MEDDOCS Open Access Publisher

Journal of Veterinary Medicine and Animal Sciences

Open Access | Review Article

Recombinant subunit vaccines against *Toxoplasma gondii*: Successful experimental trials using recombinant DNA and proteins in mice in a period from 2006 to 2018

***Ragab M Fereig*[1,2]*; Hanan H Abdelbaky*[1]*; Adel Elsayed Ahmed Mohamed*[2]*; Yoshifumi Nishikawa*[1*]**

[1]*National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan*

[2]*Department of Animal Medicine, Faculty of Veterinary Medicine, South Valley University, Qena City, Qena 83523, Egypt*

***Corresponding Author(s): Yoshifumi Nishikawa**

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

Email: nisikawa@obihiro.ac.jp

Received: July 07, 2018

Accepted: Aug 22, 2018

Published Online: Aug 30, 2018

Journal: Journal of Veterinary Medicine and Animal Sciences

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Copyright: © Nishikawa Y (2018). *This Article is distributed under the terms of Creative Commons Attribution 4.0 International License*

Keywords: Toxoplasma gondii; Vaccine; Recombinant; Toxoplasmosis; Immunization; Antigen

Abstract

Development of potent and safe vaccines is the utmost goal for all vaccinologists worldwide. Toxoplasmosis is a zoo notic disease affecting almost all the warm-blooded animals and caused by the intracellular protozoan parasite *Toxoplasma gondii.* Up to date, neither potent nor broad spectral vaccine against vulnerable hosts to *T. gondii* is available. The complexity of life cycle and various parasitic stages render the vaccine development against such parasite is far from straight forward. In the last decade, tremendous advances were achieved in the field of vaccine development against *T. gondii*. Vaccine studies against *T. gondii* were focused initially on the live, attenuated live and killed tachyzoite parasites. Although such kinds of vaccine achieved a variable degree of success, their use was restricted because of worries about the induced pathogenicity and expected high cost of manufacturing. As a result, vaccinologists shift their interest to the recombinant DNA and protein antigens. Since that time, numerous successful studies were reported indicating the effectiveness of recombinant DNA or protein as vaccine antigens. In this review, we will represent summarized information on vaccine development against toxoplasmosis and will tabulate some successful vaccine antigens using recombinant DNA or protein approach using an experimental murine model in a period from 2006 to 2018 using PubMed database.

Background

Toxoplasma gondii (*T. gondii*) is an obligatory intracellular protozoan parasite. It belongs to the family Sarcocystidae, in the phylum Apicomplexa which includes also other important parasites such as *Plasmodium* (the cause of malaria), *Eimeria* (the cause of coccidiosis) and *Neospora* (the cause of neosporosis in cattle). Four stages capable of inducing infection during the development of such parasite include tachyzoite, bradyzoite, merozoite, and sporozoite. Although *T. gondii* is a single celled-organism, it possesses a well structured and accommodated organelles rendered it as a model for studying immune responses and other aspects of host-parasite interactions. Secretory organelles such as rhoptries, micronemes, and dense granules are considered of special concern in *T. gondii* because of their role in development, invasion and survival of the parasite inside the host cell.

Cite this article: Fereig RM, Abdelbaky HH, Mohamed AEA, Nishikawa Y. Recombinant subunit vaccines against *Toxoplasma gondii*: Successful experimental trials using recombinant DNA and proteins in mice in a period from 2006 to 2018. J Vet Med Animal Sci. 2018; 1: 1005.

Toxoplasmosis in farm animals

There are several reports on abortion in sheep caused by *T. gondii* [1,2]. Sheep are considered as one of the highly susceptible animal species against toxoplasmosis. It can be infected by ingestion of contaminated food or water with sporulated oocysts. While toxoplasmosis commonly affects sheep and inducing huge economic losses, other reports of clinical toxoplasmosis in other farm animals. In pigs, *T. gondii* infection has been investigated because undercooked pork containing tissue cyst is incriminating as an important source of human toxoplasmosis. There are many reports about the prevalence of *T. gondii* infection in pigs in different countries. It has been revealed that experimental infection during pregnancy can cause vertical transmission and abortion [3,4]. In goats, natural outbreaks of toxoplasmosis were also reported. The clinical signs are mainly abortions and stillbirths. Isolation of viable parasites from the placenta and aborted kids has been detected [5]. Cattle appear to be less susceptible to toxoplasmosis than sheep, goats, and pigs. Few reports of abortion due to toxoplasmosis in cattle have been described. There is a study demonstrated the isolation of viable *T. gondii* from a naturally aborted calf [6]. However, it has been shown that experimental infection can induce transplacental transmission and abortion [7].

