

Interleukin-10 Serum Levels after Vaccination with *In Vivo* Prepared *Toxoplasma gondii* Excreted/Secreted Antigens

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Received: 04 Dec 2012 / Accepted: 14 Feb 2013
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Abstract

Objectives: *Toxoplasma gondii* is a worldwide prevalent zoonotic parasite which causes toxoplasmosis. An appropriate vaccine for animals could interrupt the circle between animals and humans. Our previous study showed that excreted/secreted antigens (E/SA), derived from the peritoneum of mice infected with *T. gondii* tachyzoites could be considered as a good candidate for animal vaccination. Interleukin-10 (IL-10) inhibits proliferation of B and T lymphocytes and induces homeostasis in immune system responses. However, since IL-10 has also been shown to suppress the killing of *T. gondii* by human macrophages, the aim of this study was to evaluate IL-10 serum levels after vaccination with *T. gondii* E/SA prepared *in vivo*.

Methods: *T. gondii* tachyzoites were inoculated in the peritoneum of mice and harvested E/SA were used as a vaccine, with and without adjuvant, in *T. gondii* infected and un-infected mice. IL-10 serum levels were evaluated using the ELISA technique.

Results: The data showed that although serum levels of IL-10 were not changed at the early phases, they were elevated at the end phases of vaccination with *T. gondii* E/SA.

Conclusion: Based on these and our previous results, it can be concluded that *in vivo* prepared *T. gondii* E/SA could be considered as a good candidate for animal vaccination.

Keywords: *Toxoplasma gondii*; Vaccination; IL-10; Excreted/secreted antigens.

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Introduction

Toxoplasma gondii (*T. gondii*) is the main cause of the toxoplasmosis.^{1,2} This parasite rarely causes any clinical presentations in infected immuno-competent individuals; however, immuno-suppressed adults or congenitally infected infants may suffer from the severe pathological effects of the disease.³ *T. gondii* can be transmitted by eating or drinking infected meat or milk, respectively, during contact with food, water or dust contaminated with cat feces, or by handling infected animals.^{4,5} Therefore, the immunization of the animals against *T. gondii* could be effective in reducing the risk of human contamination.

Purified and recombinant proteins, and DNA of *T. gondii*,⁶⁻⁸ as well as whole inactive tachyzoites,⁹ have been used to make an appropriate vaccine in animals. However, none of the vaccines have induced successful immunization against the parasite. Excreted/secreted antigens of *Toxoplasma gondii* tachyzoites (E/SA), which represent more than 90% of the parasite circulated antigens,^{2,10} might be the first target of the host immune system. Namely, it seems that E/SAs play a crucial role in inducing appropriate humoral and cellular immune responses against *T. gondii*.¹⁰

Some vaccination studies which used E/SA derived from *in vitro* *T. gondii* culture,^{9,11} reported that although it might be considered as a good candidate for immunization against *T. gondii*, it cannot achieve 100% protection.^{9,11} This perhaps may be due to a lack of appropriate glycosylation of *in vitro* prepared antigens, which has been observed (*in vivo*) in *Toxoplasma gondii*.¹² Hence, *in vivo* prepared *T. gondii* E/SA may represent a better choice for a vaccine. Our previous study already showed that *T. gondii* E/SA derived from the peritoneum of mice can be considered as a good candidate for animal vaccination.²

Interleukin-10 (IL-10) plays a key role in the regulation of many functions of the immune system.¹³ This cytokine inhibits the proliferation of B and T lymphocytes and induces homeostasis in immune system responses.¹³ Additionally, IL-10 suppresses the killing of *T. gondii* by human macrophages.¹⁴ Therefore, it is important that a vaccine against *Toxoplasma gondii* does not elevate IL-10 production, at least not during the first few days after vaccination. Hence, the aim of this study was to evaluate IL-10 serum levels in mice after vaccination with the *in vivo* prepared *T. gondii* E/SA.

Methods

The study was carried out on 8-10-week-old Balb/C female inbred mice obtained from an animal house at the Rafsanjan University of Medical Sciences and maintained under standard conditions. Each investigation group consisted of 8-10 mice and received different injections. The designed groups were: A: No injection; B: 10 µg prepared E/SA; C: 10 µg prepared E/SA and 100 µl completed Freund's adjuvant; D: 100 µl completed Freund's adjuvant; E: (*T. gondii* infected) 10 µg prepared E/SA; F: (*T. gondii* infected) 10 µg prepared E/SA and 100 µl completed Freund's adjuvant; G (*T. gondii* infected): 100 µl completed Freund's adjuvant; and H: 100 µl normal saline solution. Blood samples were collected from the tail vein 3, 7, 14, 28 and 56 days after immunization of the mice and sera were stored at -20°C until IL-10 analysis. Pre-immune serum samples (group A) were used as negative controls.

