Title: Combined immunization using DNA-Sm14 and DNA-Hsp65 increases CD8+ memory T cells, reduces chronic pathology and decreases egg viability during Schistosoma mansoni infection

Authors:

Milena S Espindola (mеспindola@usp.br)
Fabiani G Frantz (frantz@usp.br)
Luana S Soares (luanasisoares@usp.br)
Ana P Masson (masson@usp.br)
Cristiane Tefe-Silva (cristefe@usp.br)
Claudia S Bitencourt (claubitencourt@yahoo.com)
Sergio C Oliveira (scozeus1@gmail.com)
Vanderlei Rodrigues (vrodrigu@usp.br)
Simone G Ramos (sgramos@fmrp.usp.br)
Celio L Silva (cilsilva@fmrp.usp.br)
Lucia H Faccioli (faccioli@fcfrp.usp.br)

Version: 3
Date: 3 May 2014

Author's response to reviews: see over
May 2, 2014

Dear Dr.

Nathaniel Nazareno

Journal Editorial Office

BioMed Central

We appreciated the thoughtful comments from both reviewers. We have taken these comments into consideration and made modifications throughout our manuscript.

We hope our changes and new data are appropriate and that the modified version of our manuscript is now suitable for publication in BMC Infectious Diseases. If you consider necessary, we can use a professional language editing service to improve the style of written English. Thank you once again for your efforts and your interest in our work.

In the revised manuscript, our replies are outlined point-by-point in the comments below. The original reviewer comments are shown in italic. Changes to the text that addresses reviewer concerns are highlighted in yellow.

Best regards,

Dra. Lúcia Helena Faccioli
Reviewer 1.

Reviewer's report:

*This study has great relevance for research related to the control of neglected diseases. Thus, the following comments serve only to emphasize the importance of this study.*

*In Figure 3 there is no need for two subtitles 3A and 3B, because it has the same experimental groups.*

Thank you for your observation. The figure subtitle was changed as suggested.

*Furthermore, it would be very interesting to add as a demonstrative figure, by shape of photomicrographs of the histopathology*

Thank you for your suggestion, we added a figure (Figure 4) demonstrating the granuloma formation in HE, Picrosirius staining and the α-SMA marker by immunohistochemical analysis.

Reviewer 2.

Reviewer's report:

**Major Compulsory Revisions:**

*In this study carried out by Espindola et al the combination of DNA-Sm14/DNA-Hsp65 increased the numbers of CD8+ memory T cells and decreased the Schistosoma mansoni eggs viability in experimental schistosomiasis. In addition, DNA-Sm14/DNA-Hsp65 demonstrated antifibrotic property. Although this paper is valuable, there are some aspects should be improved and answered.*

**GENERAL COMMENTS:**
I suggest the inclusion of a group only with DNA-Hsp65 to evaluate all parameters, but mainly the antifibrotic effects, in comparison with DNA-Sm14/DNA-Hsp65 group.

Despite of the protective effect of DNA-Hsp65 in the granuloma model, which was a static model of 8 days with no interference of the parasite cycle [1], in the present work we could not achieve the same protection during experimental infection with DNA-Hsp65 alone. We did not observe reduction of worm burden, neither increasement of dead eggs in the intestine of mice (data not shown). Additionally, there was no enhanced production of IFN-γ in the bronchoalveolar space by the mice immunized only with DNA-Hsp65 (Figure S2).

The possible explanations are that, here we used a dynamic model of infection where the effects of the vaccines were possibly modulated by immune induction mediated by the worms during the course of infection. Therefore, taken together this preliminary data, we decided to use DNA-Hsp65 only as an adjuvant molecule that could increase protective response when used together with a specific vaccine (DNA-Sm14). In this sense, we did not perform the antifibrotic studies on DNA-Hsp65 immunized mice.

Although, using different approaches, we were able to observe the potential benefits of DNA-Hsp65 (used together with DNA-Sm14) in reducing chronic pathology in the liver of infected mice. Its antifibrotic role was observed when comparing the combined vaccination with DNA-Sm14 alone, by the reduction of soluble and tissue collagen, α-SMA accumulation around the granulomas 69 days post infection and the maintenance of soluble collagen levels between 48 and 69 days post infection.

The simultaneous vaccination with DNA-Sm14/DNA-Hsp65 is effective to control the schistomiasis after infection? The therapeutic treatment could be carried out.

This is an interesting question, especially when is taken under consideration the inclusion of a vaccine in the market and its employment in endemic areas. However, in our work we did not try a therapeutic treatment with our vaccines. We did try different approaches using prime-boost protocols with DNA-Sm14 and DNA-
Hsp65 (data not shown), but we did not have good results concerning the worm reduction.

