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: 31 May 2014

Reviewer: Dolores CD Correa

Reviewer’s report:

MAJOR COMPULSORY REVISIONS

This article deals with an important issue, i.e. control of Toxoplasma gondii infection by means of vaccination, since there is no effective treatment and prevention is difficult due to parasite’s promiscuity and presence of wild cycle. Nevertheless, there is one major issue which may preclude its publication and other important aspects that –given they properly answer the major one- must be addressed as well:

The article contains several results which demonstrate specific response against T. gondii when they inject the adjuvant plasmid alone (i.e. that which codes for IL-21 and IL-15 only); these responses include antibody synthesis and antigen driven cytokine production and lymphocyte proliferation. This induction cannot be explained by the parasite challenge, since they find these responses before it. The decrease in cyst burden or survival due to this adjuvant administration may be explained and is valuable, but the presence of the specific response might indicate a poor design, and thus all results would be questionable.

Other important aspects:

a) Introduction section

a. There is an excess of information related to infection prevalence in different animal species and poor about the variety of vaccines actually being tested, both in acquired and congenital toxoplasmosis, so the background displayed in the introduction section is insufficient, and thus the “state of the art” is not explained to the reader. Although they comment about other vaccines in the Discussion, they should be mentioned in the introduction, and what made the authors to think in this new design.

b. Likewise, there is no information regarding the general immune profile which controls parasite proliferation, but until de Discussion and thus the use of the IL-15-IL-21 plasmid as adjuvant has no fundament. The role of these cytokines in toxoplasmosis and their relation to the “Th1/TH2” responses (which they determined as a measure of specific response) should be at least mentioned and referenced.

b) Methodology

a. The authors do not give details about the mouse strain. Are these animals...
inbred? Do they have resemblance to any known resistant or susceptible strains? This is relevant, since many - if not most - studies with which they compare their results, have been performed with known - mostly inbred- mice.

b. There is lack of statistical analysis test of the survival data; this should have been done to assess difference between the group treated with the CDPK1-plasmid and that treated with both plasmids, so they could sustain the combined effect.

c) Results

a. As mentioned, it is unclear why they obtain T. gondii specific antibodies (figure 1), CD pattern, proliferation and cytokine production (table 1 and figure 2) when they inoculate the plasmid with the adjuvant cytokines alone (pVAX/IL21/IL15). Moreover, most results are equal between plasmid containing specific CDPK1 alone and IL21/IL15 alone, and when there has not been challenge infection.

b. It is also unclear why they tested IgG subclass determination at the second week, when the IgG response is suboptimal if it is compared with those found at weeks 4 or 6 (see figure 1).

c. Results on survival (figure 3) are the only which suggest CDPK1 is protective, but apparently it is so even without the need of the IL21/IL15 plasmid effect, unless proper statistics shows otherwise (see above).

d) Discussion

a. Although the Discussion addresses several aspects not introduced in the first part of the paper, their vaccine design should be more detailed compared to others (actually many) of the literature, both in terms of design and results. It would seem that a combined vaccine needs to be evaluated; so the question remains as to why they developed a new, single one and why they used the IL-21/IL-15-plasmid.

MINOR ESSENTIAL REVISIONS:

Language spelling must be carefully reviewed along the entire manuscript; even though my maternal language is not English I could detect some problems. Only as examples of missing text, lack of clarity or grammatical/spelling/typing errors are the following:

a. The last sentence of the introduction lacks text within “...and increase the of protective T. gondii immunity.”

b. It is unclear in the Methods section what were 10 mice used for: in page 5, it is stated that they used 35 mice per group; then in page 5 challenge with the RH and PRU strains are described for 15 and 10 mice respectively; thus, there are 10 animals not described, or so it seems.

c. In the Results section, the text “but there was not any significant different between three control groups....” could be “but there was no significant difference among the three control groups....”

d. In the Discussion: “The results showed that immunization intramuscularly with...” could be “The results showed that intramuscular immunization with...”
Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests