IL-17 and IL-22 Elicited by a DNA vaccine encoding ROP13 associate with protection against *Toxoplasma gondii* in BALB/c mice

Paria Alizadeh [1, 2], Ehsan Ahmadpour[*, 1], Ahmad Daryani[3], Tohid Kazemi[1], Adel Spotin[4], Mahmoud Mahami Oskouei[5], Robin J Flynn [6], Yaghob Azadi[2], Saba Rajabi [2], Siamak Sandoghchian[1]

[1] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

[3] Toxoplasmosis Research Center, Mazandaran University of Medical Sciences, Sari, Iran

[4]Infectious and Tropical Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[5] Department of Parasitology and Mycology, Tabriz University of Medical Sciences, Tabriz, Iran.

[6] Department of Infection Biology, Institute of Infection and Global Health, University of Liverpool, L3 5RF, UK

***Corresponding author:** Ehsan Ahmadpour, Ph.D

Address: Department of Parasitology and Mycology, Tabriz university of Medical Sciences, Tabriz, Iran.

Tel/ Fax: +98 41 33373745

Email address: ehsanahmadpour@gmail.com, ahmadpoure@tbzmed.ac.ir

Conflict of Interest: Authors declare there is no any conflict of interest.

Abstract:

Toxoplasma gondii, an intracellular parasitic protozoan, is capable of infecting man and all warm blooded animals. Cell-mediated immunity is vital in mounting protective responses against *T. gondii* infection. Recent studies have shown that T-helper (Th) 17 responses may play a key role in parasite control. In this current study, we constructed a DNA vaccine encoding *T. gondii* ROP13 in a pcDNA vector. Groups of BALB/c mice were immunized intramuscularly with pcROP13 or controls and challenged with the RH strain of *T. gondii*. The results showed that immunization with pcROP13 could elicit an antibody response against *T. gondii*. The expression of the canonical Th17 cytokines, IL-17 and IL-22, were significantly increased after immunization with pcROP13 compared to control groups (P<0.05). Furthermore, vaccination resulted in a significant decrease in parasite load (P<0.05). The induction of Th17 related cytokines, using a ROP13 DNA vaccine, against *T. gondii* should be considered as a potential vaccine approach for the control of toxoplasmosis.

Keywords: ROP13, Toxoplasma gondii, Th17, IL-22, IL-17, DNA vaccine, Gene Expression

1. Introduction

Toxoplasmosis is globally widespread parasitic infection caused by the intracellular protozoan pathogen, *T. gondii,* infecting humans and the other warm-blooded animals [1]. In immunocompetent people *Toxoplasma* infection is benign and mostly presents with no clinical manifestations. However, dependent on the status of host immune system it can cause serious and irreversible effects [1-2]. Toxoplasmosis in immunocompromised individuals is an opportunistic infection that may cause severe ocular and life-threatening neurological disorders [2]. Due to the high prevalence of *T. gondii* and the resulting pathogenesis of infection it is considered as a public health hazard. Despite extensive research, effective anti-*Toxoplasma* therapeutics without side effects remains a barren area [3]. Hence, the most effective strategy to reduce disease burden and clinical outcomes is the development of vaccine formulations against *T. gondii* [4].

Current approaches to immunization against *Toxoplasma* infection takes several forms including attenuated live vaccines, killed vaccines and subunit vaccines [4-5]. Owing to the safety issues with the use of attenuated or killed forms of the pathogen, subunit vaccines have attracted considerable attention [4, 6]. In particular, DNA vaccines have been developed in recent years [5]. Results from several studies have raised the possibility of developing a DNA vectored vaccine to protect against *T. gondii* infection. The most investigated compounds as vaccine candidates include excreted-secreted antigens (ESA) and surface antigens of tachyzoites [7-9]. Previous findings indicate that ESA play a significant role in disease pathogenesis, and escape of the parasite from host immunity [10]. In particular Rhoptries (ROP) are unique secretory organelles that involved in host cell penetration by *T. gondii* and parasitophorous vacuole formation allowing survival and multiplication [11-12].

The protective mechanisms against *T. gondii* involve both $CD4^+$ and $CD8^+$ T-cell responses [13]. IFN-γ is known to be the major effector as a result of T-helper 1 (Th1) cell and NK cell activation. Th17 cells are a subset of $CD4^+$ T-cells conditioned to produce the cytokines IL-17, IL-21, and IL-22 which trigger responses causing the elimination of infection [13-16]. However, T cell-dependent production of IL-17 has been implicated in both protective and pathogenic responses during infection with *T. gondii* [17]. Subsequent studies identified NK cells as the innate IL-17 secreting cells in mice challenged with *Toxoplasma* [17-18]. Moreover, IL-17 mediated signaling was reported to play an important role during the initial stages of *T. gondii* infection through neutrophil recruitment and activation [19].

