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Effect of IL-22 on DNA vaccine encoding LACK gene of *Leishmania major* in BALB/c mice

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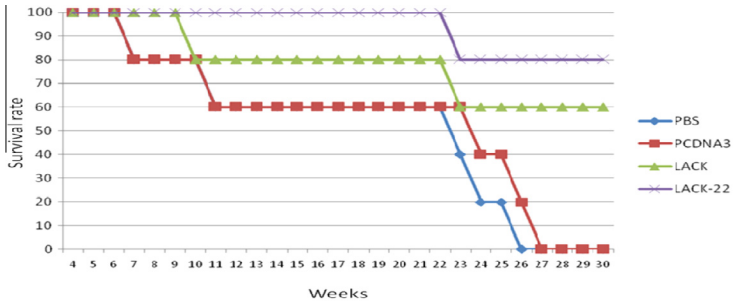
HIGHLIGHTS

- It was shown that IL-22 brought about Th1 cytokine responses.
- IFN-γ level in the LACK + IL-22 group was significantly higher than LACK group.
- IL-4 level in the LACK + IL-22 group was significantly lower than LACK group.
- Results show effectiveness of IL-22 in survival rate.

GRAPHICAL ABSTRACT

Survival rate of immunized and control groups of mice during 30 weeks after the challenge with 2×10^6 promastigotes of *Leishmania major*.



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ABSTRACT

In the present study, the effect of IL-22 together with the plasmid encoding LACK (*Leishmania* homolog of receptors for activated C-kinase) gene of *Leishmania major* on the trend of leishmaniasis in BALB/c mice was evaluated.

Evaluation of the cellular and humoral immunity was performed by measurement of IL-4 and IFN-γ, culture of splenocytes and MTT assay, and measurement of total IgG, IgG1, and IgG2a in the control and immunized groups. Clinical evaluations were also carried out by measurement of the lesion size, survival rate, and body weight of mice.

Comparison of the mean size of lesions in the LACK and LACK + IL-22 groups demonstrated that the mean size of lesions of the two groups was significantly different from week four ($p < 0.05$).

The survival rate at day 170 after challenge for the PBS, pcDNA3 (empty plasmid), pcLACK (pcDNA3 containing LACK gene), and pcLACK + IL-22 groups were 20%, 40%, 60%, and 80%, respectively.

According to the results of IFN-γ, IL-4, total IgG, IgG1, and IgG2a measurement and the MTT assay, IL-22 obviously caused an increase in IFN-γ production and a decrease in IL-4 production before and after the challenge ($p < 0.05$). The results showed the effectiveness of IL-22 in DNA vaccine. It showed that IL-22 brought about Th1 cytokine responses and high survival rate of mice.

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1. Introduction

Leishmaniasis is a widespread disease, reported from almost all countries of the world. So far, no effective vaccine or drug for control of the disease, and appropriate chemical method for definite fight against the vector is available (Reed, 2001; Duthie et al., 2012; Wolff et al., 1990).

In past decades, the results of numerous studies about the appropriate vaccine have been publicly released. DNA vaccines are powerful methods for induction of the specific cellular and humoral immune response. Sometimes, these vaccines are used in combination with plasmids encoding exogenous cytokines (such as IL-6, IL-7, or IL-12) or chemokines as the regulators of the immune response, the immune stimulator molecules against the antigen encoded by the DNA (Bhopale, 2003; Kofta and Wedrychowicz, 2001; Hoseinian Khosroshahi et al., 2012).

The LACK (Leishmania homolog of receptors for activated C-kinase) antigen of *Leishmania major* (*L. major*) is a 36-KDa protein, consisting of 313 amino acids, and is present in the promastigote and amastigote of different strains of *Leishmania* (Kemp et al., 1994; Antonio et al., 2006; Perez-Jimenez et al., 2006; Maillard and Launois, 2001). The immunogenicity of DNA of LACK/p36 against leishmaniasis was confirmed (Lopez-Fuertes et al., 2002; Ahmed et al., 2004; Tapia and Perez-Jimenez, 2003; Gurunathan and Sacks, 1997).

The LACK antigen, that is highly conserved among related *Leishmania* species, is expressed in both promastigote and amastigote forms of the parasite (Mugneau et al., 1995). LACK is a target for the early anti-parasite immune response (Julia and Glaichenhaus, 1999). In a study comparing different DNA vaccine candidates, it was demonstrated that the most promising gene is LACK (Ahmed et al., 2004). LACK vaccination trials using protein or DNA vectors show protection against cutaneous *L. major* infections by a protective Th1 response (Mugneau et al., 1995; Afonso et al., 1994). Cocktail DNA vaccine containing LACK gene increased the cellular response and survival rate and induced protection against infection with *L. major* in the mice (Ghaffarifar et al., 2012).