Antigen (E/SA) preparation was done 3 days after the infection of mice by peritoneal inoculation with *T. gondii* tachyzoites (RH strain), peritoneal fluids were taken, centrifuged (1000×g for 15 min) and E/SA supernatants were filtered and saved at -20°C for future use. The cytokine assay was done using IL-10 serum levels which were detected using ELISA technique (eBioscience, ESP), immediately after blood collection. Assays were performed according to the manufacturer's guidelines. The sensitivity of the kit

was 2 pg/ml and inter- and intra-assay assessments of reliability of the kit were conducted.

Statistical analyses were done to determine the differences between different groups with the same time interval after the immunization of mice and between same groups with different time intervals after the immunization of mice, the data was analyzed using ANOVA and repeated measures ANOVA tests, respectively. A *p* value less than 0.05 was considered significant.

Results

The (*T. gondii* infected) mice in groups E, F and G died after 5 days, while uninfected mice lived, as expected. Cytokine assays revealed that the serum levels of IL-10 did not change in groups A and H during different times intervals after the immunization of mice; while they were increased in groups E, F and G 3 days following immunization of the mice. Elevated serum levels of IL-10 were also seen in groups C and D groups on days 14, 28 and 56 after E/SA injection, while in group B, IL-10 serum levels increased following 14 and 28 days but decreased to control levels on day 56 after the immunization of mice (Table 1). The results also demonstrated that IL-10 serum levels were significantly up-regulated in groups C and D after days 14, 28 and 56 compared to groups A and H. (Table 1 & Fig. 1)

Table 1: Serum levels of IL-10 in the designated groups.

Group	Days after vaccination					<i>p</i> value
	3	7	14	28	56	
A	403.3 ± 85.3	410.35 ± 88.34	408.35 ± 76.81	450 ± 26.81	423 ± 32.1	<i>p</i> =1.000
B	447.1 ± 75.6	890 ± 160.13	1074.5 ± 170.9	968.8 ± 27.2	485.5 ± 57.18	<i>p</i> =0.04
C	621.5 ± 49.6	655.6 ± 48.29	1646.5 ± 267.23	1125.35 ± 127.04	1092.7 ± 219.4	<i>p</i> <0.001
D	695.4 ± 37.4	928.37 ± 159.82	1536.5 ± 192	1227.23 ± 83.21	1064 ± 146.21	<i>p</i> <0.001
E	2136.1 ± 285.9	-	-	-	-	-
F	2868.5 ± 249.5	-	-	-	-	-
G	1743.2 ± 278	-	-	-	-	-
H	827.57 ± 74.12	704.36 ± 171.96	689.36 ± 174.48	801.36 ± 85.75	940 ± 168.85	<i>p</i> =1.000
<i>p</i> value	<i>p</i> <0.001	<i>p</i> =0.12	<i>p</i> <0.001	<i>p</i> <0.001	<i>p</i> =0.006	-

Table shows significant differences between groups after days 14, 28 and 56 after vaccination. Data are shown as mean ± SE (pg/ml).

Discussion

Toxoplasmosis is a common parasitic infection that is transferred from animals to humans.² Therefore, the development of a good vaccine is essential for stopping the life circle of *Toxoplasma gondii* in the animal. To the best of our knowledge, the current study is the first reported investigation based on *in vivo* prepared E/SA used as a vaccine against *T. gondii*.

Our data shows that 3 days following the immunization of mice, the serum levels of IL-10 were increased only in the groups of mice infected with *T. gondii* (E, F and G groups). Previous studies demonstrated that *T. gondii* induces up-regulation of IL-10 serum

levels in order to suppress immune responses of the host.¹⁵ Hence, the results demonstrating elevated IL-10 serum levels, as well as death of mice in groups E, F and G, suggest that vaccination with *in vivo* prepared *T. gondii* E/SA is unable to suppress the infection. On the other hand, there were no differences observed in serum levels of IL-10 between groups of uninfected mice 3 days following immunization (groups B, C and D); thus, suggesting that at the early phases of vaccination with *T. gondii* E/SA, there was no change in the serum levels of IL-10 in the mice.

However, IL-10 serum levels were elevated in groups C and D groups on days 14, 28 and 56 following the immunization of mice. While in group B, serum levels of IL-10 increased on days 14 and