Given the essential roles of ROP proteins in the pathogenesis of the *Toxoplasma* infection, these critical antigens are appropriate vaccine candidates [20]. ROP13 is a relatively recently recognized antigen of *T. gondii* and in tandem few studies have evaluated the Th17 response in *T. gondii* DNA vaccine [21]. Hence, the two major objectives of the present study was to construct a DNA vaccine vector expressing *T. gondii* ROP13 for use in immunization and to thereafter analyze the protective immune responses induced by vaccination and challenge with *T. gondii* RH strain.

2. Materials and methods

2.1 Mice and parasite

The highly virulent RH strain of *T. gondii* (type I) was used in all experiments. The RH tachyzoites were provided by Toxoplasmosis Research Center in Mazandaran University of Medical Sciences, Sari, Iran. The parasite was maintained by serial passage and intraperitoneal inoculation and female 6 to 8 week-old BALB/c mice. The animals were obtained from the Pasteur Institute of Iran and maintained under standard conventional

conditions. The animal experiments were approved by the local Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (No. IR.TBZMED.REC.1395.578).

2.2 Cloning of ROP13 and construction of plasmids

DNA was extracted from tachyzoites by using an AccuPrep genomic DNA extraction kit (Bioneer, Korea) according to the manufacturer's instructions. The NCBI GenBank database was used to determine the complete sequence of ROP13 gene of RH strain and to design specific primers (GeneBank accession number: JN051278.1). The ROP13 gene was amplified using the primer pair Forward: 5' -GGATCCATGAAGAGAACAGAGCTTTG- 3', and Reverse: 5' -TCTAGATCACAATAGCCTCAAGGAATTC- 3' with six base pair, underlined, recognition sites for *Bam HI* and *Xba I* respectively in the primers. The coding sequence of ROP13 gene was 1203bp in length which was inspected by using 1% agarose gel electrophoresis to ensure the fidelity of the PCR product. The ROP13 PCR product was then inserted into the linearized pTG19-T vector (Vivantis) between the *Bam HI* and *Xba I* sites.

The pTROP13 plasmid was then transferred into competent Top10 *E. coli* cells. Transformed bacteria were plated on LB-agar plates containing ampicillin, X-gal, and IPTG and incubated overnight. Blue/white screening was used to select transformed colonies harboring pTROP13 were isolated and subjected to PCR to confirm the correct insertion was present [21-22].

To generate the vaccine plasmid pTROP13 was recovered from *E. coli* and subject to miniprep plasmid extraction (Gene All). The ROP13 coding sequence removed from the vector by double digestion cleavage using *BamHI / XbaI* (Jena Bioscience). The coding sequence was subject to gel purification and extraction (Bioneer, AccuPrep® Gel Purification Kit) before confirmation by DNA sequencing. To construct the vaccine vector, the ROP13 gene sequence was ligated into the pcDNA3, yielded the plasmid pcROP13.

2.5 Transfection of CHO cells

Chinese Hamster Ovary (CHO) cells were transfected with the pcROP13 plasmid. Cells, 1-2 $\times 10^4$ per well were plated into a 96-well tissue culture plate and used when the cells were 50-80% confluent. Transfection was performed using jetPrime (Polyplus, France) according to the manufacturer's instructions. Uptake of pcROP13 and expression from pcROP13 was detected 24-48 hours after transfection by immunofluorescence [21].

2.6 In vitro expression of pcROP13

PcROP13 plasmid expression was detected by indirect immunofluorescence assay. Serial dilutions, beginning at 1/10, of the human anti-*T. gondii* antiserum were applied to transfected cells. Anti-sera were coated on a slide where the Transfected cells, on slides, were fixed, followed by incubation with anti-sera in a humidified chamber for 30 minutes; slides were then washed with PBS and dried at room temperature. The slides were subsequently incubated with secondary antibody of goat anti-human IgG conjugated with fluorescein isothiocyanate (FITC) for 30 minutes in the dark. After washing 3 times with PBS, the cells were mounted using buffered glycerol and examined for fluorescence detection under CYTATION5 imaging reader [21].

2.7 Mice immunization and challenge

Forty female 6-8-week-old BALB/c mice were divided into four groups; group A was vaccinated with 100 μg of pcROP13 DNA plasmid suspended in PBS, by intramuscular injection. Group B received PBS, Group C received empty pcDNA3 vector in PBS, and Group D received 20 μg of TLA (*T. gondii* lysate antigen). All mice were immunized three

times, two weeks apart prior to parasite challenge. Animals were infected with *T. gondii* RH strain by intraperitoneal injection with 1×10^4 parasites.

