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Propranolol efficacy as a novel adjuvant for immunization against *Toxoplasma gondii* tachyzoites

Samad Minaei[a], Shahram Shahabi[b], Shahram Seyyedi[b], Naser Ziaali[c], Habib Mohammadzadeh Hajipirloo[a,*]

[a] Department of Parasitology and Mycology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[b] Department of Microbiology, Immunology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[c] Department of Parasitology and Mycology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

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ABSTRACT

Severe or lethal damages, caused by *Toxoplasma gondii* infection in congenital cases and immunocompromised patients implies the necessity for development of a vaccine and an appropriate adjuvant would be needed to elicit a protective Th1 biased-immune response. The adjuvant activity of propranolol was surveyed and compared with alum by immunization of BALB/c mice with protein components of *T. gondii* tachyzoites. Five groups of BALB/c mice were immunized with phosphate buffered saline (negative control), *Toxoplasma* lysate antigen (TLA), alum plus TLA, Propranolol plus TLA, and alum, propranolol and TLA. Immunization efficacy was evaluated by lymphocyte proliferation and DTH tests, challenge with live tachyzoites, IFN-γ production by spleen cells, serum TNF-α concentration and anti- *Toxoplasma* total IgG, IgG1 and IgG2a measurements. Mice of the PRP-TLA group induced significantly more IFN-γ and TNF-α production and lymphocyte proliferation than other groups. This group of mice also showed more anti-*T. gondii* IgG2a and DTH responses and showed a significantly increased survival time after challenge. These findings indicate that propranolol as an adjuvant in combination with TLA, may enhance cellular immunity against T. gondii.

1. Introduction

Toxoplasma gondii is a cosmopolitan coccidian protozoon that while its sexual stage is confined to the intestinal cells of cats, its asexual stages can infect different types of cells from almost all warm blooded animals including human (Miller et al., 2009). Despite of rare complications, primary infection in immune competent individuals leads to a benign and self-limiting illness but in immunocompromised patients or if transmitted congenitally, it may be fatal or cause severe birth defects or abortion (Jongert et al., 2009). There are many diverse routes by which *Toxoplasma* infects humans. Water, food and hands polluted with oocysts and eating raw or undercooked meat containing tissue cysts are the most important potential routes of transmission (Tenter et al., 2000; Montoya and Liesenfeld, 2004). With so many ways of transmission, vaccination against *Toxoplasma* would be an excellent alternative for usual prevention tasks (Bhopale, 2003). Though there is a commercially available live attenuated vaccine for domestic animals, no vaccine has been licensed yet to be used in humans (Liu et al., 2012). The adjuvant that is selected to be administered beside the antigen, is as important as the antigen itself (Liu et al., 2012). The role of aluminum salts as adjuvants has been described and they have been the only agents accepted by FDA to be used in human vaccines for more than 80 years (De Gregorio et al., 2008; Mohan et al., 2013) but it stimulates Th2 subset of immunological responses, rather than Th1 (Hem et al., 2007) which is essential in controlling toxoplasmosis (Tan et al., 2011). We have previously shown stimulatory effect of propranolol, a β-adrenergic receptor antagonist, on shifting immune system to Th1 pattern by using it as an adjuvant in immunization against *Salmonella typhimurium* and *Plasmodium berghei* (Mazloomi et al., 2012; Shahabi et al., 2014a). Here we report the effect of this drug when is used as adjuvant on enhancing the potency of immunization against *T. gondii*.

2. Materials and methods

2.1. Mice

Six to eight week old male BALB/c mice were obtained from Razi Institute (Karaj, Iran). One week prior to the experiments, the mice

* Corresponding author.
E-mail addresses: sm.minaei@yahoo.com (S. Minaei), shahabirabori@yahoo.com (S. Shahabi), seyedish@gmail.com (S. Seyyedi), naserzia@yahoo.com (N. Ziaali), h.mohammadzadeh@umsu.ac.ir (H. Mohammadzadeh Hajipirloo).

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were allowed to acclimate to housing conditions. All studies were performed according to the Animal Care and Use Protocol of Urmia University of Medical Sciences (Urmia, Iran).

2.2. Parasite

Cryopreserved *T. gondii* RH strain originally from France, was thawed and injected to BALB/c mice intraperitoneally.

2.3. Toxoplasma lysed antigen (TLA) preparation

Three to four days after infection, 5 ml phosphate-buffered saline (PBS - pH 7.2) was injected in peritoneal cavity of anesthetized BALB/c mice and re-aspirated after a few abdomen taps and gentle massage. The exudate was passed 5 times through a 27 G needle for the macrophages to be disrupted and tachyzoites to free and was centrifuged and washed 3 times with PBS in 750 g. The pellet was then frozen and thawed 3 times and PMSF (Sigma, Germany), EDTA (Merck, Germany), penicillin and streptomycin were added in concentrations of 5 mM, 1 μM, 100 IU/ml and 100 μg/ml, respectively. The suspension was sonicated (BANDELIN SONOPULS, HD 2200, Germany) on ice powder for 15 cycles (10 s sonication and 20 s rest for each cycle and 50 W power) and then was centrifuged in 4 °C and 12000 g for 30 min. The supernatant was dialyzed (Sigma, Germany - pore size: 12400 MWCO) against polyethylene glycol (Merck, Germany), filter sterilized (GVS, UK), and it's protein concentration was adjusted on 20 μg/50 μl and kept at −20 °C until use.