Toxoplasmosis in laboratory animals

Experimental animals can be divided into two groups according to their susceptibility to *T. gondii* infection, rats and Old World monkeys are categorized in a resistant group, whilst mice, hamsters, guinea pigs and New World monkeys in the susceptible group. The variable animal species are usually used according to the different experimental purposes because of showing different immunological and pathological aspects. However, mice are commonly used because of their small size and the adequacy for studying immunological interaction and progress. Different mouse strains can be used, such as C57BL/6, BALB/c, NMRI, Swiss-Webster, or C3H. Despite the mouse is a natural host of *T. gondii*, other species might be more suitable for the study of some properties of toxoplasmosis [8].

Target of vaccine antigens derived from *T. gondii*

Much of the vaccine studies of *T. gondii* have focused on surface membrane antigens and antigens released from secretory organelles. There are several surface antigens have been identified as antigenic and immunogenic antigens. For example, SAG1, SAG2, and SRS1 (SAG1-related sequence 1) or SRS2 (SAG1-related sequence 2) [9]. Rhoptries produce two types of proteins; rhoptry proteins (ROPs) which have numerous targets in the host cell, and another subset of rhoptry proteins are called RONs which have been demonstrated to target the moving junction [10]. Micronemes secrete a group of products which provide important keys and strategies for cellular processes, including gliding motility, active cell invasion, and migration through cells [11]. The successful establishment of infection relies on a characteristic phenomenon of some protozoan parasites including *T. gondii*, residing in a parasitophorous vacuole (PV), which is a well-protected area inside the host cell. The PV in the host cell is controlled with various proteins released from abundantly distributed organelles in the zoite cytosol called dense granules [12]. Basic organelles such as mitochondrion, Golgi bodies, endoplasmic reticulum and others are also well developed and exert their basic functions essential for growth, multiplication, and development of *T. gondii* either in vivo or in vitro [13-16].

Immune response to *T. gondii*

In the immunecompetent animals, the developed immune responses can lead to effectively controlling the infection and protecting against infection or reinfection with *T. gondii*. Generally, the cell-mediated immunity is responsible for controlling the intracellular *T. gondii*. However, antibodies also contribute in combating the infection. The cytokine gamma Interferon (IFN-γ) has been reported as an essential mediator of resistance against *T. gondii*. It stimulates the macrophages to kill intracellular parasites and activates cytotoxic T cells to destroy infected cells [17]. The crucial role of T cells against *T. gondii* infection has been demonstrated in a number of studies. It was also shown that the cytotoxic CD8+ T cells produced IFN-γ and interleukin-2 (IL-2) [18,19]. Added to the cytotoxic T cells, the helper T cells are also effective against toxoplasmosis. They are generally grouped into T Helper 1 (Th1) and T Helper 2 (Th2) subpopulations based on the type of cytokines they produce. The Th1 cells secrete IFN-γ, interleukin-2 and beta Tumor Necrosis Factor (TNF-β whereas the Th2 cells produce IL-4, IL-5, IL-10 and IL-13 [20]. Protective immunity against toxoplasmosis is predominantly attributed to a Th1 type of response [21]. However, antibodies also contribute to controlling the infection. For example, in *in vitro* study, specific antibodies against SAG1 could prevent the invasion of human fibroblast cells by tachyzoites [22]. In *in vivo*, antibodies might prevent the dissemination of extracellular stages via neutralization through opsonisation or complement activation [23,24].

Current status of vaccine development against toxoplasmosis

The complexity of life cycle and numerous developmental stages of different infective pathways, making the development of a potent vaccine against toxoplasmosis is not an easy task [25,26]. Currently, there is no large-scale, effective and safe vaccine can be used in the field. Toxovax is a live vaccine using S48 strain of *T. gondii*, it was originally developed for immunization of pregnant ewes to reduce abortion. Anyway, limited protection in sheep, the risk of infection, and inability to use in other animals restricted its field application and use [26]. In case of the first attempts of vaccine development against *T. gondii*, live or attenuated vaccines were mostly investigated. Live vaccines could elicit both humoral and cellular immunities and inducea variable degree of protection. However, worries about safety and restoring the pathogenicity are still constraint their use in field applications. In the regard to attenuated, killed or lysate antigen vaccines, they are safer than live ones, but adjuvant is required for improving the triggered immune responses [27]. Furthermore, most development of successful chemotherapy is problematic. This situation makes the development of an effective and safe vaccine against *T. gondii*is critical for controlling this parasitic infection in humans and animals.

Recombinant DNA and protein as subunit vaccine

In the last few years, numerous vaccine studies have been focused on the use of recombinant subunit vaccines (DNA and protein subunit vaccine). Such kinds of vaccines have numerous advantages such as the induction of long-lasting immunity, high safety, and low costs. In the case of DNA vaccines, the target gene of *T. gondii* is inserted into a eukaryotic vector which possesses the capacity to express the antigen inside the immunized host. While vaccination based on recombinant protein is depending on employing of a prepared parasite antigen, which is expressed in a prokaryotic or eukaryotic vector in each host cell

in a preceding stage. In the last decade, both recombinant DNA and protein vaccines have been achieved significant advances in triggering potent immune responses and inducing high levels of protection. Additionally, a tremendous advance in the manufacturing of recombinant protein vaccines has been occurred by using adjuvant substances to targeted vaccine antigens [27].