2.8 Immune responses and determination of parasite load

Serum IgG antibody levels were determined by ELISA as previously described (REF). Samples were obtained from mice at two individual time points including the pre-vaccination period (day 0) and on day 42 after immunization but prior to infection.

To evaluate parasite load 3 days after challenge, DNA was extracted from the blood using the Blood Genomic DNA Extraction kit (YTA, Iran, Cat No: YT9040) according to the manufacturer's instructions. Parasite load was determined by quantification of tachyzoites using real time PCR amplification of the highly conserved RE gene of *T. gondii* as previously described [23-24]. Briefly, forward primer: 5`AGGGACAGAAGTCGAAGGGG-3` and reverse primer: 5`GCAGCCAAGCCGGAAACATC-3` specified to amplify a 164-bp fragment of the RE gene using SYBR green chemistry, with all amplifications in triplicate. Q-PCR was performed using the following thermal cycling protocol: 10 minutes at 95°C, 40 cycles at 94°C for 30 seconds (denaturation), 55°C for 30 seconds (annealing) and 72°C for 30 seconds (amplification). Melting curve analysis was performed to verify the correct gene product ensuring the absence of side products. The threshold cycle (CT) value at which the fluorescence passes the fixed threshold was used to calculate the number of parasites in the samples according to a standard curve obtained with tachyzoites prepared for DNA samples over a range of 5×10^6 to 5×10^1/ml. The results were reported as *T. gondii* tachyzoite-equivalents per ml of blood.

Th17 cytokine gene expression was monitored using blood samples collected from animals

Subsequently, the blood RNA was extracted (YTA, Iran, Cat No: YT9075) and cDNA was synthesized (YTA, Iran, Cat No: YT4500) Real-time PCR for IL-17, IL-22 and GAPDH (as internal control) was performed using SYBR Green chemistry (YTA, Iran) on a Roche Real-time PCR system (Applied Biosystems). The primers for IL-17 and IL-22 based on real-time PCR were as follow: IL-17 Forward primer: TCTCTGATGCTGTTGCTGCT, IL-17 Reverse primer: CGTGGAACGGTTGAGGTAGT, IL-22 Forward primer: TTGAGGTGTCCAACTTCCAGCA, IL-22 Reverse primer: AGCCGGACGTCTGTGTTGTTA. The PCR cycling was carried out in a final volume of 20 µl reaction by an initial denaturation step at $95^{\circ}C$ for 3 min followed by 45 cycles at $95^{\circ}C$ for 10 seconds, $58^{\circ}C$ for 30 seconds, and $72^{\circ}C$ for 20 seconds. Relative mRNA expression was measured by the $2^{-(\Delta\Delta CT)}$ method, using GAPDH as a reference gene.

2.11 Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (San Diego, CA). Multiple comparisons between groups were conducted by 1-way ANOVA with post-hoc testing. $P < 0.05$ was reported to be statistically significant.

3. Results

3.1 Vaccine Construct

The total DNA extracted from *Toxoplasma* tachyzoites and the coding sequence of ROP13 gene was amplified using PCR, a 1203-bp PCR product corresponding to the ROP13 coding sequence was obtained (Figure 1A). This was inserted into the expression vector pcDNA3 between the *Bam HI* and *Xba I* cloning sites. The pcROP13 was transferred into CHO cells and the protein expression was confirmed using IFAT (Figure. 1).

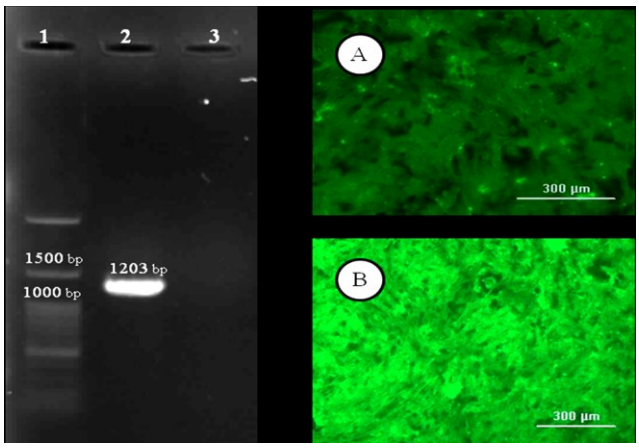
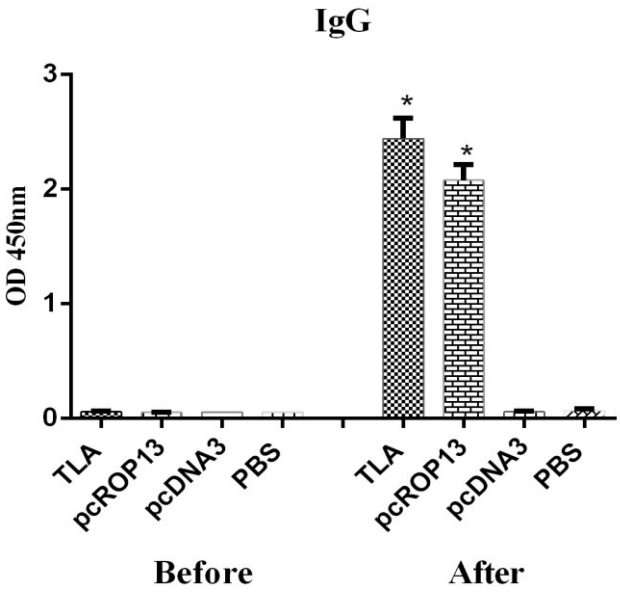