2.4. Immunization

Female 6–8 weeks old BALB/c mice were purchased and kept and treated under local Ethics Committee regulations. Mice were divided into 5 groups, 20 mice per group, and received a mixture of PBS or TLA, and aluminium hydroxide (Sigma, Germany) gel and/or propranolol (Sigma, Germany - final concentration of 6 mg/kg body weight) as adjuvants (Table 1). Buster injections were done at days 10 and 20.

2.5. Spleen cell proliferation test (MTT assay)

MTT test was performed as described before (Shahabi et al., 2014b). Briefly, spleens of 5 mice from each group were crushed separately in 2 ml of cold RPMI 1640 (Gibco, USA) and the fluid suspension was centrifuged at 2000 rpm. The supernatant was discarded and RBCs were lysed with 0.9% ammonium chloride (Mallinckrodt, USA - PH 7.2). The containing tube was centrifuged and the pellet was washed with RPMI. Live cells were counted using trypan blue dye (Sigma, Germany) and their count was adjusted to 1×10^5 cells/ml in RPMI supplemented with 10% fetal bovine serum (Gibco, USA). This suspension was divided in cells of 96-well culture microplates in 100 μl volumes and diluted with culture medium for control wells and a pre-defined concentration of TLA in RPMI for test wells. Microplates were sealed and incubated in 37 °C with 5% CO_2 for 48 h, after which 20 μl of 5 mg/ml solution of MTT powder (Sigma, Germany) in PBS was added. The microplates were incubated again for 4 h and avoiding any shake, supernatants were discarded. Formed formazan crystals were dissolved with Dimethylsulphoxide (DMSO- Sigma, Germany) and optical density was measured at 540 nm (reference wavelength: 630 nm). All tests were done in duplicate.

Table 1
Immunization protocols for groups of BALB/c mice.

Mice groups	PBS (μl)	TLA (μl)	Alum (μl)	Propranolol (μl)	Total volume (μl)
Control	150	0	0	0	150
TLA	100	50	0	0	150
Al-TLA	50	50	50	0	150
PRP-TLA	50	50	0	50	150
Al-PRP-TLA	0	50	50	50	150

2.6. IFN-γ assay

Culture microplates were prepared as mentioned above but were incubated for 72 h. The microplates were centrifuged for 10 min at 200 rpm and the supernatants were removed and stored at −80 °C until being assayed with mouse IFN-γ ELISA kit (Mabtech, Sweden- Sensitivity 2 pg/ml). The procedure was done according to the manufacturer instructions.

2.7. TNF-α assay

Two weeks after the last immunization, blood samples were taken from tails of 5 mice from each group and their sera were assayed for TNF-α concentration, using ELISA kit (eBioscience, UK− Sensitivity 4 pg/ml).

2.8. Assay for specific anti-TLA antibodies

Serum samples from 5 mice from each group that had been taken two weeks after the last immunization, were tested for anti-TLA total IgG and IgG1 and IgG2a subclasses. Microplate wells were coated with 100 μl of TLA (20 μg/ml protein concentration) in carbonate-bicarbonate buffer (H 9.6) and were incubated overnight at 4 °C and were washed three times with 0.05% tween 20 in PBS. Bovine serum albumin was used for blocking and 100 μl of 1:100 diluted serum samples were added to washed wells. HRP conjugated anti-mouse IgG (RAY BioTeck, Iran), and IgG1(AbD serotec, UK), IgG2a (AbD serotec, UK), TMB substrate (Razi Bioteck, Iran) and 5% sulfuric acid were used for the next steps in a classic ELISA test. The optical densities were measured at 450 nm wavelength (630 nm wavelength as reference). As there was no commercially available anti-TLA IgG and it's subclasses to be used as standards, the results were reported as optical densities (ODs) instead of their exact concentrations. All tests were done in duplicate.

2.9. Delayed type hypersensitivity (DTH) test

Ten days after the last immunization, 50 μl *Toxoplasma* antigen suspension containing 20 μg protein was injected in left foot pad of five mice from each group and simultaneously, 50 μl PBS was injected in the right foot pad as control. Thickness of both feet were measured 24 h later using a micrometer, and the difference of two feet for every mouse and the mean of differences for mice groups were compared.

2.10. Survival test

Three weeks after the last immunization, five mice from each group were challenged subcutaneously with 1000 live tachyzoites of *T. gondii* and their survival rates were recorded until 18 days.

2.11. Statistical analysis

Tests of variance homogeneity and normality were performed to ensure that the assumptions required for standard parametric analysis of variance were satisfied. MTT, antibody, and cytokine assays were analyzed by analysis of variance (ANOVA) followed by Tukey test and the survival rate was measured using Kaplan–Meier analysis. A p-value of 0.05 was considered as significant.