Conclusion

In conclusion, the data represented in this review are reporting promising results regarding the vaccination trials with recombinant subunit vaccines against *T. gondii*. This data can be exploited in the development of effective and safe vaccine and its implementation in large animals or clinical trials. Not only antigens derived from essential *T. gondii* organelles but also those contributed to metabolic or vital processes could be used. Numerous molecules tested as recombinant DNA or protein vaccine have elicited cellular and humoral immune responses indicating their properties as immunomodulatory molecules. Multi-component antigens consisting of antigens of various structural and functional compartments may exert optimal immune responses and prophylactic potentials and should be further investigated in the future studies.

Acknowledgments

Special thanks to the staff of National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan and of Department of Animal Medicine, Faculty of Veterinary Medicine, South Valley University, Egypt for their kind help and support.

Tables

Table 1: Vaccine studies in which recombinant DNA has been used as a vaccine candidate

***Toxoplasma* antigen**	**Experimental animal, challenge strain and protection index**	**Year [Reference]**
Bradyzoite antigen (BAG), Matrix antigen (MAG)	- C3H/HeN mice - Avirulent *T. gondii* SSI 119 strain - Reduced cyst formation	2006 [28]
Apical membrane antigen 1 (AMA1)	- BALB/c & C57BL/6 mice - Avirulent *T. gondii* Beverley strain (type II) - Survival rate (60%) & (40%) respectively	2007 [29]
Dense granule 1 (GRA1)	- BALB/c mice - RH (type I) - Prolonged survival time	2007 [30]
Rhoptry 13 (ROP13)	- Kunming mice - RH - Prolonged survival time	2012 [31]
Immune mapped protein-1 (TgIMP1)	- BALB/c mice - RH -Prolonged survival time	2012 [32]
Surface antigen 1 (SAG1) and 14-3-3	- Kunming mice - RH - Prolonged survival time	2012 [33]
AMA1	- C57BL/6 mice - PLK (type II) - 35%	2012 [34]
Cyclophilin (Cyp)	- BALB/c mice - RH - 37.5%	2013 [35]
Microneme 11 (MIC11)	- BALB/c mice - RH - 20%	2013 [36]
MIC3, ROP18	- ICR mice - RH - Prolonged survival time	2013 [37]
Calcium dependent protein kinase 3 (TgCDPK3)	- Kunming mice - RH - Prolonged survival	2013 [38]
ROP9	- Kunming mice - RH - Prolonged survival	2014 [39]
Deoxyribose Phosphate Aldolase (TgDPA)	- Swiss Webster (SW) mice - RH - Prolonged survival	2014 [40]

Glutathione reductase protein	- Swiss Webster mice - RH - Prolonged survival	2014 [41]
Glutathione-S-transferase (TgGST)	- Swiss Webster mice - RH - Prolonged survival	2015 [42]
SAG1, GRA2, GRA7 and ROP16	- BALB/c mice - RH - Prolonged survival	2015 [43]
ROP5/ROP7	- BALB/c mice - PRU (Type II) and RH strain -Reduce brain cyst number in PRU and prolonged survival in RH	2016 [44]
ROP17	- BALB/c mice - RH - Prolonged survival	2016 [45]
GRA1, MIC3	- BALB/c mice - RH - Prolonged survival	2016 [46]
ROP1	- BALB/c mice - RH - Prolonged survival	2016 [47]
GRA14	- BALB/c mice - RH - Prolonged survival	2017 [48]
Secreted protein with an altered thrombospondin repeat (TgSPATR)	- BALB/c mice - RH - Prolonged survival	2017 [49]
Superoxide dismutase (TgSOD)	- BALB/c mice - ME49 (Type II) strain - Prolonged survival	2017 [50]
Surface antigen protein 5B (SAG5B) and SAG5C	- BALB/c mice - RH - Prolonged survival	2017 [51]
GRA17 and GRA23	- BALB/c mice - RH - Prolonged survival	2017 [52]
GRA2, GRA5	- BALB/c mice - RH - Prolonged survival	2017 [53]
ROP54	- Kunming mice - PRU and RH strain -Reduce brain cyst number in PRU and prolonged survival in RH	2017 [54]
Cathepsin C protease-1 (TgCPC1)	- BALB/c mice - RH - Prolonged survival	2017 [55]
TgCDPK2	- BALB/c mice - RH - Prolonged survival	2017 [56]
Profilin	- Kunming mice - PRU strain - Reduce brain cyst number	2018 [57]
ROP18, perforin-like protein 1 (PLP1)	- Kunming mice - PRU - Prolonged survival	2018 [58]