Figure 1. (Left) Gel electrophoresis analysis on PCR product, Lane 1: DNA size marker, Lane 2: ROP13 gene with the expected band size. Lane 3: negative control. (Right) Indirect immunofluorescence (IFA) detection of *Toxoplasma gondii* ROP13 on CHO cells (A: cells were transfected with pcROP13, B: empty vector).

3.2 Immunization with pcROP13

Groups of BALB/c mice were immunized with pcROP13 or appropriate controls. Immunization resulted in the seroconversion of animals as determined by ELISA. A specific antibody response in both TLA and pcROP13 immunized groups was detected after the third immunization (Figure 2). The total IgG levels for both groups was significantly different (P<0.05) when compared to the negative control groups (PBS and pcDNA3).



192

193 **Figure 2.** Measurement of the specific anti-*Toxoplasma* IgG antbody in the sera of
194 BALB/c mice before (left) and after (right) immunization at a 1:100 dilution. The results
195 are shown as mean of the OD_{450} ± SD of three independent experiments. *$P < 0.05$
196 Statistically significant differences compared to control group were determined by a 1-
197 way anova. There were no detectable antibodies against *T. gondii* in the sera of control
198 groups.

199

200 **3.3 Blood Cytokine mRNA expression**

201 48hr after the third immunization peripheral blood was collected from tail vein to evaluate the
202 expression level of Th17 cytokines. The expression level of IL-22 mRNA in pcROP13 and
203 TLA groups was found to be respectively 4 and nearly 2.5 folds higher than that observed in
204 PBS and pcDNA3 groups (Figure 3). The expression of IL-17 was also significantly elevated
205 among pcROP13 and TLA groups (2.8 and nearly 2 folds, respectively) compared with
206 negative controls ($P<0.05$).

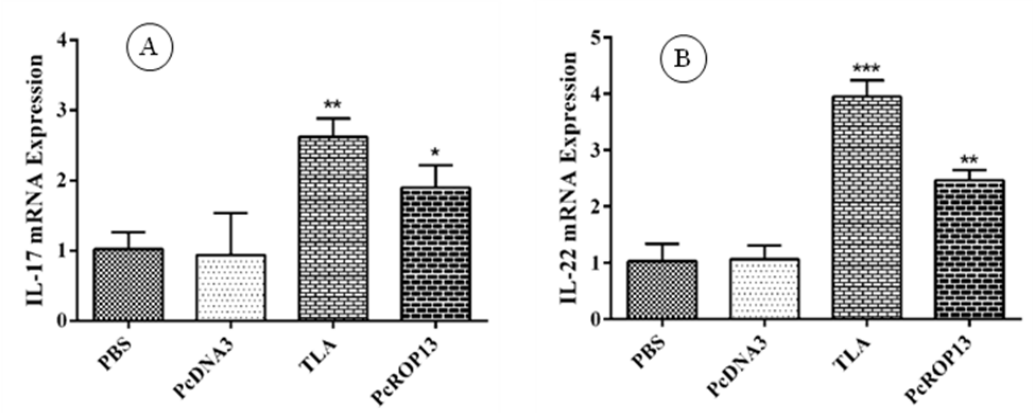


Figure 3. Relative mRNA expression of IL-17 (A) and IL-22 (B) in immunized mice with pcROP13, TLA, pcDNA3 and PBS. Results are expressed as mean and SD. *P*-value was determined using 1-way anova analysis (**P*<0.05, ** *P*<0.01, *** *P*<0.001).

3.4 **Determination of parasite load in immunized mice**

To determine the protective efficacy of the pcROP13 vaccine to induce protection against *T. gondii,* immunized mice were challenged via the intraperitoneal route with 1×10^4 tachyzoites 2 weeks after the third immunization. Blood parasite load was then determined by qPCR three days after challenge. Figure 4 clearly shows that immunization with either TLA or pcROP13 induced protection in mice as measured by the significantly different parasite burden (*P*<0.05). The PBS and pcDNA groups harbored on average 22201 and 18436 parasite/mL, respectively. The pcROP13 and TLA immunized groups harbored 1694 and 812 parasites, respectively. However no significant difference was observed between pcROP13 and TLA groups (Figure 4).