IL-22 is a cytokine belonging to the IL-10 family, is an effective inhibitor of cellular immunity and a potent enhancer of humoral immunity (Dumoutier et al., 2001). IL-22 is secreted by Th-17, particularly natural killer (NK) cells, Th22 (CCR10+), and Th1 cells immediately after activation of IL-4. IL-22 activates JAK1, TYK2, STAT1, STAT3, STAT5, and the MAP kinase pathway (Conti et al., 2003; Igwa et al., 2006; Dumoutier et al., 2001; Nagem et al., 2002).

It has been shown that cutaneous keratinocytes have receptors for this cytokine. In the skin, IL-22 induces antimicrobial peptides, enhances the proliferation and inhibits the differentiation of keratinocytes, and plays a role in wound healing and innate immunity mechanism. The role of IL-22 in skin disorders such as atopic eczema and allergic contact dermatitis is unknown (Boniface et al., 2005). The IL-22 produced by NK cells inhibits the growth of *Mycobacterium tuberculosis* via activation of macrophages and increases the phagolysosome activity (Dhiman et al., 2009).

In a study, it was demonstrated that the people with higher levels of IL-22 have a higher level of resistance to human Kala-azar. It was reported that *Leishmania donovani* stimulates the differentiation of Th17 cells for production of IL-22 and IFN-γ, and IL-22 has a protective effect against human Kala-azar (Pitta et al., 2009).

The factors related to definite treatment and eradication of *L. major* and also control of its arthropod vector are not still determined. Furthermore, the role of IL-22 in development of protection and innate immunity mechanisms particularly for intracellular bodies has been recently confirmed. Therefore, the current study was performed to know whether IL-22 influences the DNA vaccine effect or not.

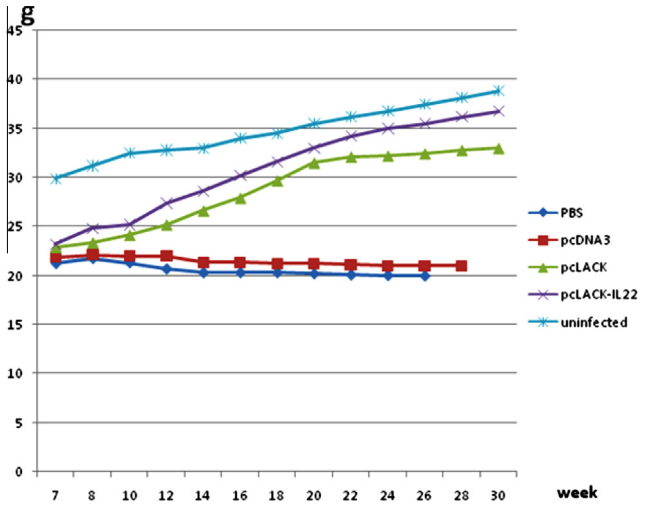

Fig. 1. Mean values of body weight (*g*) in vaccinated groups in three doses with three-week intervals with pcLACK (50 μg in 100 μl), pcLACK (50 μg in 100 μl) plus one dose of IL-22 (5 ng per g body weight of mice), and control groups, PBS (100 μl) and pcDNA3 (50 μg in 100 μl), as second control group, 7 and 30 weeks after challenge in comparison with uninfected control mice.

2. Materials and methods

2.1. Plasmid construction

We used a recombinant pcDNA3 expression plasmid that contained LACK gene (939 bp) and encodes a 36-kDa antigen. The plasmid was constructed in the Department of Parasitology, Tarbiat Modares University, Tehran, and its expression was previously evaluated by Jorjani et al. (2012).

Primary and mass culture of the plasmid containing LACK gene and pcDNA3 was carried out in TOP10 bacterium on the LB Broth culture medium (LB Broth, Miller, Luria–Bertani, Germany). The plasmid was extracted by the endo-free plasmid purification Mega Kit (QIAGEN, Germany).

2.2. Preparation and injection of IL-22

Murine recombinant IL-22 protein (Cat. Number 582-ML; R&D) was prepared in sterile PBS containing 1% BSA according to the manufacturer's protocol. IL-22 (5 ng per g body weight of mice in 100 μl) was injected in the quadriceps muscle using 30-gauge insulin syringes (Ziaee Hezarjarib et al., 2012).

Since BLAB/c mice are susceptible to promastigotes of *L. major*, we bought 60 female inbred BALB/c mice with the age of eight weeks from the Razi Vaccine and Serum Research Institute. The mice were divided into four groups, two control and two experimental groups (15 mice in each group).

Each group was divided to three subgroups, five mice in each subgroup: one subgroup was vaccinated without being infected by the parasite (20 mice) and the second and third subgroups received vaccination and then were challenged by the parasite (40 mice).

2.3. Vaccination

Sixty female BALB/c mice of eight-week age were divided into four groups. Group 1 received PBS (100 μl) in three doses with three-week intervals, as first control group; group 2 received pcDNA3 (50 μg in 100 μl) in three doses with three-week intervals, as second control group; group 3 received pcLACK (50 μg in 100 μl,