Table 2: Vaccine studies in which recombinant protein has been used as a vaccine candidate

Toxoplasma antigen	Experimental animal, challenge strain and protection index	Year [Reference]
SAG1 (Protein+ complete Freund's adjuvant)	- Dunkin Hartley guinea pigs - 76K (type II) and C56 (type III) strain - Reduced parasite burden in internal organs	2006 [59]
GRA1 (Protein + PROVAXTM adjuvant)	- BALB/c mice - RH -Prolonged survival	2007 [30]
GRA2, GRA6 (Protein + monophosphoryl lipid A adjuvant)	- CBA/J mice - PRU - Reduced cyst formation	2007 [60]
ROP2, ROP4	- C3H/HeJ mice - Low virulent DX *T. gondii* strain (type II) - Reduced cyst formation	2009 [61]
Actin depolymerizing factor protein	- BALB/c mice - RH - Prolonged survival	2012 [62]
SAG1 (Protein + poly lactide-co-glycolide)	- BALB/c mice - RH - 20%	2013 [63]
ROP5	- BALB/c mice - RH - Prolonged survival	2013 [64]
ROP18 (Protein + ginsenoside Re as adjuvant)	- ICR mice - RH - Prolonged survival	2013 [65]
Protein Disulfide Isomerase (TgPDI)	- BALB/c mice - RH (type I) - 35%	2013 [66]
Profilin (PF) (Protein + Oligomannose–coated liposome adjuvant (OML)	- C57BL/6 mice - PLK (type II) - 66.7%	2014 [67]
ROP18, ROP38 (Protein + poly (lactide-co-glycolide(PLG)	-Kunming mice -PRU - Reduce brain cyst number	2015 [68]
ROP5, ROP18 (Protein +poly I:C adjuvant)	-BALB/c and C3H/HeOuJ mice -DX and RH strain - Reduce brain cyst number in DX and prolonged survival in RH	2015 [69]
MIC1, 4, 6	-C57BL/6 - ME49 and RH strains -Reduce brain cyst number in ME49 and prolonged survival in RH	2015 [70]
Phosphoglycerate mutase 2 (TgPGAM 2)	-BALB/c mice - RH strain - Prolonged survival	2016 [71]
TgCDPK6, ROP18 (Protein + poly(lactide-co-glycolide) microspheres)	- Kunming mice - PRU and RH strains -Reduce brain cyst number in PRU and prolonged survival in RH	2016 [72]
Peroxiredoxin 3 (TgPrx3)	- C57BL/6 - PLK - 55.6%	2016 [73]
Actin depolymerizing factor (TgADF)	-BALB/c mice - RH - Prolonged survival	2016 [74]

SAG1, GRA2 (Protein +Poly (DL-lactide-co-glycolide) (PLGA) microspheres (MS))	- BALB/c mice - RH - Prolonged survival	2016 [75]
Aspartic protease 3 (ASP-3)	- BALB/c mice - RH - Prolonged survival	2017 [76]
Elongation factor 1-alpha rTgEF-1α (Protein + Freund adjuvant)	- BALB/c mice - RH - Prolonged survival	2017 [77]
TgPrx1	- C57BL/6 - PLK - 66.7%	2017 [78]
Heat shock protein 70 (TgHSP70) (Protein +Alum)	- C57BL/6 - ME49 - Reduce brain inflammation	2017 [79]
TgPI-1+ROP2, TgPI-1+GRA4, TgPI-1-+ROP2+GRA4	- C3H/HeN mice - ME49 -Reduce brain cyst number	2018 [80]
ROP2 + ROP4 + SAG1 + MAG1 (Protein + Monophosphoryl lipid A from *Salmonella enterica* and Alhydrogel (InvivoGen))	- C3H/HeOuJ mice - DX - Reduce parasite cyst in brain and neurological severity	2018 [81]