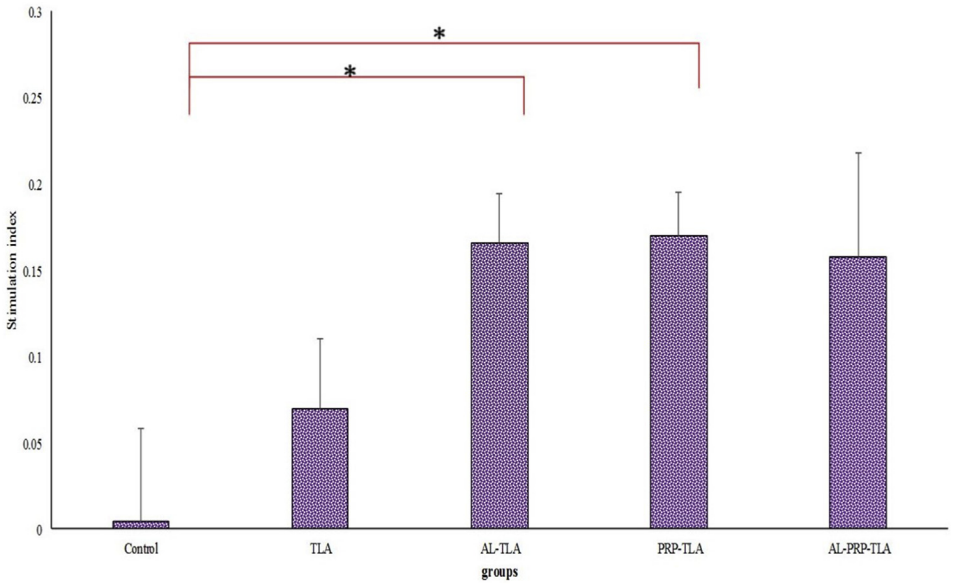

Fig. 1. Effect of administering TLA alone or with alum, PRP and AL-PRP on lymphocyte proliferation. The values are mean ± SE. *p < 0.05.

3. Results

3.1. Spleen cell proliferation

All three groups with adjuvant in their formula (Al-TLA, PRP-TLA and Al-PRP-TLA) showed much more proliferation in response to TLA antigen but even though differences with control group were statistically meaningful for Al-TLA and PRP-TLA groups, Al-PRP-TLA group could not reach that level because of higher standard deviation (Fig. 1). For the same reason, even though all three groups receiving adjuvant showed at least twofold proliferation comparing with TLA group, the difference was not meaningful.

3.2. Cytokine pattern

3.2.1. IFN-γ production

Non detectable (ND) levels of this cytokine were found in all vaccinated and control groups, except for the PRP-TLA group (Fig. 2A).

3.2.2. Serum TNF-α concentration

Serum TNF-α of mice immunized with propranolol-TLA was astonishingly higher than every other group (Fig. 2B). Comparing TNF-α concentration between TLA and Al-TLA and also PRP-TLA and Al-PRP-TLA groups, shows that alum has reduced production of this cytokine everywhere it has been used.

3.3. Specific immunoglobulin production

Mice groups with alum adjuvant in their vaccine formulations exhibited more optical densities in total specific anti-TLA IgG ELISA test. This also happened when anti-TLA IgG1 was measured, while Al-TLA group showed the least OD when surveyed for specific anti-TLA IgG2a. The proportion of IgG subclasses to total IgG was calculated by dividing the OD value of relevant IgG subclass of each mouse to its own OD for total IgG and the mean value of groups were compared (Fig. 3). All three adjuvant receiving groups showed higher IgG1/total IgG ratios but only Al-TLA group had a significant difference with control group. The highest IgG2a/total IgG was seen in PRP-TLA group following with Al-PRP-TLA group mice but because of high SD, none of them reached the significant point.

3.4. Delayed type hypersensitivity test (DTH)

All 3 adjuvant receiving groups revealed significantly higher hypersensitivity response to TLA antigen comparing with control group. The strongest response between these three groups was exhibited by propranolol and TLA received mice, though still not significant (Fig. 4).

3.5. Survival test

Mice of PRP-TLA group showed significantly higher mean survival rate (17.2 days) than all other groups when challenged with live

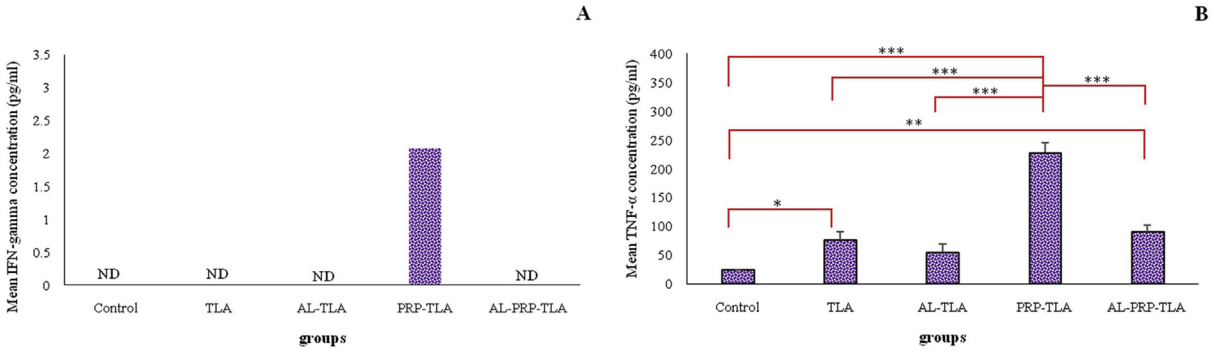

Fig. 2. IFN-γ (A) production by spleen cells of BALB/c mice exposed to *Toxoplasma* antigens and Serum TNF-α (B) concentration of BALB/c mice from different groups. The values are mean ± SE. non detectable (ND). ***p < 0.001, **p < 0.01,*p < 0.05.

