

POSTER PRESENTATION

Open Access

Cytokine expression profile in hamsters immunized with OmpL37 from *Leptospira interrogans* in different vaccine formulations

Thaís Oliveira^{1*}, André Grassmann², Rodrigo Schuch¹, Mariana Pereira¹, Daiane Hartwig³, Alan McBride², Odir Dellagostin¹

From 5th Congress of the Brazilian Biotechnology Society (SBBIOTEC)
Florianópolis, Brazil. 10-14 November 2013

Background

Pathogenic spirochetes from the genus *Leptospira* are the bacteria that cause leptospirosis, an emerging zoonosis responsible for over 500,000 human cases each year [1]. Vaccination with inactivated whole-cell preparations (bacterins) has limited efficacy due to the wide antigenic variation of the pathogen. Bacterins are reactogenic and confers serovar specific and short-term immunity [2]. The protein OmpL37 represents a potential target for vaccine development against leptospirosis since it is recognized by human and animal serum, binds human extracellular matrix components, is up-regulated in vivo and conserved among pathogenic leptospires [3]. We aimed to evaluate the immune response induced by OmpL37 from *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 in hamsters, using prime-boost, DNA, and protein-based immunizations.

Methods

The *ompL37* gene was cloned into pAE and pTarget vectors, to obtain a subunit and a DNA vaccine, respectively. The recombinant protein OmpL37 (rOmpL37) was characterized by Western blot (WB) and pTarget/*ompL37* was evaluated by transfection of CHO-K1 cells and analyzed by immunofluorescence. Groups of 6 hamsters were immunized twice with an interval of 21 days as follows: rOmpL37-Alhydrogel (2x 100 µg), pTarget-*ompL37* (2x 100 µg), prime-boost pTarget-*ompL37* (100 µg) plus

rOmpL37 (100 µg), pTarget (2x 100 µg) and PBS-Alhydrogel. Two independent experiments were conducted. Pooled blood samples, collected at days 0, 21 and 42, were processed for RNA isolation using the RiboPure-Blood Kit (Ambion). cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems). Expression profiles of IFN- γ , TNF- α , IL1- α and TGF- β were accessed by quantitative real-time PCR using SYBR Green PCR Master Mix (Applied Biosystems). The relative Ct ($\Delta\Delta C_T$) method was used to quantify cytokine gene expression. The CT of each test gene was evaluated in pooled hamster whole-blood samples, the CTs were normalized against the β -actin gene CT (ΔCT) and then compared to the same normalized gene in the respective control groups (calibrator) [4].

Results and conclusion

Considering that target genes are up or down-regulated when a 2-fold change in mRNA levels is observed [5], TNF- α was induced by rOmpL37 at day 42 (ratio = 2.84), and by pTarget/*ompL37* at days 21 and 42 (ratio > 5). In contrast, IFN- γ was down regulated in the prime-boost group at day 42 (ratio = 0.41). Similarly, down-regulation of IL1- α was observed at day 42 in the pTarget/*ompL37* (ratio = 0.28) and prime-boost (ratio = 0.19) groups. TGF- β was expressed at basal levels in all groups. Both rOmpL37 and pTarget/*ompL37* were able to induce a pro-inflammatory response, characterized by increased TNF- α expression. However, the Th1 and pro-inflammatory cytokine levels decreased in the prime-boost group.

¹Laboratório de Vacinologia, Unidade de Biotecnologia, CDTEC, Universidade Federal de Pelotas, Pelotas, RS, Brazil

Full list of author information is available at the end of the article

Acknowledgements

CNPq, CAPES and FAPERGS.

Authors' details

¹Laboratório de Vacinologia, Unidade de Biotecnologia, CDTEC, Universidade Federal de Pelotas, Pelotas, RS, Brazil. ²Laboratório de Pesquisa em Doenças Infecciosas, Unidade de Biotecnologia, CDTEC, Universidade Federal de Pelotas, Pelotas, RS, Brazil. ³Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, RS, Brazil.

Published: 1 October 2014

References

1. Adler B, de la Peña Moctezuma A: **Leptospira and leptospirosis.** *Veterinary Microbiology* 2010, **140**(3-4):287-296, doi:10.1016/j.vetmic.2009.03.012.
2. Dellagostin OA, Grassmann AA, Hartwig DD, Félix SR, da Silva ÉF, McBride AJ: **Recombinant vaccines against Leptospirosis.** *Human Vaccines* 2011, **7**(11):1215-1224, doi: 10.4161/hv.7.11.17944.
3. Pinne M, Choy HA, Haake DA: **The OmpL37 surface-exposed protein is expressed by pathogenic Leptospira during infection and binds skin and vascular elastin.** *PLoS Negl Trop Dis* 2010, **4**(9):e815, doi:10.1371/journal.pntd.0000815.
4. Livak KJ, Schmittgen TD: **Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method.** *Methods* 2001, **25**(4):402-408, doi:10.1006/meth.2001.1262.
5. Vernel-Pauillac F, Goarant C: **Differential cytokine gene expression according to outcome in a hamster model of leptospirosis.** *PLoS Negl Trop Dis* 2010, **4**(1):e582, doi:10.1371/journal.pntd.0000582.

doi:10.1186/1753-6561-8-S4-P164

Cite this article as: Oliveira et al.: Cytokine expression profile in hamsters immunized with OmpL37 from *Leptospirainterrogans* in different vaccine formulations. *BMC Proceedings* 2014 **8**(Suppl 4):P164.

Submit your next manuscript to BioMed Central
and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