References

1. Dubey JP, Schmitz JA. Abortion associated with toxoplasmosis in sheep in Oregon. Journal of the American Veterinary Medical Association. 1981; 178: 675–678.
2. Dubey JP, Welcome FL. Toxoplasma gondii-induced abortion in sheep. Journal of the American Veterinary Medical Association. 1988; 193: 697–700.
3. Dubey JP, Urban JF. Diagnosis of transplacentally induced toxoplasmosis in pigs. American Journal of Veterinary Research. 1990; 51: 1295–1299.
4. Jungersen G, Bille-Hansen V, Jensen L, Lind P. Transplacental transmission of Toxoplasma gondii in minipigs infected with strains of different virulence. Journal Parasitology. 2001; 87: 108–113.
5. Dubey JP. Epizootic toxoplasmosis associated with abortion in dairy goats in Montana. Journal of the American Veterinary Medical Association. 1981; 178: 661–670.
6. Canada N, Meireles CS, Rocha A, da Costa JM, Erickson MW, et al. Isolation of viable Toxoplasma gondii from naturally infected aborted bovine fetuses. Journal of Parasitology. 2002; 88: 1247–1248.
7. Stalheim OH, Hubbert WT, Boothe AD, Zimmermann WJ, Hughes DE, et al. Experimental toxoplasmosis in calves and pregnant cows. American Journal of Veterinary Research. 1980; 41: 10–13.
8. Darcy F, Zenner L. Experimental models of toxoplasmosis. Research in Immunology. 1993; 144: 16–23.
9. Boothroyd JC, Hehl A, Knoll LJ, Knoll LJ, Manger Id. The surface of Toxoplasma: more and less. International Journal for Parasitology. 1998; 28: 3–9.
10. Bradley PJ, Sibley LD. Rhoptries: An arsenal of secreted virulence factors. Current Opinion in Microbiology. 2007; 10: 582–587.
11. Carruthers VB, Tomley FM. Microneme proteins in apicomplexans. Sub-cellular Biochemistry. 2008; 47: 33–45.
12. Carruthers VB, Sibley LD. Sequential protein secretion from three distinct organelles of Toxoplasma gondii accompanies invasion of human fibroblasts. European Journal of Cell Biology. 1997; 73: 114–123.
13. Pieperhoff MS, Schmitt M, Ferguson DJ, Meissner M. The role of clathrin in post-Golgi trafficking in Toxoplasma gondii. PLOS ONE. 2013; 8: e77620.
14. Coffey MJ, Jennison C, Tonkin CJ, Boddey AJ. Role of the ER and Golgi in protein export by Apicomplexa. Current Opinion in Cell Biology. 2016; 41: 18–24.
15. van Dooren GG, Yeoh LM, Striepen B, McFadden GI. The import of proteins into the mitochondrion of Toxoplasma gondii. Journal of Biological Chemistry. 2016; 291: 19335–19350.
16. Tahara M, Andrabi SB, Matsubara R, Aonuma H, Nagamune K. A host cell membrane microdomain is a critical factor for organelle discharge by Toxoplasma gondii. Parasitology International. 2016; 65: 378–388.
17. Suzuki Y, Conley FK, Remington JS. Importance of endogenous IFN gamma for prevention of toxoplasmic encephalitis in mice. Journal of Immunology. 1989; 143: 2045–2050.
18. Khan IA , Ely KH, Kasper LH. A purified parasite antigen (p30) mediates CD8+ T cell immunity against fatal Toxoplasma gondii infection in mice. Journal of Immunology. 1991; 147: 3501–3506.
19. Parker SJ, Roberts CW, Alexander J. CD8+ T cells are the major lymphocyte subpopulation involved in the protective immune response to Toxoplasma gondii in mice. Clinical and Experimental Immunology. 1991; 84: 207–212.
20. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. Journal of Immunology. 1986; 136: 2348–2357.
21. Gazzinelli RT, Hakim FT, Hieny S. Synergistic role of CD4+ and CD8+ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated Toxoplasma gondii vaccine. Journal of Immunology. 1991; 146: 286–2892.
22. Mineo JR, McLeod R, Mack D, Smith J, Khan IA, et al. Antibodies

to Toxoplasma gondii major surface protein (SAG-1, P30) inhibit infection of host cells and are produced in murine intestine after peroral infection. Journal of Immunology. 1993; 150: 3951–3964.

23. Fuhrman SA, Joiner KA. Toxoplasma gondii: mechanism of resistance to complement-mediated killing. Journal of Immunology. 1989; 142: 940–947.

24. Johnson LL, Sayles PC. Deficient humoral responses underlie susceptibility to Toxoplasma gondii in CD4-deficient mice. Infection and Immunity. 2002; 70: 185–191.

25. Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS, et al. Toxoplasma gondii: epidemiology, feline clinical aspects, and prevention. Trends in Parasitology. 2010; 26: 190–196.

26. Sullivan WJ, Jeffers V. Mechanisms of Toxoplasma gondii persistence and latency. FEMS Microbiology Reviews. 2012; 36: 717–733.

27. Fereig RM, Nishikawa Y. Towards a preventive strategy for toxoplasmosis: Current trends, challenges, and future perspectives for vaccine development. Methods in Molecular Biology. 2016; 1404: 153–164.

28. Nielsen HV, Di Cristina M, Beghetto E, Spadoni A, Petersen E, et al. Toxoplasma gondii: DNA vaccination with bradyzoite antigens induces protective immunity in mice against oral infection with parasite cysts. Experimental Parasitology. 2006; 112: 274–279.

29. Dautu G, Munyaka B, Carmen G, Zhang G, Omata Y, et al. Toxoplasma gondii: DNA vaccination with genes encoding antigens MIC2, M2AP, AMA1 and BAG1 and evaluation of their immunogenic potential. Experimental Parasitology; 2007; 116: 3, 273–282.