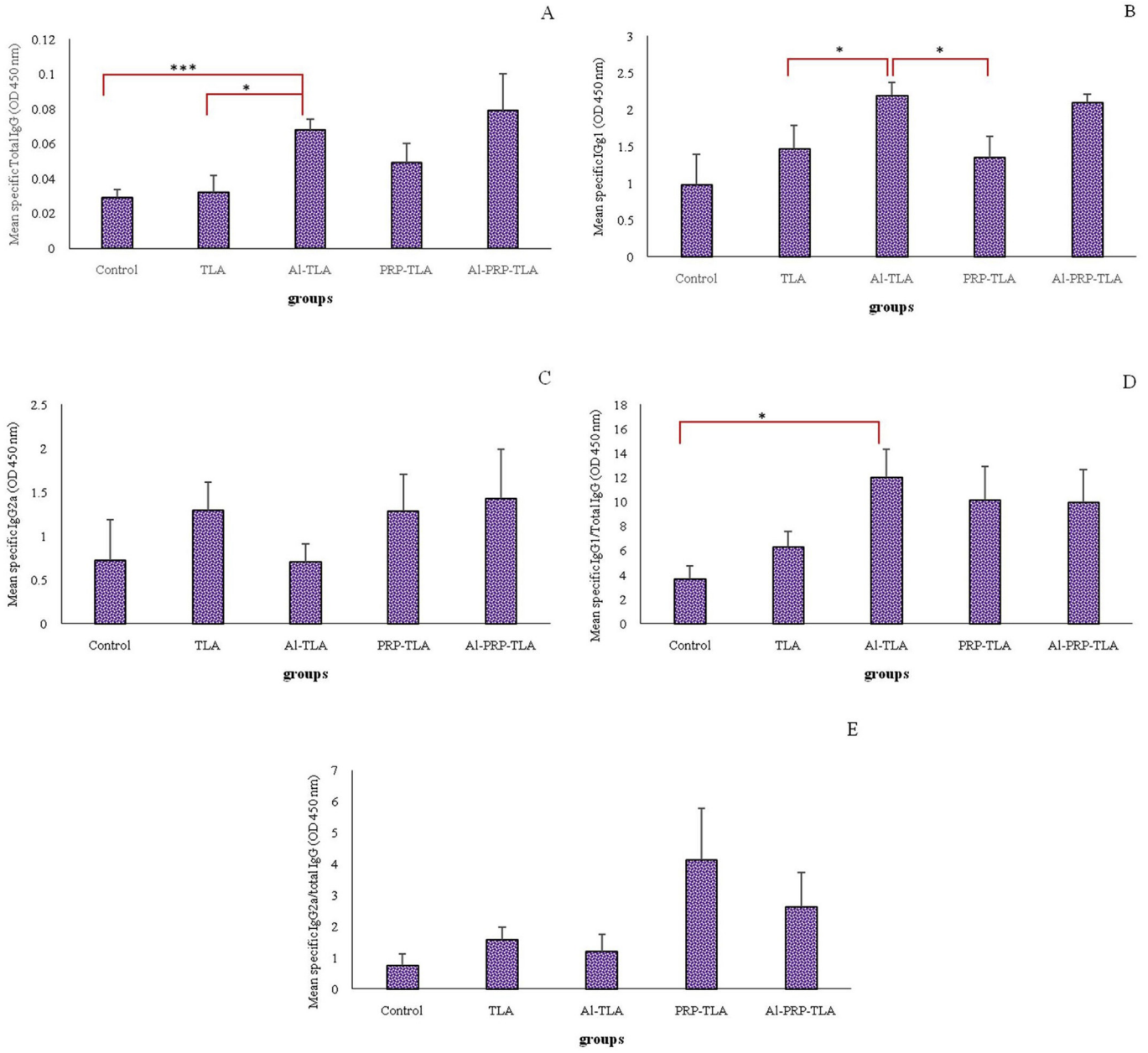

Fig. 3. Optical densities from ELISA test of mice sera for specific anti-*Toxoplasma* total IgG (A), specific anti-*Toxoplasma* IgG1 subclass (B) and specific anti-*Toxoplasma* IgG2a subclass (C). Ratio of IgG1 to Total IgG (ODs) (D) and IgG2a to Total IgG (ODs) (E) from ELISA tests of mice sera for specific anti-*Toxoplasma* antibodies. The values are mean ± SE. ***p < 0.001, *p < 0.05.

parasites (p value < 0.05). Mean survival time for control, TLA, Al-TLA, and PRP-TLA groups were 8.6, 10.8, 14.4 and 16 days respectively. The difference between survival rates of Alum-PRP and other three groups was also significant (Fig. 5).

