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

Thaís Oliveira[1*], André Grassmann[2], Rodrigo Schuch[1], Mariana Pereira[1], Daiane Hartwig[3], Alan McBride[2], Odir Dellagostin[1]

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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.

[1]Laboratório 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

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Authors' details
[1]Laboratório de Vacinologia, Unidade de Biotecnologia, CDTec, Universidade Federal de Pelotas, Pelotas, RS, Brazil. [2]Laboratório de Pesquisa em Doenças Infecciosas, Unidade de Biotecnologia, CDTec, Universidade Federal de Pelotas, Pelotas, RS, Brazil. [3]Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, RS, Brazil.

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