30. Döşkaya M, Kalantari-Dehaghi M, Walsh CM, Hiszczyńska-Sawicka E, Davies DH, et al. GRA1 protein vaccine confers better immune response compared to codon-optimized GRA1 DNA vaccine. Vaccine. 2007; 25: 1824–1837.

31. Wang PY, Yuan ZG, Petersen E, Li J, Zhang XX, et al. Protective Efficacy of a Toxoplasma gondii Rhoptry Protein 13 Plasmid DNA Vaccine in Mice. Clinical & Vaccine Immunology. 2012; 19: 1916–1920.

32. Cui X, Lei T, Yang D, Hao P, Li B, et al. Toxoplasma gondii immune mapped protein-1 (TgIMP1) is a novel vaccine candidate against toxoplasmosis. Vaccine. 2012; 30: 2282–2287.

33. Meng M, He S, Zhao G, Bai Y, Zhou H, et al. 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. 2012; 5: 273.

34. Gong P, Huang X, Yu Q, Li Y, Huang J, et al. The protective effect of a DNA vaccine encoding the Toxoplasma gondii cyclophilin gene in BALB/c mice. Parasite Immunology. 2013; 35: 140–146.

35. Tao Q, Fang R, Zhang W, Wang Y, Cheng J, et al. Protective immunity induced by a DNA vaccine-encoding Toxoplasma gondii microneme protein 11 against acute toxoplasmosis in BALB/c mice. Parasitology Research. 2013; 112: 2871–2877.

36. Qu D, Han J, Du A. Evaluation of protective effect of multiantigenic DNA vaccine encoding MIC3 and ROP18 antigen segments of Toxoplasma gondii in mice. Parasitology Research. 2013; 112; 2593–2599.

37. Zhang NZ, Huang SY, Zhou DH, Chen J, Xu Y, et al. Protective immunity against Toxoplasma gondii induced by DNA immunization with the gene encoding a novel vaccine candidate: calcium dependent protein kinase 3. BMC Infectious Diseases. 2013; 13: 512.

38. Chen J, Zhou DH, Li ZY, Petersen E, Huang SY, et al. Toxoplasma gondii: Protective immunity induced by rhoptry protein 9 (TgROP9) against acute toxoplasmosis. Experimental Parasitology. 2014; 139: 42

39. Ibrahim A Hassan, Shuai Wang, LiXin Xu, RuoFeng Yan, XiaoKai Song, et al. DNA vaccination with a gene encoding Toxoplasma gondii Deoxyribose Phosphate Aldolase (TgDPA) induces partial protective immunity against lethal challenge in mice. Parasites&Vectors. 2014; 7: 431.

40. Hassan IA, Wang S, Xu L, Yan R, Song X, et al. Immunoglobulin and cytokine changes induced following immunization with a DNA vaccine encoding Toxoplasma gondii selenium dependent glutathione reductase protein. Experimental Parasitology. 2014; 146: 1–10.

41. Cao A, Liu Y, Wang J, Li X, Wang S, et al. Toxoplasma gondii: Vaccination with a DNA vaccine encoding T- and B-cell epitopes of SAG1, GRA2, GRA7 and ROP16 elicits protection against acute toxoplasmosis in mice. Vaccine. 2015; 33: 6757–6762.

42. Wang S, Hassan IA, Liu X, Xu L, Yan R, et al. Immunological changes induced by Toxoplasma gondii glutathione-S-transferase (TgGST) delivered as a DNA vaccine. Research in Veterinary Science. 2015; 99: 157–164.

43. Wang L, Lu G, Zhou A, Han Y, Guo J, et al. Evaluation of immune responses induced by rhoptry protein 5 and rhoptry protein 7 DNAvaccines against Toxoplasma gondii. Parasite Immunology. 2016; 38: 209–217.

44. Wang HL, Wang YJ, Pei YJ, Bai JZ, Yin LT, et al. DNA vaccination with a gene encoding Toxoplasma gondii rhoptry protein 17 induces partial protective immunity against lethal challenge in mice. Parasite. 2016; 23: 4.

45. Gong P, Cao L, Guo Y, Dong H, Yuan S, et al. Toxoplasma gondii: Protective immunity induced by a DNA vaccine expressing GRA1 and MIC3 against toxoplasmosis in BALB/c mice. Experimental Parasitology. 2016; 166: 131–136.

46. Sonaimuthu P, Ching XT, Fong MY, Kalyanasundaram R, Lau YL. Induction of protective immunity against toxoplasmosis in BALB/c mice vaccinated with Toxoplasma gondii rhoptry-1. Frontiers in Microbiology. 2016; 7: 808.