4. Discussion

Effective immunity against *Toxoplasma* relies mostly on cellular reactions and activation of type 1 T lymphocytes (Rich et al., 2008). In mouse model, IgG1 antibodies are secreted by Th2 lymphocytes, while Th1 cells produce IgG2a antibodies and IFN-γ which directs immune system towards Th1 profile and activates cytotoxic lymphocytes (CTL) (Abbas et al., 2015). $CD4^+$ and $CD8^+$ T cells synergistically inhibit activation of tissue cysts of *Toxoplasma* during chronic phase of the disease (Rich et al., 2008).

Nervous system has an important role in shifting immune responses to Th1 or Th2 subsets. Stimulation of sympathetic system (β-adrenoreceptors) suppresses the immune responses and shifts it towards Th2 responses, while blocking them, intensifies immune responses and promotes Th1 differentiation. Norepinephrine and epinephrine inhibit pro-inflammatory cytokines and prevent cellular immunity (Elenkov et al., 2000).

Propranolol is a FDA proved drug from non-selective β blockers of β1 and β2 receptors of sympathetic system (Black et al., 1964). This study compares immunogenic potencies of this drug, as a novel adjuvant, and alum, which is a formerly known adjuvant. Our findings show that application of propranolol as an adjuvant in combination with lysed tachyzoites *of T. gondii* RH strain, significantly enhances immunogenicity of TLA which is evident in secretion of more IFN-γ and TNF-α, a tendency towards production of anti-TLA specific IgG2a, proliferation of lymphocytes, elevated DTH and longer survival of immunized mice in challenge with live tachyzoites.

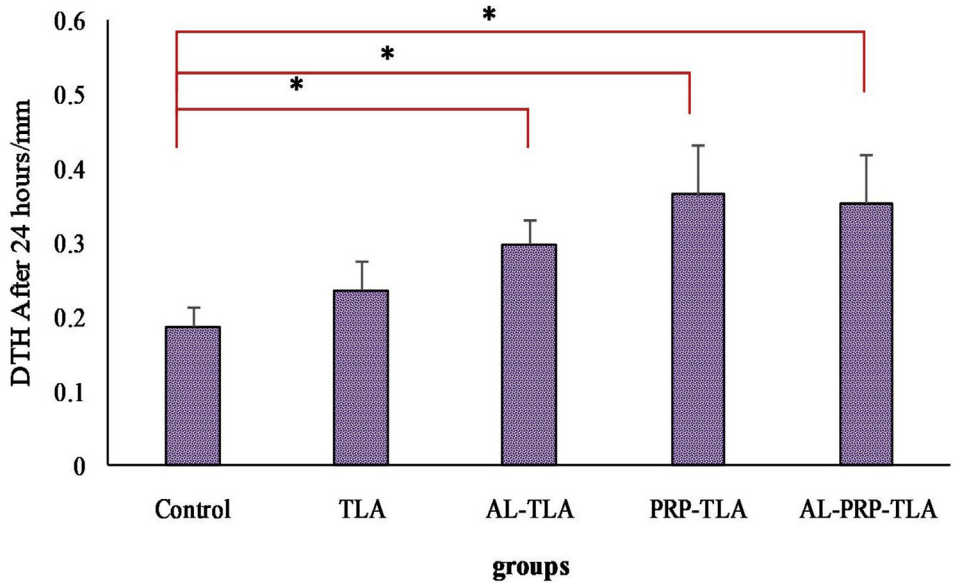

Fig. 4. Delayed type hypersensitivity of immunized mice shown as increased thickness of foot pad, 24 h after injection of *Toxoplasma* antigens. The values are mean ± SE. *p < 0.05.