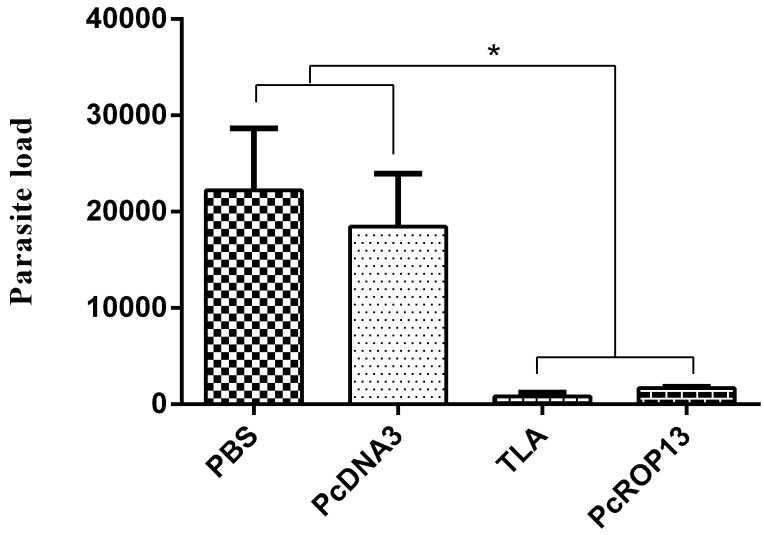


Figure 4: The parasite load, tachyzoites/mL of blood in immunized mice following a 3-day i.p. infection with RH strain of *T. gondii.* Significant differences in parasite load between groups was detected by 1-way anova (*$P < 0.05$).

4. Discussion

Immunization with *T. gondii* ROP13 gene has been previously shown to induce a strong protective humoral and cellular response against infection in the recent study when adjuvanted with IL-18 [21]. In the current study, we constructed a vaccine plasmid pcROP13 expressing protein ROP13 of *T. gondii* and evaluated the immune response induced in BALB/c mice. Our findings demonstrated that in addition to the induction of a humoral response, there is also an increased gene expression of Th17 cytokines (IL-17 and IL-22). In agreement with previous studies we found that immunization with pcROP13, as a DNA vaccine, successfully decreased the parasite load of blood in immunized mice.

In the past decade, DNA vaccines have been widely studied and have been shown to elicit an efficient immune response against target antigens in various animal models [5, 25]. Various antigens of *T. gondii* have been assessed as potential candidates for vaccine development [8,

22, 26-27]. Rhoptries are found in apical secretory organelle and function in the establishment of infection through formation of specific compartments known as parasitophorous vacuoles in which parasite evades intracellular killing [11]. ROP13 is a unique soluble effector protein known to implicate in host cell invasion that can be detected in the cytoplasm of host cells [21]. A previous study evaluated the immunogenicity of a DNA vaccine expressing ROP13 of *T. gondii*, pVAX-ROP13, in Kunming mice. The pVAX-ROP13 could induce humoral and cellular immunity against *T. gondii* [21]. The mice were assessed for production of cytokines specific for Th1 (IFN-γ and IL-2) and Th2 (IL-4 and IL-10) after immunization and the results showed that the protective efficacy of the DNA vaccine expressing *T. gondii* ROP13 were related to Th1-driven immune response in Kunming mice, confirming the importance of the cellular immune response.

Elimination of intracellular parasites is mainly conferred by Th1 immunity leading to the secretion of cytokines interferon-gamma (IFN-γ), interleukin-12 (IL-12) and tumor necrosis factor-alpha (TNF-α) [16, 18]. IL17-producing T cells termed Th17 cells secrete a set of anti-microbial cytokines including IL-22 and IL-21 and also mediate host protection against parasites and other pathogens [28-29]. They are involved in immunity to intracellular infections including *Cryptosporidium*, *Plasmodium* spp. and *Trypanosoma cruzi* [30-31]. However, pathogenic roles of Th17 responses have also been reported in the context of some parasitic infections [30, 32]. Among the complex network of cytokines that have been described in the immune responses to *T. gondii*, the pro-inflammatory cytokine, IFN-γ was shown to block intracellular development of the parasite and is considered as the main mediator of resistance to *T. gondii* [33]. There is evidence indicating that IL-22 has anti-parasite effects during infection with intracellular parasite, *Eimeria falciformis* that belongs to the same phylum with *T. gondii* [34]. In contrast with these findings, IL-22 but not IL-17 is

