors instead trying to indicate a significant difference between pVAX/IL-21/IL-15 and pVAX/CDPK1 + pVAX/IL-21IL-15 immunization?

Major Revisions:
1. There are a number of typos, spelling errors and grammatical errors throughout the manuscript that need to be corrected before resubmission. Also,
the authors have paragraphs that are only one sentence in length (paragraph 3 in discussion).

2. The authors need to provide stronger rationale as to why the TgCDPK1 protein would make a good vaccination candidate. Is this protein unique to this protozoan spp. and not expressed by mammals? Are serum Abs specific for this protein detected in infected humans and animals? Just because the protein is involved in motility and egress does not mean it will induce a protective Ab response upon challenge infection.

3. The expression of TgCDPK1 in the Marc-145 cells should be shown and control non-transfected Marc-145 cells should also be shown to confirm the specificity of the TgCDPK1 stain. This data is important because it helps address my concern in point #2, as the serum derived from the goat infected or immunized with T. gondii (specify) must contain antibodies specific for TgCDPK1 if it is able to stain cells transfected with the plasmid DNA. Thus, suggesting that animals infected naturally with T. gondii may make Abs against this protein.

4. The authors need to include rationale in the various sections within the results explaining why they are evaluating certain aspects of the immune response after vaccination.

5. In figure 1 the authors are only measuring total serum IgG or IgG1/IgG2c Ab levels after vaccination. However, this should be complimented with TgPDCK1-specific ELISAs primarily, because vaccination with the IL-15/IL-21 plasmid induced IgG production by itself and these Abs are not specific for CDPK1. Therefore, the majority of the Ab produced with the pVAX/CDPK1 + pVAX/IL-21/IL-15 co-vaccination could also be primarily non-specific IgG Ab, or it could in fact be specific for CDPK1.

6. The conclusions the authors are trying to make in the discussion section are often not expressed with any clarity to the reader. For example, I’m not sure what point the author is trying to make in paragraph three. Why would the activation of a Th1 response by the vaccine prevent severe immunopathology during acute or chronic T. gondii infection? The authors never show this in the manuscript.

7. In the discussion the authors should elaborate/speculate on the function of IL-15 and IL-21 during vaccination as well as reference their previous work and findings using this cytokine expression plasmid (Li et al., Vaccine 2014). What are the normal functions of these cytokines in an immune response? What cell types are they acting on after vaccination?

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

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I have no competing interest to declare.