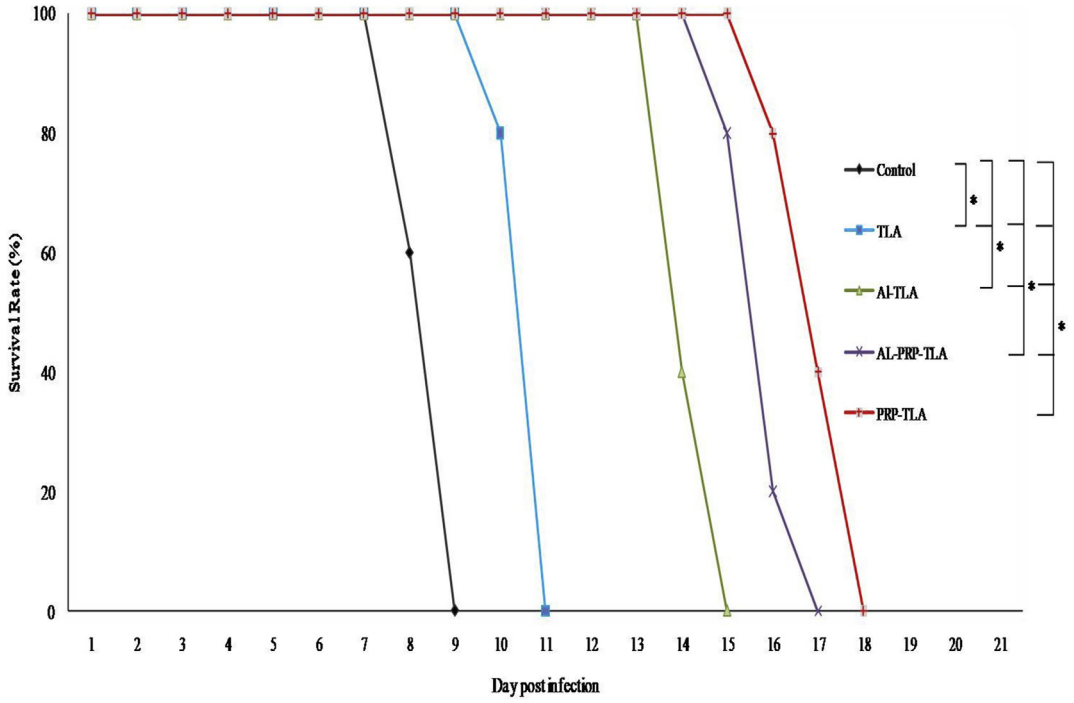

Fig. 5. Survival rate of immunized mice after subcutaneously challenging with 1000 live tachyzoites of *Toxoplasma*. *p < 0.05.

Potent adjuvanticity of propranolol seen in this study, is in agreement with our previous studies with *Salmonella typhimurium* and *Plasmodium berghei* (Mazloomi et al., 2012; Shahabi et al., 2014a). While the best result of current study was achieved with propranolol group, in the study with *S. typhimurium* it was the alum plus propranolol group that revealed the highest survival of immunized mice. This can be attributed to the fact that control of *S. typhimurium* infection needs both Th1 and Th2 mechanisms (Khalili et al., 2013).

These findings are also in coordination with effectiveness of propranolol, clenbuterol (β2-AR) and yohimbine (α2-AR) in control of BALB/c mice infection with *Leishmania mexicana* (Mittrucker and Kaufmann, 2000). In recent study, propranolol improved $CD4^+$ and $CD8^+$ responses and reduced recovery time. Treatment of influenza A virus infected mice with nadolol and ICI118,551 (non-selective β-blockers of β1 and β2 receptors), also increased anti-viral activities of $CD8^+$ T cells (Garcia-Miss Mdel et al., 2015). Immunogenicity of propranolol in treatment of herpes simplex infection and prevention of opportunistic infections in severely burned children has been shown (Peuschel, 2011; Kobayashi et al., 2011).

It has been shown that propranolol reduces recurrence of thick cutaneous melanoma in patients (De Giorgi et al., 2018) and size of pancreatic tumors in mice (Partecke et al., 2016). Application of propranolol as adjuvant in a breast tumor microenvironment, showed anti-cancer effects and increased secretion of IFN-γ and Il-2, IL-12 and IL-17 cytokines (Ashrafi et al., 2017). Use of propranolol as a drug in acute and chronic toxoplasmosis, increases anti-Toxoplasma activities and

survival of mice (Montazeri et al., 2015, 2016).

As propranolol has a short half-life time inside the body (Ware, 2007), its stimulatory effect on immune system may be due to effect on innate immunity (Scanzano and Cosentino, 2015) which in turn, promotes specific immunity. Dendritic cells, macrophages, and natural killer cells, respond to norepinephrine and express β-ARs (Maestroni, 2006) and by this way, propranolol can induce specific immunity without attaching to its receptors on effector B and T cells. In human DC β2-AR may affect Th1 or Th2 differentiation of $CD4^+$ T cells (Scanzano and Cosentino, 2015).

It is probable that, propranolol enhances local inflammation by blocking β-2 adrenergic receptors and hence inhibits the effects of catecholamines from APCs or other sources like sympathetic system or adrenal glands (Panina-Bordignon et al., 1997; Woiciechowsky et al., 1998; Engler et al., 2005; Sitkauskiene and Sakalauskas, 2005; Steinman, 2004). Catecholamines inhibit migration of neutrophils, expression of Mac1 and oxidative mechanisms (Scanzano and Cosentino, 2015). Propranolol, also recruits leukocytes and creates pro-inflammatory environment by enhancing the secretion of chemokines like CCL2 and expressing CCR2 (Guo et al., 2014).

The next possible mechanism for propranolol activity as an adjuvant, is the inhibitory effect on regulatory T lymphocytes (Treg) (Malec et al., 1988; Peek et al., 2005; Vida et al., 2011) that down regulate immune responses. Treg cells in absence of APCs, weakly suppress effector T cells and it means that APCs are important targets for Tregs. Therefore, blocking Tregs by propranolol, would allow APCs to stimulate immune system more efficiently (Houot et al., 2006; Bayry et al., 2007). Evidences show that propranolol with selective β2-AR antagonists, suppresses secretion of IL-10 (Garcia-Miss Mdel et al., 2015; Maestroni and Mazzola, 2003).

In the present study, injection of TLA alone, or along with alum adjuvant, generated weak protecting responses, comparing with other groups and it is not surprising, as alum is a weak stimulator for Th1 responses. In contrast, propranolol when used as an adjuvant with TLA, elicited stronger Th1 reactions and improved cellular immunity. Whole tachyzoite antigens were used in this study and it is predictable that applying specific antigens or epitopes may produce better responses. To our knowledge, this is the first study to survey adjuvant efficacy of a β adrenergic antagonist receptor along with *T. gondii* tachyzoite proteins as a vaccine. As propranolol is an approved and safe drug for human use, if the other surveys prove its efficacy in immunogenicity, it can be considered as a candidate to be used not only for *T. gondii* but also for most of pathogens and tumors that protective immunity against them needs Th1 responses and cellular immunity.