261 shown to drive inflammation and tissue injury following mice infection with *T. gondii* [35].
262 Furthermore, recently published data from a mouse model of rickettsial infection, an obligate
263 intracellular bacterium, demonstrates that either Th1 or Th17 responses can have protective
264 effects. Surprisingly cells producing IL-17A or IL-22 are as protective as IFN-γ producing
265 Th1 cells, if the immunopathologic effects of TNF-α are controlled [36]. This compliments
266 recent novel findings that Th17 cells provide stronger protection, compared with Th1
267 responses, against the intracellular microorganism *T. cruzi* [32]. These findings open the
268 possibility that Th17 mediated protection during *T. gondii* is a prospect for vaccination.

269 In the present study, we found significantly raised levels of both IL-17 and IL-22 mRNA in
270 mice immunized with pcROP13 compared with control mice immediately prior to infection.
271 These elevated levels of IL-17 and IL-22 in pcROP13 immunized mice associated with lower
272 parasite burdens ($P< 0.05$) compared with PBS and pcDNA3 treated mice. This set of
273 responses also provoked secretion of specific IgG antibodies detected in the sera of mice
274 immunized with pcROP13 after the last immunization compared to control groups ($P<0.05$).
275 Early reports supporting our data indicating a key role for IL-17 in the recruitment of
276 neutrophils which is required for resistance to *T. gondii* [37]. Neutrophils are critical for
277 successful host protection during early *T. gondii* infection [38]and experimental models have
278 demonstrated that IL-17R$^{-/-}$ mice show significantly decreased migration of neutrophils into
279 the peritoneal cavity after *T. gondii* infection [19], indicating that neutrophil response is
280 dependent on IL-17-induced signaling. Studies on the NK response demonstrated that the
281 need for Il-6 in driving IL-17 responses against T. gondii was conserved between across the
282 T-cell populations [17].

283 In contrast to the perception that Th17 cells only function against extracellular pathogens, we
284 have demonstrated that Th17 effectors, IL-17 and IL-22, may be important in the defense

against *T. gondii* infection as conferred by a ROP13 DNA-based vaccine. Multiple subtypes of innate and adaptive immune cells such as NK cells, γδ and CD4 T cells have been found as a source of IL-17; what subset of IL-17 producers is specifically implicated in pathogenic or protective immunity to *T. gondii* remained unclear. Further research is required to achieve a more detailed understanding of the exact correlates of protection against *T. gondii* infection in our system. This may enable us to revise the previously described harmful effects of IL-17 and IL-22 producing T cells during infection with intracellular pathogens in particular *T. gondii* infection.

Acknowledgment

This study was prepared from Paria Alizadeh's MSc thesis and supported by Immunology research center, Tabriz University of Medical Sciences, Tabriz, Iran (Grant No. 94/2-5/7). The sponsor or funding organization had no role in the design or conduct of this research.

References

[1] J.G. Montoya, O. Liesenfeld, Toxoplasmosis, The Lancet 363(9425) (2004) 1965-1976.

[2] E. Ahmadpour, A. Daryani, M. Sharif, S. Sarvi, M. Aarabi, A. Mizani, M.T. Rahimi, A. Shokri, Toxoplasmosis in immunocompromised patients in Iran: a systematic review and meta-analysis, The Journal of Infection in Developing Countries 8(12) (2014) 1503-1510.

[3] M. Montazeri, M. Sharif, S. Sarvi, S. Mehrzadi, E. Ahmadpour, A. Daryani, A systematic review of in vitro and in vivo activities of anti-Toxoplasma drugs and compounds (2006–2016), Frontiers in microbiology 8 (2017) 25.

[4] N.-Z. Zhang, J. Chen, M. Wang, E. Petersen, X.-Q. Zhu, Vaccines against Toxoplasma gondii: new developments and perspectives, Expert review of vaccines 12(11) (2013) 1287-1299.

[5] E. Hiszczyńska-Sawicka, L. Holec-Gasior, J. Kur, DNA vaccines and recombinant antigens in prevention of Toxoplasma gondii infections--current status of the studies, Wiadomosci parazytologiczne 55(2) (2009) 125-139.

[6] F. Azmi, A.A.H. Ahmad Fuaad, M. Skwarczynski, I. Toth, Recent progress in adjuvant discovery for peptide-based subunit vaccines, Human vaccines & immunotherapeutics 10(3) (2014) 778-796.

[7] M. Meng, S. He, G. Zhao, Y. Bai, H. Zhou, H. Cong, G. Lu, Q. Zhao, X.-Q. Zhu, Evaluation of protective immune responses induced by DNA vaccines encoding Toxoplasma gondii surface antigen 1 (SAG1) and 14-3-3 protein in BALB/c mice, Parasites & vectors 5(1) (2012) 273.