47. Ahmadpour E, Sarvi S, Hashemi Soteh MB, Sharif M, Rahimi MT, et al. Evaluation of the immune response in BALB/c mice induced by a novel DNA vaccine expressing GRA14 against Toxoplasma gondii. Parasite Immunology. 2017; 39.

48. Bin Zheng, Jianzu Ding, Xiaoheng Chen, Haijie Yu, Di Lou, et al. Immuno-efficacy of a T. gondii secreted protein with an altered thrombospondin repeat (TgSPATR) as a novel DNA vaccine candidate against acute toxoplasmosis in BALB/c mice. Frontiers in Microbiology. 2017; 8: 2016.

49. Yuan Liu, Aiping Cao, Yawen Li, Xun Li, Hua Cong, et al. Immunization with a DNA vaccine encoding Toxoplasma gondiisuperoxide dismutase (TgSOD) induces partial immune protection against acute toxoplasmosis in BALB/c mice. BMC Infectious Diseases. 2017; 17: 403.

50. Lu G, Zhou J, Zhou A, Han Y, Guo J, et al. SAG5B and SAG5C combined vaccine protects mice against Toxoplasma gondii infection. Parasitology International. 2017; 66: 596–602.

51. Zhu WN, Wang JL, Chen K, Yue DM, Zhang XX, et al. Evaluation of protective immunity induced by DNA vaccination with genes encoding Toxoplasma gondii GRA17 and GRA23 against acute toxoplasmosis in mice. Experimental Parasitology. 2017; 179: 20–27.

52. Ching XT, Fong MY, Lau YL. Evaluation of immunoprotection con-

ferred by the subunit vaccines of GRA2 and GRA5 against acute toxoplasmosis in BALB/c mice. Frontiers in Microbiology. 2016; 7: 609.

53. Yang WB, Zhou DH, Zou Y, Chen K, Liu Q, et al. Vaccination with a DNA vaccine encoding Toxoplasma gondii ROP54 induces protective immunity against toxoplasmosis in mice. Acta Tropica. 2017; 176: 427–432.

54. Han Y, Zhou A, Lu G, Zhao G, Sha W, et al. DNA vaccines encoding Toxoplasma gondii cathepsin C 1 induce protection against toxoplasmosis in mice. Korean Journal of Parasitology. 2017; 55: 505–512.

55. Chen K, Wang JL, Huang SY, Yang WB, Zhu WN, et al. Immune responses and protection after DNA vaccination against Toxoplasma gondii calcium-dependent protein kinase 2 (TgCDPK2). Parasite. 2017; 24: 41.

56. Qi Gao, Nian-Zhang Zhang, Fu-Kai Zhang, Meng Wang, Ling-Ying Hu, et al. Immune response and protective effect against chronic Toxoplasma gondii infection induced by vaccination with a DNA vaccine encoding profilin. BMC Infectious Diseases. 2018; 18: 117.

57. Chen Y, Yu M, Hemandez JA, Li J, Yuan ZG, et al. Immuno-efficacy of DNA vaccines encoding PLP1 and ROP18 against experimental Toxoplasma gondii infection in mice. Experimental Parasitology. 2018; 188: 73–78.

58. Flori P, Tardy L, Jacquet A, Bellete B, Hafid J, et al. Effect of rSAG-1(P30) immunisation on the circulating and tissue parasites in guinea pigs as determined by quantitative PCR. Parasitology Research. 2006; 98: 511–518.

59. Golkar M, Shokrgozar MA, Rafati S, Musset K, Assmar M, et al. Evaluation of protective effect of recombinant dense granule antigens GRA2 and GRA6 formulated in monophosphoryl lipid A (MPL) adjuvant against Toxoplasma chronic infection in mice. Vaccine. 2007; 25: 4301–4311.

60. Dziadek B, Gatkowska J, Brzostek A, Dziadek J, Dzitko K, et al. Toxoplasma gondii: the immunogenic and protective efficacy of recombinant ROP2 and ROP4 rhoptry proteins in murine experimental toxoplasmosis. Experimental Parasitology. 2009; 123: 81–89.

61. Huang X, Li J, Zhang G, Gong P, Yang J, et al. Toxoplasma gondii: Protective immunity against toxoplasmosis with recombinant actin depolymerizing factor protein in BALB/c mice. Experimental Parasitology. 2012; 130: 218–222.

62. Shu-Chun Chuang, Jing-Chun Ko, Chaio-Ping Chen, Jia-Tze Du, Chung-Da Yang. Induction of long-lasting protective immunity against Toxoplasma gondii in BALB/c mice by recombinant surface antigen 1 protein encapsulated in poly (lactide-co-glycolide) microparticles. Parasites&Vectors. 2013; 6: 34.

63. Zheng B, Lu S, Tong Q, Kong Q, Lou D. The virulence-related rhoptry protein 5 (ROP5) of Toxoplasma gondii is a novel vaccine candidate against toxoplasmosis in mice. Vaccine. 2013; 31: 4578– 4584.