Disclosures

The authors declare no financially or commercially conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exppara.2018.09.014.

References

Abbas, A.K., Lichtman, A.H.H., Pillai, S., 2015. Cellular and Molecular Immunology. Saunders, pp. 218–225.
Ashrafi, S., Shapouri, R., Mahdavi, M., 2017. Immunological consequences of immunization with tumor lysate vaccine and propranolol as an adjuvant: a study on cytokine profiles in breast tumor microenvironment. Immunol. Lett. 181, 63–70.
Bayry, J., Triebel, F., Kaveri, S.V., Tough, D.F., 2007. Human dendritic cells acquire a semimature phenotype and lymph node homing potential through interaction with CD4+CD25+ regulatory T cells. J. Immunol. 178 (7), 4184–4193.
Bhopale, G.M., 2003. Development of a vaccine for toxoplasmosis: current status. Microb. Infect./Institut Pasteur. 5 (5), 457–462.
Black, J.W., Crowther, A.F., Shanks, R.G., Smith, L.H., Dornhorst, A.C., 1964. A new adrenergic betareceptor antagonist. Lancet 1 (7342), 1080–1081.
De Giorgi, V., Grazzini, M., Benemei, S., Marchionni, N., Botteri, E., Pennacchioli, E., Geppetti, P., Gandini, S., 2018. Propranolol for off-label treatment of patients with melanoma: results from a cohort study. JAMA Oncol. 4 (2), e172908.
De Gregorio, E., Tritto, E., Rappuoli, R., 2008. Alum adjuvanticity: unraveling a century old mystery. Eur. J. Immunol. 38 (8), 2068–2071.
Elenkov, I.J., Wilder, R.L., Chrousos, G.P., Vizi, E.S., 2000. The sympathetic nerve–an integrative interface between two supersystems: the brain and the immune system. Pharmacol. Rev. 52 (4), 595–638.
Engler, K.L., Rudd, M.L., Ryan, J.J., Stewart, J.K., Fischer-Stenger, K., 2005. Autocrine actions of macrophage-derived catecholamines on interleukin-1 beta. J. Neuroimmunol. 160 (1–2), 87–91.
Garcia-Miss Mdel, R., Mut-Martin, M.C., Gongora-Alfaro, J.L., 2015. beta-Adrenergic blockade protects BALB/c mice against infection with a small inoculum of *Leishmania mexicana mexicana* (LV4). Int. Immunopharm. 24 (1), 59–67.
Guo, Y.L., Zhou, J.Q., Xiang, C.Q., Yang, W.H., Zhang, B., Dai, W.J., Liu, J.H., Zheng, S.J., 2014. Monocyte/macrophage β2-AR as a target of antisympathetic excitation-induced atherosclerotic progression. Genet. Mol. Res. 13 (4), 8080–8088.
Hem, S.L., HogenEsch, H., 2007. Aluminum-containing adjuvants: properties, formulation, and use. In: Singh, M. (Ed.), Vaccine Adjuvants and Delivery Systems. John Wiley & Sons, Inc., United States of America.
Houot, R., Perrot, I., Garcia, E., Durand, I., Lebecque, S., 2006. Human CD4+CD25high regulatory T cells modulate myeloid but not plasmacytoid dendritic cells activation. J. Immunol. 176 (9), 5293–5298.
Jongert, E., Roberts, C.W., Gargano, N., Forster-Waldl, E., Petersen, E., 2009. Vaccines against *Toxoplasma gondii*: challenges and opportunities. Mem. Inst. Oswaldo Cruz 104 (2), 252–266.
Khalili, A., Hassan, Z.M., Shahabi, S., Pourfathollah, A.A., Ostad, S.N., Noori, S., Mahdavi, M., Haybar, H., Langroudi, L., 2013. Long acting propranolol and HSP-70 rich tumor lysate reduce tumor growth and enhance immune response against fibrosarcoma in Balb/c mice. Iran. J. Immunol.: IJI 10 (2), 70–82.
Kobayashi, M., Jeschke, M.G., Asai, A., Kogiso, M., Yoshida, S., Herndon, D.N., Suzuki, F., 2011. Propranolol as a modulator of M2b monocytes in severely burned patients. J. Leukoc. Biol. 89 (5), 797–803.
Liu, Q., Singla, L.D., Zhou, H., 2012. Vaccines against *Toxoplasma gondii*: status, challenges and future directions. Hum. Vaccines Immunother. 8 (9), 1305–1308.
Maestroni, G.J., 2006. Sympathetic nervous system influence on the innate immune response. Ann. N. Y. Acad. Sci. 1069, 195–207.
Maestroni, G.J., Mazzola, P., 2003. Langerhans cells beta 2-adrenoceptors: role in migration, cytokine production, Th priming and contact hypersensitivity. J. Neuroimmunol. 144 (1–2), 91–99.
Malec, P., Markiewicz, K., Tchórzewski, H., Zeman, K., Baj, Z., Nowak, Z., Majewska, E., 1988. The stimulatory effect of a single intravenous dose of propranolol of some immune parameters in humans. Allergol. Immunopathol. 16 (2), 85–89.
Mazloomi, E., Jazani, N.H., Shahabi, S., 2012. A novel adjuvant, mixture of alum and the beta-adrenergic receptor antagonist propranolol, elicits both humoral and cellular immune responses for heat-killed *Salmonella typhimurium* vaccine. Vaccine 30 (16), 2640–2646.
Miller, C.M., Boulter, N.R., Ikin, R.J., Smith, N.C., 2009. The immunobiology of the innate response to *Toxoplasma gondii*. Int. J. Parasitol. 39 (1), 23–39.
Mittrucker, H.W., Kaufmann, S.H., 2000. Immune response to infection with *Salmonella typhimurium* in mice. J. Leukoc. Biol. 67 (4), 457–463.
Mohan, T., Verma, P., Rao, D.N., 2013. Novel adjuvants & delivery vehicles for vaccines development: a road ahead. Indian J. Med. Res. 138 (5), 779–795.
Montazeri, M., Daryani, A., Ebrahimzadeh, M., Ahmadpour, E., Sharif, M., Sarvi, S., 2015. Effect of propranolol alone and in combination with pyrimethamine on acute murine toxoplasmosis. Jundishapur J. Microbiol. 8 (9).
Montazeri, M., Ebrahimzadeh, M.A., Ahmadpour, E., Sharif, M., Sarvi, S., Daryani, A., 2016. Evaluation of propranolol effect on experimental acute and chronic toxoplasmosis using quantitative PCR. Antimicrob. Agents Chemother. 60 (12), 7128–7133.
Montoya, J.G., Liesenfeld, O., 2004. Toxoplasmosis. Lancet 363 (9425), 1965–1976.
Panina-Bordignon, P., Mazzeo, D., Lucia, P.D., D'Ambrosio, D., Lang, R., Fabbri, L., Self, C., Sinigaglia, F., 1997. Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. J. Clin. Invest. 100 (6), 1513–1519.
Partecke, L.I., Speerforck, S., Käding, A., Seubert, F., Kühn, S., Lorenz, E., Schwandke, S., Sendler, M., Keßler, W., Trung, D.N., 2016. Chronic stress increases experimental pancreatic cancer growth, reduces survival and can be antagonised by beta-adrenergic receptor blockade. Pancreatology 16 (3), 423–433.
Peek, E.J., Richards, D.F., Faith, A., Lavender, P., Lee, T.H., Corrigan, C.J., Hawrylowicz, C.M., 2005. Interleukin-10-secreting "regulatory" T cells induced by glucocorticoids and beta2-agonists. Am. J. Respir. Cell Mol. Biol. 33 (1), 105–111.
Peuschel, K.E., 2011. Some clinical evidence of the hypothesis of an indirect antiviral effect of propranolol through immunoactivation. Med. Hypotheses 76 (5), 689–691.
Rich, R.R., Fleisher, T.A., Shearer, W.T., Schroeder, H.W., Frew, A.J., Weyand, C.M., 2008. Clinical Immunology: Principles and Practice, third ed. Elsevier, pp. 440–441.
Scanzano, A., Cosentino, M., 2015. Adrenergic regulation of innate immunity: a review. Front. Pharmacol. 6, 171.
Shahabi, S., Mohammadzadeh Hajipirloo, H., Keramati, A., Hazrati Tappeh, K.,