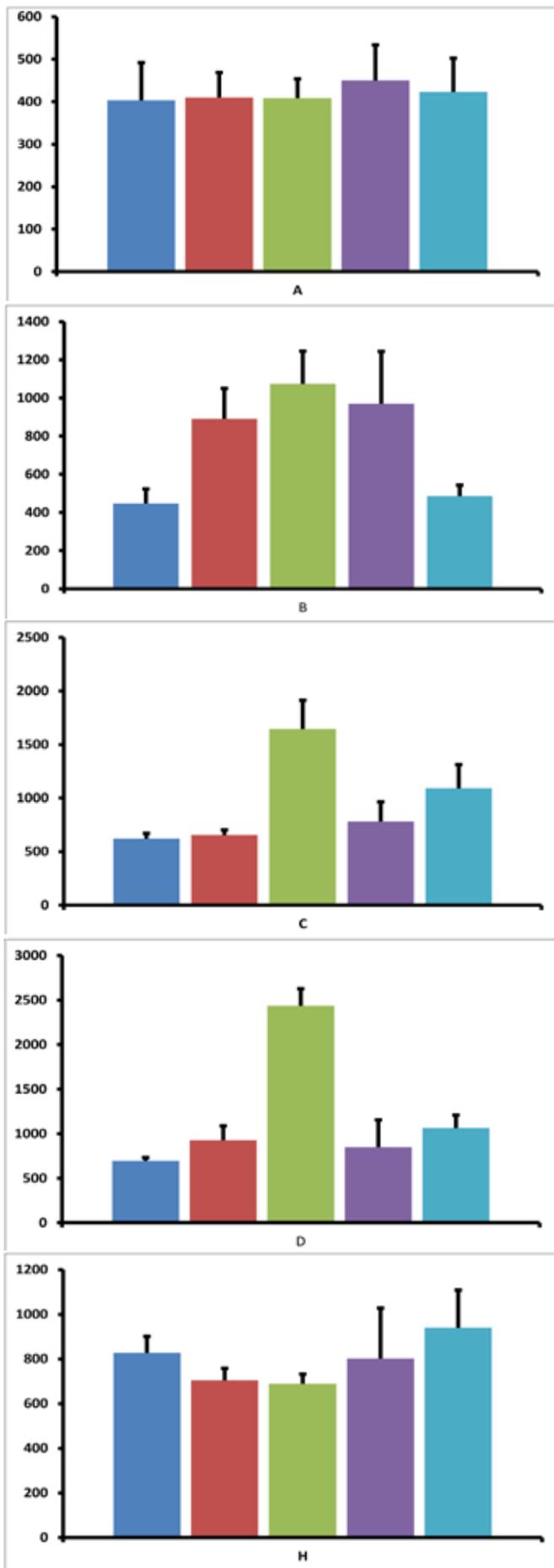


Figure 1: Serum levels of IL-10 were increased significantly on the 14th day after antigen injection in groups B, C and D.

*=significant differences between groups ($p < 0.001$).

28 after the mice were immunized, but the levels returned to control levels on day 56. In groups B, C and D, the elevated serum levels of IL-10 14 or more days after immunization represent the normal immune response in order to induce homeostasis. Namely, the increased IL-10 serum levels suppress elevated immune responses against *T. gondii* E/SA or following completed Freund's adjuvant injection, in order to prevent or to inhibit hypersensitivity of the immune system and autoimmunity processes. Interestingly, our previous study showed that like IL-10 serum levels, the serum levels of TGF- β (another anti-inflammatory cytokine) were also unchanged during the early phases following *in vivo* prepared *T. gondii* E/SA injection, although IL-10 levels in the serum of mice increased at the later phases.

Although serum levels of the cytokine IL-10 were high on day 56 in groups C and D of mice receiving completed Freund's adjuvant, they decreased to the normal range in the mice that were not injected with completed Freund's adjuvant (B group). Hence, these results suggest that the return of IL-10 serum levels from elevated to the control levels on day 56 may be due to the absence of completed Freund's adjuvant.

Rosenberg et al. showed that the serum levels of IL-10 in mice were increased following DNA vaccination with recombinant chimeric tachyzoite antigens.¹⁶ Moreover, increased serum levels of IL-10 were demonstrated 23 days after vaccination of mice against *T. gondii* with Mic1-3KO tachyzoites.¹⁷ On the other hand, DNA vaccine of plasmid encoding the rhoptry protein 1 gene combined with the genetic adjuvant of pcIFN- γ , applied against *T. gondii* did not induced IL-10 production in mice after 30, 50 and 70 days.¹⁸ These results suggest that this vaccination probably produced immune hypersensitivity or autoimmunity in the mice. Some studies which have used other types of the vaccination, including DNA vaccine,¹⁹⁻²¹ purified protein,²² and subunit vaccines,²² against *T. gondii* have demonstrated increases in the inflammatory and decreases in the anti-inflammatory cytokines.¹⁹⁻²³ Additionally, based on the fact that so many tests were done in the analysis, hence, the multiple testing was as a limitation to discuss the results.

Conclusion

The results of this study and our previous study,² suggest that *in vivo* prepared *T. gondii* E/SA can be considered as a potentially good vaccine for the animals, due to a good anti-E/SA induction, no enhancement of IL-10 serum levels at the early phases, as well as the increase in the serum levels of IL-10 during the final phases of vaccination. Although the advantages of *T. gondii* E/SA prepared *in vivo* include the induction of anti-E/SA with higher avidity, as well as the preparation being cheaper and easier E/SA in the mice peritoneum; more aspects of this method of vaccination, such as cellular immunity and animal survival need to be evaluated in order to overcome the mechanisms used by *T. gondii* to escape from the host immune responses.

Acknowledgements

The authors of this article would like to take this opportunity to thank all the staff at the Molecular and Medicine Research Center and of the animal house at Rafsanjan University of Medical Sciences, who warmly helped this research project. This project was supported by a grant from Rafsanjan University of Medical Sciences.

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