[8] M.T. Rahimi, S. Sarvi, M. Sharif, S. Abediankenari, E. Ahmadpour, R. Valadan, M. Fasihi-Ramandie, S.-A. Hosseini, A. Daryani, Immunological evaluation of a DNA cocktail vaccine with co-delivery of calcium phosphate nanoparticles (CaPNs) against the Toxoplasma gondii RH strain in BALB/c mice, Parasitology research 116(2) (2017) 609-616.

[9] H. Waldeland, J. Frenkel, Live and killed vaccines against toxoplasmosis in mice, The Journal of parasitology (1983) 60-65.

[10] K.N. Deilami, A. Daryani, E. Ahmadpour, M. Sharif, Y. Dadimoghaddam, S. Sarvi, A. Alizadeh, Excretory–secretory antigens: A suitable candidate for immunization against ocular toxoplasmosis in a murine model, Comparative immunology, microbiology and infectious diseases 37(5-6) (2014) 369-374.

[11] H. Dlugonska, Toxoplasma rhoptries: unique secretory organelles and source of promising vaccine proteins for immunoprevention of toxoplasmosis, BioMed Research International 2008 (2008).

[12] P.-Y. Wang, Z.-G. Yuan, E. Petersen, J. Li, X.-X. Zhang, X.-Z. Li, H.-X. Li, Z.-C. Lv, T. Cheng, D. Ren, Protective efficacy of a Toxoplasma gondii rhoptry protein 13 plasmid DNA vaccine in mice, Clinical and Vaccine Immunology 19(12) (2012) 1916-1920.

[13] R. Gazzinelli, Y. Xu, S. Hieny, A. Cheever, A. Sher, Simultaneous depletion of CD4+ and CD8+ T lymphocytes is required to reactivate chronic infection with Toxoplasma gondii, The Journal of Immunology 149(1) (1992) 175-180.

[14] E.Y. Denkers, R.T. Gazzinelli, Regulation and function of T-cell-mediated immunity during Toxoplasma gondii infection, Clinical microbiology reviews 11(4) (1998) 569-588.

[15] T. Korn, E. Bettelli, M. Oukka, V.K. Kuchroo, IL-17 and Th17 Cells, Annual review of immunology 27 (2009) 485-517.

[16] M. Munoz, O. Liesenfeld, M.M. Heimesaat, Immunology of Toxoplasma gondii, Immunological reviews 240(1) (2011) 269-285.

[17] S.T. Passos, J.S. Silver, A.C. O'Hara, D. Sehy, J.S. Stumhofer, C.A. Hunter, IL-6 promotes NK cell production of IL-17 during toxoplasmosis, The Journal of Immunology 184(4) (2010) 1776-1783.

[18] J. Alves Silva, K. Rezende-Oliveira, M.V. da Silva, mez-Hern, C. ndez, sar, B. Crema Peghini, nea, N.M. Silva, J. Mineo, Roberto, R. J, V. nior, IL-17-Expressing CD4+ and CD8+ T Lymphocytes in Human Toxoplasmosis, Mediators of Inflammation 2014 (2014) 7.

[19] M.N. Kelly, J.K. Kolls, K. Happel, J.D. Schwartzman, P. Schwarzenberger, C. Combe, M. Moretto, I.A. Khan, Interleukin-17/interleukin-17 receptor-mediated signaling is important for generation of an optimal

polymorphonuclear response against Toxoplasma gondii infection, Infection and immunity 73(1) (2005) 617-621.

[20] J.C. Boothroyd, J.-F. Dubremetz, Kiss and spit: the dual roles of Toxoplasma rhoptries, Nature reviews. Microbiology 6(1) (2008) 79.

[21] J.M. Turetzky, D.K. Chu, B.E. Hajagos, P.J. Bradley, Processing and secretion of ROP13: A unique Toxoplasma effector protein, International journal for parasitology 40(9) (2010) 1037-1044.

[22] E. Ahmadpour, S. Sarvi, M.B.H. Soteh, M. Sharif, M.T. Rahimi, R. Valadan, M. Tehrani, A. Khalilian, M. Montazeri, M. Fasihi-Ramandi, Enhancing immune responses to a DNA vaccine encoding Toxoplasma gondii GRA14 by calcium phosphate nanoparticles as an adjuvant, Immunology letters 185 (2017) 40-47.

[23] Y. Dadimoghaddam, A. Daryani, M. Sharif, E. Ahmadpour, Z. Hossienikhah, Tissue tropism and parasite burden of Toxoplasma gondii RH strain in experimentally infected mice, Asian Pacific Journal of Tropical Medicine 7(7) (2014) 521-524.