Observing the immunobiology of *Schistosoma* infection and elimination, it is well known that the inflammatory response that occurs initially in the lung preferentially eliminates the schistosomula stage, in which the blood capillaries of the lungs are known to be the largest site of natural and immune elimination of schistosomula [2, 3]. In the other hand, adult worms develop different mechanisms to survive and often scape from immune system effector response [4], but they are the targets of Praziquantel, the drug of choice in schistosomiasis that does not eliminate immature worms.

Furthermore, it is also known that the ideal anti-schistosome vaccine must be able to reduce parasite load and that the schistosomula migrating is considered the main target in order to obtain protective immunity [5, 6]. Different schistosome vaccine models focuses on a strong Th1 immune response in the beginning of the infection, in order to eliminate the schistosomula, including DNA-Sm14 vaccine. Therefore, we don’t believe that an immunization protocol after the establishment of the disease would reduce the parasite load and the immunopathological injuries at the liver.

*How is the inflammatory response in the liver? Histological analysis of hepatic tissues could be provided.*

Thank you for your suggestion, we added a figure (Figure 4) demonstrating the granuloma formation in HE staining, Picrosirius staining and the α-SMA marker by immunohistochemical analysis.

*How are Th1/Th2 cytokines profiles of vaccinated animals?*

According with Fonseca et al. (2006), the immunological profile generated following immunization with DNA-Sm14 is directed to Th1 response with high production of IFN-γ and the regulatory cytokine IL-10 by splenic cells restimulated with rSm14 protein *in vitro*, and also increased production of IgG anti-Sm14 [7].
The immunological profile generated after immunization with DNA-Hsp65 is mediated by antigen-specific T cells producing IFN-γ and cytotoxic products, but not IL-4, indicating a Th1 response, with increased production of IgG1 and IgG2a [8-10].

In our study, we observed that immunization with DNA-Sm14 induced a higher Th1 response compared to DNA-Sm14/DNA-Hsp65 according to INF-γ production on the Bronchoalveolar lavage fluid 15 days after infection (Figure S2).

Additionally, we calculated the IgG1/IgG2a ratio, in order to demonstrate the T helper profile generated. In mice, IgG1 is preferentially produced during a Th2 response and the isotype IgG2a is preferentially produced during a Th1 response. Therefore, the lower is the IgG1/IgG2a ratio, the higher is the Th1 profile. In our results, we observed that immunization with DNA-Sm14 alone induced a lower ratio compared to DNA-Sm14/DNA-Hsp65. Finally, we concluded that the immunological profile of DNA-Sm14/DNA-Hsp65 did not overlap the immune Th1 induction of DNA-Sm14. These findings are probably directly linked to the enhanced worm reduction achieved by DNA-Sm14 alone, but not by DNA-Sm14/DNA-Hsp65.

Abstract
The information about statistical analyses should be removed.
More details about DNA-Hsp65 should be given.

Material and methods
References in the topic "Immunization Procedures" should be added.

I suggest to exchange "control group" to other such as "non-immunized and infected group" in all text.
All suggestions above were made. Thank you.

Results
The combined vaccination induced the differentiation of CD8+ memory T cells. Is it different from DNA-sm14? More clarification about this in the text is needed.
Yes. The statistical analysis was missing and it was now correct. DNA-Sm14/DNA-Hsp65 differentiates significantly more CD8+ memory T cells/Total CD8+ comparing to Control group. In the other hand, DNA-Sm14 did not statistically enhance CD8+ memory T cells/Total CD8+ comparing to Control group. This statement was clarified in the text.

Discussion

In the fifth paragraph is written “However, we observed that DNA-Hsp65 enhanced the number of dead eggs compared to DNA-Sm14 alone.” I believe that should be “However, we observed that DNA-Sm14/DNA-Hsp65 enhanced the number of dead eggs compared to DNA-Sm14 alone.”

Thank you, we have corrected this sentence.

References

More current references should be added

We now tried to accomplish this issue.

References


7. Fonseca CT, Pacifico LG, Barsante MM, Rassi T, Cassali GD, Oliveira SC: Co-administration of plasmid expressing IL-12 with 14-kDa Schistosoma mansoni fatty acid-binding protein cDNA alters immune response profiles and fails to enhance protection induced by Sm14 DNA vaccine alone. *Microbes and infection / Institut Pasteur* 2006, **8**(9-10):2509-2516.