64. Qu D, Han J, Du A. Enhancement of protective immune response to recombinant Toxoplasma gondii ROP18 antigen by ginsenoside Re. Experimental Parasitology. 2013; 135: 234–239.

65. Wang HL, Li YQ, Yin LT, Meng XL, Guo M, et al. Toxoplasma gondii protein disulfide isomerase (TgPDI) is a novel vaccine candidate against toxoplasmosis. PLOS ONE. 2013; 8: 8.

66. Tanaka S, Kuroda Y, Ihara F, Nishimura M, Hiasa J, et al. Vaccination with profilin encapsulated in oligomannose-coated liposomes induces significant protective immunity against Toxoplasma gondii. Vaccine. 2014; 32: 1781–1785.

67. Xu Y, Zhang NZ, Wang M, Dong H, Feng SY, et al. A long-lasting protective immunity against chronic toxoplasmosis in mice induced byrecombinant rhoptry proteins encapsulated in poly (lactide-co-glycolide) microparticles. Parasitology Research. 2015; 114: 4195–4203.

68. Grzybowski MM, Dziadek B, Gatkowska JM, Dzitko K, Długońska H. Towards vaccine against toxoplasmosis: evaluation of the immunogenic and protective activity of recombinant ROP5 and ROP18 Toxoplasma gondii proteins. Parasitology Research. 2015; 114: 4553–4563.

69. Pinzan CF, Sardinha-Silva A, Almeida F, Lai L, Lopes CD, et al. Vaccination with recombinant microneme proteins confers protection against experimental toxoplasmosis in mice. PLOS ONE. 2015; 10: e0143087.

70. Wang HL, Wen LM, Pei YJ, Wang F, Yin LT, et al. Recombinant Toxoplasma gondii phosphoglycerate mutase 2 confers protective immunity against toxoplasmosis in BALB/c mice. Parasite. 2016; 23: 12.

71. Zhang NZ, Xu Y, Wang M, Chen J, Huang SY, et al. Vaccination with Toxoplasma gondii calcium-dependent protein kinase 6 and rhoptry protein 18 encapsulated in poly (lactide-co-glycolide) microspheres induces long-term protective immunity in mice. BMC Infectious Diseases. 2016; 16: 168.

72. Fereig RM, Nishikawa Y. Peroxiredoxin 3 promotes IL-12 production from macrophages and partially protects mice against infection with Toxoplasma gondii. Parasitology International. 2016; 65: 741–748.

73. Zhuanzhuan Liu, Litian Yin, Yaqing Li, Fei Yuan, Xiaofan Zhang, et al. Intranasal immunization with recombinant Toxoplasma gondii actin depolymerizing factor confers protective efficacy against toxoplasmosis in mice. BMC Immunology. 2016; 17: 37.

74. Allahyari M, Mohabati R, Amiri S, Esmaeili Rastaghi AR, Babaie J, et al. Synergistic effect of rSAG1 and rGRA2 antigens formulated in PLGA microspheres in eliciting immune protection against Toxoplasma gondii. Experimental Parasitology. 2016; 170: 236–246.

75. Zhao G, Song X, Kong X, Zhang N, Qu S, et al. Immunization with Toxoplasma gondii aspartic protease 3 increases survival time of infected mice. Acta Tropica. 2017; 171: 17–23.

76. Wang S, Zhang Z, Wang Y, Gadahi JA, Xu L, et al. Toxoplasma gondiielongation factor 1-alpha (TgEF-1α) is a novel vaccine candidate antigen against toxoplasmosis. Frontiers in Microbiology. 2017; 8: 168.

77. Fereig RM, Kuroda Y, Terkawi MA, Mahmoud ME, Nishikawa Y. Immunization with Toxoplasma gondii peroxiredoxin 1 induces protective immunity against toxoplasmosis in mice. PLOS ONE. 2017; 12: e0176324.

78. Czarnewski P, Araújo ECB, Oliveira MC, Mineo TWP, Silva NM. Recombinant TgHSP70 immunization protects against Toxoplasma gondii brain cyst formation by enhancing inducible nitric oxide expression. Frontiers in Cellular and Infection Microbiology. 2017; 7: 142.

79. Picchio MS, Sánchez VR, Arcon N, Soto AS, Perrone Sibilia M, et al. Vaccine potential of antigen cocktails composed of recombinant Toxoplasma gondii TgPI-1, ROP2 and GRA4 proteins against chronic toxoplasmosis in C3H mice. Experimental Parasitology. 2018; 185: 62–70.

80. Gatkowska J, Wieczorek M, Dziadek B, Dzitko K, Dziadek J, et al. Assessment of the antigenic and neuroprotective activity of the subunit anti-Toxoplasma vaccine in T. gondii experimentally infected mice. Veterinary Parasitology. 2018; 254: 82–94.