[24] A. Daryani, M. Sharif, Y. Dadimoghaddam, M.B.H. Souteh, E. Ahmadpour, A. Khalilian, S. Sarvi, T. Farazmand, H. Kalani, M. Rasouli, Determination of parasitic load in different tissues of murine toxoplasmosis after immunization by excretory–secretory antigens using Real time QPCR, Experimental parasitology 143 (2014) 55-59.

[25] A. Xenopoulos, P. Pattnaik, Production and purification of plasmid DNA vaccines: is there scope for further innovation?, Expert review of vaccines 13(12) (2014) 1537-1551.

[26] P. Sonaimuthu, X.T. Ching, M.Y. Fong, R. Kalyanasundaram, Y.L. Lau, Induction of protective immunity against toxoplasmosis in BALB/c mice vaccinated with Toxoplasma gondii rhoptry-1, Frontiers in microbiology 7 (2016) 808.

[27] H.-K. Yan, Z.-G. Yuan, H.-Q. Song, E. Petersen, Y. Zhou, D. Ren, D.-H. Zhou, H.-X. Li, R.-Q. Lin, G.-L. Yang, Vaccination with a DNA vaccine coding Perforin-like Protein 1 (TgPLP1) and MIC6 induces significant protective immunity against Toxoplasma gondii, Clinical and Vaccine Immunology (2012) CVI. 05578-11.

[28] G.F. Sonnenberg, L.A. Monticelli, M.M. Elloso, L.A. Fouser, D. Artis, CD4+ lymphoid tissue-inducer cells promote innate immunity in the gut, Immunity 34(1) (2011) 122-134.

[29] G. Zhao, Y. Fang, U. Ryan, Y. Guo, F. Wu, S. Du, D. Chen, Q. Lin, Dynamics of Th17 associating cytokines in Cryptosporidium parvum-infected mice, Parasitology research 115(2) (2016) 879-887.

[30] T. Keswani, S. Sarkar, A. Sengupta, A. Bhattacharyya, Role of TGF-β and IL-6 in dendritic cells, Treg and Th17 mediated immune response during experimental cerebral malaria, Cytokine 88 (2016) 154-166.

[31] G.-H. Zhao, W.-Y. Cheng, W. Wang, Y.-Q. Jia, Y.-Q. Fang, S.-Z. Du, S.-K. Yu, The expression dynamics of IL-17 and Th17 response relative cytokines in the trachea and spleen of chickens after infection with Cryptosporidium baileyi, Parasites & vectors 7(1) (2014) 212.

[32] C.W. Cai, J.R. Blase, X. Zhang, C.S. Eickhoff, D.F. Hoft, Th17 cells are more protective than Th1 cells against the intracellular parasite Trypanosoma cruzi, PLoS pathogens 12(10) (2016) e1005902.

[33] C.D. Dupont, D.A. Christian, C.A. Hunter, Immune response and immunopathology during toxoplasmosis, Seminars in immunopathology, Springer, 2012, pp. 793-813.

[34] J. Stange, M.R. Hepworth, S. Rausch, L. Zajic, A.A. Kühl, C. Uyttenhove, J.-C. Renauld, S. Hartmann, R. Lucius, IL-22 mediates host defense against an intestinal intracellular parasite in the absence of IFN-γ at the cost of Th17-driven immunopathology, The Journal of Immunology 188(5) (2012) 2410-2418.

[35] M. Muñoz, M.M. Heimesaat, K. Danker, D. Struck, U. Lohmann, R. Plickert, S. Bereswill, A. Fischer, I.R. Dunay, K. Wolk, Interleukin (IL)-23 mediates Toxoplasma gondii–induced immunopathology in the gut via matrixmetalloproteinase-2 and IL-22 but independent of IL-17, Journal of Experimental Medicine 206(13) (2009) 3047-3059.

[36] K. Moderzynski, L. Heine, J. Rauch, S. Papp, S. Kuehl, U. Richardt, B. Fleischer, A. Osterloh, Cytotoxic effector functions of T cells are not required for protective immunity against fatal Rickettsia typhi infection in a murine model of infection: Role of TH1 and TH17 cytokines in protection and pathology, PLoS neglected tropical diseases 11(2) (2017) e0005404.

[37] E.Y. Denkers, B.A. Butcher, L. Del Rio, S. Bennouna, Neutrophils, dendritic cells and Toxoplasma, International journal for parasitology 34(3) (2004) 411-421.

404 [38] S.K. Bliss, L.C. Gavrilescu, A. Alcaraz, E.Y. Denkers, Neutrophil depletion during Toxoplasma gondii
405 infection leads to impaired immunity and lethal systemic pathology, Infection and immunity 69(8) (2001) 4898-
406 4905.
407
408

409