Bozorgomid, A., 2014a. Evaluation of the adjuvant activity of propranolol, a Beta-adrenergic receptor antagonist, on efficacy of a malaria vaccine model in BALB/c mice. Iran. J. Allergy, Asthma Immunol. 13 (5), 307–316.
Shahabi, S., Azizi, H., Mazloomi, E., Tappeh, K.H., Seyedi, S., Mohammadzadeh, H., 2014b. A novel adjuvant, the mixture of alum and naltrexone, augments vaccine-induced immunity against Plasmodium berghei. Immunol. Invest. 43 (7), 653–666.
Sitkauskiene, B., Sakalauskas, R., 2005. The role of beta(2)-adrenergic receptors in inflammation and allergy. Curr. Drug Targets - Inflamm. Allergy 4 (2), 157–162.
Steinman, L., 2004. Elaborate interactions between the immune and nervous systems. Nat. Immunol. 5 (6), 575–581.
Tan, F., Hu, X., Luo, F.J., Pan, C.W., Chen, X.G., 2011. Induction of protective Th1 immune responses in mice by vaccination with recombinant *Toxoplasma gondii* nucleoside triphosphate hydrolase-II. Vaccine 29 (15), 2742–2748.
Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30 (12–13), 1217–1258.
Vida, G., Peña, G., Kanashiro, A., Thompson-Bonilla Mdel, R., Palange, D., Deitch, E.A., Ulloa, L., 2011. β2-Adrenoreceptors of regulatory lymphocytes are essential for vagal neuromodulation of the innate immune system. Faseb. J. 25 (12), 4476–4485.
Ware, W.A., 2007. Cardiovascular Disease in Small Animal Medicine, first ed. Manson Publishing, London.
Woiciechowsky, C., Asadullah, K., Nestler, D., Eberhardt, B., Platzer, C., Schoning, B., Glockner, F., Lanksch, W.R., Volk, H.D., Docke, W.D., 1998. Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced by brain injury. Nat. Med. 4 (7), 808–813.